

Preface

This third edition of *Endocrine Physiology* provides comprehensive

coverage of the

fundamental concepts of hormone biological action. The content has been

revised

and edited to enhance clarity and understanding mostly
based on comments

and

questions received from medical students. In addition, the test

questions at the

end of the chapter have been modified to reflect clinical scenarios that

require

the integration of the endocrine physiology concepts
presented in the

respective

chapter. New figures have been added to enhance the understanding of

important

endocrine physiology concepts.

The concepts herein provide the basis by which first and second year

medical

students will better grasp the physiologic mechanisms
involved in

neuroendocrine

regulation of organ function. The information presented is also meant to

serve as

a reference for residents and fellows. The objectives listed at the

beginning of each

chapter follow those established by the American
Physiological Society

for each

hormone system and are the topics tested in Step I of the United States

Medical

Licensing Exam (USMLE).

As with any discipline in science and medicine, our understanding of

endocrine

molecular physiology has changed and continues to evolve to encompass

neural, immune, and metabolic regulation and interaction.
The suggested

readings

have been updated to provide guidance for more in-depth understanding of

the concepts presented. They are by no means all inclusive, but were

found by the

author to be of great help in putting the information together.

The first chapter describes the organization of the endocrine system, as

well

as general concepts of hormone production and release,
transport and

metabolic

fate, and cellular mechanisms of action. Chapters 2–9
discuss specific

endocrine

systems. The specific hormone produced by each system is described in

the context

of the regulation of its production and release, the target physiologic

actions, and

the implications of excess or deficiency in its production of hormone

action. Each

chapter starts with a short description of the functional anatomy of the

organ,

highlighting important features pertaining to circulation, location, or

cellular

composition that have a direct impact on its endocrine function.

Understanding

the mechanisms underlying normal endocrine physiology is essential in

order

to understand the transition from health to disease and the rationale

involved

in pharmacological, surgical, or genetic interventions. Thus,
the

salient features

involved in determination of abnormal hormone production,
regulation or

function

are also described. Each chapter includes simple diagrams illustrating

some

of the key concepts presented and concludes with sample questions

designed to

test the overall assimilation of the information given. The key concepts

provided

in each chapter correspond to the particular section of the chapter that

describes

them. Chapter 10 illustrates how the individual endocrine systems

described

throughout the book dynamically interact in maintaining homeostasis. A

special

emphasis is placed on adipose tissue and its newly recognized role as an

endocrine

organ.

As with the previous two editions of this book, the modifications are

driven

by the questions raised by my students during lecture or
when studying

for an

exam. Those questions have been the best way of gauging the clarity of

the writing

and they have also alerted me when unnecessary description
complicated

or

obscured the understanding of a basic concept. Improved learning and

understanding

of the concepts by our students continues to be my
inspiration. I would

like to thank them, as well as all the faculty of the Department
of

Physiology at

LSUHSC for their dedication to the teaching of this discipline.

As

always, I thank

my friend, colleague and husband, Miguel F. Molina, MD, for
his

unconditional

support and constant reminder of what is really important in life.

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Authors

Patricia E. Molina, MD, PhD

Richard Ashman, PhD Professor and Head

Department of Physiology

Louisiana State University Health Sciences Center

New Orleans, Louisiana

OBJECTIVES

- ▶ *Contrast the terms endocrine, paracrine, and autocrine.*
- ▶ *Define the terms hormone, target cell, and receptor.*
- ▶ *Understand the major differences in mechanisms of action of peptides, steroid, and thyroid hormones.*
- ▶ *Compare and contrast hormone actions exerted via plasma membrane receptors with those mediated via intracellular receptors.*
- ▶ *Understand the role of hormone-binding proteins.*
- ▶ *Understand the feedback control mechanisms of hormone secretion.*
- ▶ *Explain the effects of secretion, degradation, and excretion on plasma hormone concentrations.*
- ▶ *Understand the basis of hormone measurements (eg, radioimmunoassay, immunometric assay) and their interpretation.*

GENERAL PRINCIPLES OF ENDOCRINE PHYSIOLOGY: INTRODUCTION

The function of the endocrine system is to coordinate and integrate cellular activity within the whole body by regulating cellular and organ function throughout life and maintaining **homeostasis**. Homeostasis, or the maintenance of a constant internal environment, is critical to ensuring appropriate cellular function.

THE ENDOCRINE SYSTEM: PHYSIOLOGIC FUNCTIONS & COMPONENTS

Some of the key functions of the endocrine system include:

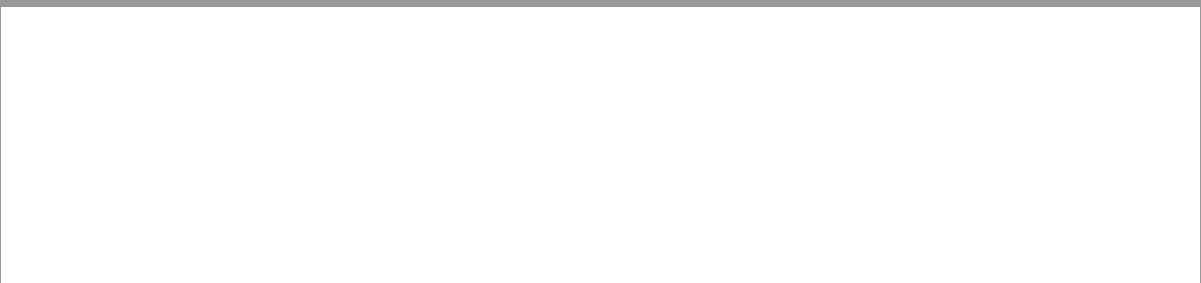
- Regulation of sodium and water balance and control of blood volume and pressure
- Regulation of calcium and phosphate balance to preserve extracellular fluid concentrations required for cell membrane integrity and intracellular signaling

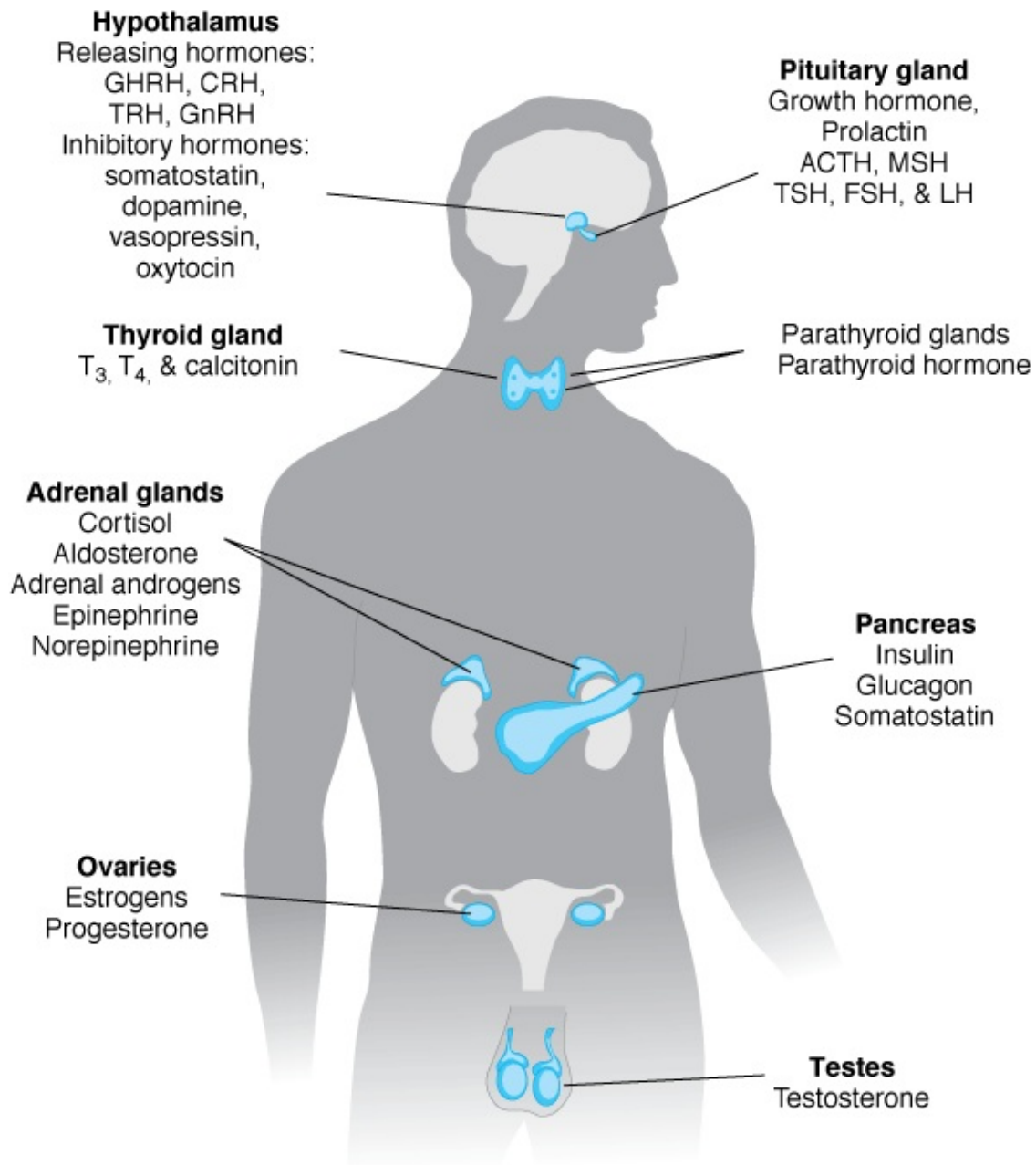
- Regulation of energy balance and control of fuel mobilization, utilization, and storage to ensure that cellular metabolic demands are met
- Coordination of the host hemodynamic and metabolic counterregulatory responses to stress
- Regulation of reproduction, development, growth, and senescence

In the classic description of the endocrine system, a chemical messenger or **hormone** produced by an organ is released into the circulation to produce an effect on a distant target organ. Currently, the definition of the endocrine system is that of an integrated network of multiple organs derived from different embryologic origins that release hormones ranging from small peptides to glycoproteins, which exert their effects either in neighboring or distant target cells. This endocrine network of organs and mediators does not work in isolation, and is closely integrated with the central and peripheral nervous systems as well as with the immune systems, leading to currently used terminologies such as "neuroendocrine" or "neuroendocrine-immune" systems for describing their interactions. Three basic components make up the core of the endocrine system.

Endocrine glands—The classic endocrine glands are ductless and secrete their chemical products (hormones) into the interstitial space from where they reach the circulation. Unlike the cardiovascular, renal, and digestive systems, the endocrine glands are not anatomically connected and are scattered throughout the body (Figure 1–1). Communication among the different organs is ensured through the release of hormones or neurotransmitters.

Figure 1–1.





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The endocrine system. Endocrine organs are located throughout the body, and their function is controlled by hormones delivered through the circulation or produced locally or by direct neuroendocrine stimulation. Integration of hormone production from endocrine organs is under regulation by the hypothalamus. ACTH, adrenocorticotrophic hormone; CRH, corticotropin-releasing hormone; FSH, follicle-

stimulating hormone; GHRH, growth hormone–releasing hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; MSH, melanocyte-stimulating hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; T_3 , triiodothyronine; T_4 , thyroxine.

Hormones—Hormones are chemical products, released in very small amounts from the cell that exert a biologic action on a target cell. Hormones can be released from the endocrine glands (ie, insulin, cortisol), the brain (ie, corticotropin-releasing hormone, oxytocin, and antidiuretic hormone), and other organs such as the heart (atrial natriuretic peptide), liver (insulin-like growth factor-I), and adipose tissue (leptin).

Target organ—The target organ contains cells that express hormone-specific receptors and that respond to hormone binding by a demonstrable biologic response.

HORMONE CHEMISTRY & MECHANISMS OF ACTION

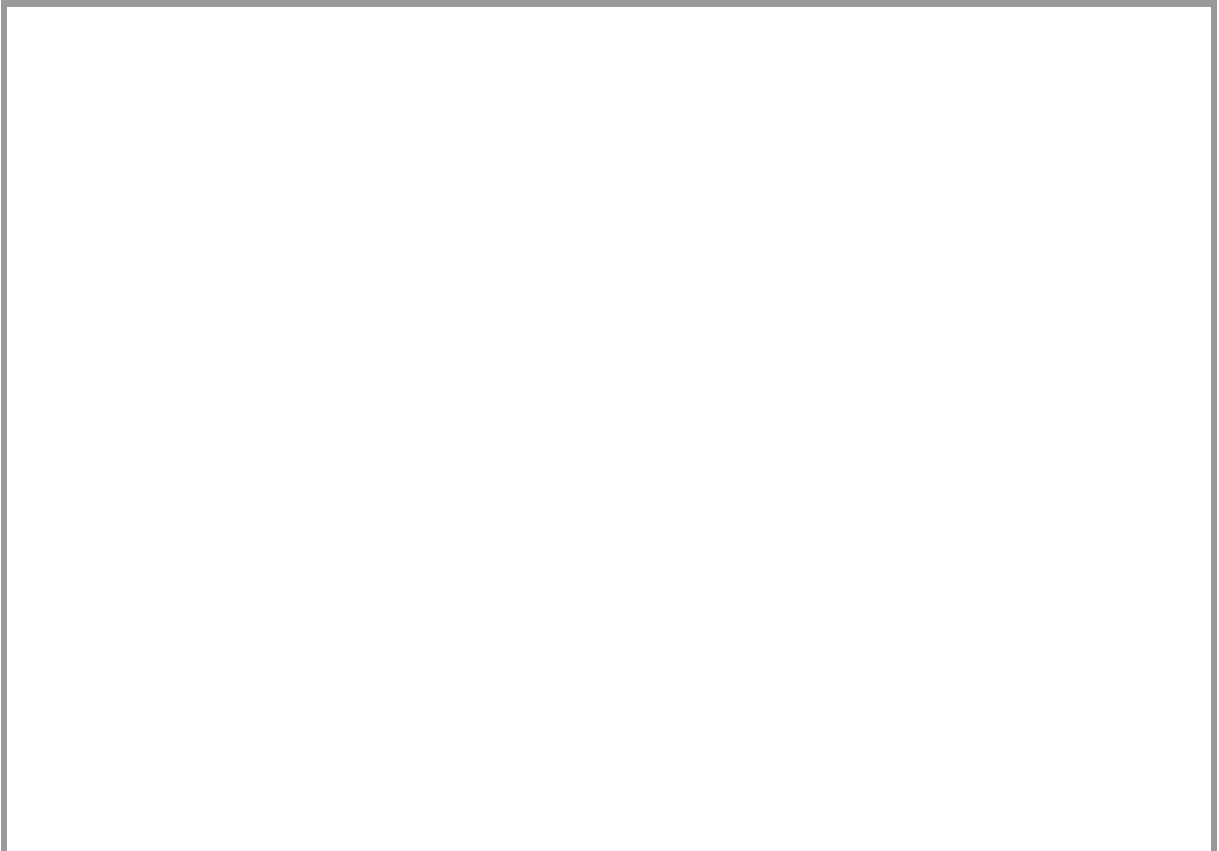


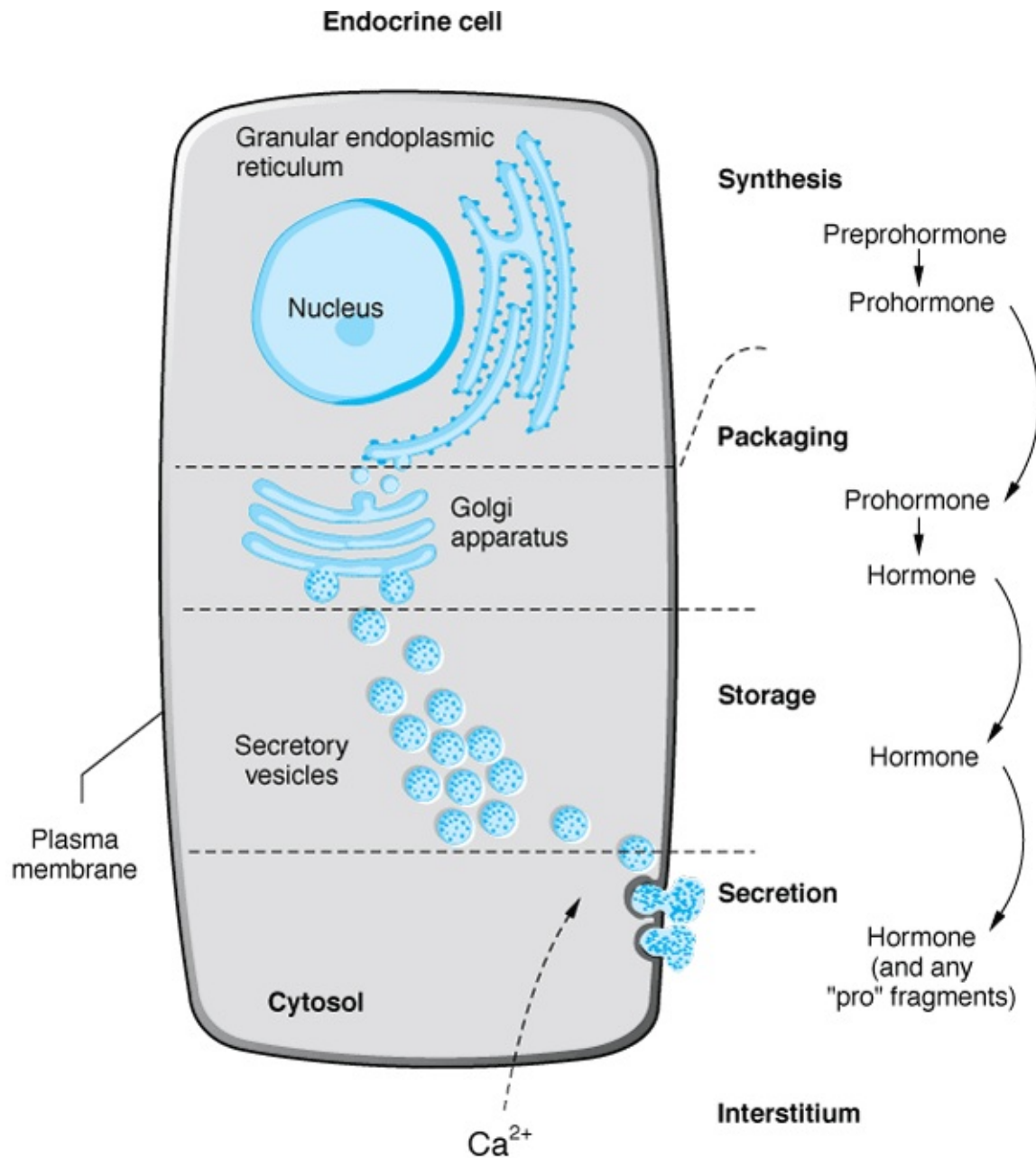
Based on their chemical structure, hormones can be classified into proteins (or peptides), steroids, and amino acid derivatives (amines). Hormone structure, to a great extent, dictates the location of the hormone receptor, with amines and peptide hormones binding to receptors in the cell surface and steroid hormones being able to cross plasma membranes and bind to intracellular receptors. An exception to this generalization is thyroid hormone, an amino acid–derived hormone, which is transported into the cell in order to bind to its nuclear receptor. Hormone structure influences the half-life of the hormone as well. Amines have the shortest half-life (2–3 minutes), followed by polypeptides (4–40 minutes), steroids and proteins (4–170 minutes), and thyroid hormones (0.75–6.7 days).

Protein or Peptide Hormones

Protein or peptide hormones constitute the majority of hormones. These are molecules ranging from 3 to 200 amino acid residues. They are synthesized as pre-prohormones and undergo post-translational processing. They are stored in secretory granules before being released by exocytosis (Figure 1–2), in a manner reminiscent of how neurotransmitters get released from nerve terminals. Examples of peptide hormones include insulin, glucagon, and adrenocorticotrophic hormone (ACTH). Some hormones in this category, such as the gonadotropic hormone, luteinizing hormone, and follicle-stimulating hormone, together with thyroid-stimulating hormone (TSH) and human chorionic gonadotropin, contain carbohydrate moieties, leading to their designation as glycoproteins. The carbohydrate moieties play important roles in determining the biologic activities and circulating clearance rates of glycoprotein hormones.

Figure 1–2.





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Peptide hormone synthesis. Peptide hormones are synthesized as pre-prohormones in the ribosomes and processed to prohormones in the endoplasmic reticulum (ER). In the Golgi apparatus, the hormone or prohormone is packaged in secretory vesicles, which are released from the cell in response to an influx of Ca^{2+} . The rise in cytoplasmic Ca^{2+} is required for docking of the secretory vesicles in the plasma

membrane and for exocytosis of the vesicular contents. The hormone and the products of the post-translational processing that occurs inside the secretory vesicles are released into the extracellular space. Examples of peptide hormones are adrenocorticotrophic hormone (ACTH), insulin, growth hormone, and glucagon.

Steroid Hormones

Steroid hormones are derived from cholesterol and are synthesized in the adrenal cortex, gonads, and placenta. They are lipid soluble, require binding proteins to circulate in plasma, and cross the plasma membrane to bind to intracellular cytosolic or nuclear receptors. Vitamin D and its metabolites are also considered steroid hormones. Steroid hormone synthesis is described in Chapters 5 and 6.

Amino Acid–Derived Hormones

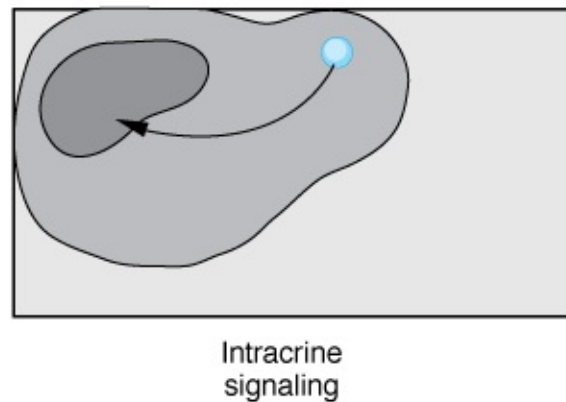
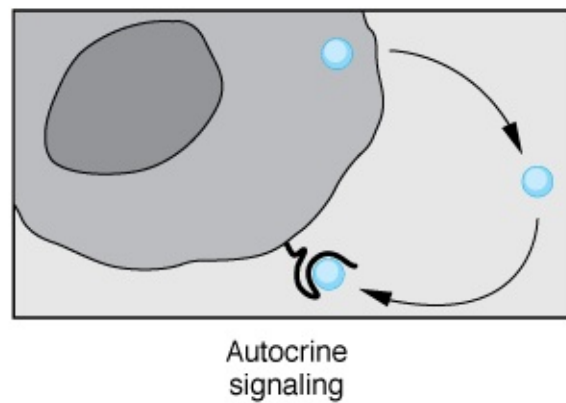
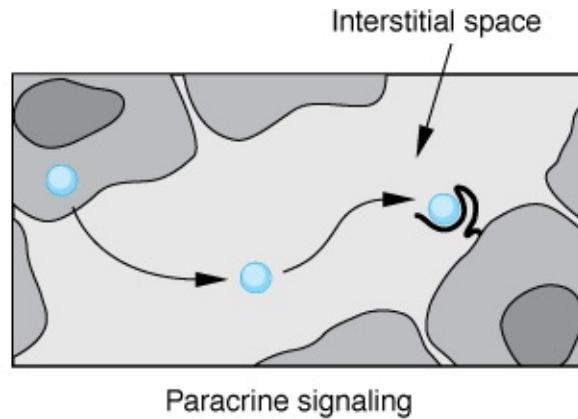
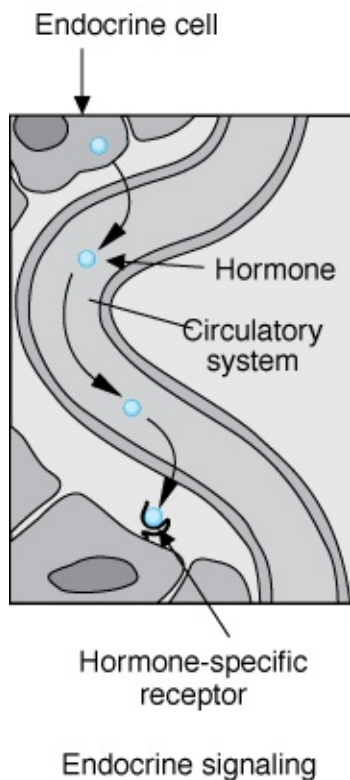
Amino acid–derived hormones are those hormones that are synthesized from the amino acid tyrosine and include the catecholamines, norepinephrine, epinephrine, and dopamine as well as the thyroid hormones derived from the combination of 2 iodinated tyrosine amino acid residues. The synthesis of thyroid hormone and catecholamines is described in Chapters 4 and 6, respectively.

Hormone Effects

Depending on where the biologic effect of a hormone is elicited in relation to where the hormone was released, its effects can be classified in 1 of 3 ways (Figure 1–3). The effect is **endocrine** or telecrine when a hormone is released into the circulation and then travels in the blood to produce a biologic effect on distant target cells. The effect is **paracrine** when a hormone released from one cell produces a biologic effect on a neighboring cell, which is frequently a cell in the same organ or tissue. The effect is **autocrine** when a hormone produces a biologic effect on the same cell that released it.

Figure 1–3.





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Mechanisms of hormone action. Depending on where hormones exert their effects, they can be classified into endocrine, paracrine, and autocrine mediators. Hormones that enter the bloodstream and bind to hormone receptors in target cells in distant organs mediate endocrine effects. Hormones that bind to cells near the cell that

released them mediate paracrine effects. Hormones that produce their physiologic effects by binding to receptors on the same cell that produced them mediate autocrine effects.

Recently, an additional mechanism of hormone action has been proposed in which a hormone is synthesized and acts intracellularly in the same cell. This mechanism has been termed **intracrine** and has been identified to be involved in the effects of parathyroid hormone–related peptide in malignant cells and in some of the effects of androgen-derived estrogen (Chapter 9).

Hormone Transport



Hormones released into the circulation can circulate either freely or bound to carrier proteins, also known as binding proteins. The binding proteins serve as a reservoir for the hormone and prolong the hormone's **half-life**, the time during which the concentration of a hormone decreases to 50% of its initial concentration. The free or unbound hormone is the active form of the hormone, which binds to the specific hormone receptor. Thus, hormone binding to its carrier protein serves to regulate the activity of the hormone by determining how much hormone is free to exert a biologic action. Most carrier proteins are globulins and are synthesized in the liver. Some of the binding proteins are specific for a given protein, such as cortisol-binding proteins. However, proteins such as globulins and albumin are known to bind hormones as well. Because for the most part, these proteins are synthesized in the liver, alterations in hepatic function may result in abnormalities in binding protein levels and may indirectly affect total hormone levels. In general, the majority of amines, peptides, and protein (hydrophilic) hormones circulate in their free form. However, a notable exception to this rule is the binding of the insulin-like growth factors to 1 of 6 different high-affinity binding proteins. Steroid and thyroid hormones circulate bound to specific transport proteins.

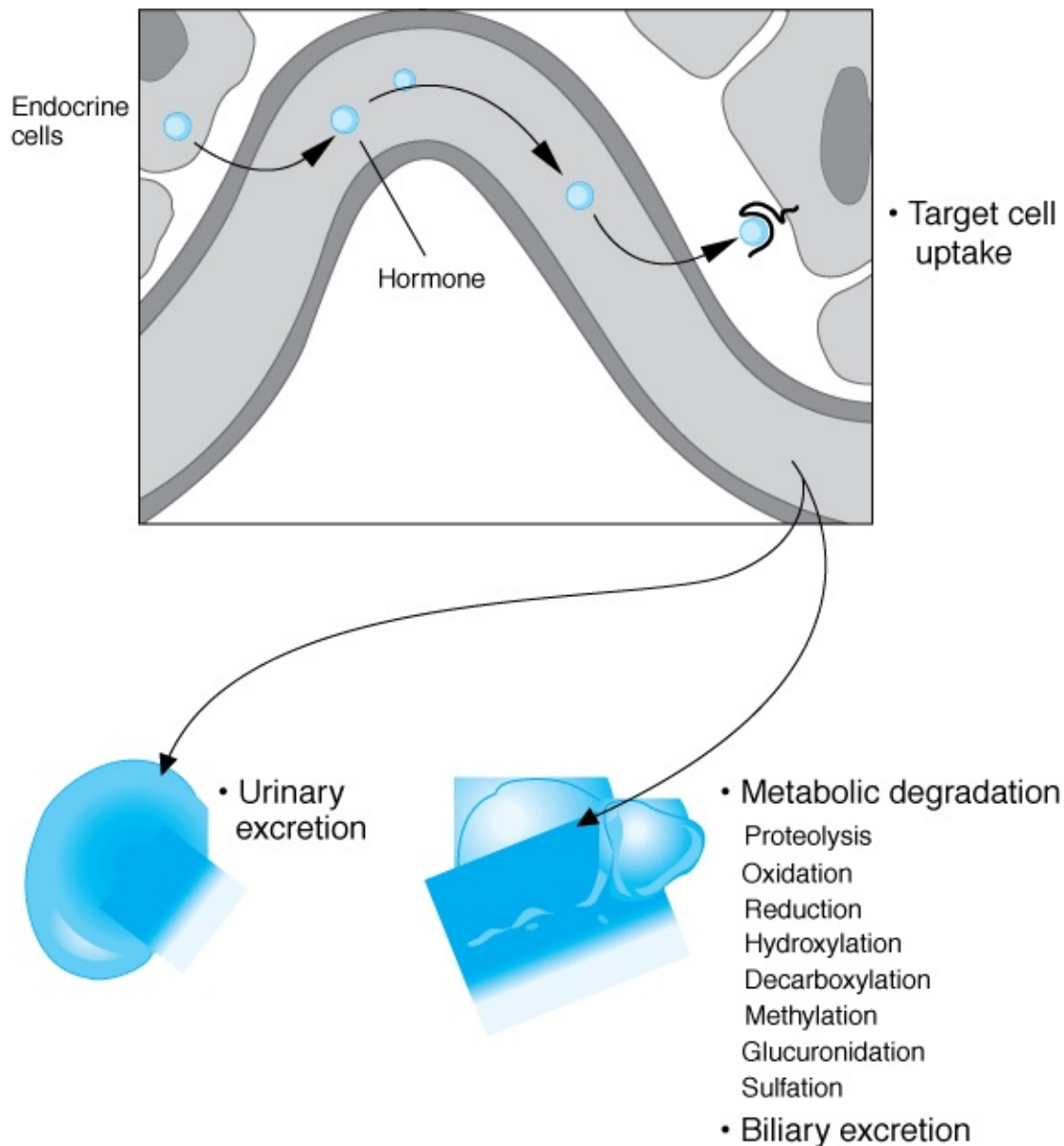
The interaction between a given hormone and its carrier protein is in a **dynamic equilibrium** and allows adjustments that prevent clinical manifestations of hormone deficiency or excess. Secretion of the hormone is adjusted rapidly following changes in the levels of carrier proteins. For example, plasma levels of cortisol-binding protein increase during pregnancy. Cortisol is a steroid hormone produced by the adrenal cortex (Chapter 6). The increase in circulating levels of cortisol-binding protein leads to an increased binding capacity for cortisol and a resulting decrease in free cortisol levels. This decrease in free cortisol stimulates the hypothalamic release of corticotropin-releasing hormone, which stimulates ACTH release from the anterior pituitary and consequently cortisol synthesis and release from the

adrenal glands. The cortisol, released in greater amounts, binds to the cortisol-binding protein. This **feedback mechanism** restores free cortisol levels and prevents manifestation of cortisol deficiency.

As already mentioned, the binding of a hormone to a binding protein prolongs its half-life. The half-life of a hormone is inversely related to its removal from the circulation. Removal of hormones from the circulation is also known as the **metabolic clearance rate**: the volume of plasma cleared of the hormone per unit of time. Once hormones are released into the circulation, they can bind to their specific receptor in a target organ, they can undergo metabolic transformation by the liver, or they can undergo urinary excretion (Figure 1–4). In the liver, hormones can be inactivated through Phase I (hydroxylation or oxidation) and/or Phase II (glucuronidation, sulfation, or reduction with glutathione) reactions, and then excreted by the liver through the bile or by the kidney. In some instances, the liver can actually activate a hormone precursor, as is the case for vitamin D synthesis, discussed in Chapter 5. Hormones can be degraded at their target cell through internalization of the hormone-receptor complex followed by lysosomal degradation of the hormone. Only a very small fraction of total hormone production is excreted intact in the urine and feces.

Figure 1–4.





$$\text{MCR} = \frac{\text{mg/min (removed)}}{\text{mg/mL (plasma)}} = \frac{\text{mL plasma (cleared)}}{\text{min}}$$

Volume of plasma cleared of hormone per unit of time

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Hormone metabolic fate. The sum of the hormone's removal from the organism is referred to as the metabolic clearance rate (MCR). This process includes metabolic degradation, which occurs mainly in the

kidney and the liver through enzymatic processes such as proteolysis, oxidation, reduction, hydroxylation, decarboxylation, and methylation. Excretion can also be achieved by glucuronidation and sulfation and bile or urinary excretion. In addition, the target cell may internalize the hormone and degrade it. The kidney has an important role in eliminating hormone and its degradation products from the body. In some cases, urinary determinations of a hormone or its metabolite are used to assess function of a particular endocrine organ based on the assumption that renal function and handling of the hormone are normal.

HORMONE CELLULAR EFFECTS



The biologic response to hormones is elicited through binding to hormone-specific receptors at the target organ. Hormones circulate in very low concentrations (10^{-7} to 10^{-12} M), so the receptor must have high affinity and specificity for the hormone to produce a biologic response.

Affinity is determined by the rates of dissociation and association for the hormone-receptor complex under equilibrium conditions. The equilibrium dissociation constant (K_d) is defined as the hormone concentration required for binding 50% of the receptor sites. The lower the K_d the higher the affinity of binding. Basically, affinity is a reflection of how tight the hormone-receptor interaction is. **Specificity** is the ability of a hormone receptor to discriminate among hormones with related structures. This is a key concept that has clinical relevance as will be discussed in Chapter 6 as it pertains to cortisol and aldosterone receptors.

The binding of hormones to their receptors is saturable, with a finite number of hormone receptors to which a hormone can bind. In most target cells, the maximal biologic response to a hormone can be achieved without reaching 100% hormone-receptor occupancy. The receptors that are not occupied are called spare receptors. Frequently, the hormone-receptor occupancy needed to produce a biologic response in a given target cell is very low; therefore, a decrease in the number of receptors in target tissues may not necessarily lead to an immediate impairment in hormone action. For example, insulin-mediated cellular effects occur when less than 3% of the total number of receptors in adipocytes is occupied.

Abnormal endocrine function is the result of either excess or deficiency in hormone action. This can result from abnormal production of a given hormone (either in excess or in insufficient amounts) or from decreased receptor number or function. Hormone-receptor agonists and antagonists are widely used clinically to restore endocrine function in patients with hormone deficiency or excess. Hormone-receptor

agonists are molecules that bind the hormone receptor and produce a biologic effect similar to that elicited by the hormone. Hormone-receptor antagonists are molecules that bind to the hormone receptor and inhibit the biologic effects of a particular hormone.

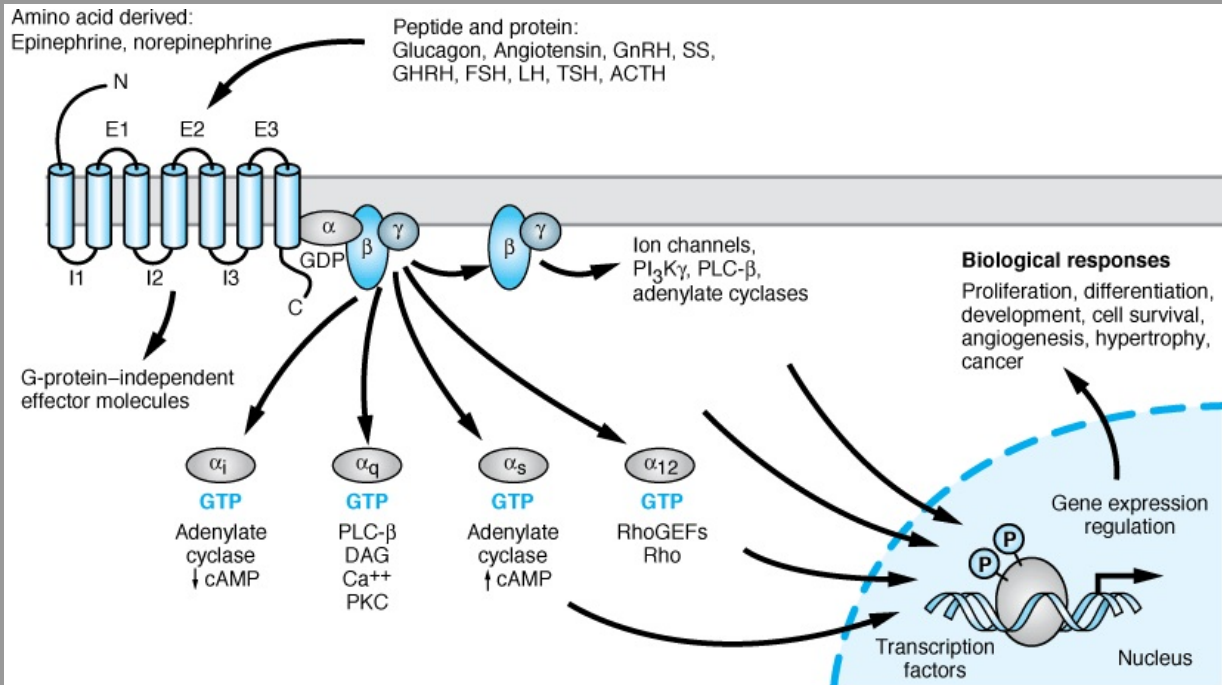
HORMONE RECEPTORS & SIGNAL TRANSDUCTION

As mentioned previously, hormones produce their biologic effects by binding to specific hormone receptors in target cells, and the type of receptor to which they bind is largely determined by the hormone's chemical structure. Hormone receptors are classified depending on their cellular localization, as **cell membrane** or **intracellular receptors**. Peptides and catecholamines are unable to cross the cell membrane lipid bilayer and in general bind to cell membrane receptors, with the exception of thyroid hormones as mentioned above. Thyroid hormones are transported into the cell and bind to nuclear receptors. Steroid hormones are lipid soluble, cross the plasma membrane, and bind to intracellular receptors.

Cell Membrane Receptors

These receptor proteins are located within the phospholipid bilayer of the cell membrane of target cells (Figure 1–5). Binding of hormones (ie, catecholamines, peptide and protein hormones) to cell membrane receptors and formation of the **hormone-receptor complex** initiates a signaling cascade of intracellular events, resulting in a specific biologic response. Functionally, cell membrane receptors can be divided into ligand-gated ion channels and receptors that regulate activity of intracellular proteins.

Figure 1–5.



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G protein–coupled receptors. Peptide and protein hormones bind to cell-surface receptors coupled to G proteins. Binding of the hormone to the receptor produces a conformational change that allows the receptor to interact with the G proteins. This results in the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) and activation of the G protein. The second-messenger systems that are activated vary depending on the specific receptor, the α -subunit of the G

protein associated with the receptor, and the ligand it binds. Examples of hormones that bind to G protein–coupled receptors are thyroid hormone, arginine vasopressin, parathyroid hormone, epinephrine, and glucagon. ACTH, adrenocorticotrophic hormone; ADP, adenosine diphosphate; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; FSH, follicle-stimulating hormone; GHRH, growth hormone–releasing hormone; IP₃, inositol trisphosphate; LH, luteinizing hormone; PI₃K, phosphatidylinositol-3-kinase; PIP₂, phosphatidylinositol bisphosphate; PKC, protein kinase C; PLC, phospholipase C; RhoGEFs, Rho guanine-nucleotide exchange factors; TSH, thyroid-stimulating hormone.

LIGAND-GATED ION CHANNELS

These receptors are functionally coupled to ion channels. Hormone binding to this receptor produces a conformational change that opens ion channels on the cell membrane, producing ion fluxes in the target cell. The cellular effects occur within seconds of hormone binding.

RECEPTORS THAT REGULATE ACTIVITY OF INTRACELLULAR PROTEINS

These receptors are transmembrane proteins that transmit signals to intracellular targets when activated. Ligand binding to the receptor on the cell surface and activation of the associated protein initiate a signaling cascade of events that activates intracellular proteins and enzymes and that can include effects on gene transcription and expression. The main types of cell membrane hormone receptors in this category are the **G protein–coupled receptors** and the **receptor protein tyrosine kinases**. An additional type of receptor, the receptor-linked kinase receptor, activates intracellular kinase activity following binding of the hormone to the plasma membrane receptor. This type of receptor is used in producing the physiologic effects of growth hormone (Figure 1–5).

G protein–coupled receptors—G protein–coupled receptors are single polypeptide chains that have 7 transmembrane domains and are coupled to heterotrimeric guanine-binding proteins (G proteins) consisting of 3 subunits: α , β , and γ . Hormone binding to the G protein–

coupled receptor produces a conformational change that induces interaction of the receptor with the regulatory G protein, stimulating the release of guanosine diphosphate (GDP) in exchange for guanosine triphosphate (**GTP**), resulting in activation of the G protein (Figure 1–5). The activated G protein (bound to GTP) dissociates from the receptor **followed by dissociation of the α from the $\beta\gamma$ subunits**. The subunits activate intracellular targets, which can be either an ion channel or an enzyme. Hormones that use this type of receptor include TSH, vasopressin or antidiuretic hormone, and catecholamines.

The 2 main enzymes that interact with G proteins are adenylate cyclase and phospholipase C, and this selectivity of interaction is dictated by the type of G protein with which the receptor is associated. On the basis of the G_α subunit, G proteins can be classified into 4 families associated with different effector proteins. The signaling pathways of 3 of these have been extensively studied. The G_{α_s} activates adenylate cyclase, G_{α_i} inhibits adenylate cyclase, and G_{α_q} activates phospholipase C; the second-messenger pathways used by $G_{\alpha_{12}}$ have not been completely elucidated.

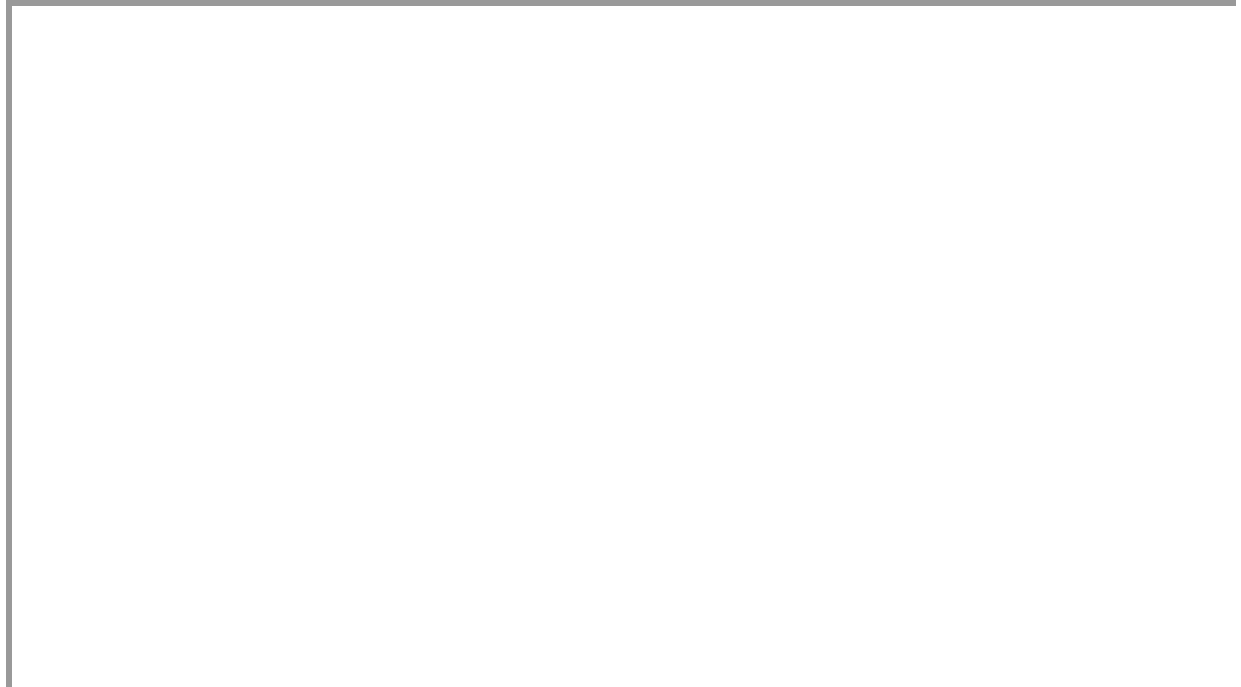
The interaction of G_{α_s} with adenylate cyclase and its activation result in increased conversion of adenosine triphosphate to cyclic 3', 5'-adenosine monophosphate (cAMP), with the opposite response elicited by binding to G_{α_i} -coupled receptors. The rise in intracellular cAMP activates protein kinase A, which in turn phosphorylates effector proteins, responsible for producing cellular responses. The action of cAMP is terminated by the breakdown of cAMP by the enzyme phosphodiesterase. In addition, the cascade of protein activation can also be controlled by phosphatases; which dephosphorylate proteins. Phosphorylation of proteins does not necessarily result in activation of an enzyme. In some cases, phosphorylation of a given protein results in inhibition of its activity.

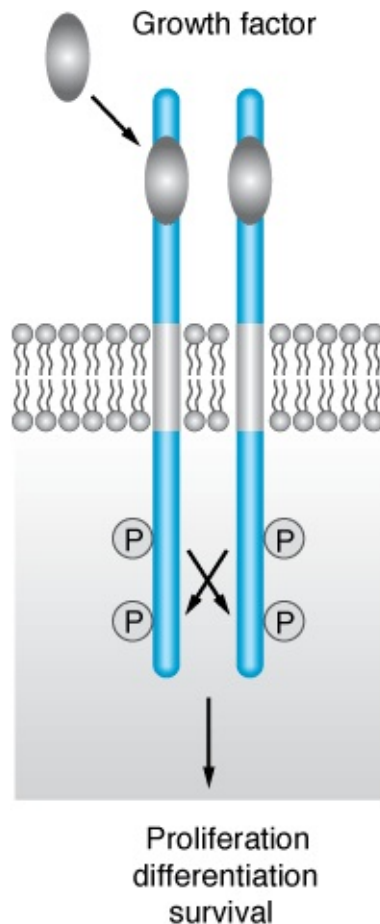
G_{α_q} activation of phospholipase C results in the hydrolysis of phosphatidylinositol bisphosphate and the production of diacylglycerol (DAG) and inositol trisphosphate (IP_3). DAG activates protein kinase C, which phosphorylates effector proteins. IP_3 binds to calcium channels in the endoplasmic reticulum, leading to an increase of Ca^{2+} influx into the

cytosol. Ca^{2+} can also act as a second messenger by binding to cytosolic proteins. One important protein in mediating the effects of Ca^{2+} is calmodulin. Binding of Ca^{2+} to calmodulin results in the activation of proteins, some of which are kinases, leading to a cascade of phosphorylation of effector proteins and cellular responses. An example of a hormone that uses Ca^{2+} as a signaling molecule is oxytocin discussed in Chapter 2.

Receptor protein tyrosine kinases—Receptor protein tyrosine kinases are usually single transmembrane proteins that have intrinsic enzymatic activity (Figure 1–6). Examples of hormones that use these types of receptors are insulin and growth factors. Hormone binding to these receptors activates their intracellular kinase activity, resulting in phosphorylation of tyrosine residues on the catalytic domain of the receptor itself, increasing its kinase activity. Phosphorylation outside the catalytic domain creates specific binding or docking sites for additional proteins that are recruited and activated, initiating a downstream signaling cascade. Most of these receptors consist of single polypeptides, although some, like the insulin receptor, are dimers consisting of 2 pairs of polypeptide chains.

Figure 1–6.





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Receptor kinase and receptor-linked kinase receptors. Receptor kinases have intrinsic tyrosine or serine kinase activity, which is activated by binding of the hormone to the amino terminal of the cell membrane receptor. The activated kinase recruits and phosphorylates downstream proteins, producing a cellular response. One hormone that uses this receptor pathway is insulin. Receptor-linked tyrosine kinase receptors do not have intrinsic activity in their intracellular domain. They are closely associated with kinases that are activated with binding of the hormone. Examples of hormones using this mechanism are growth hormone and prolactin.

Hormone binding to cell-surface receptors results in rapid activation of cytosolic proteins and cellular responses. Through protein phosphorylation, hormone binding to cell-surface receptors can also alter the transcription of specific genes through the phosphorylation of

transcription factors. An example of this mechanism of action is the phosphorylation of the transcription factor cAMP response element-binding protein (CREB) by protein kinase A in response to receptor binding and adenylate cyclase activation. This same transcription factor (CREB) can be phosphorylated by calcium-calmodulin following hormone binding to receptor tyrosine kinase and activation of phospholipase C. Therefore, hormone binding to cell-surface receptors can elicit immediate responses when the receptor is coupled to an ion channel or through the rapid phosphorylation of preformed cytosolic proteins, and it can also activate gene transcription through phosphorylation of transcription factors.

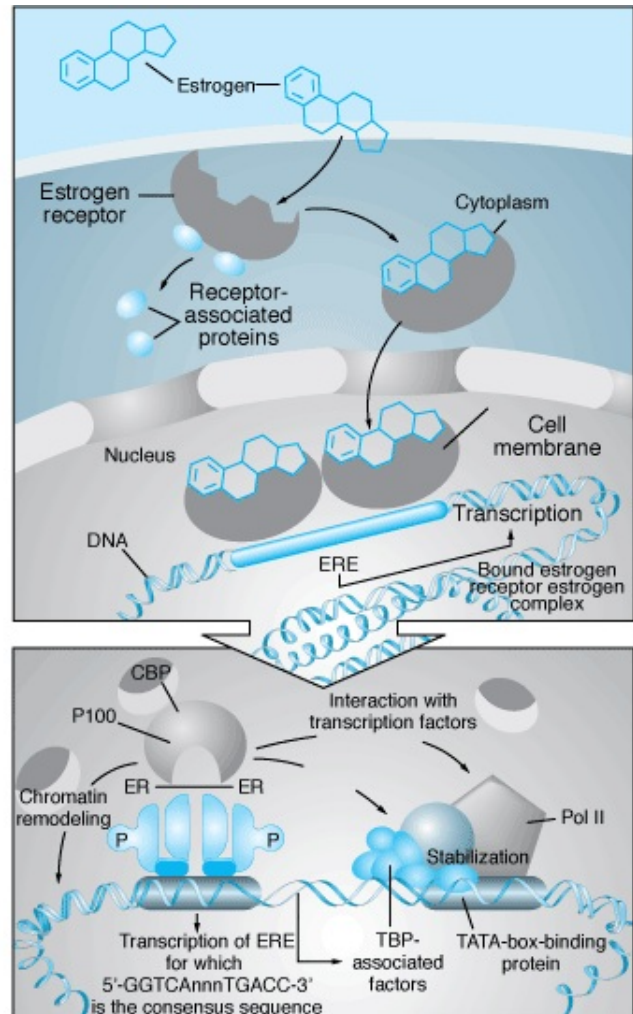
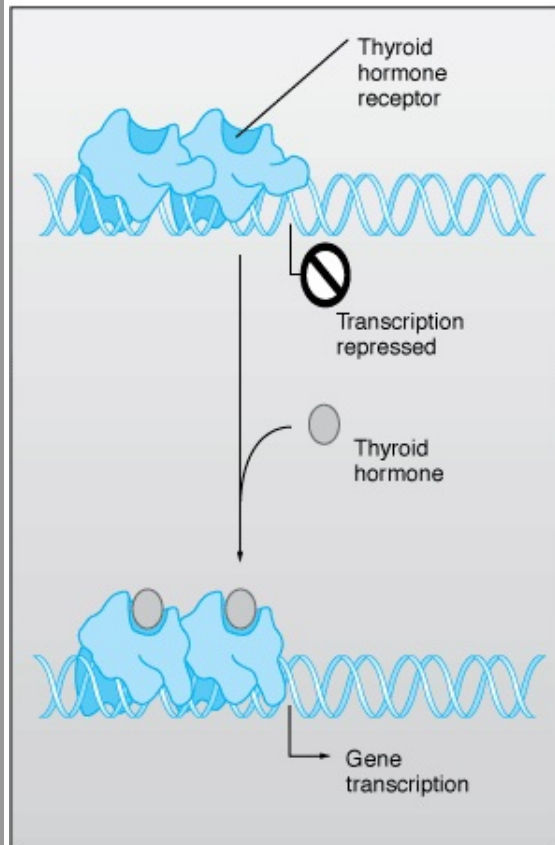
Intracellular Receptors

Receptors in this category belong to the **steroid receptor superfamily** (Figure 1–7). These receptors are transcription factors that have binding sites for the hormone (ligand) and for DNA and function as ligand (hormone)-regulated transcription factors. Hormone-receptor complex formation and binding to DNA result in either activation or repression of gene transcription. Binding to intracellular hormone receptors requires that the hormone be hydrophobic and cross the plasma membrane. Steroid hormones and the steroid derivative vitamin D₃ fulfill this requirement (Figure 1–7). Thyroid hormones must be actively transported into the cell.

Figure 1–7.



Intracellular receptors



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Intracellular receptors. Two general types of intracellular receptors can be identified. The unoccupied thyroid hormone receptor is bound to DNA and it represses transcription. Binding of thyroid hormone to the receptor allows for gene transcription to take place. Therefore, thyroid hormone receptor, acts as a repressor in the absence of the hormone, but hormone binding converts it to an activator that stimulates transcription of thyroid-hormone inducible genes. The steroid receptor, such as that used by estrogen, progesterone, cortisol, and aldosterone, is not able to bind to DNA in the absence of the hormone. Following steroid hormone binding to its receptor (estrogen shown in the figure) the receptor dissociates from receptor-associated chaperone proteins. The hormone-receptor complex translocates to the nucleus, where it binds to its specific responsive element on the DNA and initiates gene

transcription. (Reproduced, with permission, from Gruber *et al.* Mechanisms of disease: Production and actions of estrogens. *NEJM*. 2002;346(5):340. Copyright © Massachusetts Medical Society. All rights reserved.)

The distribution of the unbound intracellular hormone receptor can be cytosolic or nuclear. Hormone-receptor complex formation with cytosolic receptors produces a conformational change that allows the hormone-receptor complex to enter the nucleus and bind to specific DNA sequences to regulate gene transcription. Once in the nucleus, the receptors regulate transcription by binding, generally as dimers, to hormone response elements normally located in regulatory regions of target genes. In all cases, hormone binding leads to a nearly complete nuclear localization of the hormone-receptor complex. Unbound intracellular receptors may be located in the nucleus, as in the case of thyroid hormone receptors. The unoccupied thyroid receptor represses transcription of genes. Binding of thyroid hormone to the receptor activates gene transcription.

Hormone Receptor Regulation

Hormones can influence responsiveness of the target cell by modulating receptor function. Target cells are able to detect changes in hormone signal over a very wide range of stimulus intensities. This requires the ability to undergo a reversible process of adaptation or **desensitization**, whereby a prolonged exposure to a hormone decreases the cells' response to that level of hormone. This allows cells to respond to *changes* in the concentration of a hormone (rather than to the absolute concentration of the hormone) over a very wide range of hormone concentrations. Several mechanisms can be involved in desensitization to a hormone. Hormone binding to cell-surface receptors, for example, may induce their endocytosis and temporary sequestration in endosomes. Such hormone-induced receptor endocytosis can lead to the destruction of the receptors in lysosomes, a process that leads to *receptor downregulation*. In other cases, desensitization results from a rapid inactivation of the receptors for example, as a result of a receptor phosphorylation. Desensitization can also be caused by a change in a protein involved in signal transduction following hormone binding to the receptor or by the production of an inhibitor that blocks the transduction process. In addition, one hormone can downregulate or decrease the expression of receptors for another hormone and reduce that hormone's effectiveness. Hormone receptors can also undergo upregulation.

Upregulation of receptors involves an increase in the number of receptors for the particular hormone and frequently occurs when the prevailing levels of the hormone have been low for some time. The result is an increased responsiveness to the physiologic effects of the hormone at the target tissue when the levels of the hormone are restored or when an agonist to the receptor is administered. A hormone can also upregulate the receptors for another hormone, increasing the effectiveness of that hormone at its target tissue. An example of this type of interaction is the upregulation of cardiac myocyte adrenergic receptors following sustained elevations in thyroid hormone levels.

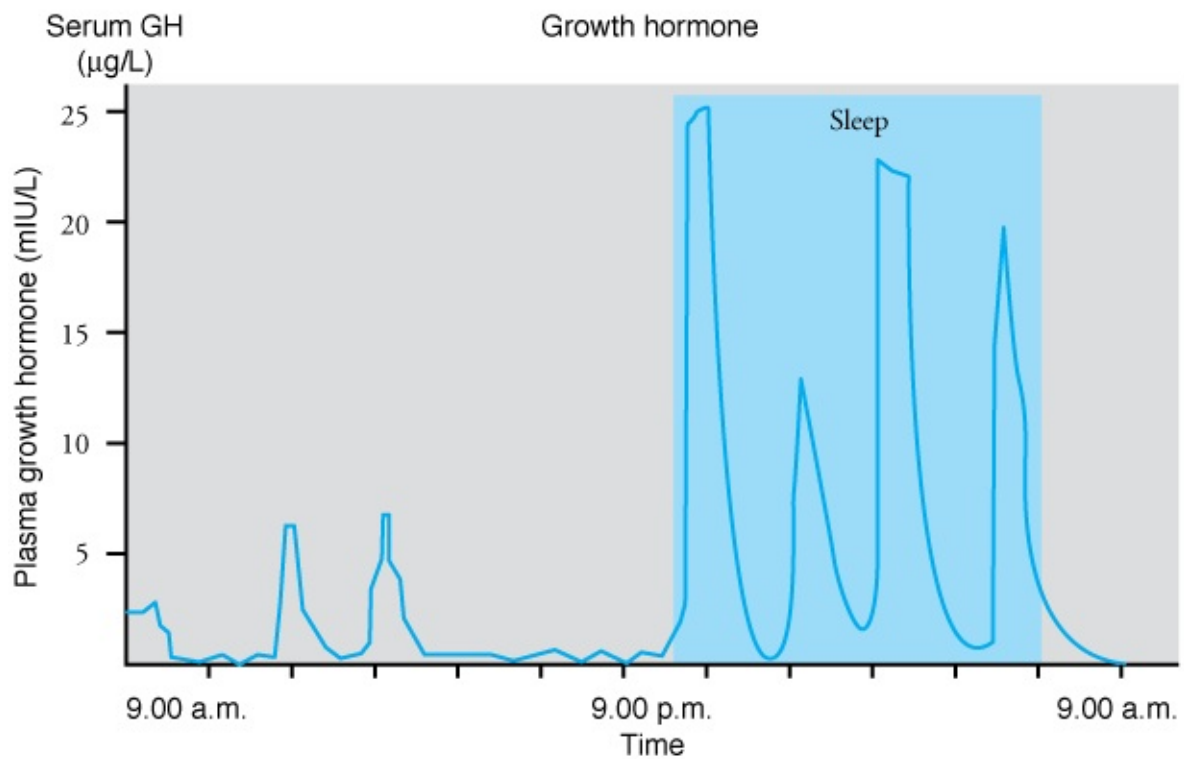
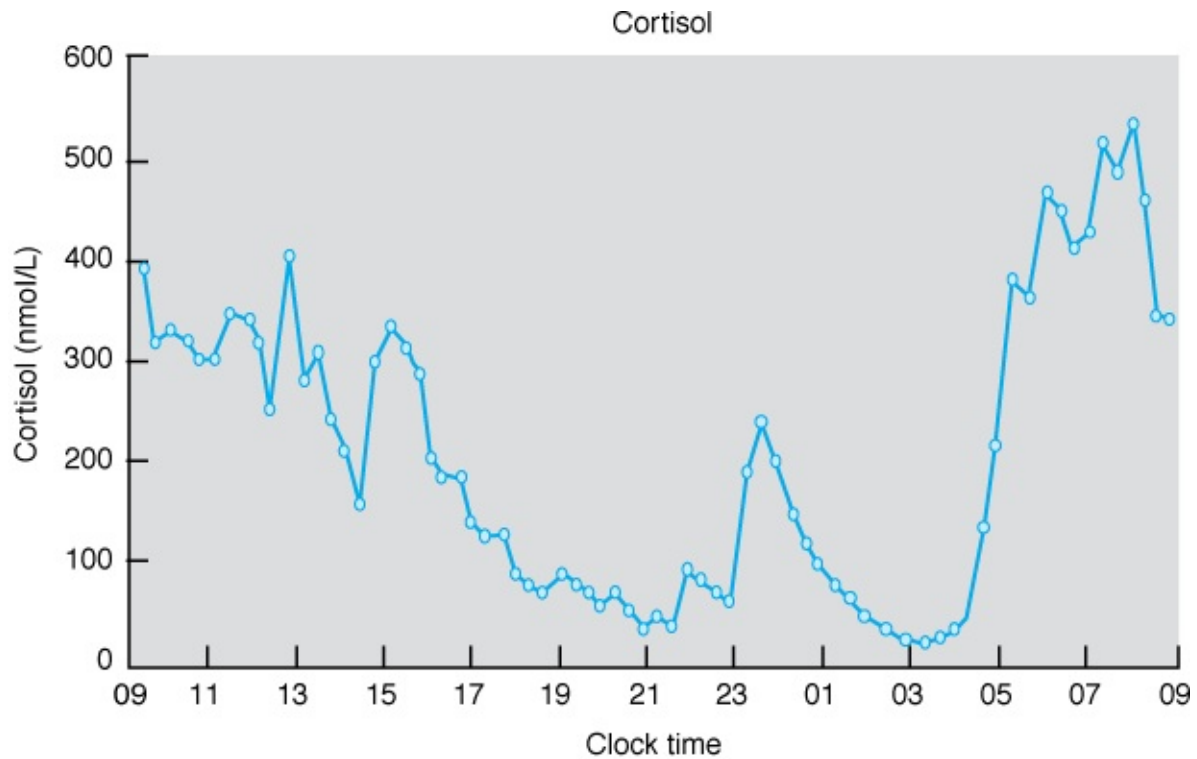
CONTROL OF HORMONE RELEASE



The secretion of hormones involves synthesis or production of the hormone and its release from the cell. In general, the discussion of regulation of hormone release in this section refers to both synthesis and secretion; specific aspects pertaining to the differential control of synthesis and release of specific hormones will be discussed in the respective chapters when they are considered of relevance.

Plasma levels of hormones oscillate throughout the day, showing peaks and troughs that are hormone specific (Figure 1–8). This variable pattern of hormone release is determined by the interaction and integration of multiple control mechanisms, which include hormonal, neural, nutritional, and environmental factors that regulate the constitutive (basal) and stimulated (peak levels) secretion of hormones. The periodic and pulsatile release of hormones is critical in maintaining normal endocrine function and in exerting physiologic effects at the target organ. The important role of the hypothalamus, and particularly of the photo-neuro-endocrine system in control of hormone pulsatility is discussed in Chapter 2. Although the mechanisms that determine the pulsatility and periodicity of hormone release are not completely understood for all the different hormones, 3 general mechanisms can be identified as common regulators of hormone release.

Figure 1–8.



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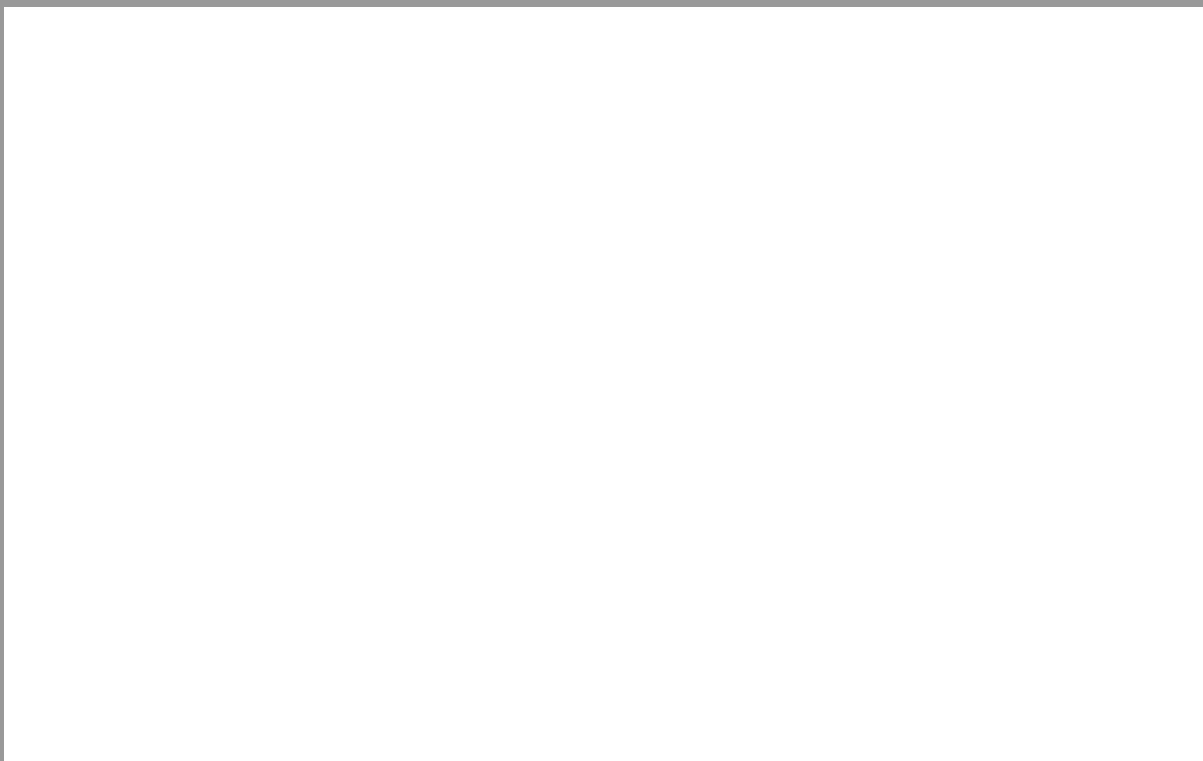
Patterns of hormone release. Plasma hormone concentrations

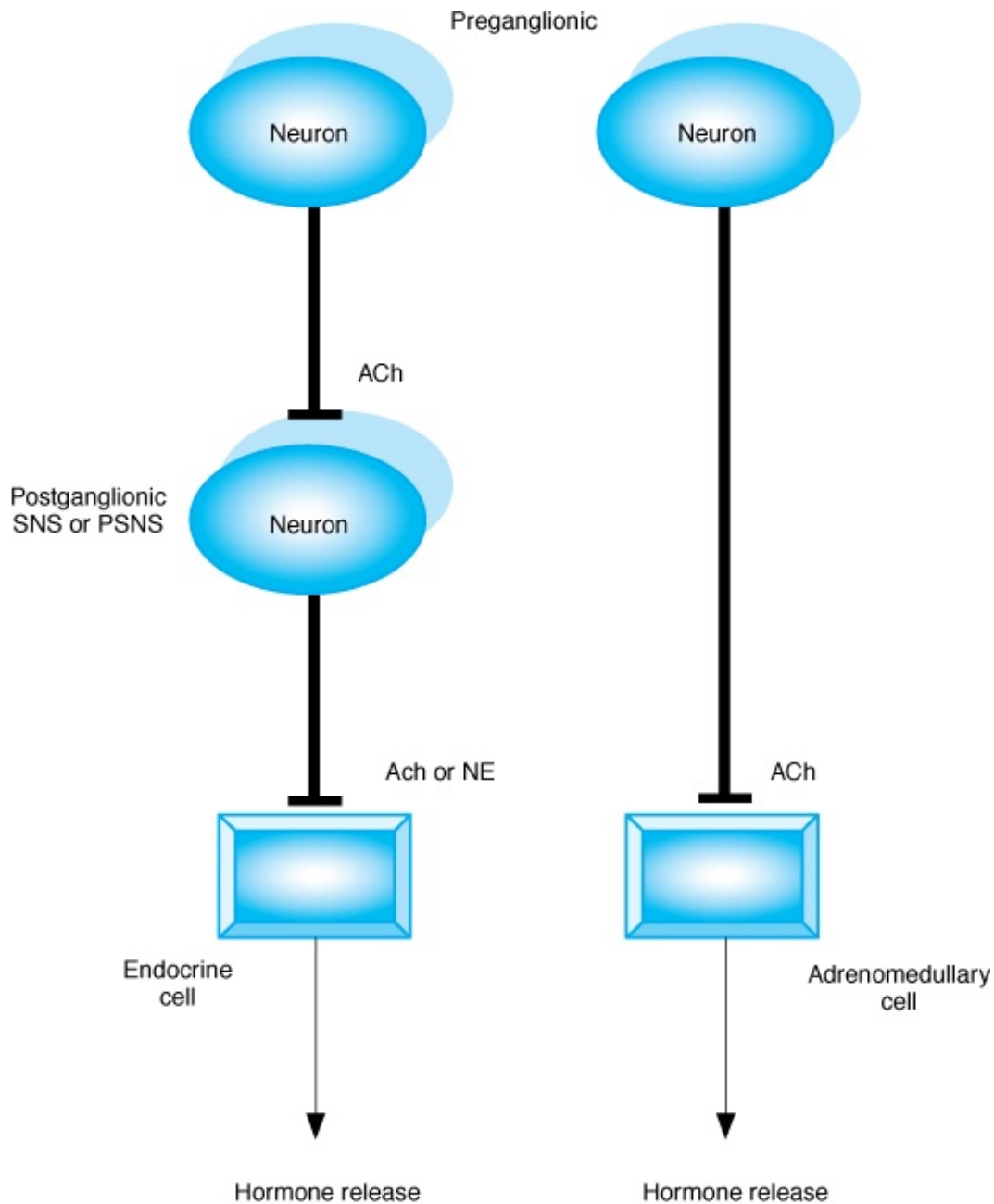
fluctuate throughout the day. Therefore, plasma hormone measurements do not always reflect the function of a given endocrine system. Both cortisol and growth hormone show considerable variations in blood levels throughout the day. These levels can also be affected by sleep deprivation, light, stress, and disease, and are dependent on their secretion rate, rates of metabolism and excretion, metabolic clearance rate, circadian pattern, fluctuating environmental stimuli, and internal endogenous oscillators. Biologic influences include illness, night work, sleep patterns, changes in longitude, and prolonged bed rest. (Reproduced, with permission, from Melmed S. Acromegaly. *NEJM*. 2006;355(24):2558. Copyright © Massachusetts Medical Society. All rights reserved.)

Neural Control

Control and integration by the central nervous system is a key component of hormonal regulation and is mediated by direct neurotransmitter control of endocrine hormone release (Figure 1–9). The central role of the hypothalamus in neural control of hormone release is discussed in Chapter 2 and is exemplified by dopaminergic control of pituitary prolactin release. Neural control also plays an important role in the regulation of peripheral endocrine hormone release. Endocrine organs such as the pancreas receive sympathetic and parasympathetic input, which contributes to the regulation of insulin and glucagon release. The neural control of hormone release is best exemplified by the sympathetic regulation of the adrenal gland, which functions as a modified sympathetic ganglion receiving direct neural input from the sympathetic nervous system. Release of acetylcholine from preganglionic sympathetic nerve terminals at the adrenal medulla stimulates the release of epinephrine into the circulation (Figure 1–9).

Figure 1–9.





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Neural control of hormone release. Endocrine function is under tight regulation by the nervous system, hence the term "neuroendocrine." Hormone release by endocrine cells can be modulated by postganglionic neurons from the sympathetic nervous system (SNS) or parasympathetic nervous system (PSNS) using acetylcholine (ACh) or norepinephrine (NE) as neurotransmitters, or by preganglionic neurons using acetylcholine as a neurotransmitter. Therefore,

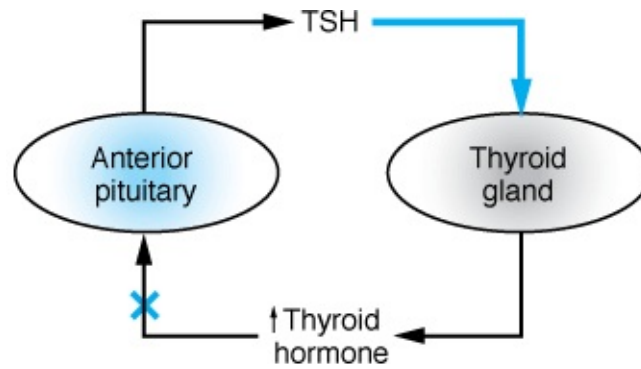
pharmacologic agents that interact with the production or release of neurotransmitters will affect endocrine function.

Hormonal Control

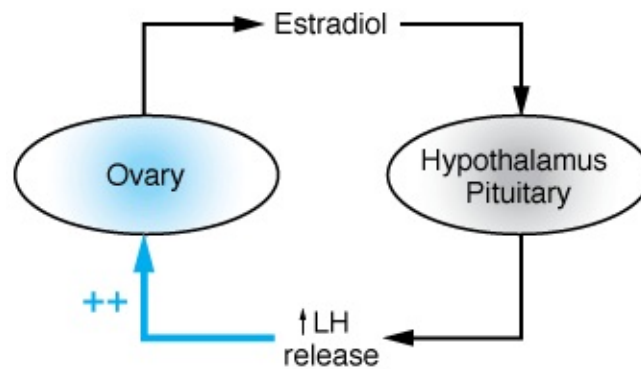
Hormone release from an endocrine organ is frequently controlled by another hormone. When the outcome is stimulation of hormone release, the hormone that exerts that effect is referred to as a tropic hormone (Figure 1–10), as is the case for most of the hormones produced and released from the anterior pituitary. One example of this type of hormone release control is the regulation of glucocorticoid release by ACTH. Hormones can also suppress another hormone's release. An example of this is the inhibition of growth hormone release by somatostatin.

Figure 1–10.

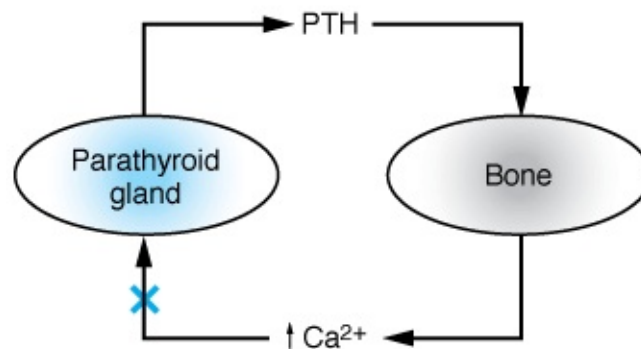




A. Negative feedback



B. Positive feedback



C. Product regulation

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A. Negative feedback regulation. In some cases, the endocrine gland is itself a target organ for another hormone. In this case, endocrine cells from organ 1 produce a hormone that stimulates the target organ to produce another hormone (hormone 2). Hormone 2 decreases the production and release of the hormone that stimulated its release, also known as a tropic hormone. An example is the

regulation of anterior pituitary release of thyroid-stimulating hormone (TSH) by thyroid hormones produced by the thyroid gland. **B.** Positive feedback regulation, also known as feed-forward control of hormone release, occurs when the release of a hormone feeds forward the system ultimately resulting in stimulation of its own release. An example is the stimulation of luteinizing hormone (LH) release by estradiol released during midcycle. **C.** Product control of hormone release. The production and release of a hormone can be regulated by the circulating levels of the substrate that it controls. An example is the regulation of parathyroid hormone (PTH) release from the parathyroid glands by the prevailing serum levels of Ca^{2+} .



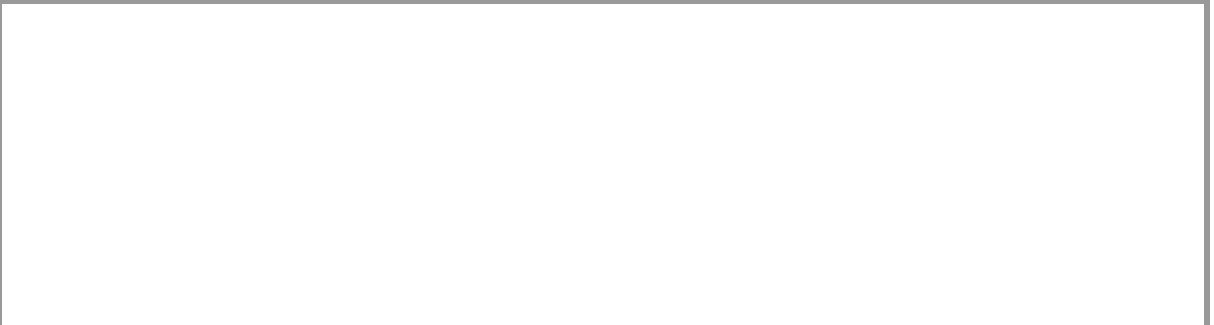
Hormonal inhibition of hormone release plays an important role in the process of **negative feedback regulation** of hormone release (described below and in Figure 1–10). In addition, hormones can stimulate the release of a second hormone in what is known as a feed-forward mechanism (Chapter 9).

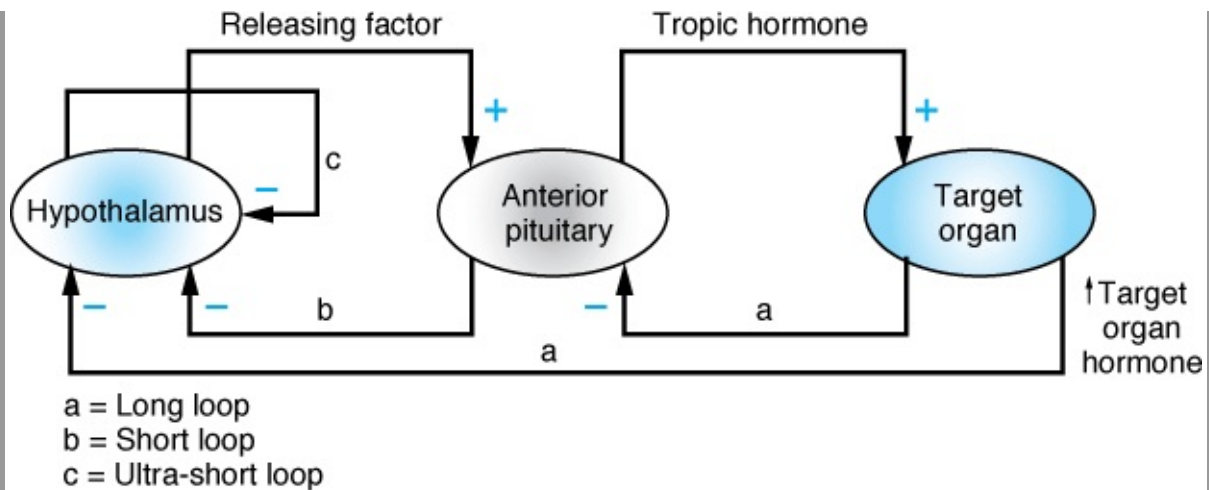
Nutrient or Ion Regulation

Plasma levels of nutrients or ions can also regulate hormone release (Figure 1–10). In all cases, the particular hormone regulates the concentration of the nutrient or ion in plasma either directly or indirectly. Examples of nutrient and ion regulation of hormone release include the control of insulin release by plasma glucose levels and the control of parathyroid hormone release by plasma calcium and phosphate levels.

In several instances, release of one hormone can be influenced by more than one of these mechanisms. For example, insulin release is regulated by nutrients (plasma levels of glucose and amino acids), neural (sympathetic and parasympathetic stimulation), and hormonal (somatostatin) mechanisms. The ultimate function of these control mechanisms is to allow the neuroendocrine system to adapt to a changing environment, integrate signals, and maintain homeostasis. The responsiveness of target cells to hormonal action leading to regulation of hormone release constitutes a **feedback** control mechanism. A dampening or inhibition of the initial stimulus is called **negative feedback**. Stimulation or enhancement of the original stimulus is called **positive feedback** (Figure 1–10). Negative feedback is the most common control mechanism regulating hormone release. The integrity of the system ensures that adaptive changes in hormone levels do not lead to pathologic conditions. Furthermore, the control mechanism plays an important role in short-and long-term adaptations to changes in the environment. Three levels of feedback can be identified: long loop, short loop, and ultra-short loop. These are depicted in Figure 1–11.

Figure 1–11.





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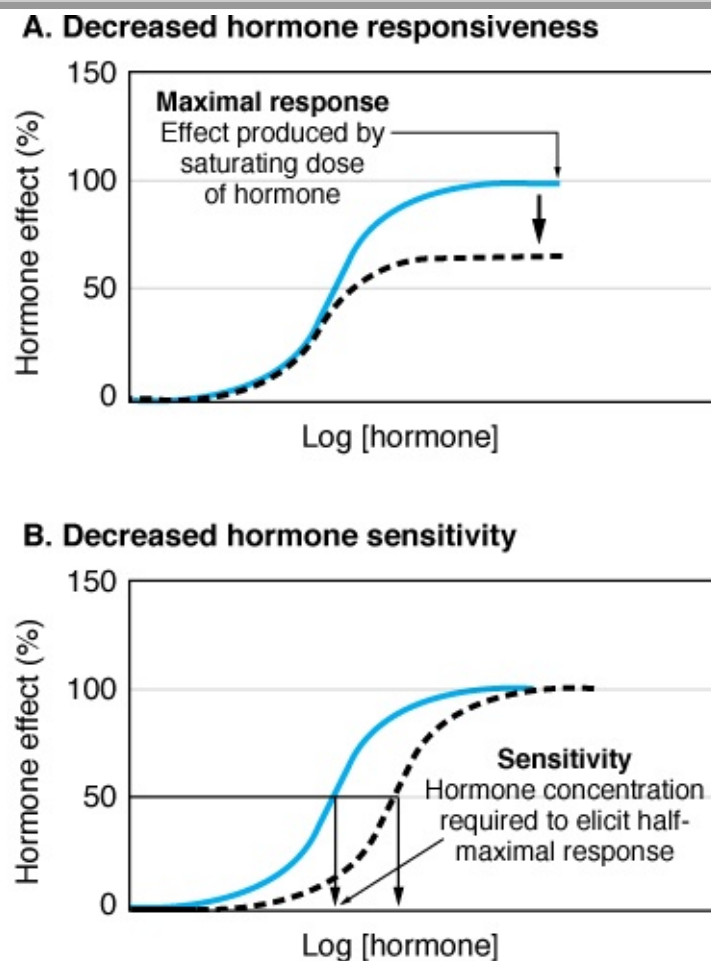
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Levels of feedback mechanisms. Three levels of feedback mechanisms controlling hormone synthesis can be identified: long loop, short loop, and ultra-short loop. Hormones under negative feedback regulation stimulate the production of another hormone by their target organ. The increase in circulating levels of that hormone then inhibits further production of the initial hormone. Hypothalamic releasing factors stimulate the release of tropic hormones from the anterior pituitary. The tropic hormone stimulates the production and release of hormone from the target organ. The hormone produced by the target organ can inhibit the release of the tropic hormone and of the hypophysiotropic factor by a long-loop negative feedback. The tropic hormone can inhibit the release of the hypothalamic factor in a short-loop negative feedback. The hypophysiotropic factor can inhibit its own release in an ultra-short negative feedback mechanism. The accuracy of this control mechanism allows the use of circulating levels of hormones, tropic hormones, and nutrients for assessment of the functional status of the specific endocrine organ in question.

ASSESSMENT OF ENDOCRINE FUNCTION

In general, disorders of the endocrine system result from alterations in hormone secretion or target cell responsiveness to hormone action. Alterations in target cell response can be due to increased or decreased biologic responsiveness to a particular hormone (Figure 1–12). The initial approach to assessment of endocrine function is measurement of plasma hormone levels.

Figure 1–12.



Receptor function. **A.** Hormone responsiveness. Decreased responsiveness to hormone effects can be due to a decreased number of hormone receptors, a decreased concentration of enzyme

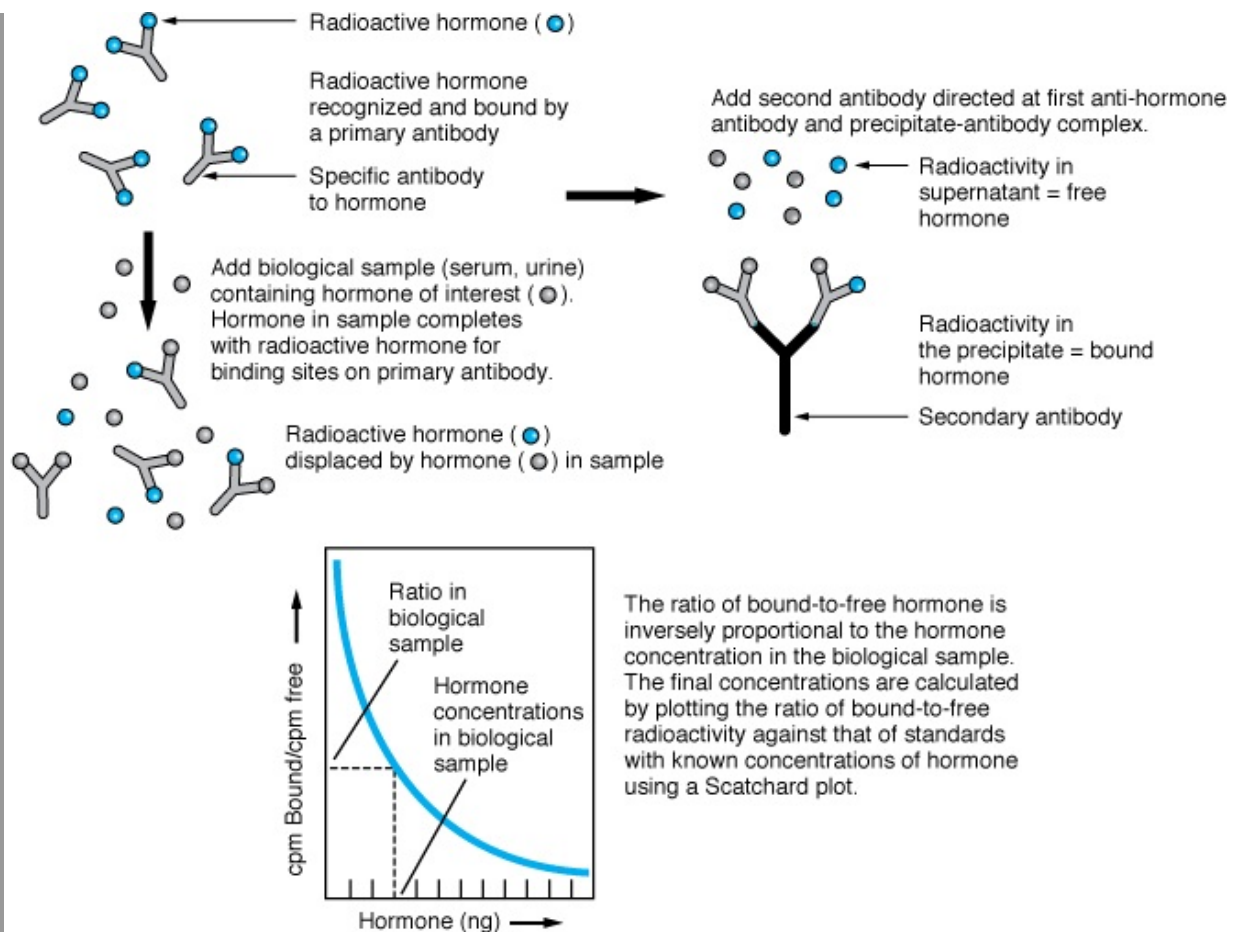
activated by the hormone, an increased concentration of noncompetitive inhibitor, or a decreased number of target cells. When responsiveness is decreased, then no matter how high the hormone concentration, a maximal response will not be achieved. **B. Hormone sensitivity.** A decrease in hormone sensitivity requires higher hormone concentrations to produce 50% of the maximal response. Decreased sensitivity can be due to decreased hormone-receptor affinity, decreased hormone-receptor number, increased rate of hormone degradation, and increased levels of antagonistic or competitive hormones.

Hormone Measurements

Hormone concentrations in biologic fluids (most commonly plasma and urine) are measured using radioimmunoassays (RIAs) and immunometric assays or adaptations of these. The principle behind these analytical approaches is the competitive inhibition of binding of a radiolabeled antigen (hormone) to an antibody (usually of the immunoglobulin G class) by an unlabeled antigen (hormone in biologic sample) (Figure 1–13). The higher the concentration of hormone in the biologic sample the lower the amount of radiolabeled hormone that is bound to the antibody will be. The ratio of the bound hormone to free hormone as a function of the bound hormone is then plotted in a Scatchard plot. For most RIAs, the Scatchard plot yields a curvilinear relationship because of the heterogeneity of the antibodies in the assay.

Figure 1–13.





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A radiolabeled (radioactive) hormone is incubated with the biologic sample containing the hormone of interest (serum, plasma, or urine) and with a specific primary antibody to the hormone. Hormone and radiolabeled hormone compete for binding to the antibody. Addition of a secondary antibody directed at the primary antibody precipitates the hormone-antibody complex. The amount of hormone that binds to the antibody is directly related to the total amount of hormone in the biologic sample analyzed. The concentration of the hormone of interest is determined by plotting the ratio of bound:unbound radioactivity against known concentrations of hormone in standard samples. Newer methods use two antibodies directed to different sites of the hormone, a procedure that enhances the specificity of the assay.

Among the limitations of these assays is the lack of specificity due to cross-reactivity of the antibody with more than one hormone. Some

hormones (ie, luteinizing hormone, follicle-stimulating hormone, and TSH) share homology that can lead to nonspecific recognition by the antibody (cross-reactivity). In addition, heterogeneity in the form of the hormone found in plasma may cause a lack of specificity of the assay. Hormones with different biologic activities, such as insulin and proinsulin may be recognized equally by the antibody. Constant developments and improvements in analytical approaches have led to enhanced sensitivity of the assays used in clinical laboratories. Confounding factors in evaluation of plasma hormone levels include the possibility of degradation of the hormone by enzymes in serum, which may alter hormone levels following sample collection. Furthermore, circulating endogenous antibodies to the hormone may interfere with the assay by binding the labeled hormone, resulting in detection of an artificially high hormone level.

These limitations of RIA have led to the development of more sensitive approaches to measuring hormone levels, such as the newer immunometric assays. The principle behind immunometric assay is the binding of the hormone by a monoclonal antibody followed by binding by a second monoclonal antibody to a different antigenic site in the hormone, "sandwiching" the hormone. The second antibody is labeled and is fixed to a solid phase to allow separation. These assays, use the same principle of antibody recognition, but have greater sensitivity for the hormone because the antibodies recognize 2 antigenic sites of the hormone, and circulating hormone antibodies do not interfere with measurements. In addition, the concentrations of the antibodies used can be increased dramatically, speeding the reaction time and completion of the assay.

Interpretation of Hormone Measurements



Because of the variability in circulating hormone levels resulting from pulsatile release, circadian rhythms, sleep/wake cycle, and nutritional status, interpretation of isolated plasma hormone measurements should always be done with caution and with understanding of the integral components of the hormone axis in question. These will be identified for each of the hormone systems discussed. Plasma hormone measurements reflect endocrine function only when interpreted in the right context. An abnormality in endocrine function is identified through measurements of hormone levels, hormone-nutrient or hormone-tropic hormone pairs, or by functional tests of hormone status together with the clinical assessment of the individual. It is important to keep in mind that the circulating levels of a particular hormone reflect the immediate state of the individual. Regulation of hormone release is a dynamic process that is constantly changing to adapt to the needs of the individual to maintain homeostasis. For example, plasma insulin levels reflect the fed or fasted state; estrogen and progesterone levels reflect the stage of the menstrual cycle. In addition, hormone levels can reflect the time of day during which they were obtained. For example, because of the circadian rhythm of cortisol release, cortisol levels will be higher early in the morning than in late afternoon. Age, health status, gender, and sleep patterns are among the many factors that influence hormone levels. Diseases and 24-hour light periods like those in an intensive care unit alter the pulsatility and rhythm of hormone release.

Some general aspects that should be considered when interpreting hormone measurements are as follows:

- Hormone levels should be evaluated with their appropriate regulatory factors (eg, insulin with glucose, calcium with parathyroid hormone, thyroid hormone with TSH, etc).
- Simultaneous elevation of pairs (elevation of both the hormone and the substrate that it regulates; such as elevated plasma glucose and insulin levels) indicates a hormone-resistance state.

- Urinary excretion of hormone or hormone metabolites over 24 hours may be a better estimate of hormone secretion than one-time plasma-level measurement. However, these measures rely on adequate renal function.
- Target hormone excess should be evaluated with the appropriate tropic hormone to rule out ectopic hormone production, which is usually due to a hormone-secreting tumor.

The possible interpretations of altered hormone and regulatory factor pairs are summarized in Table 1–1. Increased tropic hormone levels with low target hormone levels indicate primary failure of the target endocrine organ. Increased tropic hormone levels with increased target gland hormone levels indicate autonomous secretion of tropic hormone or inability of target gland hormone to suppress tropic hormone release (impaired negative feedback mechanisms). Low tropic hormone levels with low target gland hormone levels indicate a tropic hormone deficiency, as seen with pituitary failure. Low tropic hormone levels with high target gland hormone levels indicate autonomous hormone secretion by the target endocrine organ.

Table 1–1. Interpretation of Hormone Levels

Pituitary hormone level	Target hormone level		
	Low	Normal	High
High	Primary failure of target endocrine organ		Autonomous secretion of pituitary hormone or resistance to target hormone action
Normal		Normal range	
Low	Pituitary failure		Autonomous secretion by target endocrine organ

Dynamic Measurements of Hormone Secretion

In some cases, detection of abnormally high or low hormone concentrations may not be sufficient to conclusively establish the site of endocrine dysfunction. Dynamic measures of endocrine function provide more information than that obtained from hormone-pair measurements and rely on the integrity of the feedback control mechanisms that regulate hormone release. These tests of endocrine function are based on either stimulation or suppression of the endogenous hormone production.

STIMULATION TESTS

Stimulation tests are designed to determine the capacity of the target gland to respond to its control mechanism, either a tropic hormone or a substrate that stimulates its release. Examples of these tests are the use of ACTH to stimulate cortisol release (Chapter 6) and the use of an oral glucose load to stimulate insulin release (Chapter 7).

SUPPRESSION TESTS

Suppression tests are used to determine whether the negative feedback mechanisms that control that hormone's release are intact. Examples are the use of dexamethasone, a synthetic glucocorticoid, to suppress ACTH secretion and cortisol release from the adrenal glands.

Hormone-Receptor Measurements

The measurement of hormone-receptor presence, number, and affinity has become a useful diagnostic tool, particularly in instituting hormone therapy for the treatment of some tumors. Receptor measurements made in tissue samples obtained surgically allow determinations of tissue responsiveness to hormone and prediction of tumor responsiveness to hormone therapy. An example is the assessment of estrogen receptors in breast tumors to determine the applicability of hormone therapy.

KEY CONCEPTS



Hormones are classified into protein, amino acid–derived, and steroid based on their chemistry.



Binding proteins regulate hormone availability and physiologic function.



Physiologic effects of hormones require binding to specific receptors in target organs



Hormone release is under neural, hormonal, and product regulation.



Hormones can control their own release through feedback regulation.



Interpretation of hormone levels requires consideration of hormone pairs or of the nutrient or factor controlled by the hormone.

SUGGESTED READINGS

Aranda A, Pascual A. Nuclear hormone receptors and gene expression. *Physiol Rev.* 2001;81:1269. [PMID: 11427696]

Morris AJ, Malbon CC. Physiological regulation of G protein-linked signaling. *Physiol Rev.* 1999;79:1373. [PMID: 10508237]

OBJECTIVES

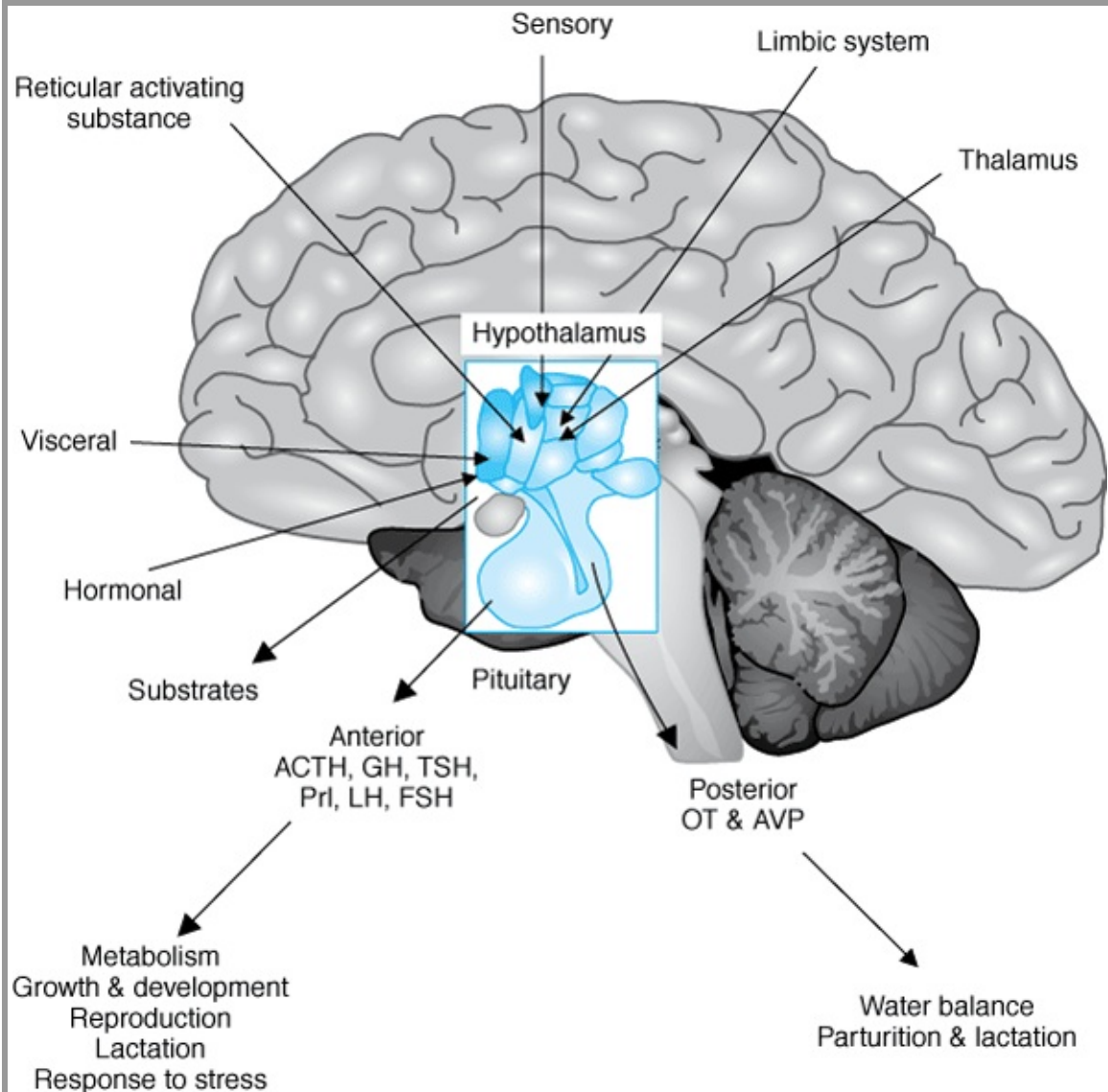
- ▶ *Describe the physiologic and anatomic relationships between the hypothalamus and the anterior and the posterior pituitary.*
- ▶ *Understand the integration of hypothalamic and pituitary function and identify the 2 different pathways used for hypothalamic-pituitary interactions.*
- ▶ *Identify the appropriate hypothalamic releasing and inhibitory factors controlling the secretion of each of the anterior pituitary hormones.*
- ▶ *Differentiate between the routes of transport of hypothalamic neuropeptides to the posterior and anterior pituitary.*
- ▶ *Identify the mechanisms that control the release of oxytocin and arginine vasopressin.*
- ▶ *Understand the physiologic target-organ responses and the cellular mechanisms of oxytocin and arginine vasopressin action.*

THE HYPOTHALAMUS & POSTERIOR PITUITARY GLAND: INTRODUCTION

The hypothalamus is the region of the brain involved in coordinating the physiologic responses of different organs that together maintain homeostasis. It does this by integrating signals from the environment, from other brain regions, and from visceral afferents and then stimulating the appropriate neuroendocrine responses. In doing so, the hypothalamus influences many aspects of daily function, including food intake, energy expenditure, body weight, fluid intake and balance, blood pressure, thirst, body temperature, and the sleep cycle. Most of these hypothalamic responses are mediated through hypothalamic control of pituitary function (Figure 2–1). This control is achieved by 2 mechanisms: (1) release of hypothalamic neuropeptides synthesized in hypothalamic neurons and transported through the hypothalamo-hypophyseal tract to the posterior pituitary and (2) neuroendocrine control of the anterior pituitary through the release of peptides that

mediate anterior pituitary hormone release (hypophysiotropic hormones) (Figure 2–2). Because of this close interaction between the hypothalamus and the pituitary in the control of basic endocrine physiologic function, they are presented as an integrated topic.

Figure 2–1.



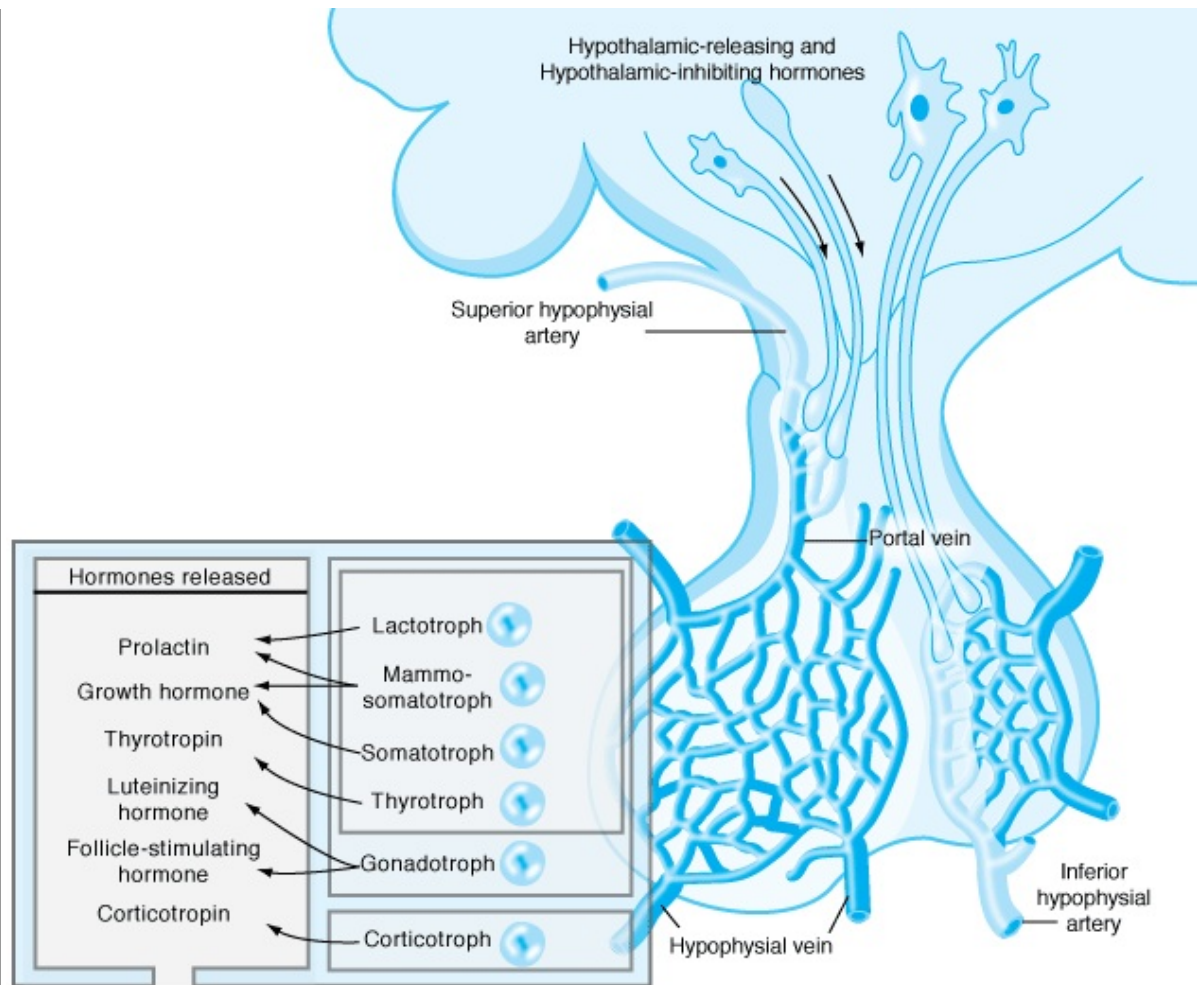
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Neuroendocrine regulation of homeostasis. The release of hypothalamic neuropeptides is regulated by afferent signals from other brain regions, from visceral afferents, and by circulating levels of

substrates and hormones. The sleep/awake state of the individual, variations in light, noise, fear, anxiety, and visual images all are examples of signals that are integrated by the hypothalamus and that are involved in the regulation of hypothalamic neuropeptide release and control of pituitary function. The hormones released from the anterior and posterior pituitary regulate vital body functions to maintain homeostasis. ACTH, adrenocorticotrophic hormone; AVP, arginine vasopressin; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; OT, oxytocin; Prl, prolactin; TSH, thyroid-stimulating hormone.

Figure 2–2.



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Anatomical and functional relationship between the hypothalamus and the pituitary. The hypothalamus is anatomically and functionally linked with the anterior and posterior pituitary. They are closely related because of the portal system of blood supply. The superior (SHA), medial and inferior hypophyseal (IHA) arteries provide arterial blood supply to the median eminence and the pituitary. Magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei have long axons that terminate in the posterior pituitary. The axons of parvicellular neurons terminate in the median eminence where they release their neuropeptides. The long portal veins drain the median eminence, transporting the peptides from the primary capillary plexus to the secondary plexus that provides blood supply to the anterior pituitary. (Adapted, with permission, from Melmed S. Acromegaly.

FUNCTIONAL ANATOMY

The hypothalamus is the part of the diencephalon located below the thalamus and between the lamina terminalis and the mamillary bodies forming the walls and the floor of the third ventricle. At the floor of the third ventricle, the 2 halves of the hypothalamus are rejoined to form a bridge-like region known as the median eminence (Figure 2–2). The median eminence is important because this is where axon terminals of hypothalamic neurons release neuropeptides involved in the control of anterior pituitary function. In addition, the median eminence is traversed by the axons of hypothalamic neurons ending in the posterior pituitary. The median eminence funnels down to form the infundibular portion of the neurohypophysis (also called the pituitary or infundibular stalk). In practical terms, the neurohypophysis or posterior pituitary can be considered an extension of the hypothalamus.

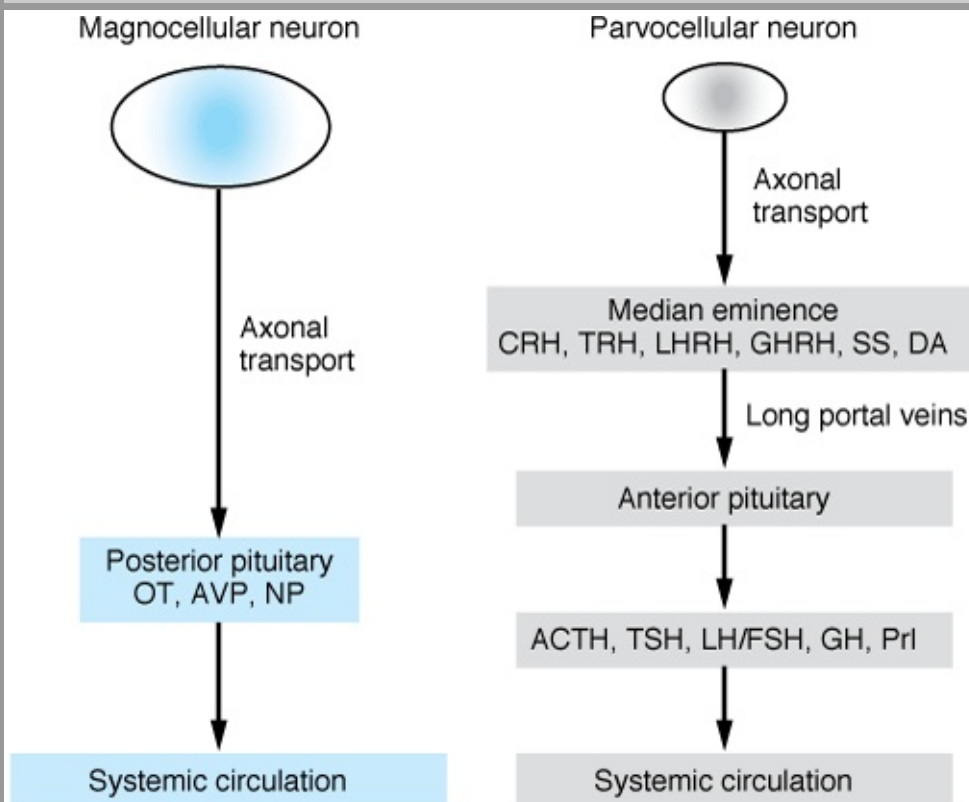
Hypothalamic Nuclei

In the hypothalamus, the neuronal bodies are organized in nuclei. These are clusters or groups of neurons that have projections reaching other brain regions as well as ending in other hypothalamic nuclei. This intricate system of neuronal connections allows continuous communication between the hypothalamic neurons and other brain regions. The hypothalamic nuclei can be classified on the basis of their anatomic location or the principal neuropeptide that their cells produce. However, these are not discrete definitions of cell groups; some hypothalamic nuclei may contain more than 1 neuronal cell type. It is best to consider the groups of neurons as clusters of neurons and not as well-defined and delineated nuclei made of a single neuronal type.

Some of the neurons that make up the hypothalamic nuclei are neurohormonal in nature. Neurohormonal refers to the ability of these neurons to synthesize neuropeptides that function as hormones and to release these neuropeptides from axon terminals in response to neuronal depolarization. Two types of neurons are important in mediating the endocrine functions of the hypothalamus: the

magnocellular and the **parvocellular** neurons (Figure 2–3). The magnocellular neurons are predominantly located in the paraventricular and supraoptic nuclei of the hypothalamus and produce large quantities of the neurohormones **oxytocin** and **arginine vasopressin (AVP)**. The unmyelinated axons of these neurons form the **hypothalamo-hypophysial tract**, the bridge-like structure that traverses the median eminence and ends in the posterior pituitary. Oxytocin and AVP are released from the posterior pituitary in response to an action potential. Parvocellular neurons have projections that terminate in the median eminence, brainstem, and spinal cord. These neurons release small amounts of releasing or inhibiting neurohormones (**hypophysiotropic hormones**) that control anterior pituitary function.

Figure 2–3.



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Magnocellular neurons are larger in size and produce large quantities of neurohormones. Located predominantly in the paraventricular and

supraoptic nuclei of the hypothalamus, their unmyelinated axons form the hypothalamo-hypophyseal tract that traverses the median eminence ending in the posterior pituitary. They synthesize the neurohormones oxytocin and vasopressin which are transported in neurosecretory vesicles down the hypothalamo-hypophyseal tract and stored in varicosities at the nerve terminals in the posterior pituitary. Parvocellular neurons are small in size and have projections that terminate in the median eminence, brain stem, and spinal cord. They release small amounts of releasing or inhibiting neurohormones (hypophysiotrophic hormones) that control anterior pituitary function will be discussed in the next chapter. These are transported in the long portal veins to the anterior pituitary where they stimulate the release of pituitary hormones into the systemic circulation.

Blood Supply

The specialized capillary network that supplies blood to the median eminence, infundibular stalk, and pituitary plays an important role in the transport of hypophysiotropic neuropeptides to the anterior pituitary. Hypophysiotropic peptides released near the median eminence are transported down the infundibular stalk to the anterior pituitary, where they exert their physiologic effects. Branches from the internal carotid artery provide the blood supply to the pituitary. The superior hypophyseal arteries form the primary capillary plexus that supplies blood to the median eminence. From this capillary network, the blood is drained in parallel veins called long hypophyseal portal veins down the infundibular stalk into the secondary plexus (Figure 2–2). The hypophysiotropic peptides released at the median eminence enter the primary plexus capillaries. From there, they are transported to the anterior pituitary via the long hypophyseal portal veins to the secondary plexus. The secondary plexus is a network of fenestrated sinusoid capillaries that provides the blood supply to the **anterior pituitary** or adenohypophysis. Because of the fenestrated architecture of these capillary vessels, the neuropeptides easily diffuse out of the circulation to reach the cells of the anterior pituitary. The cells of the anterior pituitary express specific cell surface G protein–coupled receptors (Chapter 1, Figure 1–5) that bind the neuropeptides, activating intracellular second-messenger cascades that produce the release of

anterior pituitary hormones.

The blood supply to the posterior pituitary and to the pituitary stalk is provided mostly by the middle and inferior hypophysial arteries and, to a lesser extent, by the superior hypophysial arteries. Short portal vessels provide venous connections that originate in the neural lobe and pass across the intermediate lobe of the pituitary to the anterior lobe. This structure allows neuropeptides released from the posterior pituitary to have access to cells in the anterior pituitary, so that the functions of the 2 main regions of the pituitary cannot be dissociated from each other. Blood from the anterior and posterior pituitary drains into the intercavernous sinus and then into the internal jugular vein, entering the systemic venous circulation.

Hypothalamic Neuropeptides



As described earlier, 2 general types of neurons constitute the endocrine hypothalamus: the magnocellular neurons, with axons terminating in the posterior pituitary; and the parvicellular neurons, with axons terminating in the median eminence. The neuropeptides released from the parvicellular neuron terminals in the median eminence (corticotropin-releasing hormone [CRH], growth hormone (GH)–releasing hormone, thyrotropin-releasing hormone, dopamine, luteinizing hormone–releasing hormone, and somatostatin) control anterior pituitary function (Table 2–1). The hypothalamic hypophysiotropic peptides stimulate the release of anterior pituitary hormones. The products released from both the anterior pituitary (**adrenocorticotrophic hormone [ACTH], prolactin, GH, luteinizing hormone [LH], follicle-stimulating hormone [FSH], and thyroid-stimulating hormone [TSH]**) and the posterior pituitary (**oxytocin and AVP**) are transported in the venous blood draining the pituitary that enters the intercavernous sinus and the internal jugular veins to reach the systemic circulation. Their control and regulation will be discussed repeatedly throughout this book whenever the specific hormone systems to which they belong are described. Several neuropeptides have been isolated from the hypothalamus, and many continue to be discovered. However, only those that have been demonstrated to control anterior pituitary function (hypophysiotropic hormones) and

therefore play an important role in endocrine physiology will be discussed.

Table 2–1. Key Aspects of Hypophysiotropic Hormones

Hypophysiotropic hormone	Predominant hypothalamic nuclei	Anterior pituitary hormone controlled	Target cell
Thyrotropin-releasing hormone	Paraventricular nuclei	Thyroid-stimulating hormone and prolactin	Thyrotroph
Luteinizing hormone–releasing hormone	Anterior and medial hypothalamus; preoptic septal areas	Luteinizing hormone and follicle-stimulating hormone	Gonadotroph
Corticotropin-releasing hormone	Medial parvocellular portion of paraventricular nucleus	Adrenocorticotrophic hormone	Corticotroph
Growth hormone–releasing hormone	Arcuate nucleus, close to median eminence	Growth hormone	Somatotroph
Somatostatin or growth hormone–inhibiting hormone	Anterior paraventricular area	Growth hormone	Somatotroph
Dopamine	Arcuate nucleus	Prolactin	Lactotroph

The 6 recognized hypophysiotropic factors and the predominant locations of their cells of origin are listed in the left columns. The right columns list the anterior pituitary hormone that each hypophysiotropic factor regulates and the cell that releases the specific hormones.

Regulation of Hormone Release



Because the hypothalamus receives and integrates afferent signals from multiple brain regions, it does not function in isolation from the rest of the central nervous system (Figure 2–1). Some of these afferent signals convey sensory information about the individual's environment such as light, heat, cold, and noise. Among the environmental factors, light plays an important role in generating the circadian rhythm of hormone secretion. This endogenous rhythm is generated through the interaction between the retina, the hypothalamic suprachiasmatic nucleus and the pineal gland through the release of melatonin. Melatonin is a hormone synthesized and secreted by the pineal gland at night. Its rhythm of secretion is entrained to the light/dark cycle. Melatonin conveys information concerning the daily cycle of light and darkness to body physiology and participates in the organization of circadian rhythms. Other signals perceived by the hypothalamus are visceral afferents that provide information to the central nervous system from peripheral organs such as the intestines, the heart, the liver, and the stomach. One can think of the hypothalamus as a center for integration of the information that the body is continuously processing. The neuronal signals are transmitted by various neurotransmitters released from the afferent fibers, including glutamate, norepinephrine, epinephrine, serotonin, acetylcholine, histamine, γ -aminobutyric acid, and dopamine. In addition, circulating hormones produced by endocrine organs and substrates such as glucose can regulate hypothalamic neuronal function. All of these neurotransmitters, substrates, and hormones can influence hypothalamic hormone release. Therefore, hypothalamic hormone release is under environmental, neural, and hormonal regulation. The ability of the hypothalamus to integrate these signals makes it a center of command for regulating endocrine function and maintaining homeostasis.

Hormones can signal the hypothalamus to either inhibit or stimulate hypophysiotropic hormone release. This control mechanism of negative (or positive) feedback regulation, discussed in detail in Chapter 1, consists of the ability of a hormone to regulate its own cascade of release (Figure 1–11). For example, as discussed in greater detail in Chapter 6, cortisol produced from the adrenal gland can inhibit the

release of CRH, thus inhibiting the production of proopiomelanocortin and ACTH and consequently decreasing adrenal gland synthesis of cortisol. This loop of hormonal control and regulation of its own synthesis is critical in maintaining homeostasis and preventing disease. A shorter loop of negative feedback inhibition also exists, which depends on the inhibition of hypophysiotropic neuropeptide release by the pituitary hormone that it stimulates. In this case, an example would be the ability of ACTH to inhibit CRH release by the hypothalamus. Some neuropeptides also possess an ultra-short feedback loop, in which the hypophysiotropic neuropeptide itself is able to modulate its own release. As an example, oxytocin stimulates its own release, creating a positive feedback regulation of neuropeptide release. These feedback loops are illustrated in Figure 1–11, Chapter 1.

This continuous regulation of hormonal release is dynamic; it is continuously adapting to changes in the environment and in the internal milieu of the individual. Throughout a given day, the hypothalamus integrates a multitude of signals to ensure that the rhythms of hormone release are kept in pace with the needs of the organism. Disruption of these factors can alter the patterns of hormone release. For example, a patient in the intensive care unit, where the lights are on throughout the 24 hours of the day, will have a disrupted cycle of hormone release. Other situations that disrupt the normal cycles of hormone release are travel across time zones and night-shift employment.

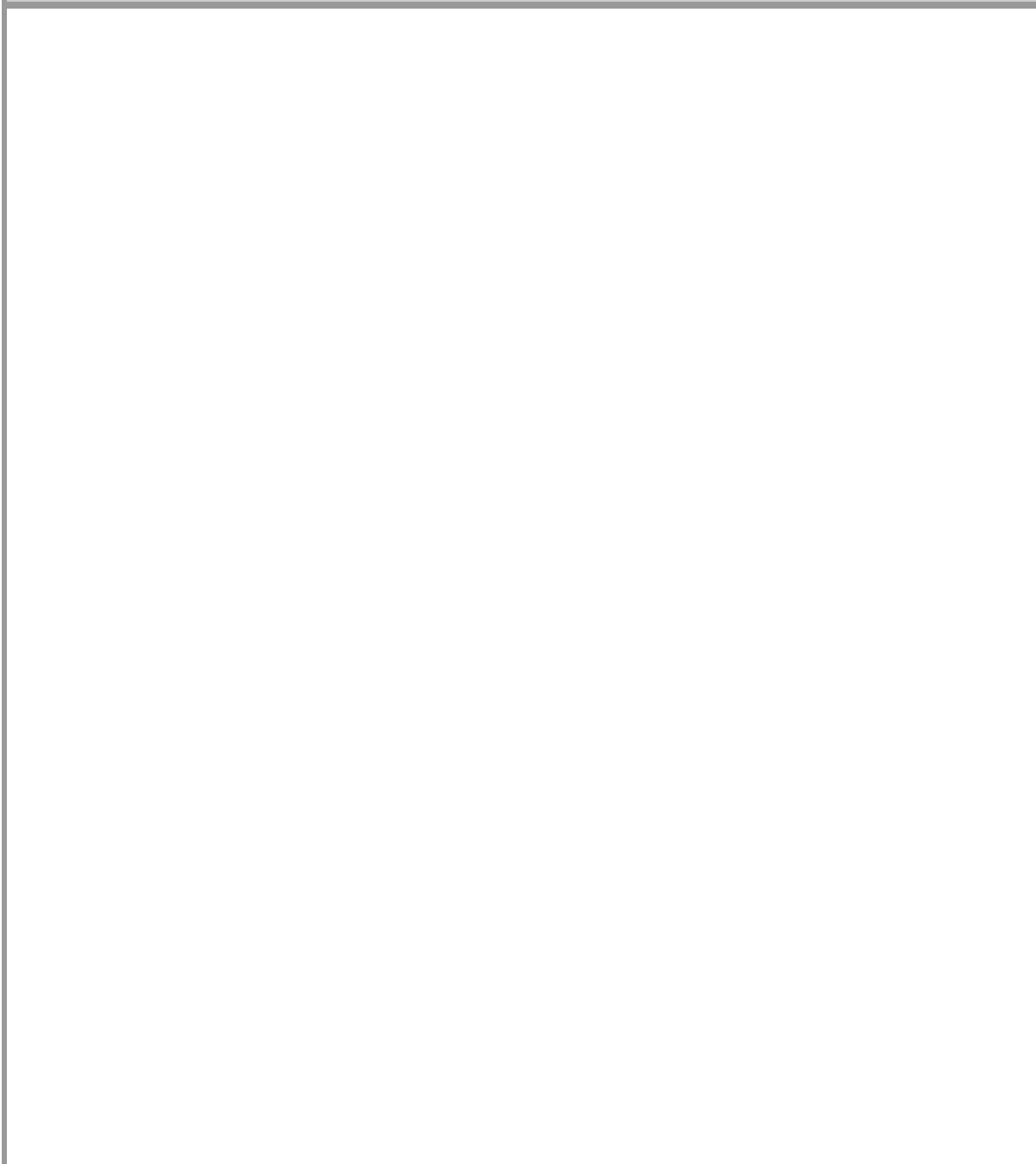
HORMONES OF THE POSTERIOR PITUITARY

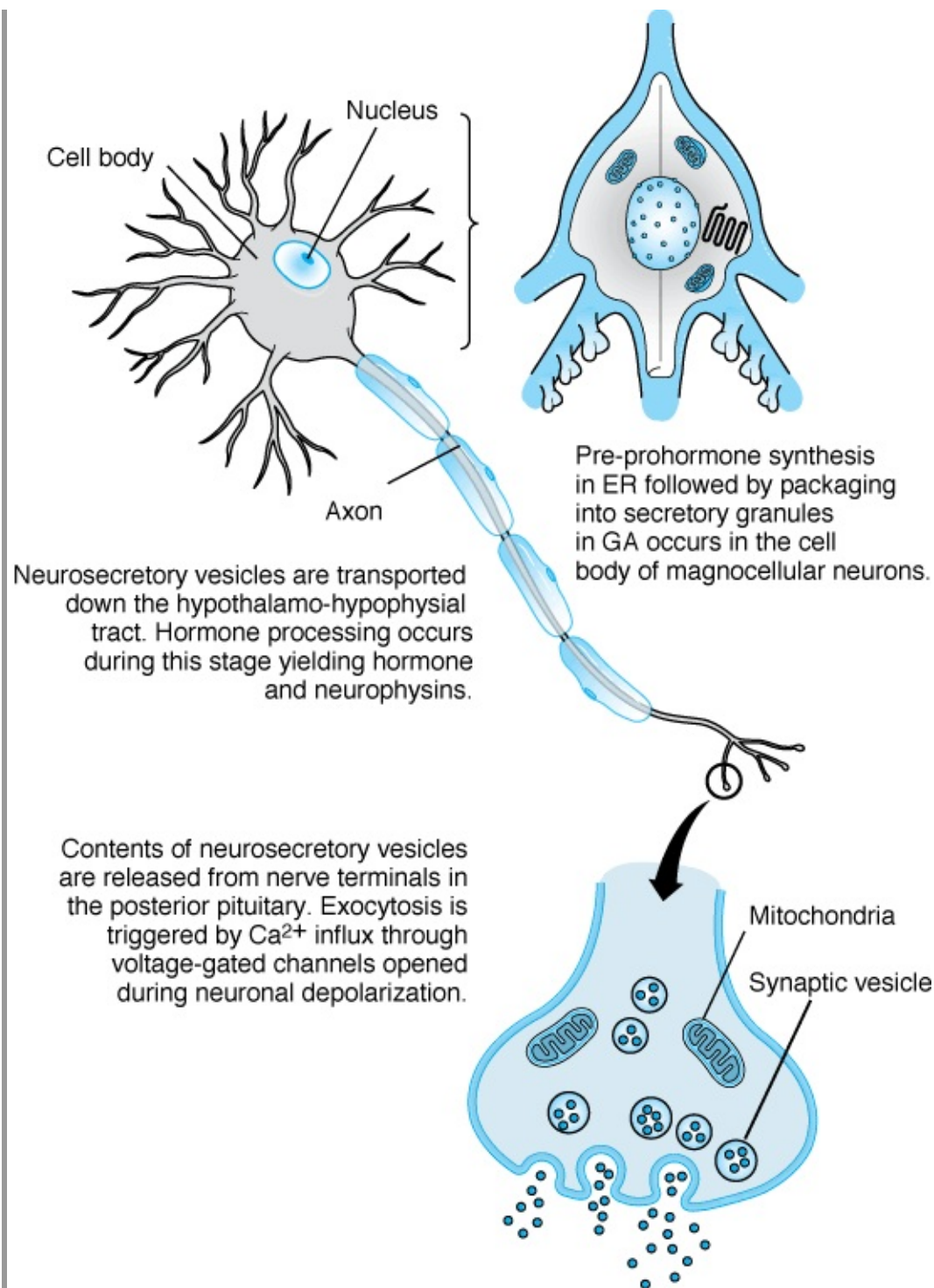


As mentioned above, the posterior pituitary is an extension of the hypothalamus and contains the axon terminals of magnocellular neurons located in the supraoptic and paraventricular nuclei (Figure 2–2). These neurons generate and propagate action potentials, producing membrane depolarization and exocytosis of the contents of their secretory granules. The neuropeptides produced by the magnocellular neurons, and consequently released from the posterior pituitary, are oxytocin and AVP. As the axons leave the supraoptic and paraventricular nuclei, they give rise to collaterals, some of which terminate in the median eminence.

Oxytocin and AVP are closely related peptides consisting of 9 amino acids (nonapeptides) with ring structures. They are synthesized as large precursor molecules in the ribosomes of the magnocellular neurons, after which they are transferred to the Golgi apparatus and packaged into neurosecretory vesicles (Figure 2–4).

Figure 2–4.





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Synthesis and processing of oxytocin and AVP. Oxytocin (OT) and arginine vasopressin (AVP) are synthesized in the endoplasmic reticulum (ER) of hypothalamic magnocellular neurons as pre-

prohormones. In the Golgi apparatus (GA), they are packaged in secretory granules and are transported down the hypothalamo-hypophyseal tract. During transport, the precursor hormones are processed, yielding the final hormone and the respective neurophysins. The contents of the neurosecretory vesicles are released by exocytosis from the axon terminals in the posterior pituitary. Exocytosis is triggered by the influx of Ca^{2+} through voltage-gated channels that are opened during neuronal depolarization. The rise in Ca^{2+} allows docking of the secretory vesicles on the axonal plasma membrane and release of the neuropeptides into the interstitial space.



Within the neurosecretory vesicles, the precursor hormone undergoes additional posttranslational processing during axonal transport, producing the biologically active peptides AVP and oxytocin as well as small peptide products of hormone processing called neurophysins (defined below). The release of the contents of the vesicles is triggered by the neuronal influx of calcium ions through voltage-gated calcium channels, which open as the wave of depolarization reaches the axon terminals. The rise in intracellular calcium triggers the movement and docking of the secretory vesicles on the plasma membrane, resulting in exocytosis of the vesicle contents into the extracellular space. These neuropeptides enter the systemic circulation through venous drainage of the posterior pituitary into the intercavernous sinus and internal jugular vein. In the systemic circulation, oxytocin and AVP circulate unbound. They are rapidly cleared from the circulation by the kidney and, to a lesser extent, by the liver and brain. Their half-life is short and is estimated to range between 1 and 5 minutes.

Neurophysins

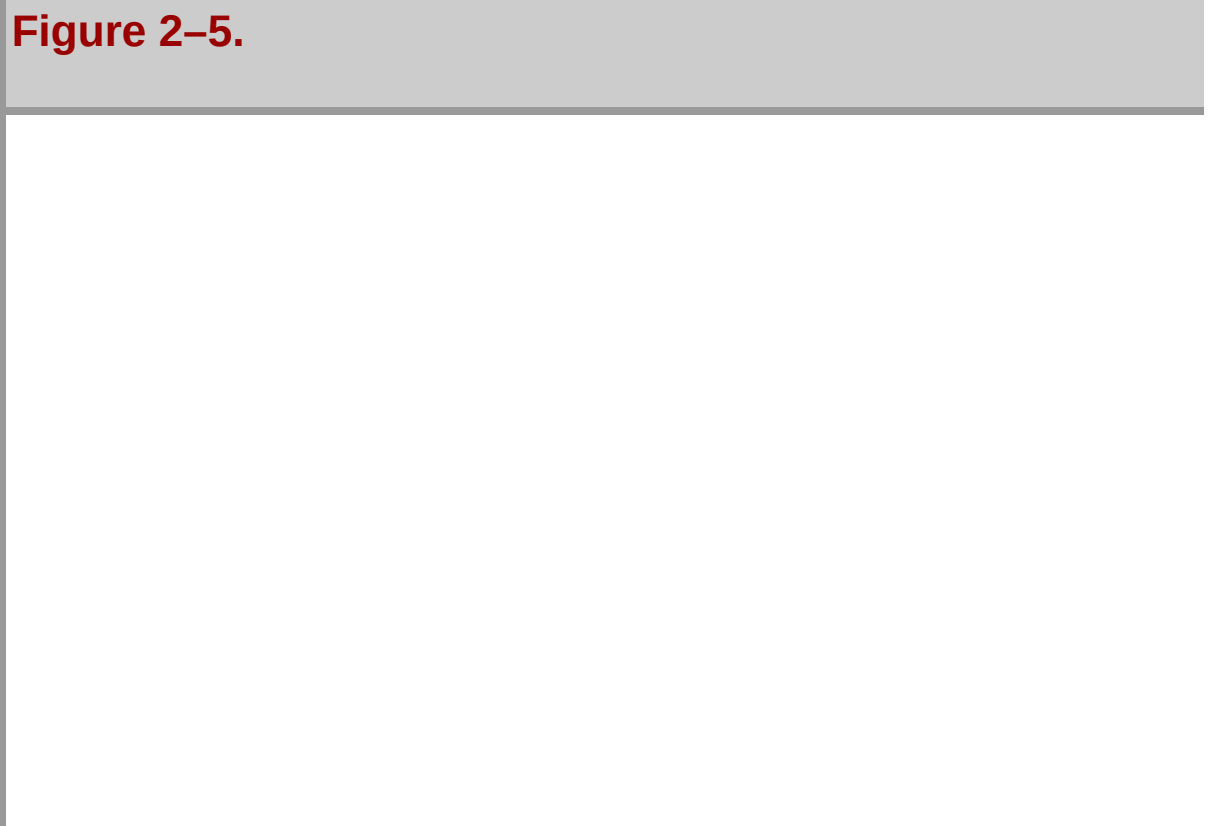
Neurophysins are byproducts of posttranslational prohormone processing in the secretory vesicles. The release of AVP and oxytocin is accompanied by the release of neurophysins from the secretory granules. Originally, neurophysins were considered to have a carrier function as the neuropeptide is transported down its trajectory through

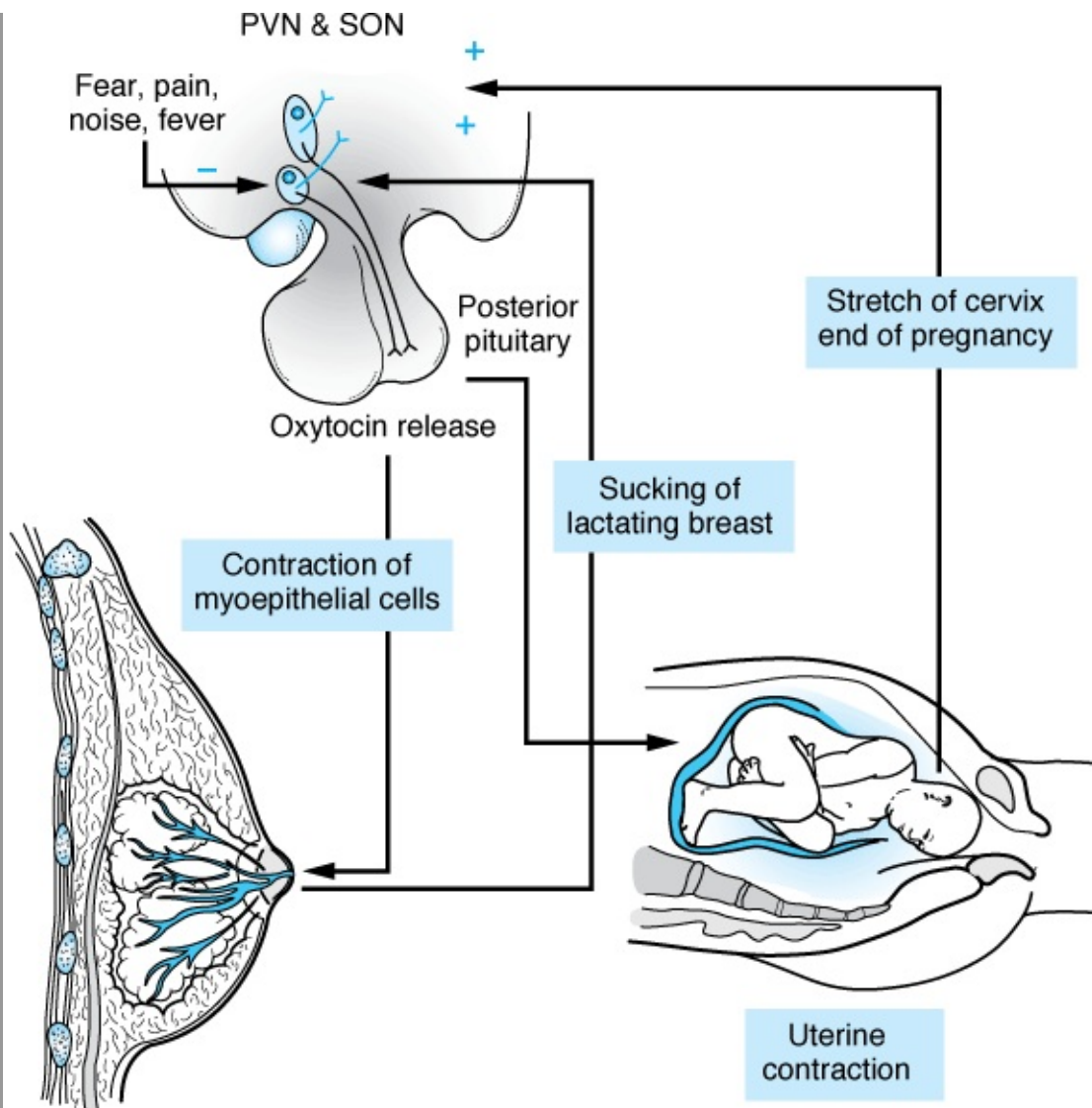
the hypothalamo-hypophysial tract. Although the exact function of these byproducts is not clear, it appears that neurophysins play a central role in AVP synthesis. This role has become more evident since the identification of the inherited disease of familial neurogenic diabetes insipidus. This disease is characterized by AVP deficiency due to mutations in neurophysins and improper targeting of the hormone to neurosecretory granules. Neurophysins thus have an important role in the transport of AVP from the cell bodies of magnocellular neurons to their final release from the posterior pituitary. Impairment in hormone targeting leads to retention of the mutated neuropeptide precursor in the endoplasmic reticulum of the magnocellular neurons, and these cells progress to programmed cell death (apoptosis).

Oxytocin

The neuropeptide oxytocin is synthesized by magnocellular neurons in the supraoptic and paraventricular nuclei of the hypothalamus and is released from the posterior pituitary into the peripheral circulation. The release of oxytocin is stimulated during breast-feeding (lactation) and childbirth (parturition) (Figure 2–5).

Figure 2–5.





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Physiologic effects and regulation of oxytocin release. The release of oxytocin is stimulated by the distention of the cervix at term of pregnancy and by the contraction of the uterus during parturition. The signals are transmitted to the paraventricular nuclei (PVN) and supraoptic nuclei (SON) of the hypothalamus, where they provide a positive feedback regulation of oxytocin release. The increased number of oxytocin receptors, the increased number of gap junctions between smooth muscle cells, and the increased synthesis of prostaglandins enhance the responsiveness of the uterine muscle. Oxytocin release causes an increase in uterine contractility, aiding in

the delivery of the baby, and involution of the uterus after parturition. Suckling of the nipple of the lactating breast also stimulates oxytocin release. The afferent sensory signals elicit a rise in oxytocin release into the circulation. Oxytocin produces contraction of the myoepithelial cells lining the breast ducts, resulting in milk ejection.

PHYSIOLOGIC EFFECTS OF OXYTOCIN

The 2 main target organs for oxytocin's physiologic effects are the lactating breast and the uterus during pregnancy (Figure 2–5). In the lactating breast, oxytocin stimulates milk ejection by producing contraction of the myoepithelial cells that line the alveoli and ducts in the mammary gland. In the pregnant uterus, oxytocin produces rhythmic contractions to help induce labor and to promote regression of the uterus following delivery (Chapter 9). Oxytocin is used clinically during labor and delivery to promote uterine contractions and during the postpartum period to help decrease bleeding and return the uterus to its normal size (uterine involution) (Table 2–2).

Table 2–2. Key Aspects of Posterior Pituitary Hormones

	Oxytocin	Arginine vasopressin
Receptor	G _{q/11} protein–coupled	G protein–coupled (V ₁ R, G _{q/11} , V ₂ R, G _s)
Second messenger	Phospholipase C ↑ [Ca ²⁺] _i	(V ₁ R) Phospholipase C ↑ [Ca ²⁺] _i (V ₂ R) Adenylate cyclase

cAMP		
Target organ or cells	Uterus Mammary myoepithelial cells	V ₂ R, kidney collecting ducts V ₁ R, smooth muscle cells
Physiologic effects	Uterine contraction Milk ejection	Increased H ₂ O permeability Vasoconstriction

cAMP, cyclic adenosine monophosphate.

The physiologic effects of oxytocin in the pregnant uterus are augmented by a dramatic increase in sensitivity to the hormone during the onset of labor. This increased sensitivity to oxytocin is due to an increased density (up-regulation) of oxytocin receptors in the uterine muscle. Receptor levels can be 200 times greater at the onset of labor than in the nonpregnant uterus. Because of this increase, levels of oxytocin that would normally not be effective can produce forceful uterine contractions toward the end of pregnancy. The increased density of oxytocin receptors is mediated by steroid hormone regulation of oxytocin-receptor synthesis. Responsiveness of the uterus is also enhanced by increased gap-junction formation between smooth muscle cells, facilitating conduction of action potentials between one cell and the next; and by increased synthesis of prostaglandin, a known stimulator of uterine contraction toward the end of gestation. All of these factors enhance myometrial contractile activity in response to oxytocin at term (Chapter 9). Additional secondary effects that have been attributed to oxytocin are potentiation of the release of ACTH by CRH, interaction with the AVP receptor to produce vasoconstriction, stimulation of prolactin release, and an influence on maternal behavior and amnesia.

CONTROL OF OXYTOCIN RELEASE

The principal stimulus for oxytocin release is mechanical stimulation of the uterine cervix by the fetus near the end of gestation. Oxytocin

release is also stimulated by the forceful contractions of the uterus during the fetal expulsion reflex. Hence, the contractile activity of the uterus acts through positive feedback mechanisms during parturition to stimulate oxytocin neurons, and this further increases the secretion of oxytocin. Before the oxytocin neurons can secrete oxytocin, they must be released from inhibition by other neurons containing endogenous opioids, nitric oxide, and γ -aminobutyric acid. This modulation of oxytocin release is partly due to the declining blood levels of progesterone and increasing levels of estrogen during late pregnancy. The neurotransmitters involved in stimulating oxytocin release are thought to be acetylcholine and dopamine.

Oxytocin release is also triggered by stimulation of tactile receptors in the nipples of the lactating breast during suckling (Figure 2–5). Breast-feeding generates sensory impulses that are transmitted to the spinal cord and then to the oxytocin-producing neurons in the hypothalamus. The information transmitted by these sensory afferents produces intermittent synchronized burst firing of oxytocin neurons, resulting in pulsatile release of oxytocin and increases in blood oxytocin concentrations.

In addition to its secretion from neurohypophyseal terminals in the posterior pituitary, oxytocin is released within the hypothalamic supraoptic and paraventricular nuclei. The function of this intrahypothalamic release of oxytocin is to control the activity of oxytocin neurons in an autocrine fashion by a positive feedback mechanism, increasing the neurohypophyseal release of oxytocin. The release of oxytocin is inhibited by severe pain, increased body temperature, and loud noise. Note how the environmental, hormonal, and neural mechanisms of hypothalamic hormone regulation are in play to regulate oxytocin release at the appropriate time of gestation and in response to the relevant stimuli. The role of oxytocin in males is not clear, although recent studies have suggested that it may participate in ejaculation.

OXYTOCIN RECEPTORS

The physiologic effects of oxytocin are achieved by binding to cell membrane $G_{q/11}$ protein–coupled oxytocin receptors expressed in the uterus, mammary glands, and brain. Binding of oxytocin to the receptor

activates phospholipase C, producing an increase in inositol trisphosphate and 1,2-diacylglycerol, which in turn results in an increase in cytosolic calcium concentrations. The rise in cytosolic calcium concentrations produces contraction of smooth muscle cells.

DISORDERS OF OXYTOCIN PRODUCTION

Diseases resulting from oxytocin excess have not been described. Although oxytocin deficiency causes difficulty with nursing because of impaired milk ejection, it is not associated with altered fertility or delivery in animals. Normal oxytocin levels have been detected in women with diabetes insipidus (AVP deficiency).

Arginine Vasopressin

AVP, also known as antidiuretic hormone (ADH), is the other neuropeptide produced by magnocellular neurons of the hypothalamus and released from the posterior pituitary. The principal effect of AVP is to increase water reabsorption by enhancing permeability to water in the distal convoluted tubules and the medullary collecting ducts in the kidney. The result is the production of smaller volumes of concentrated urine. In addition, AVP increases vascular resistance. This function of AVP may be important during periods of severe lack of responsiveness to other vasoconstrictors, as may occur during severe blood loss (hemorrhagic shock) or systemic infection (sepsis). The circulating concentrations of AVP range from 1.5 to 6 ng/L.

PHYSIOLOGIC EFFECTS OF AVP



The main target site of ADH is the collecting duct in the kidney. Water permeability in the collecting duct is relatively low compared with that in the proximal tubule and the thin descending limb of Henle's loop. In the proximal tubule and the descending limb of Henle's loop, the water channel protein aquaporin 1 (AQP1) is constitutively expressed both in the apical (luminal) and basolateral (interstitial) membranes of epithelial cells (Table 2–3). The proximal tubule is responsible for reabsorbing about 90% of filtered water. Reabsorption of the remaining 10% of filtered water in the distal collecting ducts is under tight control by AVP. Although it may seem that only a small fraction of the total filtered water

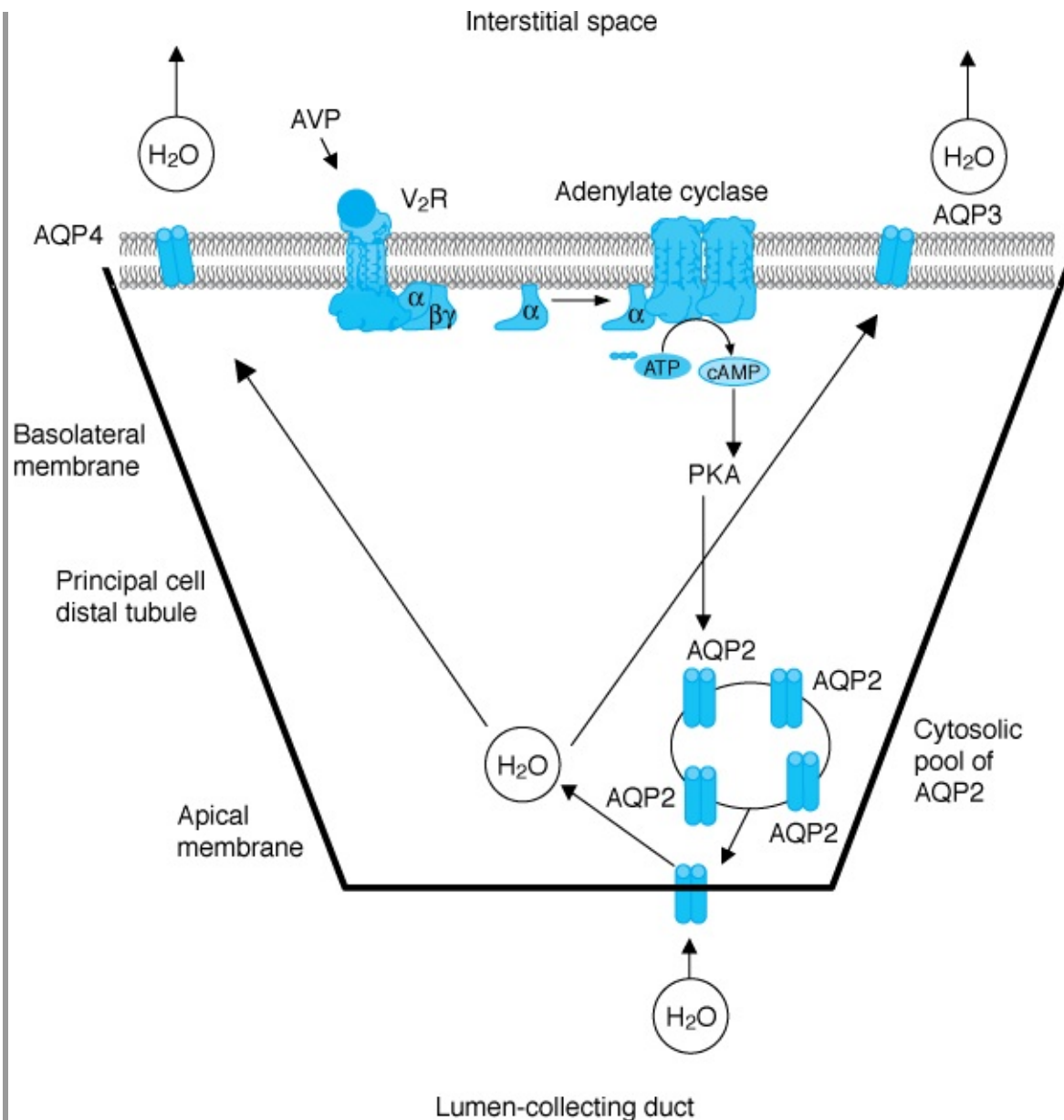
reabsorption is under AVP control, water permeability of the collecting duct can be dramatically increased (within a few minutes) through the production of cyclic adenosine monophosphate (cAMP) following AVP binding to V_2 receptors in the basolateral membrane of the principal cells (Figure 2–6).

Table 2–3. Key Features of Aquaporins

Aquaporin	Features
AQP1	Constitutively expressed in apical and basolateral membranes of epithelial cells of proximal tubules and descending limb of Henle's loop. Involved in 90% of water reabsorption.
AQP2	Exclusively expressed in the collecting ducts. The only aquaporin directly regulated by ADH. Binding to the V_2 AVP receptor stimulates insertion into the luminal membrane.
AQP3, AQP4	Constitutively expressed in the basolateral membranes of epithelial cells in the collecting ducts. Enhance water reabsorption following AQP2 insertion into the luminal membrane.

ADH, antidiuretic hormone; AQP, aquaporin; AVP, arginine vasopressin.

Figure 2–6.



Source: Molina PE: *Endocrine Physiology, 3rd Edition*:
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Cellular mechanism of AVP water conservation. The principal function of arginine vasopressin (AVP) is to increase water reabsorption and to conserve water. AVP binds to the V₂ G protein–coupled receptor (V₂R) in the principal cells of the distal tubule. This triggers the activation of adenylate cyclase and the formation of cyclic adenosine monophosphate (cAMP), leading to activation of protein kinase A (PKA). Protein kinase A phosphorylates the water channel aquaporin 2 (AQP2), leading to the insertion of aquaporin 2 into the luminal cell

membrane. The insertion of water channels into the membrane increases the permeability to water. Water reabsorbed through these water channels leaves the cell through aquaporins 3 (AQP3) and 4 (AQP4), which are constitutively expressed in the basolateral membrane of the principal cells.

The importance of AVP is better understood in terms of the total amount of urine that would be excreted in its absence. For example, in a healthy individual an average of 180 L of glomerular filtrate is formed per day. Thus, without AVP-mediated reabsorption of 10% of filtered water in the distal collecting ducts, urine output would be close to 18 L/d. This is 10-fold higher than the volume of urine output (1.5–2 L/d) under normal conditions.

The increase in cAMP that is stimulated by AVP binding to the receptor located in the basolateral membrane activates protein kinase A and subsequently the phosphorylation of aquaporin 2 (AQP2), another protein. Phosphorylation of AQP2 is essential for its movement from cytoplasmic pools and its insertion in the luminal (apical) epithelial cell membrane of the collecting-duct cells. The result is an increase in the number of functional water channels in the luminal membrane, making it more permeable to water. This event is a short-term regulation of water permeability in response to an increase in circulating levels of AVP. In addition, AVP is thought to regulate water permeability over hours to days as a result of an increase in the total cellular amount of AQP2 due to increased protein synthesis.

AQP2, one of several members of the aquaporin family, is exclusively expressed in the collecting ducts of the kidney. It is the only aquaporin that is directly regulated by ADH via the V_2 AVP receptor. Aquaporins 3 (AQP3) and 4 (AQP4) are constitutively expressed in the basolateral membranes of the collecting ducts and contribute to the enhanced water reabsorption following AQP2 insertion into the luminal membrane. Water that enters the epithelial cell through AQP2 on the apical membrane leaves the cell through AQP3 and AQP4, located in the basolateral membranes of these cells, eventually entering the vasculature. This AVP-mediated insertion of AQP2 into the luminal membrane results in water conservation and urine concentration.

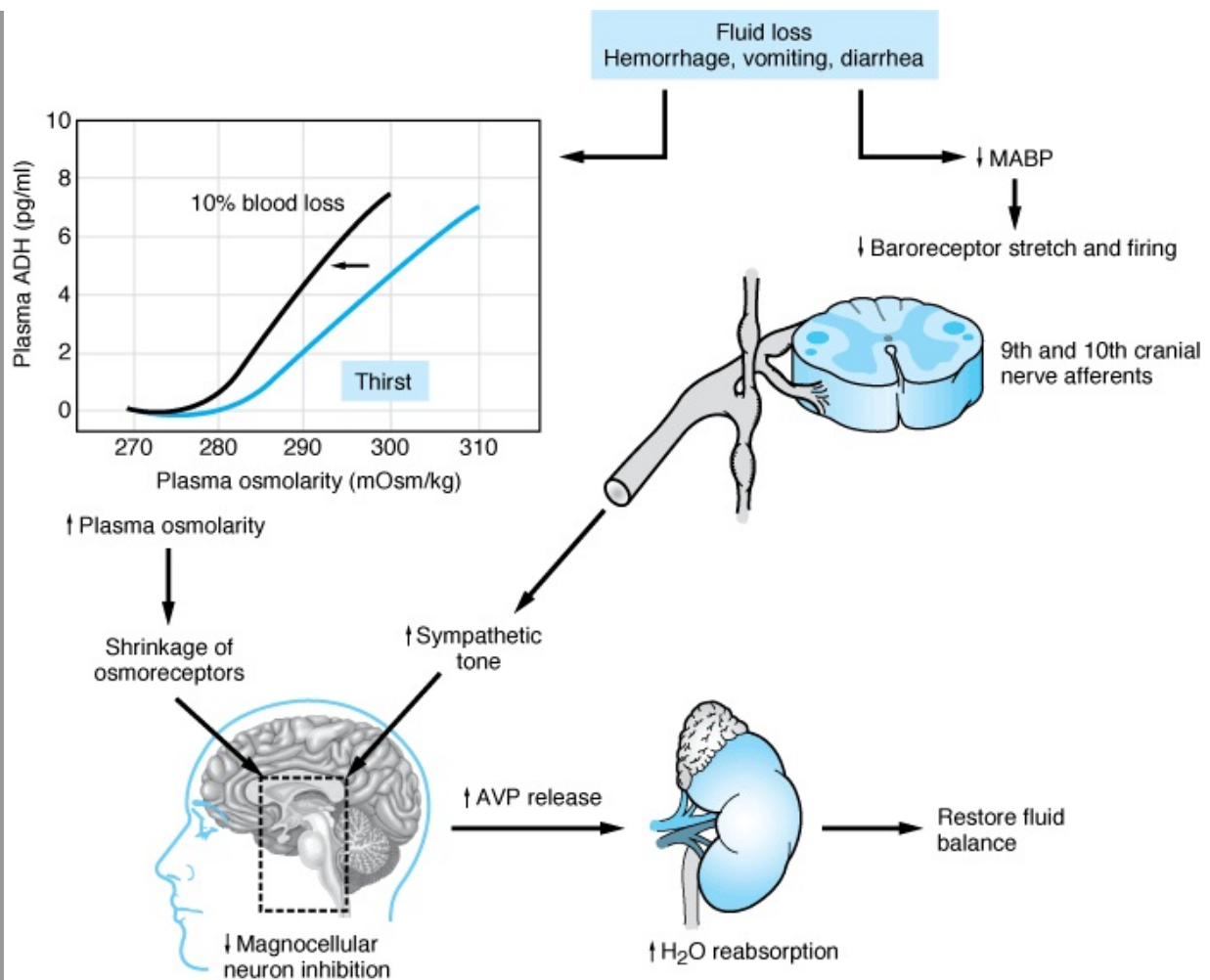
AVP also binds the V_1 receptor, found in the vascular smooth muscle, producing contraction and increasing peripheral vascular resistance. The hormone is known as vasopressin because of these vasoconstrictor effects. In particular, renal medullary blood flow has been demonstrated to be under AVP regulation. AVP circulates unbound and is distributed in a volume approximately equal to that of the extracellular space. Because of its relatively low molecular weight, AVP permeates peripheral and glomerular capillaries readily, so the urinary excretion rate of AVP is extraordinarily high.

CONTROL OF AVP RELEASE



AVP is released into the circulation following either an increase in plasma osmolality or a decrease in blood volume (Figure 2–7). Under physiologic conditions, the most important stimulus for AVP release is the "effective" plasma osmolality. The changes in osmotic pressure are detected by special osmoreceptor neurons located in the hypothalamus and in 3 structures associated with the lamina terminalis: the subfornical organ, the median preoptic nucleus, and the organum vasculosum lamina terminalis. Dehydration produces loss of intracellular water from the osmoreceptors, resulting in cell shrinkage. This decrease in the stretch of the cell signals the AVP magnocellular neurons to stimulate AVP release. Release of AVP occurs even before the sensation of thirst. Therefore, on a hot day, AVP has already been released and is at work conserving fluid even before one feels the desire to drink water. In contrast, hypotonic stimuli, such as excess fluid intake or intravenous fluid administration in a hospital setting, result in cell swelling and stretching and hyperpolarization of the magnocellular neurons, which result in decreased depolarization and firing and consequently decreased release of AVP. The sensitivity of this system is quite high. That is, very small changes in plasma osmolality (as little as 1% change) above the osmotic threshold of 280–284 mOsm/kg water produce significant increases in AVP release.

Figure 2–7.



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Integration of signals that trigger arginine vasopressin (AVP) release. Release of AVP is stimulated by an increase in plasma osmolality and by a decrease in blood volume. Small changes in plasma osmolality above a threshold of 280–284 mOsm/kg produce an increase in antidiuretic hormone (ADH) release before the stimulation of thirst. A decrease in blood volume sensitizes the system and increases the responsiveness to small changes in plasma osmolality. Blood loss and a decrease in mean arterial blood pressure (MABP) greater than 10% signal the hypothalamus to increase the release of AVP. The afferent signals are transmitted by the 9th and 10th cranial nerves. These signals increase sympathetic tone, therefore decreasing magnocellular neuron inhibition and stimulating AVP release.

AVP secretion is also stimulated by a decrease in blood pressure of greater than 10%. Decreases in blood volume or blood pressure are detected by pressure-sensitive receptors in the cardiac atria, aorta, and carotid sinus. Factors that reduce cardiac output, such as a decrease in blood volume greater than 8%, orthostatic hypotension, and positive-pressure breathing, are all potent stimuli for AVP release. A decrease in blood pressure decreases the stretch of the baroreceptors and decreases their firing rate. These signals are transmitted to the central nervous system by neurons of the vagus and glossopharyngeal nerves. The reduced stimulation produces a decrease in tonic inhibition of AVP release, leading to an increase in AVP release from the magnocellular neurosecretory neurons. In addition to signaling the brain to stimulate the release of AVP, the decrease in blood pressure is also perceived by the macula densa in the kidney. This results in stimulation of renin release from the juxtaglomerular apparatus in the kidney. Renin catalyzes the conversion of angiotensinogen produced in the liver to angiotensin I, which is then converted to angiotensin II, by angiotensin-converting enzyme. The resulting rise in circulating levels of angiotensin II sensitizes the osmoreceptors, leading to enhanced AVP release. This is another example of hormonal regulation of hypothalamic neuropeptide release.

The volume-induced sensitization of AVP release results in a more accentuated AVP response to changes in plasma osmolality. However, AVP secretion is far more sensitive to small changes in plasma osmolality than to changes in blood volume. AVP is barely detectable below a certain plasma osmolality (287 mOsm/kg) threshold. Above this threshold, the plasma AVP concentration rises steeply in direct proportion to plasma osmolality. An increase in osmolality of only 1% will change AVP by an average of 1 pg/mL, an amount sufficient to significantly alter urine concentration and output. Circulating levels of AVP may reach 15–20 pg/mL under a strong osmotic stress. Because of this extraordinary sensitivity, the osmoreceptor plays the primary role in mediating the antidiuretic response to changes in water balance. In contrast, the response to pressure-volume changes is exponential. Reductions in plasma volume by 5–10% usually have little effect on AVP levels, whereas decreases of 20–30% result in intense hormone secretion, bringing AVP concentrations to a level many times higher than

that required to produce maximum antidiuresis (up to 50–100 pg/mL). In other words, small changes in plasma osmolality are more effective than small changes in blood pressure and volume in stimulating AVP release. Other factors that can increase AVP release are estrogen and progesterone, opiates, and nicotine. Alcohol and atrial natriuretic factor inhibit AVP release.

AVP RECEPTORS

The cellular effects of AVP are mediated by binding to G protein–coupled membrane receptors. Three AVP receptors have been characterized thus far, which differ in terms of where they are expressed as well as in the specific G proteins to which they are coupled and, thus, in the second-messenger systems that they activate.

V_1R (also known as V_{1a}) is coupled to $G_{q/11}$ and is specific for AVP. It is found in the liver, smooth muscle, brain, and adrenal glands. It activates phospholipases C, D, and A_2 and stimulates the hydrolysis of phosphatidylinositol, resulting in an increase in intracellular calcium concentrations.

V_2R is coupled to G_s and is expressed in the kidney. Binding of AVP to the V_2R receptor activates adenylate cyclase and increases cAMP formation. The main effects of AVP are mediated through this receptor.

V_3R (also known as V_{1b}) is coupled to $G_{q/11}$ and is expressed in the majority of anterior pituitary corticotroph cells and in several tissues, including the kidney, thymus, heart, lung, spleen, uterus, and breast. Binding of AVP to the V_3R receptor stimulates the activity of phospholipase C, resulting in an increase in intracellular calcium.

DISORDERS OF AVP PRODUCTION



Either excess or deficiency of AVP can result in clinical disease. The concentrations of AVP may be altered in various chronic pathophysiologic conditions, including congestive heart failure, liver cirrhosis, and nephritic syndrome. The most frequent abnormality is a decrease in AVP release in diabetes insipidus, a clinical syndrome resulting from the inability to form concentrated urine.

Diabetes insipidus—Diabetes insipidus is characterized by the excretion of abnormally large volumes (30 mL/kg of body weight per day for adult subjects) of dilute (<250 mmol/kg) urine and excessive thirst. Three basic defects have been identified in the etiology. Only the first 2 pertain to alterations related to components of the AVP system itself.

Decreased AVP release—Neurogenic (central or hypothalamic) diabetes insipidus is the most common defect and is due to a decrease in AVP release from the posterior pituitary, resulting from diseases affecting the hypothalamic-neurohypophyseal axis. Three causes can be identified: traumatic, inflammatory or infectious, and cancer related.

Decreased renal responsiveness to AVP—Renal (nephrogenic) diabetes insipidus results from renal insensitivity to the antidiuretic effect of AVP. AVP production and release are not affected, but responsiveness at the distal tubule is impaired. Nephrogenic diabetes insipidus can be inherited or acquired and is characterized by an inability to concentrate urine despite normal or elevated plasma concentrations of AVP. About 90% of cases are males with the X-linked recessive form of the disease who have mutations in the AVP type-2 receptor gene. A small number of cases of inherited nephrogenic diabetes insipidus are due to mutations in the AQP2 water channel gene. Acquired nephrogenic diabetes insipidus can result from lithium treatment, hypokalemia, and postobstructive polyuria.

Excess water intake—Finally, the third possible cause of diabetes insipidus is excess water intake. This cause does not involve dysfunction of the AVP system.

Differential diagnosis—The differential diagnosis of diabetes insipidus relies on an understanding of the physiologic regulation of AVP release and its effects on the kidney. As in screening for other endocrine pathologies, plasma levels of AVP are determined using radioimmunoassay. The values obtained are interpreted together with the indirect assessment of antidiuretic activity triggered by a dehydration test. This test determines the ability of the body to increase the production and release of AVP during water deprivation. Fluids are withheld from the individual, and the rise in urine osmolality, which indicates the response of the body to conserve fluid, is measured. Normal function consists of an increase in urine osmolality and a

decrease in urine output during water deprivation alone.

Another way of testing the system is by a challenge test with synthetic AVP. Individuals with normal pituitary function do not exhibit a further increase in urine osmolality following the administration of a synthetic AVP analog (desmopressin). Individuals with central diabetes insipidus have a $>9\%$ increase in urine osmolality following desmopressin administration, indicating that the body is not capable of producing maximal release of AVP and consequently does a better job when a synthetic analog of AVP is administered. Another approach to diagnosing central diabetes insipidus is also based on the physiologic regulation of AVP release and uses the response to osmotic stimulation produced by the intravenous infusion of hypertonic (5%) saline solution. These examples illustrate that understanding the etiology of the disease requires an understanding of the normal physiologic regulation of the endocrine system in question.

Syndrome of inappropriate ADH secretion—An increase or excess in the release of ADH, also known as the syndrome of inappropriate ADH secretion, may be the result of tumor production of AVP. The tumor can be located in the brain, but malignancies of other organs such as the lung have also been shown to produce high levels of AVP. The excess production of AVP results in the production of very small volumes of concentrated urine. Retention of water may lead to hyponatremia. Management of this condition entails fluid restriction and in some cases the use of saline solutions to restore adequate plasma sodium levels.

KEY CONCEPTS



The parvocellular neurons of the hypothalamus produce neuropeptides that are released at the median eminence and are transported to the anterior pituitary, where they regulate the release of anterior pituitary hormones.



The hypothalamus integrates information from various brain regions, the environment, and peripheral organs and mediates systemic responses that help maintain homeostasis.



The magnocellular neurons of the hypothalamus produce oxytocin and AVP, hormones that are released from the posterior pituitary into the systemic circulation.



Prohormone posttranslational modification and processing of oxytocin and AVP occur inside the secretory granules during axonal transport.



AVP binds to the V_2 receptor and increases water reabsorption by stimulating the insertion of aquaporin 2 into the apical (luminal) membrane of tubular collecting-duct epithelial cells.



The release of AVP is more sensitive to small changes in plasma osmolality than to small changes in blood volume.



Deficiency of AVP results in the production of large quantities of dilute urine.

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OBJECTIVES

- ▶ *Identify the 3 families of anterior pituitary hormones and their main structural differences.*
- ▶ *Understand the mechanisms that regulate anterior pituitary hormone production and describe the actions of tropic hormones on target organs.*
- ▶ *Diagram the short-and long-loop negative feedback control of anterior pituitary hormone secretion.*
- ▶ *Predict the changes in secretory rates of hypothalamic anterior pituitary and target gland hormones caused by oversecretion or undersecretion of any of these hormones or receptor deficit for any of these hormones.*
- ▶ *Explain the importance of pulsatile and diurnal hormone secretion.*

ANTERIOR PITUITARY GLAND: INTRODUCTION

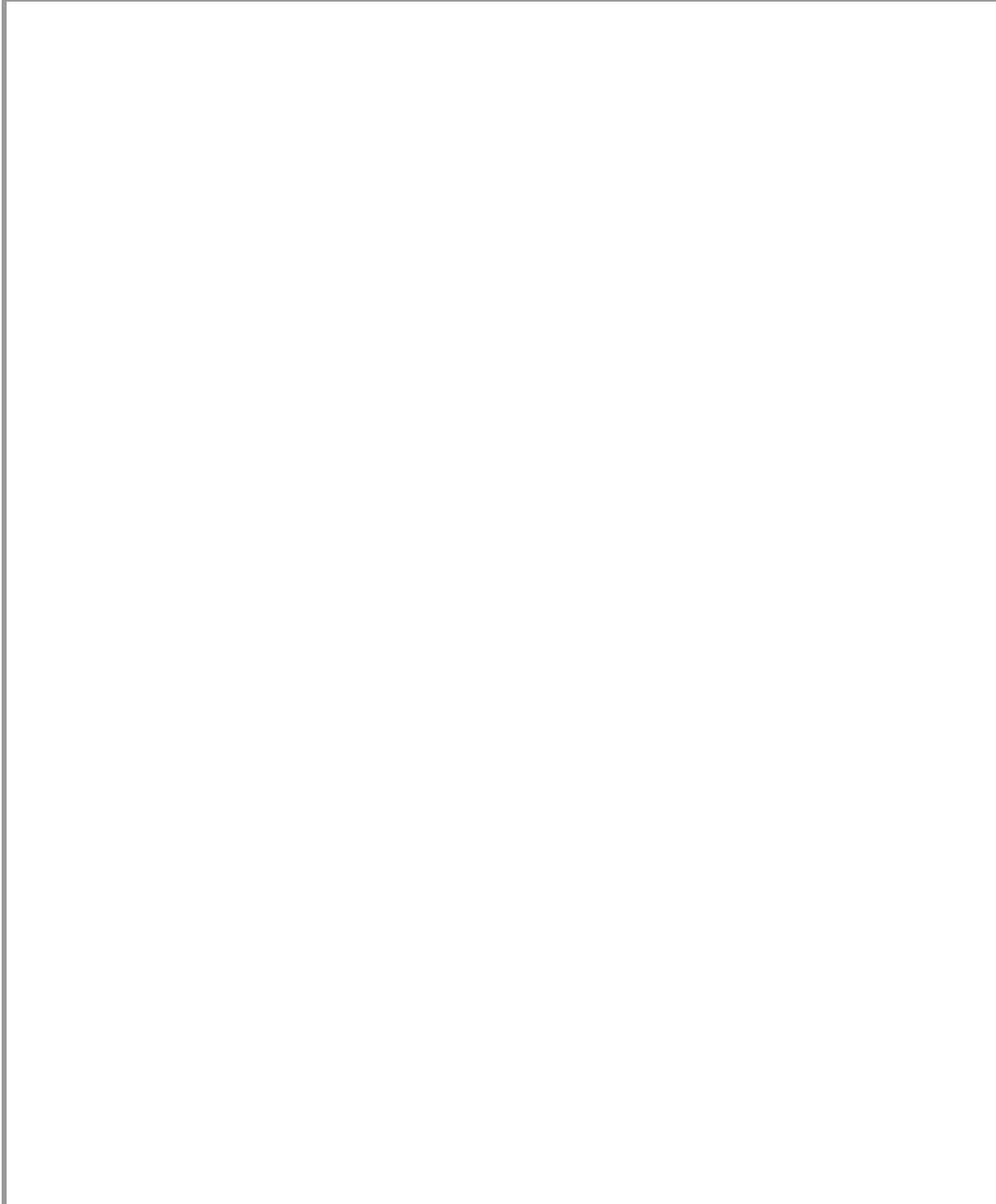


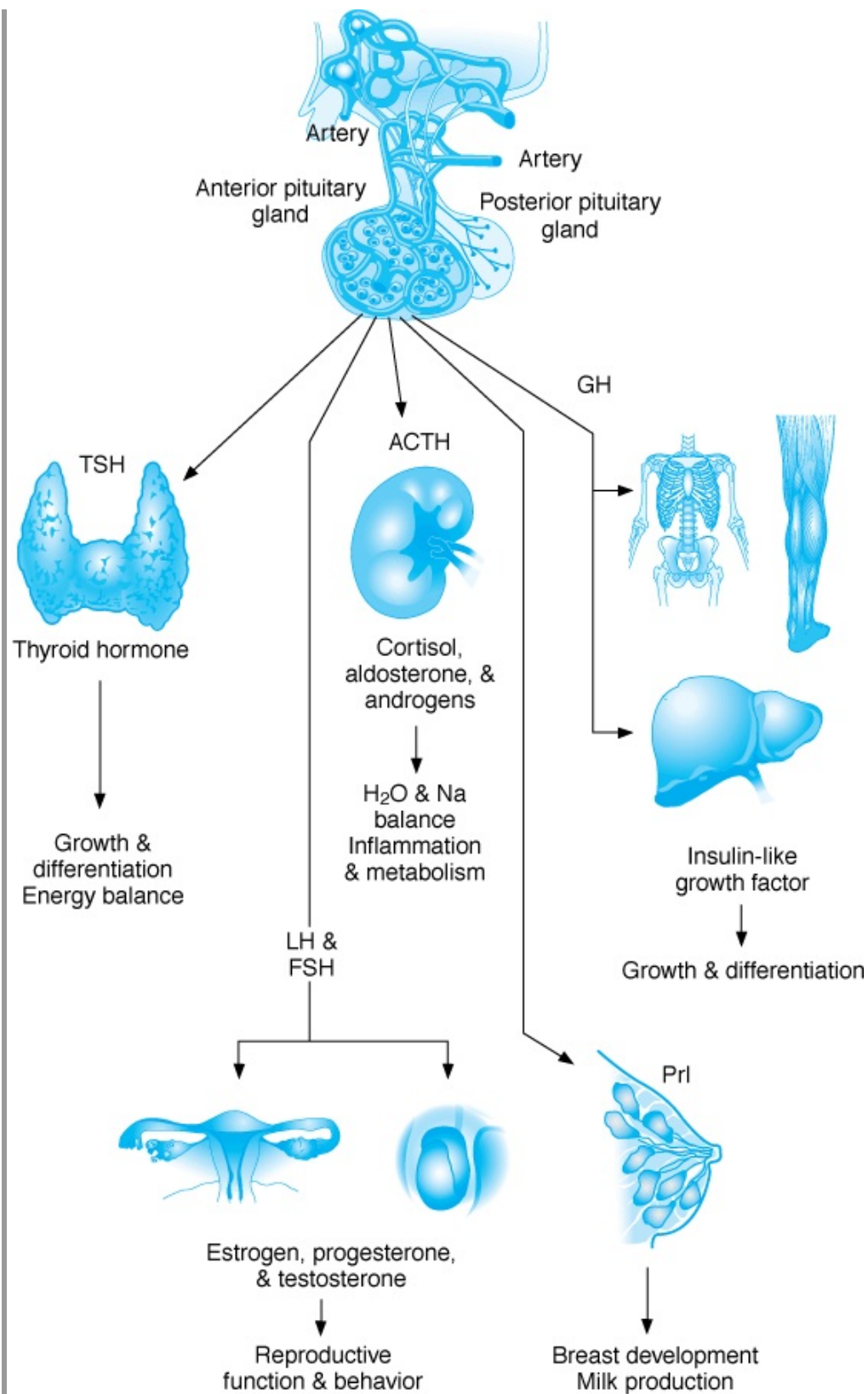
The anterior pituitary, or adenohypophysis, plays a central role in the regulation of endocrine function through the production and release of **tropic hormones** (Figure 3–1). The function of the anterior pituitary, and thereby the production of tropic hormones, is under hypothalamic regulation by the hypophysiotropic neuropeptides released in the median eminence, as discussed in Chapter 2 and summarized in Table 3–1. The tropic hormones produced by the anterior pituitary are released into the systemic circulation, from where they reach their target organs to produce a physiologic response, most frequently involving the release of a target organ hormone (Figure 3–1).

The hormones produced by the target organs affect anterior pituitary function as well as the release of hypophysiotropic

neuropeptides, maintaining an integrated feedback control system of endocrine function (Chapter 1, Figure 1–11).

Figure 3–1.





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Anterior pituitary hormones, target organs, and physiologic effects. Thyroid-stimulating hormone (TSH) stimulates the thyroid gland to produce and release thyroid hormones that regulate growth, differentiation, and energy balance. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) stimulate gonadal production of sex steroids, which mediate reproductive function and behavior. Adrenocorticotrophic hormone (ACTH) stimulates the adrenal glands to produce steroid hormones, which regulate water and sodium balance, inflammation, and metabolism. Prolactin (Prl) stimulates breast development and milk production. Growth hormone (GH) exerts direct effects on tissue growth and differentiation and indirect effects through the stimulation of insulin-like growth factor-I production, which mediates some of the growth and differentiation effects of GH.

Table 3–1. Anterior Pituitary Cell Type, Regulatory Hypothalamic Factor, and Hormone Product

Anterior pituitary cells	Hypothalamic factor	Pituitary hormone produced
Lactotrophs	Dopamine	Prolactin
Corticotrophs	CRH	POMC: ACTH, β -LPH, α -MSH, β -endorphin
Thyrotrophs	TRH	Thyroid-stimulating hormone
Gonadotrophs	GnRH	LH and FSH
Somatotrophs	GHRH	Growth hormone

ACTH, adrenocorticotrophic hormone; CRH,

corticotropin-releasing hormone; FSH, follicle-stimulating hormone; GHRH, growth hormone–releasing hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; LPH, lipotropin; MSH,

melanocyte-stimulating hormone; POMC,

proopiomelanocortin; TRH, thyrotropin-releasing hormone.

FUNCTIONAL ANATOMY

The pituitary, or hypophysis, consists of an anterior and a posterior lobe that differ from one another in their embryologic origin, mode of development, and structure. The anterior lobe, also known as the **adenohypophysis**, is the larger and consists of a pars anterior and a pars intermedia, or intermediate lobe, separated from each other by a narrow cleft, the remnant of Rathke's pouch. The pars intermedia is of minor importance in human physiology.

The anterior pituitary is a highly vascularized structure consisting of epithelial cells derived from the ectodermal lining of the roof of the mouth. The pituitary cells that line the capillaries produce the tropic hormones: adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH), growth hormone (GH), prolactin, and the gonadotropins luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Figure 3–1). All of these hormones are released into the systemic circulation.

The cells of the anterior

pituitary are named according to the hormone that they produce.

According to their specific distribution, they may be more or less susceptible to traumatic injury. For example, the gonadotrophs and somatotrophs (GH-producing cells) are more numerous in the

posterolateral region of the anterior pituitary, making them vulnerable to mechanical damage of the pituitary. The corticotrophs (ACTH-producing cells) and the thyrotrophs

(TSH-producing cells) are located predominantly in the anteromedial region, making them more resilient to traumatic injury. The lactotrophs (prolactin-producing cells) are dispersed throughout the pituitary, and this too is a resilient cell population. The posterior pituitary is of nervous origin. It consists of unmyelinated nerve fibers and axon terminals of magnocellular hypothalamic neurons, with bodies located primarily in the supraoptic and paraventricular hypothalamic nuclei. The neurohormones released from the posterior pituitary have been discussed in Chapter 2. This chapter will focus on the endocrine function of the anterior pituitary.

HYPOTHALAMIC CONTROL OF ANTERIOR PITUITARY HORMONE RELEASE



The production of pituitary tropic hormones is under direct regulation by the hypothalamic neurohormones released from neuronal terminals in the median eminence. The neurohormones are delivered to the anterior pituitary through a specialized capillary network, described in Chapter 2 and illustrated in Figure 2–2. The hypothalamic

neuropeptides travel down the long hypophyseal portal veins to the anterior pituitary, where they bind to specific cell-surface G

protein–coupled receptors and activate intracellular second-messenger cascades, resulting in the release of pituitary hormone from the respective target cells.

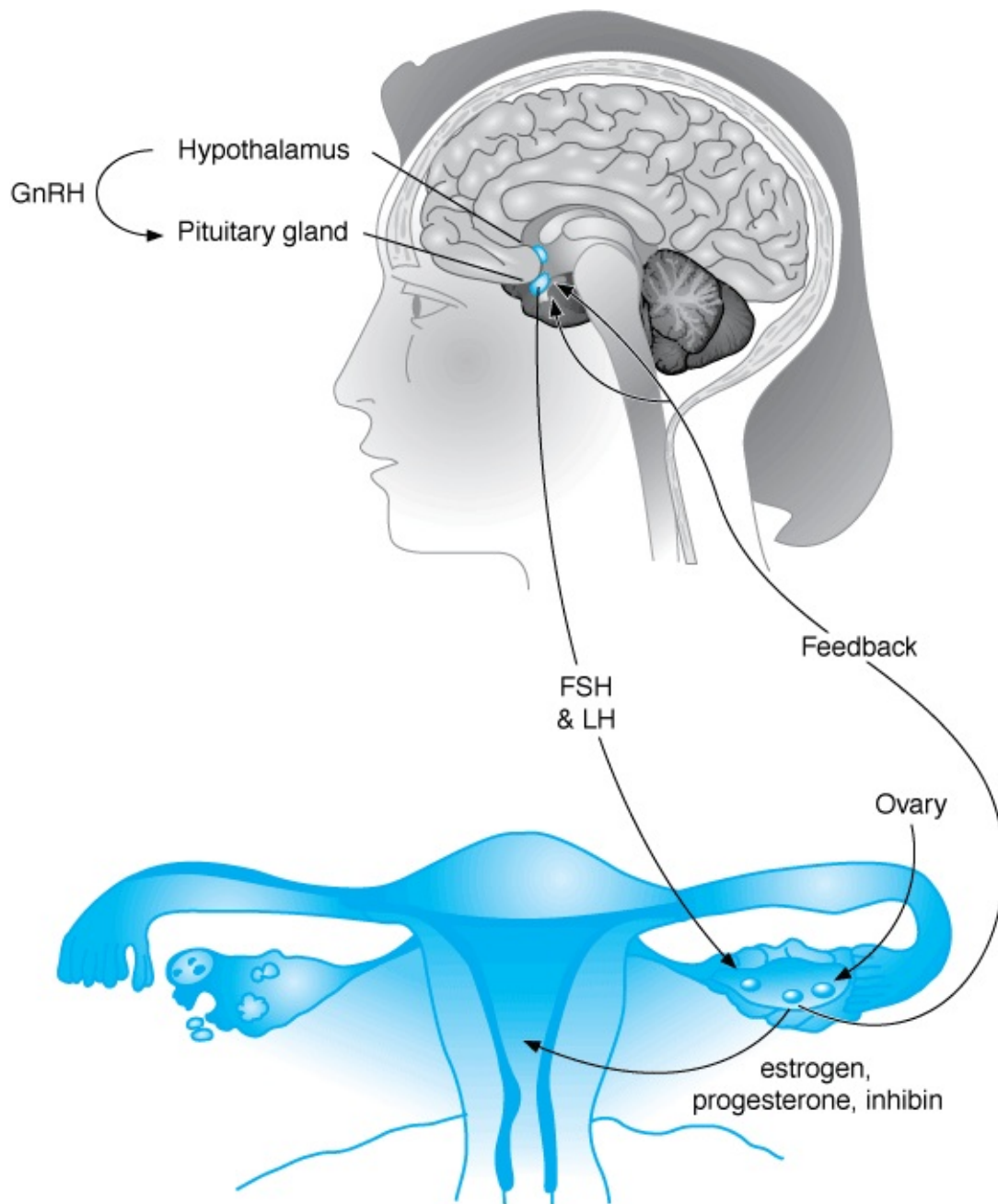
The

responsiveness of the anterior pituitary to the inhibitory or stimulatory effects of hypophysiotropic neurohormones can be

modified by several factors, including hormone levels, negative feedback inhibition, and circadian rhythms (Figure 3-2). The release of anterior pituitary hormones is cyclic in nature, and this cyclic pattern of hormone release is governed by the nervous system. Most rhythms are driven by an internal biologic clock located in the hypothalamic **suprachiasmatic nucleus**; this clock is synchronized by external signals such as light and dark periods. Both sleep and circadian effects interact to produce the overall rhythmic pattern of pituitary hormone release and the associated responses. Some of the 24-hour hormonal rhythms depend on the circadian clock (ie, ACTH, cortisol, and melatonin) and some are sleep related (ie, prolactin, GH, and TSH). For example, GH secretion is influenced by the first slow-wave sleep episode at the beginning of the night. Pulses of prolactin and GH are positively linked to increases in delta-wave activity, present during the deepest phases of sleep and occurring primarily during the first third of the night. Pulses of TSH

and cortisol are related to superficial phases of sleep.

Figure 3-2.



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Feedback regulation of pituitary hormone release.

Hypothalamic neurohormones (eg, gonadotropin-releasing hormone [GnRH]) stimulate the anterior pituitary to produce

and release tropic hormones (eg, follicle-stimulating hormone [FSH] and luteinizing hormone [LH]).

Tropic hormones bind to receptors in target organs and elicit a physiologic response. In most cases, the response involves the production of a target organ hormone, which in turn mediates physiologic effects at the target organ (eg, uterus). In addition, the target organ hormone is involved in feedback mechanisms (negative or positive) that regulate the production and release of the tropic hormone and the hypothalamic factor that regulates pituitary hormone release.



Although the regulation of the patterns of hormone release is not well understood, it is clear that the respective patterns of anterior pituitary hormone release play a crucial role in achieving their physiologic effects and, thus, in maintaining homeostasis. The importance of this regulation has become evident because constant or continuous exogenous hormone administration produces effects that differ from the hormone's natural physiologic effects. These observations have highlighted the importance of trying to simulate as much as possible the endogenous cyclic patterns of hormone release when giving hormone replacement therapy to a patient. In addition, disruption of the cyclic patterns of hormone release has been identified in disease states and is thought to play an important role in the impaired endocrine function that occurs with aging. Therefore, the natural cyclic pattern of hypothalamic, pituitary, and target organ hormone release is of central importance to normal endocrine function.

HORMONES OF THE ANTERIOR PITUITARY

The hormones of the anterior

pituitary can be classified into 3 families: the **glycoproteins**, those derived from proopiomelanocortin (**POMC**), and those belonging to the **GH and prolactin** family.

Glycoproteins

Glycoprotein hormones are among the largest hormones known to date. They include TSH, FSH, LH, and human chorionic gonadotropin produced by the placenta. These hormones are heterodimeric glycoproteins consisting of a common α -subunit and a unique β -subunit, which confers the biologic specificity of each hormone.

THYROID-STIMULATING HORMONE

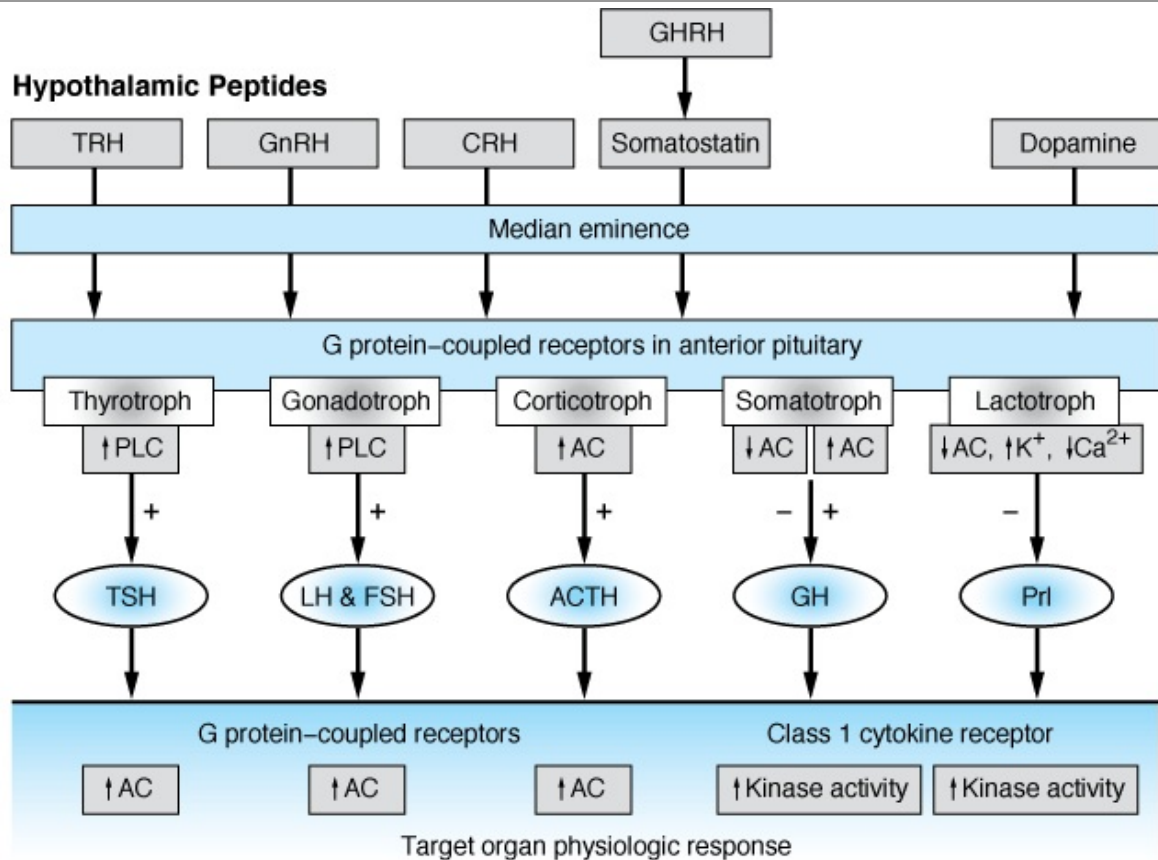
TSH is a glycoprotein

synthesized and secreted from thyrotrophs of the anterior pituitary gland. Thyrotrophs constitute approximately 5% of all adenohypophyseal cells. They synthesize and release TSH in response to

thyrotropin-releasing hormone (TRH) stimulation. TRH is synthesized in the paraventricular nuclei of the hypothalamus, predominantly by parvocellular neurons, and is released from nerve terminals in the median eminence. TRH binds to a $G_{q/11}$ protein-coupled receptor, which activates phospholipase C, leading to increased phosphoinositide turnover, calcium mobilization, and release of TSH into the circulation (Figure 3–3). TSH binds to a G_s

protein-coupled receptor in the thyroid gland, activating adenylate cyclase and leading to increased intracellular cAMP formation and stimulation of the protein kinase A signaling pathway. TSH stimulates all the events involved in thyroid hormone synthesis and release (Chapter 4). In addition, it acts as a growth and survival factor for the thyroid gland. The release of TSH from the anterior pituitary gland is under negative feedback inhibition by thyroid hormone, particularly triiodothyronine, as discussed in detail in Chapter 4.

Figure 3–3.



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Cellular signaling pathways involved in hypothalamo-pituitary hormone-mediated effects. All hypothalamic releasing and inhibiting factors mediate their effects predominantly via G protein–coupled receptors. Anterior pituitary hormones bind to either G protein–coupled receptors (thyroid-stimulating hormone [TSH], luteinizing hormone [LH], follicle-stimulating hormone [FSH], adrenocorticotrophic hormone [ACTH]) or class-1 cytokine receptors (growth hormone [GH] and prolactin [Prl]). Most of the cellular responses elicited by anterior pituitary hormones that bind to G

protein–coupled receptors are mediated by modulation of adenylate cyclase activity. The cellular responses evoked by anterior pituitary binding to class-1 cytokine receptors are mediated through protein

kinase activation. AC, adenylate cyclase; CRH, corticotropin-releasing hormone; GHRH, GH-releasing hormone; GnRH, gonadotropin-releasing hormone; PLC, phospholipase C; TRH, thyrotropin-releasing hormone.

GONADOTROPINS (FSH AND LH)

The gonadotropic hormones LH

and FSH are synthesized and secreted by gonadotrophs of the anterior pituitary in response to stimulation by gonadotropin-releasing hormone (GnRH). Gonadotrophs constitute about 5–10% of the pituitary cells. Most of the gonadotrophs (60%) produce both LH and FSH. The remainder of the gonadotroph population produces LH (18%) or FSH (22%) exclusively. GnRH

is synthesized and secreted by the hypothalamus in a pulsatile manner.

GnRH binds to the GnRH $G_{q/11}$ protein–coupled receptor on pituitary gonadotrophs and produces activation of phospholipase C, leading to phosphoinositide turnover and Ca^{2+} mobilization and influx (Figure 3–3). This signaling cascade increases the transcription of the FSH and LH α -subunit and β -subunit genes and increases the release of FSH and LH into the circulation.

FSH and

LH exert their physiologic effects on multiple cells of the reproductive system by binding to G_s protein–coupled receptors and activation of adenylate cyclase. Among the target cells for gonadotropins are ovarian granulosa cells,

theca interna cells, testicular Sertoli cells, and Leydig cells. The physiologic responses produced by the gonadotropins include stimulation of sex hormone synthesis (steroidogenesis), spermatogenesis, folliculogenesis, and ovulation. Therefore, their central role is the control of reproductive function in both males and females. GnRH controls the synthesis and secretion of both FSH and LH by the pituitary gonadotroph cell.

Gonadotropin synthesis and release, as well as differential expression, is under both positive and negative feedback control by gonadal steroids and gonadal peptides (Figure 3–2). Gonadal hormones can decrease gonadotropin release both by decreasing GnRH release from the hypothalamus and by affecting the ability of GnRH to stimulate gonadotropin secretion from the pituitary itself. Estradiol enhances LH

and inhibits FSH release, whereas inhibins A and B, gonadal glycoprotein hormones, reduce FSH secretion (Chapter 9).

The complexity of the regulation of synthesis and release of anterior pituitary hormones is best illustrated by the cyclic nature of FSH and LH release. The pattern of GnRH pulses changes during the menstrual cycle in women, as summarized in Table 3–2 and discussed in detail in Chapter 9. During the luteal to follicular phase transition, pulses of GnRH release occur every 90–120 minutes, and FSH

secretion predominates. In the mid-to late-follicular phase, GnRH

frequency increases to 1 pulse every 60 minutes, favoring LH secretion over FSH. After ovulation, ovarian progesterone production predominates.

Progesterone increases hypothalamic opioid activity and

slows GnRH

pulse secretion. This slower GnRH pulse pattern (1 pulse per 3–5 hours) favors FSH production. However, at the same time, estradiol and inhibin A produced by the corpus luteum inhibit FSH release, leading to increased FSH stores. With involution of the corpus luteum and the sharp decline in estradiol, inhibin A, and progesterone, the frequency of GnRH pulse secretion is increased. In the absence of estradiol and inhibin A (inhibitors of FSH release), a selective FSH release predominates and initiates the next wave of follicular development.

Table 3–2. Regulation

of Gonadotropin Release in Ovulating Females

Phase of menstrual cycle	Gonadal hormones	GnRH pulses	Gonadotropin release
Luteal to follicular transition	Low estradiol Low inhibin	90–120 minutes	FSH > LH
Mid-to late-follicular phase	Increasing estradiol and inhibin B	Increased pulsatility; 60 minutes	LH > FSH
Post ovulation	Increased estradiol, inhibin A, and progesterone	Decreased GnRH pulsatility	Increased FSH synthesis; inhibited release
Corpus luteum involution	Decreased estradiol, inhibin A, and progesterone	Increased GnRH pulsatility	FSH

FSH, follicle-stimulating hormone;

GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

Proopiomelanocortin-Derived

Hormones

POMC is a

precursor prohormone produced by the corticotrophs of the anterior pituitary. Corticotrophs account for 10% of the secretory cells of the anterior pituitary. The production and secretion of POMC-derived hormones from the anterior pituitary are regulated predominantly by corticotropin-releasing hormone (CRH) produced in the paraventricular nucleus of the hypothalamus and released in the median eminence. CRH

binds to a G_s protein–coupled receptor whose actions are mediated through activation of adenylate cyclase and elevation of cAMP

production (Figure 3–3). Two types of remarkably homologous (about 70%

amino acid identity) CRH receptors have been identified. Both CRH-1 and CRH-2 receptors belong to the family of transmembrane receptors that signal by coupling to G proteins and use cAMP as a second messenger.

Stimulation of POMC synthesis and peptide release is mediated by the CRH-1 receptor, which is expressed in many

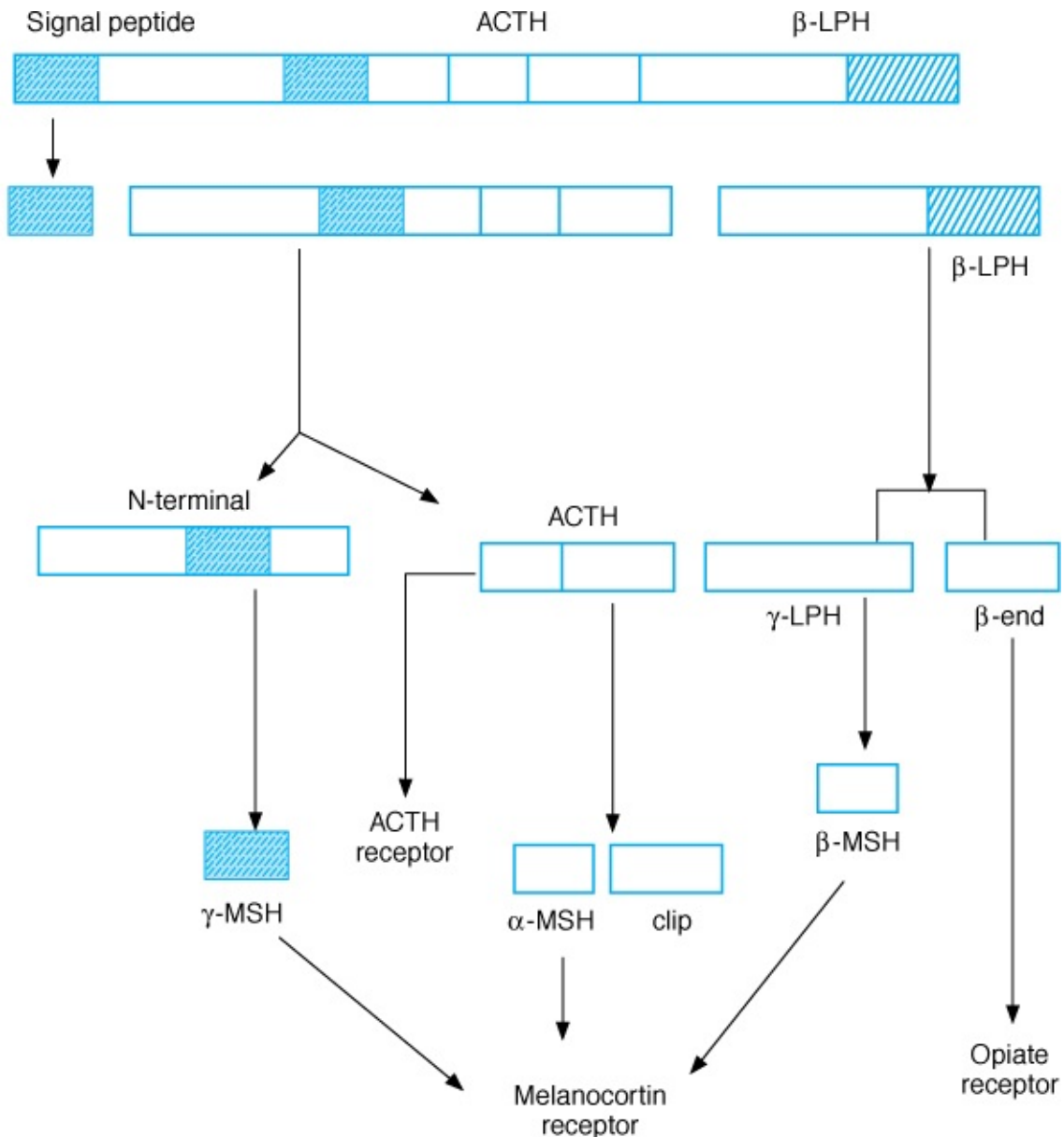
areas of the brain as well as in the pituitary, gonads, and skin. CRH-2 receptors are expressed on brain neurons located in neocortical, limbic, and brainstem regions of the central nervous system and on pituitary corticotrophs and in peripheral tissues (eg, cardiac myocytes, gastrointestinal tract, lung, ovary, and skeletal muscle). The role of CRH-2 receptors is not completely understood. CRH hypothalamic neurons also produce

vasopressin, and this neuropeptide also stimulates the release of POMC-derived peptides from the anterior pituitary.

POMC is post-translationally cleaved to ACTH; β -endorphin, an endogenous opioid peptide; and α -, β -, and γ -melanocyte-stimulating hormones (MSHs) (Figure 3–4). The biologic effects of POMC-derived peptides are largely mediated through melanocortin receptors (MCRs), of which 5 have been described. MC1R, MC2R, and MC5R have defined roles in the skin, adrenal steroid hormone production, and thermoregulation, respectively. MC4R is expressed in the brain and has been implicated in feeding behavior and appetite regulation. The role of MC3R is not well defined.

Figure 3–4.





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Proopiomelanocortin (POMC) processing.

Corticotropin-releasing hormone stimulates the production, release, and processing of POMC, a pre-prohormone synthesized in the anterior pituitary. POMC is post-translationally cleaved to adrenocorticotrophic hormone (ACTH); β -endorphin, an endogenous opioid peptide; and α -, β -, and γ -melanocyte-stimulating hormones (MSHs). The

cellular effects of these peptides are mediated via melanocortin (ACTH and MSH) or opiate (β -endorphin) receptors. β LPH, β -lipotropin.

ADRENOCORTICOTROPIC HORMONE

The main hormone of interest

produced by the cleavage of POMC is ACTH. The release of ACTH is stimulated by psychological and physical stress such as infection, hypoglycemia, surgery, and trauma and is considered critical in mediating the stress or the adaptive response of the individual to stress (Chapter 10). ACTH is released in small amounts, and circulating levels average 2–19 pmol/L in healthy individuals. The hormone is released in pulses, with the highest concentrations occurring at approximately 4:00 AM and the lowest concentrations in the afternoon.

ACTH released into the systemic circulation binds to a G_s

protein–coupled receptor, part of the MCR superfamily (MC2R), and activates adenylate cyclase, increases cAMP formation, and activates protein kinase A (Figure 3–3). The physiologic effects of ACTH at the adrenal cortex are to stimulate the production and release of glucocorticoids (cortisol) and, to a lesser extent, mineralocorticoids (aldosterone) (see Chapter 6). Although all 5 MCRs can bind ACTH to some extent, MC2R binds ACTH with the highest affinity and is expressed almost exclusively in the adrenal cortex; thus, it is considered the physiologic ACTH receptor. The release of cortisol follows the same diurnal rhythm as that of ACTH (Chapter 1, Figure 1–8). The feedback inhibition of ACTH and of CRH release by cortisol is mediated by

glucocorticoid receptor binding in the hypothalamus and in the anterior pituitary.

MELANOCYTE-STIMULATING

Hormone

-MSH

is produced by the proteolytic cleavage of POMC, mainly in the pars intermedia of the pituitary gland (Figure 3–4). Only small amounts of α -MSH

are produced in the pituitary under normal conditions.

Melanocortin peptides exert their effects through MC1R found in melanocytes, which are key components of the skin's pigmentary system, endothelial cells, monocytes, and keratinocytes. Binding of α -MSH

to MC1R activates adenylate cyclase, which in turn causes an increase in intracellular cAMP. This is the classic pathway by which α -MSH

is believed to increase melanin synthesis in melanocytes. The peripheral production of α -MSH

by nonendocrine cells, particularly by melanocytes, has been described.

The involvement of this paracrine system in the development of skin cancer has received considerable attention because of the localized production and paracrine actions of this peptide and the greater expression of MC1R in melanoma than in normal skin.

β -ENDORPHIN

β -Endorphin, the most abundant endogenous opioid peptide, is another product of POMC

processing in the pituitary (Figure 3–4). The physiologic

effects of this opioid peptide are mediated by binding to opiate receptors. Because these receptors are expressed in multiple cell types in the brain as well as in peripheral tissues, their effects are pleiotropic. The physiologic actions of endorphins include analgesia, behavioral effects, and neuromodulatory functions. Among the effects on endocrine function is inhibition of GnRH release. Endogenous opioids have also been implicated in the mechanisms involved in alcohol and drug addiction and have led to therapies such as the use of naltrexone, an opiate receptor antagonist, in the management of alcohol dependency.

GH & Prolactin Family

GROWTH HORMONE

GH, is a 191–amino acid

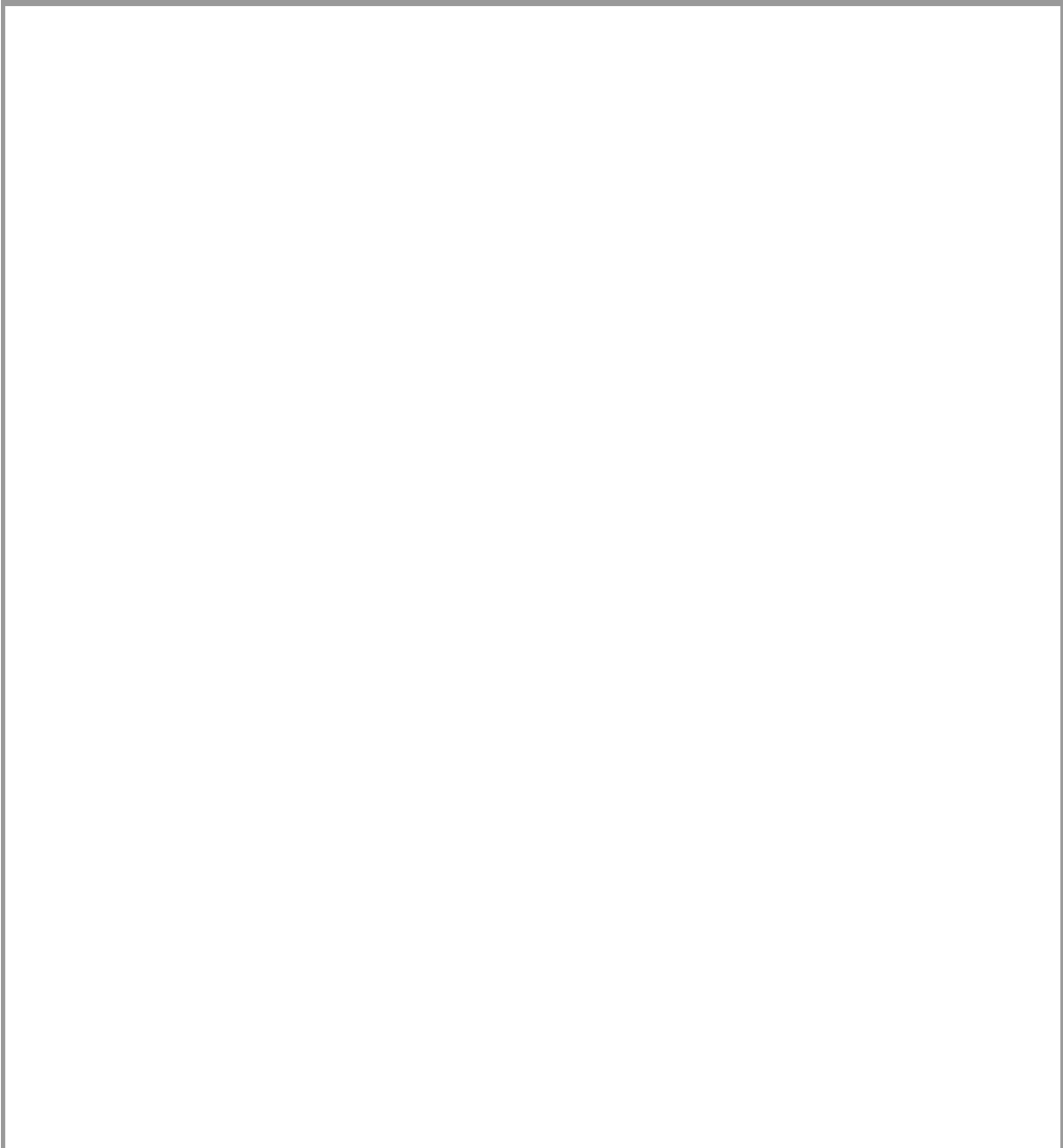
peptide hormone, with a molecular weight of ~22 kDa and structural similarity to prolactin and chorionic somatomammotropin, a

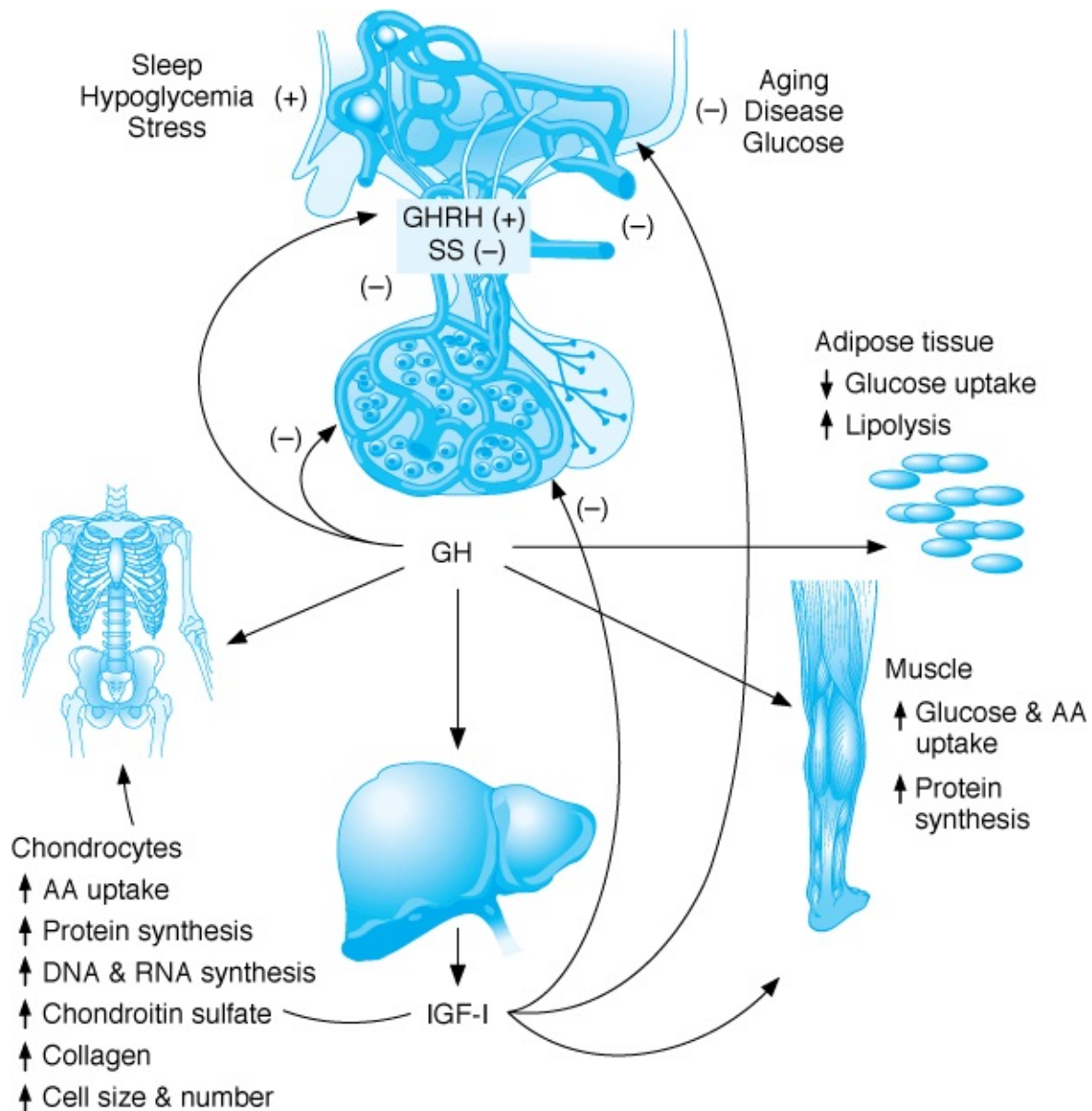
placental-derived hormone. GH exists in various molecular isoforms and this heterogeneity is reflected in the wide variability in GH levels determined by different immunoassays. However, it is the 22 kDa that is the principal form with physiological effects found in humans. GH is released from the somatotrophs, an abundant (50%) cell type in the anterior pituitary. GH is released in pulsatile bursts, with the majority of secretion occurring nocturnally in association with slow-wave sleep (Figure 3–5). The basis of the pulsatile release of GH

and the function of this pattern are not fully understood; however, nutritional, metabolic, and age-related sex steroid

mechanisms, adrenal glucocorticoids, thyroid hormones, and renal and hepatic functions are all thought to contribute to the pulsatile release of GH and appear to be essential in achieving optimal biologic potency of the hormone. Most of the GH in the circulation is bound to GH-binding protein.

Figure 3–5.





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Control of growth hormone (GH) release. Production of GH

is modulated by several factors, including stress, exercise, nutrition, sleep, and GH itself. The primary controllers of GH release are 2

hypothalamic peptide hormones: GH-releasing hormone (GHRH), which stimulates both the synthesis and secretion

of GH, and somatostatin (SS), which inhibits GH release in response to GHRH and to other stimulatory factors such as low blood glucose concentration. GH

secretion is also part of a negative feedback loop involving insulin-like growth factor-I (IGF-I). High blood levels of IGF-I lead to decreased secretion of GH not only by directly suppressing the lactotroph, but also by stimulating the release of somatostatin from the hypothalamus. GH also feeds back to inhibit GHRH secretion and probably has a direct (autocrine) inhibitory effect on secretion from the lactotroph. Integration of all the factors that affect GH synthesis and secretion leads to a pulsatile hormone release pattern. Basal concentrations of GH in blood are very low. In children and young adults, the most intense period of GH release is shortly after the onset of deep sleep. AA, amino acid; FFA, free fatty acid.

Regulation

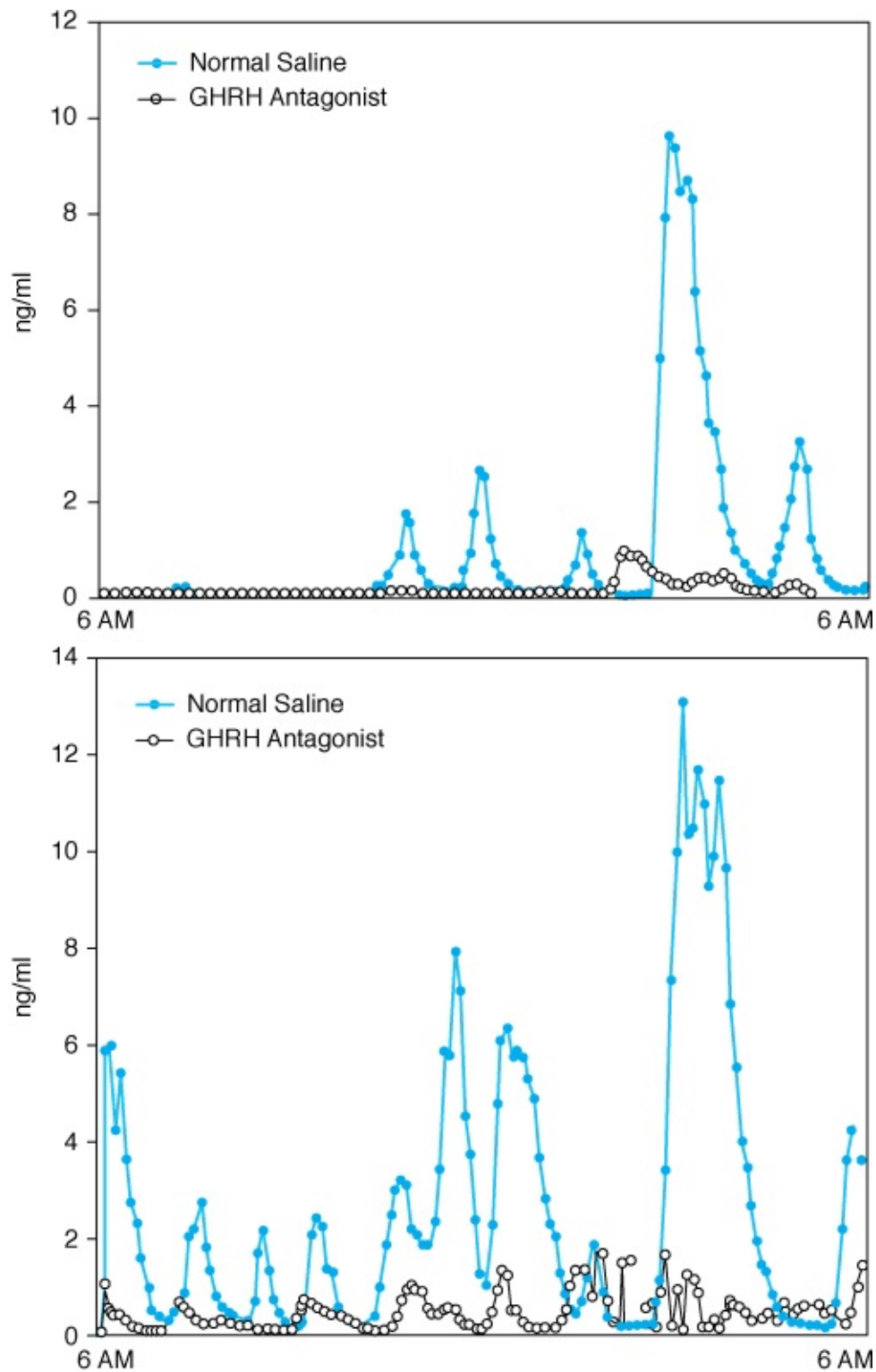
of GH release—The 2 principal hypothalamic regulators of GH or somatotropin release from the anterior pituitary are GH-releasing hormone (GHRH) and somatostatin, which exert stimulatory and inhibitory influences, respectively, on the somatotrophs (Figure 3–6). GH release is also inhibited by insulin-like growth factor-I (IGF-I), the hormone produced in the periphery, particularly in the liver, in response to GH

receptor stimulation, in a classic negative feedback mechanism of hormone control. More recently, ghrelin has been identified as an additional GH secretagogue. Ghrelin is a peptide released predominantly from stomach, but also

expressed in the pancreas, kidney, liver, and in the arcuate nucleus of the hypothalamus (Chapter 10). The overall contribution of ghrelin to regulation of GH release in humans is still not fully elucidated.

Figure 3–6.





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Pulsatile release of growth hormone (GH). The circadian rhythm of

GH release in normal human males (top) and females (bottom) with and without treatment with a GH-releasing hormone (GHRH) antagonist. The majority of the daily production of GH in children and young adults occurs during the early night time hours that follow the onset of deep sleep. GH release during has increased amplitude and duration of pulses. While basal GH release and frequency of GH peaks is not different between males and females, females have higher secretory burst mass per peak. (Reproduced, with permission, from Goldenberg N, Barkan A. Factors regulating GH secretion in humans. *Endocrinol Metabol Clin N Am*. 2007;36:37–55. Copyright © Elsevier.)

GH-releasing hormone—GHRH

stimulates GH secretion from somatotrophs through increases in GH gene transcription and biosynthesis and somatotroph proliferation. GHRH binds to G_s protein–coupled receptors on anterior pituitary somatotrophs, activating the catalytic subunit of adenylate cyclase. The stimulation of adenylate cyclase by G_s protein leads to intracellular cAMP accumulation and activation of the catalytic subunit of protein kinase A (Figure 3–3). Protein kinase A phosphorylates cAMP

response element–binding protein (CREB), leading to CREB activation and enhanced transcription of the gene encoding pituitary-specific transcription factor (Pit-1). Pit-1 activates transcription of the GH

gene, leading to increased GH mRNA and protein and replenishing cellular stores of GH. Pit-1 also stimulates transcription of GHRH receptor gene, resulting in increased numbers of GHRH receptors on the responding somatotroph cell.

Somatostatin—The

stimulated release of GH is inhibited by somatostatin, a peptide synthesized in most brain regions, predominantly in the periventricular nucleus, arcuate nucleus, and ventromedial nucleus of the hypothalamus.

Somatostatin is also produced in peripheral organs, including the endocrine pancreas, where it also plays a role in the inhibition of hormone release. Axons from somatostatin neurons run caudally through the hypothalamus to form a discrete pathway toward the midline that enters the median eminence. Somatostatin produces its physiologic effects through binding to the G_i protein–coupled

somatostatin receptors, resulting in decreased activity of adenylate cyclase, intracellular cAMP, and Ca^{2+} concentrations and stimulation of protein tyrosine phosphatase (Figure 3–3). In addition, somatostatin binding to receptors coupled to K^+ channels causes hyperpolarization of the membrane, leading to cessation of spontaneous action potential activity and a secondary reduction in intracellular Ca^{2+} concentrations. The expression of somatostatin receptors is modulated by hormones and by the nutritional state of the individual.

Other

regulators—In addition to regulation by GHRH and somatostatin, GH

is regulated by other hypothalamic peptides and neurotransmitters, which act by regulation of GHRH and somatostatin release, as summarized in Table 3–3. Catecholamines, dopamine, and excitatory amino acids

increase GHRH and decrease somatostatin release, resulting in an increase in GH

release (Figure 3–6). Hormones such as cortisol, estrogen, androgens, and thyroid hormone can also affect somatotroph responsiveness to GHRH

and somatostatin and consequently GH release. Metabolic signals such as glucose and amino acids can affect GH release. Decreased blood glucose concentrations (hypoglycemia) stimulate GH secretion in humans. In fact, insulin-induced hypoglycemia is used as a clinical test to provoke GH

secretion in GH-deficient children and adults. Glucose and nonesterified fatty acids decrease GH release, whereas amino acids, particularly arginine, increase GH release by decreasing somatostatin release.

Consequently, arginine administration is also an effective challenge to elicit an increase in GH release in the clinical setting.

Table 3–3. Factors That Regulate Growth Hormone Release

Stimulation of GH release	Inhibition of GH release
GHRH	Somatostatin
Dopamine	IGF-I
Catecholamines	Glucose
Excitatory amino acids	FFA
Thyroid hormone	

FFA,

free fatty acids; GH, growth hormone; GHRH, GH-releasing hormone; IGF-I, insulin-like growth factor-I.

GH is released from the

anterior pituitary into the systemic circulation. Circulating GH levels are <3 ng/mL, and the majority (60%) is bound to GH-binding protein.

This protein is derived from proteolytic cleavage of the GH membrane receptor by metalloproteases and serves as a reservoir for GH, prolonging its half-life by decreasing its rate of degradation. The half-life of the hormone averages 6–20 minutes. GH is degraded in the lysosomes following binding to its receptor and internalization of the hormone-receptor complex.

Physiologic effects of GH—GH can have direct effects on cellular responses, by binding to the GH receptor at target tissues and indirectly, by stimulating the production and release of IGF-I, a mediator of several of GH's effects at target tissues. IGF-I is a small peptide (about 7.5 kDa) structurally related to proinsulin that mediates several of the anabolic and mitogenic effects of GH in peripheral tissues. The most important physiologic effect of GH is stimulation of postnatal longitudinal growth. GH also plays a role in regulation of substrate metabolism, adipocyte differentiation, maintenance and development of the immune system, and regulation of brain and cardiac function.

GH receptor—In the peripheral tissues, GH

binds to specific cell-surface receptors belonging to the class-
1

cytokine receptor superfamily (Figure 3–3). This family of receptors includes those for prolactin, erythropoietin, leptin, interferons, granulocyte colony–stimulating factor, and interleukins. GH receptors are present in many biologic tissues and cell types, including liver, bone, kidney, adipose tissue, muscle, eye, brain, heart, and cells of the immune system. The GH molecule exhibits 2 binding sites for the GH

receptor, resulting in dimerization of the receptor, a step that is required for biologic activity of the hormone. Receptor dimerization is followed by activation of a receptor-associated kinase, Janus-kinase 2.

This kinase acts via special signal transducers and activators of transcription proteins (STATs), which dimerize and translocate to the nucleus, transmitting signals to specific regulatory DNA response elements.



GH effects at target organs—Bone—GH stimulates longitudinal growth by increasing the formation of new bone and cartilage. The growth effects of GH are not critical during the gestational period, but begin gradually during the first and second years of life and peak at the time of puberty. Before the epiphyses in long bones have fused, GH stimulates chondrogenesis and widening of the cartilaginous epiphysal plates, followed by bone matrix deposition. In addition to its effects on linear growth stimulation, GH plays a role in regulating the normal physiology of bone formation in the adult by increasing bone turnover, with increases in bone formation and, to a lesser extent, bone resorption. The effects of GH at the epiphysal growth plate are thought to be mediated directly through stimulation of chondrocyte precursor differentiation and indirectly through enhancement of the local production of and responsiveness to IGF-I, which, in turn, acting in an autocrine or paracrine fashion, stimulates clonal expansion of differentiating chondrocytes.

Adipose tissue—GH stimulates release and oxidation of free fatty acids, particularly during fasting. These effects are mediated by a reduction in the activity of lipoprotein lipase, the enzyme involved in clearing triglyceride-rich chylomicrons and very low-density lipoprotein particles from the bloodstream. Thus, GH favors the availability of free fatty acids for adipose tissue storage and skeletal muscle oxidation.

Skeletal muscle—GH has anabolic actions on skeletal muscle tissue. GH stimulates amino acid uptake and

incorporation into protein, cell proliferation, and suppression of protein degradation.

Liver—GH

stimulates hepatic IGF-I production and release. GH stimulates hepatic glucose production.

Immune

system—GH affects multiple aspects of the immune response, including B-cell responses and antibody production, natural killer cell activity, macrophage activity, and T-lymphocyte function.

GH also has central nervous

system effects by modulating mood and behavior. Overall, GH counteracts the action of insulin on lipid and glucose metabolism, by decreasing skeletal muscle glucose utilization, increasing lipolysis, and stimulating hepatic glucose production.

Key aspects of GH physiology can be summarized as follows:

- GH

is produced and stored in somatotrophs in the anterior pituitary.

- The production of GH is pulsatile, mainly nocturnal, and is controlled mainly by GHRH and somatostatin.
-

Circulating levels of GH increase during childhood, peak during puberty, and fall with aging.

- GH

stimulates lipolysis, amino acid transport into cells, and protein synthesis.

- GH

stimulates the production of IGF-I, which is responsible for many of the activities attributed to GH.

INSULIN-LIKE GROWTH

Factors

Many of the

growth and metabolic effects of GH are mediated by the IGFs, or somatomedins. These small-peptide hormones are members of a family of insulin-related peptides including relaxin, insulin, IGF-I, and IGF-II.

The IGFs are synthesized primarily by the liver and, unlike insulin, retain the C peptide; they circulate at higher concentrations than insulin and act as a mitogen, stimulating DNA, RNA, and protein synthesis. IGF-I circulates in blood either free (half-life is about 15–20 minutes) or bound to one

of several specific binding proteins that prolong the half-life of the peptide. These binding proteins, like the IGFs, are synthesized primarily in the liver and are produced locally by several tissues, where they act in an autocrine or paracrine manner.

Both of the IGFs are essential to embryonic development, and nanomolar concentrations of both persist in the circulation into adult life. After birth, however, IGF-I appears to have the predominant role in regulating growth, whereas the physiologic role of IGF-II is unknown. In adults, IGF-II occurs in quantities threefold higher than those of IGF-I, is minimally GH dependent, and decreases modestly with age.

The principal site for IGF-I

secretion is the liver; from where it is transported to other tissues to function as an endocrine hormone. However, IGF-I is secreted by a variety of tissues and thus exerts tissue-specific paracrine effects. In addition, IGF-I is thought to have autocrine effects and to act as an oncogene. Many of the anabolic effects of GH are mediated by IGF-I.

Regulation of IGF-I

production—As mentioned previously, IGF-I is secreted primarily by the liver and is transported to other tissues, acting as an endocrine hormone. IGF-I secreted by extrahepatic tissues, including cartilaginous cells, acts locally as a paracrine hormone. GH, parathyroid hormone, and sex steroids regulate the production of IGF-I in bone, whereas sex steroids are the main regulators of local production of IGF-I in the reproductive system. The functions of circulating IGF-I are becoming clearer, but the actions of locally produced IGFs

have yet to be defined. IGF-I is an important GH, mediating the anabolic and linear growth-promoting effects of pituitary GH protein. In cartilage cells, IGF-I has synergistic effects with GH. IGF-I concentrations are low at birth, increase substantially during childhood and puberty, and begin to decline in the third decade, paralleling the secretion of GH. The binding proteins regulate the biologic actions of the IGFs.

IGF-binding proteins—Six

IGF-I-binding proteins (IGFBPs) have been identified and constitute an elaborate system for regulating IGF-I activity. IGFBPs regulate the availability of IGF-I to its receptor in target tissues. IGFBPs generally inhibit IGF-I action by binding competitively to it and thereby reducing its bioavailability; however, in some cases, they appear to enhance IGF-I activity or to act independently of IGF-I. In humans, almost 80% of circulating IGF-I is carried by IGFBP-3, a ternary complex consisting of 1 molecule of IGF-I, 1 molecule of IGFBP-3, and 1

molecule of a protein named acid-labile subunit. In this form, IGFBP-3

sequesters IGF-I in the vascular system, increasing its half-life and providing an IGF-I reservoir. Other IGFBPs form binary complexes with IGF-I that may cross the capillary boundary, allowing selective transport of IGF-I to various tissues. The interaction between IGFBPs and IGFs is controlled by 2 different mechanisms: (1) proteolytic cleavage by a family of specific serine proteases, which decreases IGF

binding affinity and (2) binding to the extracellular matrix, which potentiates IGF actions. Cleavage of IGFBPs by their

specific proteases also influences IGF-I bioavailability by reducing the amount of bioavailable IGFBPs. Overall IGF-I bioactivity *in vivo*, therefore, represents the combined effect of interactions involving endocrine, autocrine, and paracrine sources of IGF-I, IGFBPs, and IGFBP

proteases.

The IGFBPs

are produced by a variety of different tissues through complex regulatory processes. Hepatic IGFBP-3 and production of its acid-labile subunit are under stimulation by GH. Insulin is the primary regulator of hepatic IGFBP-1 production. Relatively little is known about the principal regulatory mechanisms that control the expression of IGFBP-2, IGFBP-4, IGFBP-5, and IGFBP-6.

Physiologic effects of IGF-I—IGF-I is a small 70–amino acid peptide that exerts its physiologic effects by binding to specific cell-surface receptors. Although IGF-I binds primarily to the IGF-I receptor, some effects may be mediated through the IGF-II and insulin receptors. The similarity in structure to insulin explains the ability of IGF-I to bind (with low affinity) to the insulin receptor.

The main effects of IGF-I are regulation of somatic growth, cell proliferation, transformation, and apoptosis. The importance of this hormone in linear growth is clearly demonstrated by the severe growth failure in children with congenital IGF-I deficiency.

IGF-I stimulates bone

formation, protein synthesis, glucose uptake in muscle, neuronal survival, and myelin synthesis. IGF-I also reverses

negative nitrogen balance during food deprivation and inhibits protein degradation in muscle. IGF-I increases replication of cells of the osteoblastic lineage, enhances osteoblastic collagen synthesis and matrix apposition rates, and decreases collagen degradation in calvariae. IGF-I is also thought to stimulate bone resorption by enhanced osteoclastic recruitment, thus acting on both bone formation and resorption, possibly coupling the 2 processes.

IGF receptor—IGF-I and IGF-II bind specifically to 2 high-affinity membrane-associated receptors that are tyrosine kinases. A third receptor, the IGF-II mannose-6-phosphate receptor, binds IGF-II but has no known intracellular signaling actions.

The IGF receptors are heterotetramers that belong to the same family of receptors as insulin. They are ligand-activated receptor kinases that become autophosphorylated upon hormone binding. The receptor for IGF is composed of 2 extracellular spanning α -subunits and transmembrane β -subunits.

The α -subunits have binding sites for IGF-I and are linked by disulfide bonds. The β -subunit has a short extracellular domain, a transmembrane domain, and an intracellular domain. The intracellular part contains a tyrosine kinase domain, which constitutes the signal transduction mechanism. Ligand binding results in autophosphorylation of the receptor, increasing the kinase activity and allowing it to phosphorylate multiple substrate proteins, such as insulin receptor substrate 1. This produces a continued cascade of enzyme activation via phosphatidylinositol-3

kinase, Grb2 (growth factor receptor-bound protein 2), Syp (a phosphotyrosine phosphatase), Nck (an oncogenic protein),

and Shc (src homology domain protein), in association with Grb2. This signaling cascade leads to activation of protein kinases including Raf, mitogen-activated protein kinase, 5 G kinase, and others involved in mediating growth and metabolic responses.

The insulin and IGF-I receptors, although similar in structure and function, play different physiologic roles *in vivo*.

In healthy individuals, the insulin receptor is primarily involved in metabolic functions, whereas the IGF-I receptor mediates growth and differentiation. The separation of these functions is controlled by several factors, including the tissue distribution of the respective receptors, the binding with high affinity of each ligand to its respective receptor, and the binding of IGF to IGFBPs.

PROLACTIN

Prolactin is a polypeptide

hormone synthesized and secreted by lactotrophs in the anterior pituitary gland. The lactotrophs account for approximately 15–20% of the cell population of the anterior pituitary gland. However, this percentage increases dramatically in response to elevated estrogen levels, particularly during pregnancy. Prolactin levels are higher in females than in males, and the role of prolactin in male physiology is not completely understood. Plasma concentrations of prolactin are highest during sleep and lowest during the waking hours in humans.



Regulation of prolactin release—Prolactin release is predominantly under tonic inhibition by dopamine derived from

dopaminergic neurons of the periventricular and arcuate nuclei of the medial–basal hypothalamus. In addition, prolactin release is also under inhibitory control by somatostatin (SST), and γ -aminobutyric acid. However, the overall regulation of prolactin release is complex and involves not only inhibition by dopamine, but also stimulation by serotonergic and opioidergic pathways, GnRH, and possibly galanin. Dopaminergic inhibition of

lactotroph release of prolactin is mediated by dopaminergic (D_2) G_i protein–coupled receptors, resulting in inhibition of adenylate cyclase and inositol phosphate metabolism (Figure 3–3). In addition, activation of the D_2 receptor modifies at least 5

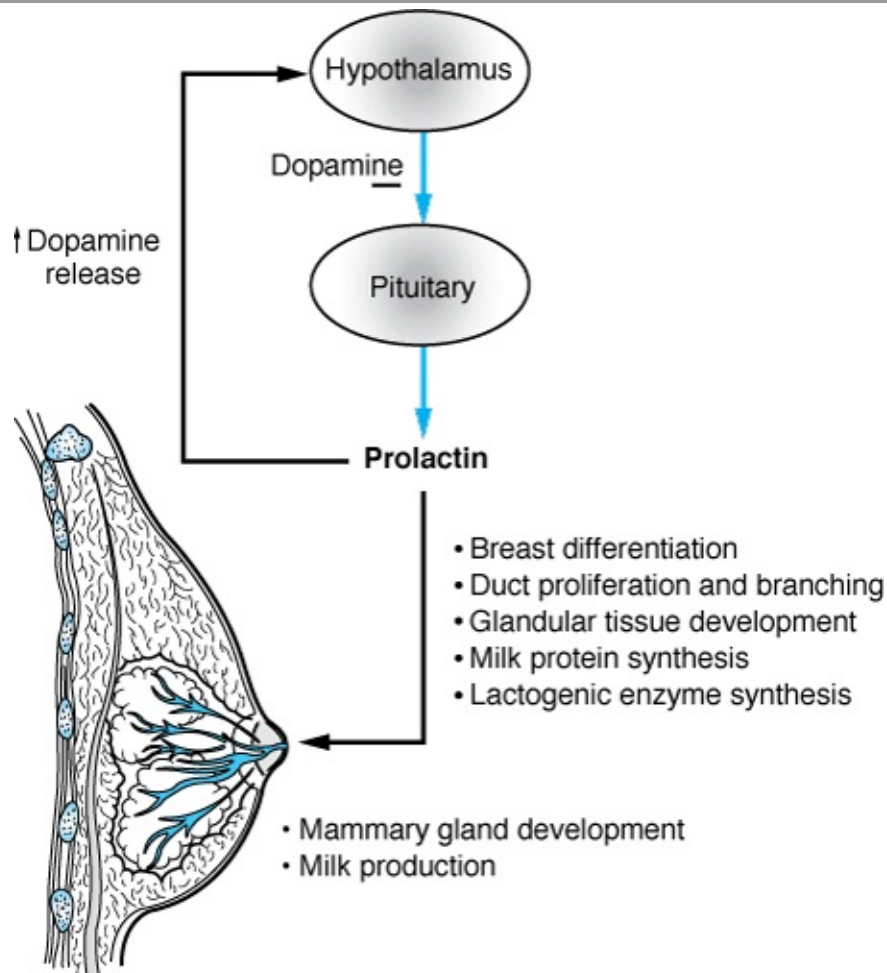
different ion channels. In particular, dopamine activates potassium current that induces plasma membrane hyperpolarization while decreasing voltage-activated calcium currents. Therefore, dopamine-induced inhibition of prolactin secretion is a function of the inhibition of adenylate cyclase activity, activation of voltage-sensitive potassium channels, and inhibition of voltage-sensitive calcium channels.

Prolactin release is affected

by a large variety of stimuli provided by the environment and the internal milieu, the most important being suckling, increased levels of ovarian steroid hormones, primarily estrogen and stress. The release of prolactin in response to suckling is a classical neuroendocrine reflex also referred to as a stimulus-secretion reflex (Figure 3–7). This surge in prolactin release in response to a suckling stimulus is mediated by a decrease in the amount of dopamine released at the median eminence, relieving the lactotroph from tonic inhibition. Estrogen stimulates growth of the lactotrophs

during pregnancy as well as prolactin gene expression and release.

Figure 3–7.



Source: Molina PE: *Endocrine Physiology, 3rd Edition*:
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Physiologic effects of prolactin. Prolactin plays an important role in the normal development of mammary tissue and in milk production. Prolactin release is predominantly under negative control by hypothalamic dopamine. Suckling stimulates the release of prolactin.

Prolactin inhibits its own release by stimulating dopamine release from the hypothalamus.

Several neuropeptides have

been identified as prolactin-releasing factors. These include TRH, oxytocin, vasoactive intestinal peptide, and neurotensin. These releasing factors fall into 2 categories of peptides: those which are active in the presence of the physiologic inhibitory tone of dopamine and those that have an effect only when dopamine inhibitory tone has been removed. TRH belongs to the first category and is a potent stimulus for the release of prolactin through stimulation of TRH receptors on the lactotroph cell membrane. However, the physiologic role of TRH-induced prolactin release is unclear. Furthermore, it is important to note that release of TSH and prolactin do not always occur hand in hand under physiologic conditions. Nevertheless, exogenous

administration of TRH can elevate levels of prolactin.

Prolactin regulates its own

secretion through a short-loop feedback mechanism by binding to prolactin receptors located in neuroendocrine dopaminergic neurons; this binding leads to increased hypothalamic dopamine synthesis (Figure 3–7). When the concentration of dopamine in the hypothalamo-hypophysial portal blood rises, the release of prolactin from the lactotrophs is suppressed.

Physiologic

effects of prolactin—The physiologic effects of prolactin are mediated by the prolactin receptor; a single membrane-bound protein that belongs to class 1 of the cytokine receptor superfamily (Figure 3–3).

Prolactin receptors are found in the mammary gland and the ovary, 2 of the best-characterized sites of prolactin actions in mammals, as well as in various regions of the brain.

Activation of the prolactin receptor involves ligand-induced sequential receptor dimerization. Each prolactin molecule contains 2 binding sites. Prolactin-mediated activation of prolactin receptor results in tyrosine phosphorylation of numerous cellular proteins, including the receptor itself.

The main physiologic effects of prolactin are stimulation of growth and development of the mammary gland, synthesis of milk, and maintenance of milk secretion (Figure 3–7; Chapter 9).

Prolactin stimulates glucose and amino acid uptake and synthesis of the milk proteins β -casein and α -lactalbumin, the milk sugar lactose, and milk fats by the mammary epithelial cells. During pregnancy, prolactin prepares the breast for lactation. The production of milk is prevented during pregnancy by the high progesterone levels. Additional effects of prolactin include inhibition of GnRH release, progesterone biosynthesis, and luteal cell hypertrophy during pregnancy. Prolactin also modulates reproductive and parental behavior. Systemically, prolactin is a component of the immunoneuroendocrine network, by which the nervous, endocrine, and immune systems communicate with each other to modulate the host inflammatory responses. Prolactin modulates immune cell function through endocrine mechanisms. Finally, prolactin can act as a cytokine because it is released by cells of the immune system and regulates the lymphocyte responses by both paracrine and autocrine mechanisms.

DISEASES OF THE ANTERIOR PITUITARY

As with most endocrine organs,

alterations in function of the anterior pituitary can be due to excess or deficient production of pituitary hormones or to altered

responsiveness to hormone effects at the target organ.

Hormone-Producing Pituitary

Adenomas

The most common

cause of excess production of pituitary hormones is a hormone-producing pituitary adenoma. Prolactinomas are the most common (40–45%) pituitary tumors, followed by somatotroph (20%), corticotroph (10–12%), gonadotroph (15%), and rarely thyrotroph (1–2%) adenomas. Small pituitary adenomas can cause manifestations of excess tropic hormone production, whereas larger tumors can produce neurologic symptoms by mass effect in the sellar area.

Patients with a prolactinoma present with elevated levels of prolactin (hyperprolactinemia), milk secretion (galactorrhea), and reproductive dysfunction. In males, prolactinomas may cause infertility by producing hypogonadism. In most cases, dopamine agonists are extremely effective in lowering serum prolactin levels, restoring gonadal function, decreasing tumor size, and improving visual fields. Hyperprolactinemia can also be due to drug-induced inhibition of dopamine release. GH-secreting adenomas can be associated with acromegaly or bone and soft-tissue overgrowth in adults, and with gigantism in children. Corticotropin-releasing adenomas are associated with excess cortisol production or Cushing syndrome; patients

present with central obesity, proximal myopathy, hypertension, mood changes, dorso-cervical fat pads, and hyperglycemia, among other clinical signs and symptoms. Gonadotroph pituitary adenomas are frequently inefficient in hormone production. Thyrotrophin-secreting tumors are rare and are frequently large when diagnosed.

Hypopituitarism

Hypopituitarism, or deficiency of anterior pituitary hormones, can be congenital or acquired. Isolated GH and gonadotropin deficiencies are the most common. The most frequent cause of pituitary insufficiency is trauma, such as that associated with surgery, penetrating injury, or automobile accidents. Severe blood loss and hypoperfusion of the pituitary can also lead to pituitary insufficiency.

Ischemic damage to the pituitary gland or hypothalamic-pituitary stalk during the peripartum period leads to Sheehan syndrome, manifested as hypothyroidism, adrenal insufficiency, hypogonadism, GH deficiency, and hypoprolactinemia.

GH

deficiency and retarded growth may result from impaired release of GH

from the pituitary gland because of diseases of the hypothalamus or pituitary gland or genetic predisposition. Alternatively, mutations in the gene for the GH receptor can cause insensitivity to GH and growth retardation with low serum IGF-I concentrations.

GH Insensitivity

Growth failure can be the result of decreased GH

release, decreased GH action, or GH insensitivity syndrome, also known as Laron syndrome. The syndrome is characterized by deletions or mutations in the GH receptor gene, resulting in failure to generate IGF-I and IGFBP-3. The typical manifestation is short stature or dwarfism, which can be prevented by IGF-I treatment. The study of these patients has provided much of our understanding of the differential effects of GH and IGF-I.

Evaluation of Anterior Pituitary Function

Measurements of anterior

pituitary hormone concentrations and of the respective target gland hormone levels are used to assess the functional status of the system (Table 3–4). For example, paired measures of TSH and thyroid hormone, FSH and estradiol, and ACTH and cortisol are used to evaluate the integrity of the respective systems. In addition, stimulation

and inhibition tests can be used to assess the functional status of the pituitary gland. These tests are based on the normal physiologic feedback mechanisms that control tropic hormone release. For example, insulin-induced hypoglycemia and administration of the amino acid arginine can be used to elicit an increase in GH release in patients with suspected GH deficiency. In contrast, suppression tests can be used to diagnose Cushing syndrome, a clinical state resulting from prolonged, inappropriate exposure to excessive endogenous secretion of cortisol (discussed in Chapter 6). Cushing syndrome is characterized by loss of the normal feedback mechanism of the

hypothalamo-pituitary-adrenal axis and the normal circadian rhythm of cortisol secretion. The basis of the test is that, in most situations, the corticotroph tumor cells in Cushing disease retain some

responsiveness to the negative feedback effects of glucocorticoids, whereas tumors ectopically secreting ACTH do not.

Table 3–4. Pituitary

Tropic Hormone and Target Organ Hormone Pairs

Pituitary tropic hormone	Target organ hormone
ACTH (8:00 AM)	Cortisol (8:00 AM)
<80 pg/mL (<80 pmol/L)	140–690 nmol/L (5–25 µg/dL)
GH	IGF-I
2–6 ng/mL (<5 µg/L)	140–400

	ng/mL
LH (adult, premenopausal)	Estradiol
Female: 5–25 IU/L (3–35 mIU/mL)	Female: 70–220 pmol/L (20–60 pg/mL)
Male: 5–20 IU/L (5–20 mIU/mL)	Male: <180 pmol/L (50 pg/mL)
FSH	Progesterone
Female: 5–20 IU/L (20–50 mIU/mL)	Female: luteal peak >16 nmol/L (75 ng/mL)
Male: 5–20 IU/L (20–50 mIU/mL)	Male: <6 nmol/L (<2 ng/mL)
	Testosterone
	Female: <3.5 nmol/L (<1 ng/mL)
	Male: 10–35 nmol/L (3–10 ng/mL)
TSH	Thyroxine
0.4–5 mU/L (0.4–5 μ U/mL)	64–154 nmol/L (5–12 μ g/dL)
	Triiodothyronine
	1.1–2.9 nmol/L (70–190 ng/dL)
Prolactin 2–15 μ g/L	

(2–5 ng/mL)	

ACTH, adrenocorticotrophic hormone;

FSH, follicle-stimulating hormone; GH, growth hormone;
IGF-I, insulin-like growth factor-I; LH, luteinizing hormone;
TSH,

thyroid-stimulating hormone.

KEY CONCEPTS



Anterior pituitary function is under regulation by the hypothalamus.



Anterior pituitary hormone release is under feedback regulation by peripheral hormone levels.



Pulsatile release of hypothalamic, pituitary, and target organ hormones plays an important role in endocrine function.



GH exerts direct and indirect (IGF-I) effects on linear growth and metabolism.



With the exception of prolactin, adenohypophysial hormones are under stimulatory hypothalamic control.

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OBJECTIVES

- ▶ *Identify the steps and control factors of thyroid hormone biosynthesis, storage, and release.*
- ▶ *Describe the distribution of iodine and the metabolic pathway involved in thyroid hormone synthesis.*
- ▶ *Explain the importance of thyroid hormone binding in blood for free and total thyroid hormone levels.*
- ▶ *Understand the significance of the conversion of tetraiodothyronine (T_4) to triiodothyronine (T_3) and reverse T_3 (rT_3) in extrathyroidal tissues.*
- ▶ *Understand how thyroid hormones produce their cellular effects.*
- ▶ *Describe their effects on development and metabolism.*
- ▶ *Understand the causes and consequences of excess and deficiency of thyroid hormones.*

THYROID GLAND: INTRODUCTION

Thyroid hormones play important roles in maintaining energy homeostasis and regulating energy expenditure. Their physiologic effects, mediated at multiple target organs, are primarily to stimulate cell metabolism and activity. The vital roles of these hormones, particularly in development, differentiation, and maturation, are underscored by the severe mental retardation observed in infants with deficient thyroid hormone function during gestation. Thyroid hormones are derived from the amino acid tyrosine and are produced by the thyroid gland in response to stimulation by thyroid-stimulating hormone (TSH) produced by the anterior pituitary. TSH, in turn, is regulated by the hypophysiotropic peptide thyrotropin-releasing hormone (TRH). Thyroid hormone production is also under regulation by dietary iodine.

FUNCTIONAL ANATOMY

The thyroid gland is a highly vascular, ductless alveolar (acinar) gland located in the anterior neck in front of the trachea. The gland weighs 10–25 g and consists of a right and left lobe connected by the isthmus. The cellular composition of the thyroid gland is diverse, which includes the following:

- Follicular (epithelial) cells, involved in thyroid hormone synthesis
- Endothelial cells lining the capillaries that provide the blood supply to the follicles
- Parafollicular or C cells, involved in the production of calcitonin, a hormone involved in calcium metabolism
- Fibroblasts, lymphocytes, and adipocytes.

Thyroid Follicle

The main function of the thyroid gland is the synthesis and storage of thyroid hormone. The secretory or functional unit of the thyroid gland is the thyroid follicle, consisting of a layer of thyroid epithelial cells arranged around a large central cavity filled with colloid. Colloid makes up about 30% of the thyroid gland mass and contains a protein called **thyroglobulin** (Tg). Tg plays a central role in the synthesis and storage of thyroid hormone, as discussed later. The thyroid epithelial cells are morphologically and functionally polarized. That is, each side or compartment of the cell has specific functions pertaining to the synthesis of thyroid hormones and their release. The apical surface of the follicular cell faces the follicular lumen, where colloid is stored. The basolateral surface faces the interstitium and is therefore exposed to the bloodstream.

The polarity of the thyroid follicular cells is important in the overall function of the cell. Thyroid hormone synthesis requires the iodination of tyrosine residues on the protein Tg, which is stored in the colloid. This process occurs in the colloid at the apical plasma membrane, yet iodine is obtained from the circulation. Whereas thyroid hormone synthesis occurs on the apical side of the thyroid epithelial cell, hormone release occurs at the basolateral side of the cell. Thus, the polarity of the thyroid epithelial cells is critical for maintaining basolaterally located iodide uptake and tetraiodothyronine (T_4) deiodination, and apically located iodide efflux and iodination mechanisms.

The parafollicular cells surround the follicles. Most parafollicular cells synthesize and secrete the hormone **calcitonin**, and therefore they are frequently referred to as **C cells**. They are preferentially located in central regions of the thyroid gland lobes, where follicular cell activity is the greatest. Parafollicular cells secrete regulatory factors other than calcitonin that modulate follicular cell activity in a paracrine fashion.

Blood & Nerve Supply

The thyroid gland is highly vascularized with arterial blood derived from the superior and inferior thyroid arteries. The veins draining the thyroid

gland form a plexus on the surface of the gland and on the front of the trachea and give rise to the superior, middle, and inferior thyroid veins, which drain into the internal jugular and innominate veins. The lymphatic vessels end in the thoracic and right lymphatic trunks. Innervation to the thyroid gland is provided through the middle and inferior cervical ganglia of the sympathetic nervous system.

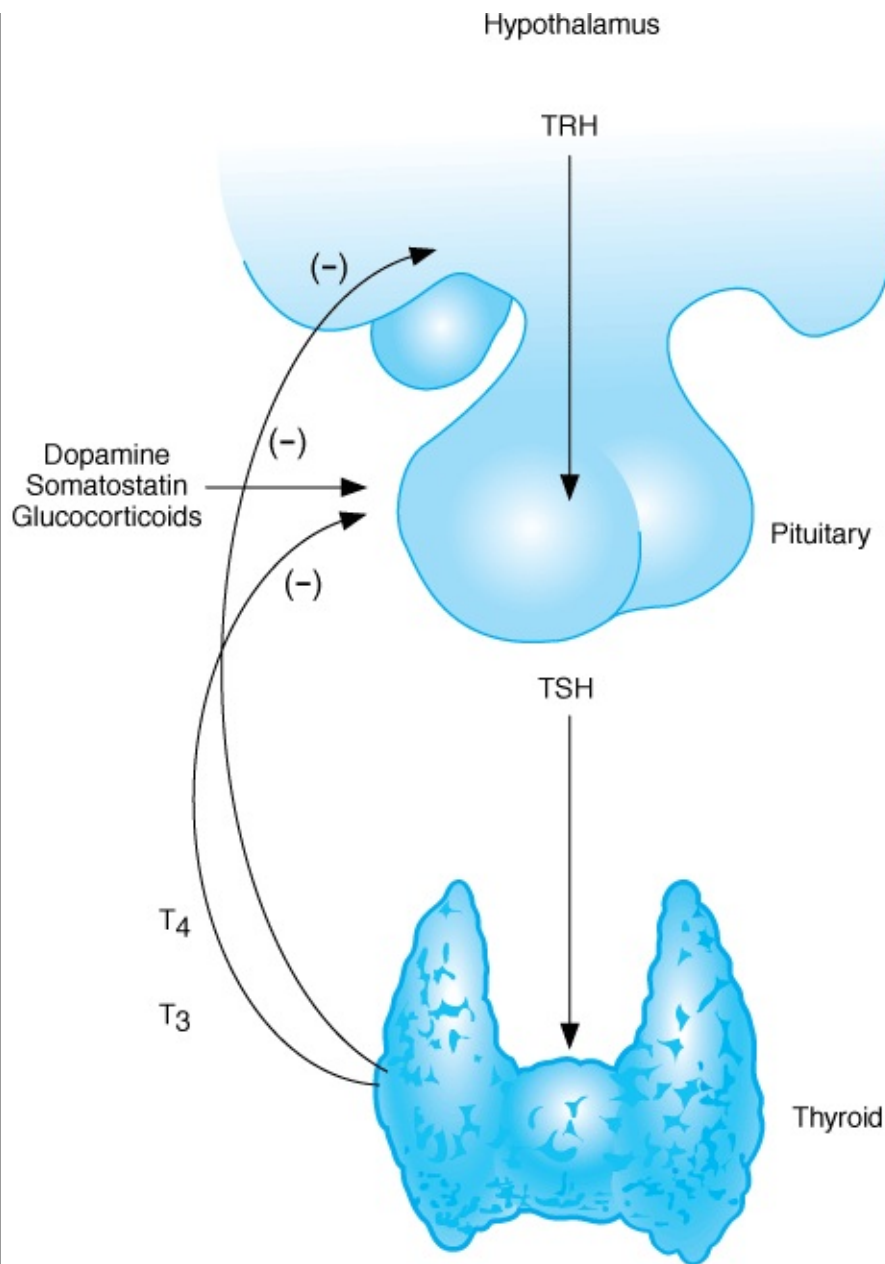
REGULATION OF BIOSYNTHESIS, STORAGE, & SECRETION OF THYROID HORMONES

Hypothalamic Regulation of TSH Release



Thyroid hormone synthesis and release are under negative feedback regulation by the **hypothalamic-pituitary-thyroid axis** (Figure 4–1). TRH is a tripeptide synthesized in parvocellular neurons of the paraventricular nucleus of the hypothalamus. TRH is released from nerve terminals in the median eminence and is transported through the portal capillary plexus to the anterior pituitary. TRH binds to cell membrane $G_{q/11}$ receptors on thyrotrophs (basophilic cells of the anterior pituitary gland), where it activates phospholipase C (PLC), resulting in the hydrolysis of phosphatidylinositol biphosphate and the generation of inositol triphosphate and diacylglycerol. This process leads to an increase in the intracellular Ca^{2+} concentration, resulting in stimulation of exocytosis and release of TSH into the systemic circulation.

Figure 4–1.



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The hypothalamic-pituitary-thyroid axis. Thyrotropin-releasing hormone (TRH) is synthesized in parvocellular neurons of the paraventricular nucleus of the hypothalamus and released from nerve terminals in the median eminence, from where it is transported via the portal capillary plexus to the anterior pituitary. TRH binds to a G protein–coupled receptor in the anterior pituitary, leading to an increase in intracellular Ca^{2+} concentration, which in turn results in

stimulation of exocytosis and release of thyroid-stimulating hormone (TSH) into the systemic circulation. TSH stimulates the thyroid gland to increase the synthesis and secretion of tetraiodothyronine (T_4) and triiodothyronine (T_3) into the circulation. T_4 and T_3 inhibit the secretion of thyrotropin (TSH) both directly and indirectly by inhibiting the secretion of TRH. Additional factors that inhibit TSH release are glucocorticoids, somatostatin, and dopamine.

TSH is transported in the bloodstream to the thyroid gland, where it binds to the TSH receptor located on the basolateral membrane of thyroid follicular epithelial cells. The TSH receptor is a cell membrane G protein–coupled receptor. Adequate function of the TSH receptor is critical in the development, growth, and function of the thyroid gland. Binding of TSH to its receptor initiates signaling through cAMP, PLC and the protein kinase A signal transduction systems. Activation of adenylate cyclase, formation of cAMP, and activation of protein kinase A regulate iodide uptake and transcription of Tg, thyroid peroxidase (TPO), and the activity of the sodium-iodide symporter. Signaling through PLC and intracellular Ca^{2+} regulate iodide efflux, H_2O_2 production, and Tg iodination. The TSH receptor is an important antigenic site involved in thyroid autoimmune disease. Autoantibodies to the receptor may act as agonists mimicking the actions of TSH in the case of Graves disease; or agonists antagonists in the case of autoimmune hypothyroidism (Hashimoto thyroiditis).

TSH receptor activation results in stimulation of all of the steps involved in thyroid hormone synthesis, including iodine uptake and organification, production and release of iodothyronines from the gland, and promotion of thyroid growth. As part of this process, TSH regulates thyroidal uptake of nutrients as well as intracellular transport of specific proteins involved in thyroid hormone synthesis, storage, and release. These cellular events involve regulation of cellular metabolic processes, morphologic differentiation, cell proliferation, and protection from apoptosis. Specifically, the biologic effects of TSH include stimulation of gene transcription of the following:

- Na^+/I^- symporter, the protein involved in transporting and concentrating iodide in the thyroid epithelial cell

- Tg, the glycoprotein that serves as a scaffold for tyrosine iodination and thyroid hormone synthesis, as well as storage of thyroid hormone
- TPO, the enzyme involved in catalyzing the oxidation of iodide and its incorporation into tyrosine residues of Tg
- Thyroid hormones T_4 and triiodothyronine (T_3).

As mentioned earlier, both growth and function of the thyroid are stimulated by the rise in cAMP produced by TSH stimulation. Therefore, continued stimulation of the cAMP pathway by binding to the TSH receptor causes hyperthyroidism and thyroid hyperplasia.

Thyroid Hormone Regulation of TSH Release

The production and release of thyroid hormones are under negative feedback regulation by the hypothalamic-pituitary-thyroid axis. The release of TSH is inhibited mainly by T_3 , produced by conversion of T_4 to T_3 in the hypothalamus, and in the anterior pituitary by type II deiodinase. The contribution of this intracellularly derived T_3 in producing the negative feedback inhibition of TSH release is greater than that of T_3 derived from the circulation. Other neuroendocrine mediators that inhibit TSH release include dopamine, somatostatin, and glucocorticoids at high levels, which produce partial suppression of TSH release (Chapter 10).

Thyroid Hormone Synthesis

Tg, plays an important role in the synthesis and storage of thyroid hormone. Tg is a glycoprotein containing multiple tyrosine residues. It is synthesized in the thyroid follicular epithelial cells and secreted through the apical membrane into the follicular lumen, where it is stored in the colloid. A small amount of noniodinated Tg is also secreted through the basolateral membrane into the circulation. Although circulating levels of Tg can be detected under normal conditions, levels are elevated in diseases such as thyroiditis and Graves disease.

Tg can be considered a scaffold upon which thyroid hormone synthesis takes place. Once Tg is secreted into the follicular lumen, it undergoes major post-translational modification during the production of thyroid hormones. At the apical surface of the thyroid follicular epithelial cells, multiple tyrosine residues of Tg are iodinated, followed by coupling of some of the iodotyrosine residues to form T_3 and T_4 .



The iodide required for thyroid hormone synthesis is readily absorbed from dietary sources, primarily from iodized salt, but also from seafood and plants grown in soil that is rich in iodine. Following its absorption, iodide is confined to the extracellular fluid ($1 \mu\text{g/dL}$), from which it is removed primarily by the thyroid and the kidneys. The total excretion of iodide by the kidneys is approximately equal to daily intake. The balance between dietary intake and renal excretion preserves the total extracellular pool of iodide at about $250 \mu\text{g}$.

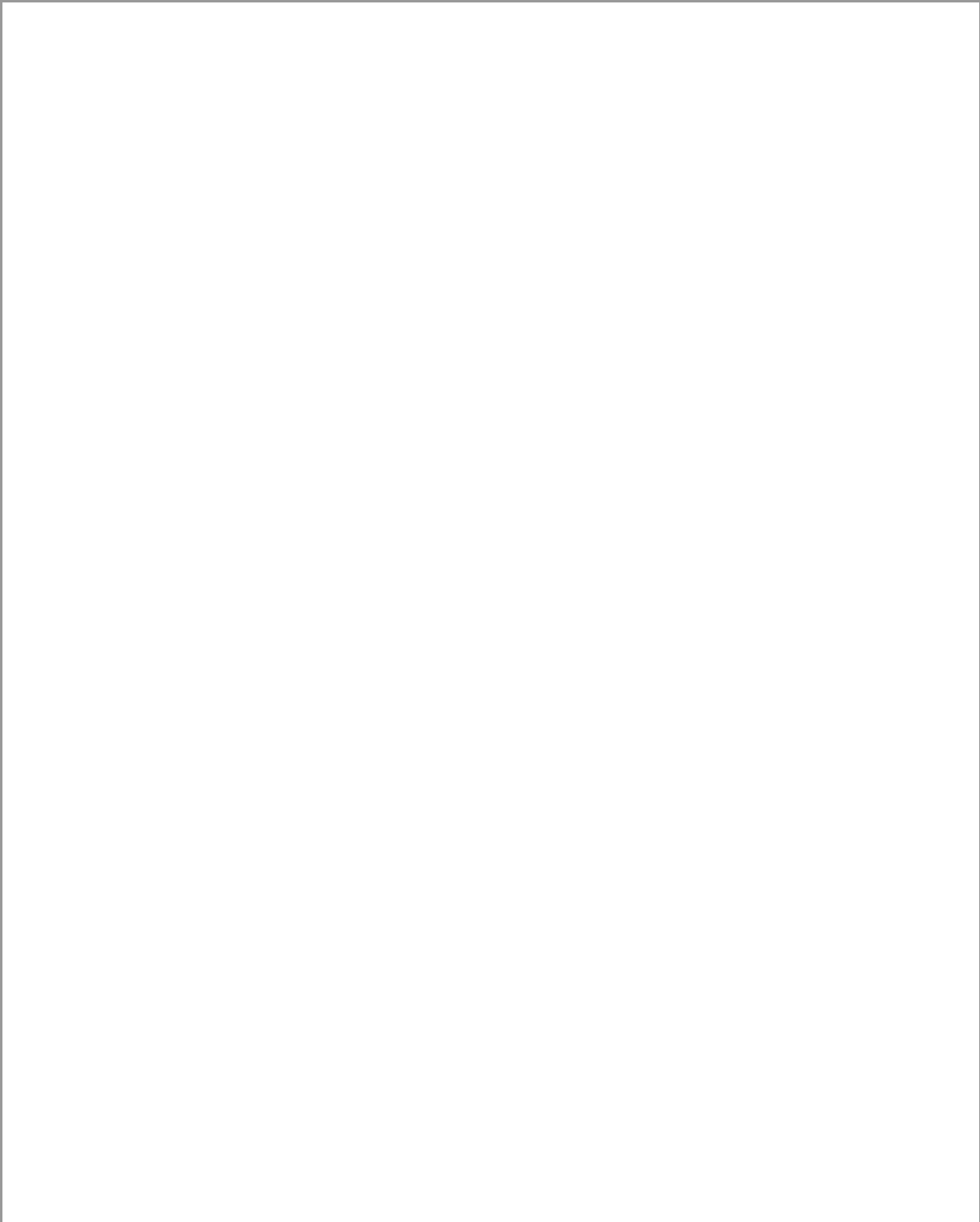
Regulation of Iodine Metabolism in the Thyroid Follicular Cell

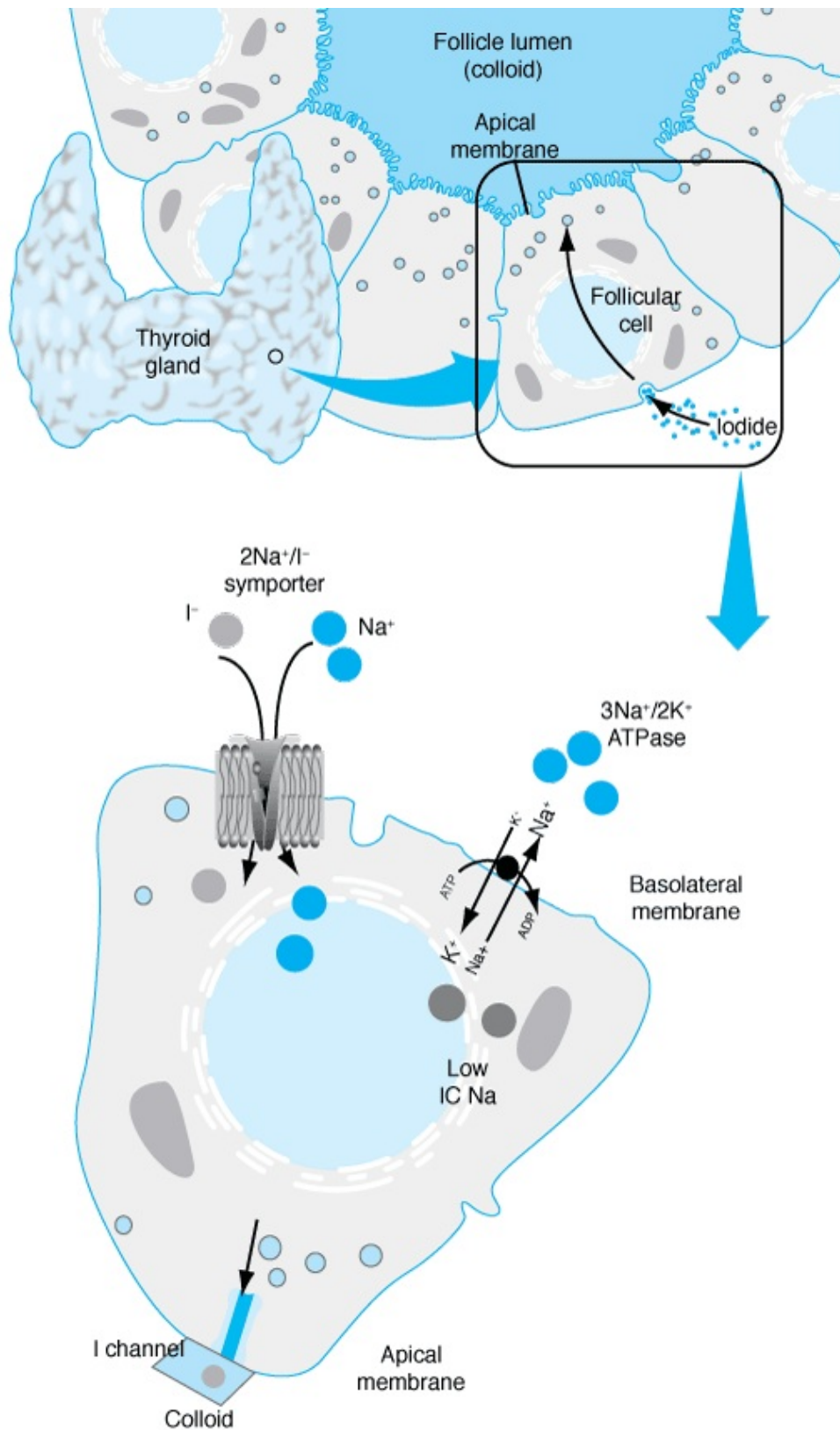


Iodide is concentrated in thyroid epithelial cells by an active, saturable, energy-dependent process mediated by a sodium-iodide (Na^+/I^-) symporter located in the basolateral plasma membrane of the follicular cell (Figure 4–2). This ability of the thyroid gland to accumulate iodine allows therapeutical administration of radioactive

iodine for the treatment of Graves disease, toxic nodules, and to ablate the thyroid in the treatment of malignancy.

Figure 4–2.





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Mechanism of iodide concentration by the thyroid gland. Iodide is transported into the cytosol of the follicular cell by active transport against a chemical and electrical gradient. The energy is derived from the electrochemical gradient of sodium. Two sodium ions are transported inside the thyroid follicular cell with each iodide molecule. Sodium moves down its concentration gradient, which is maintained by the Na^+/K^+ -ATPase that constantly pumps sodium out of the cytoplasm of the thyroid follicular epithelial cell, maintaining the low intracytoplasmic concentrations of sodium. Iodide must reach the colloid space, where it is used for organification of thyroglobulin. This process is achieved by efflux through the iodide channel. One of the early effects of TSH binding to its receptor is the opening of these channels, which facilitate the leakage of iodide into the extracellular space. This transcellular transport of iodide relies on the functional and morphologic polarization of the thyroid follicular epithelial cell. ATP, adenosine triphosphate; IC, intracellular.

The iodination of Tg residues is a process that occurs at the apical membrane. Thus, once inside the cell, iodide must leave the follicular cell through apical efflux by an iodide-permeating mechanism consisting of a chloride-iodide-transporting protein (iodide channel) located in the apical membrane of the thyroid follicular cell. The uptake, concentration, and efflux of iodide through the iodide channel are all a function of TSH-stimulated transepithelial transport of iodide. As already mentioned, this transcellular transport of iodide is possible because of the morphologic and functional polarization of the thyroid epithelial cell.

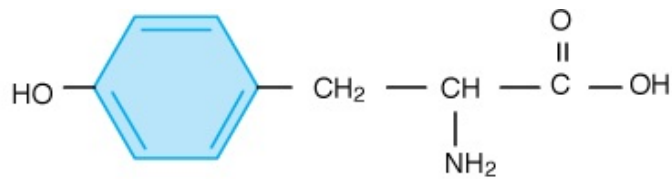
The transport of iodine through the Na^+/I^- symporter can be substituted by other substances including perchlorate and pertechnetate.

Radiolabeled pertechnetate ($\text{Tc}^{99\text{m}}$ -pertechnetate) can be used for imaging of the thyroid gland. In the follicular lumen, tyrosine residues of Tg are iodinated by iodine (I^+ ; formed by oxidation of I^- by TPO) (Figure 4–3). This reaction requires hydrogen peroxide, which is generated by a flavoprotein Ca^{2+} -dependent reduced nicotinamide adenine dinucleotide phosphate oxidase at the apical cell surface and serves as an electron acceptor in the reaction process. As shown in

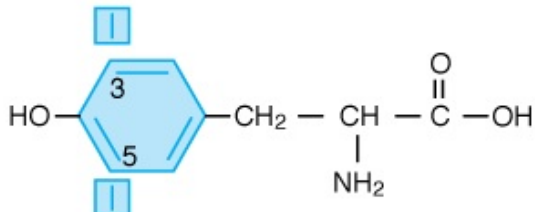
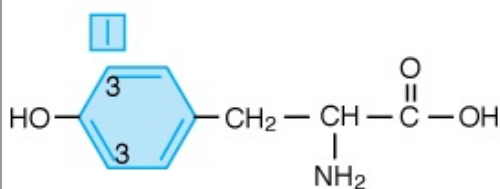
Figure 4–3, iodine bonds to carbon 3 or to carbon 5 of the tyrosine residues on Tg in a process referred to as the organification of iodine. This iodination of specific tyrosines located on Tg yields monoiodinated (MIT) and diiodinated (DIT) residues that are enzymatically coupled to form T_3 or T_4 . The coupling of iodinated tyrosine residues, either of 2 DIT residues or of 1 MIT and 1 DIT residue, is catalyzed by the enzyme **TPO**. Because not all of the iodinated tyrosine residues undergo coupling, Tg stored in the follicular lumen contains MIT and DIT residues as well as formed T_3 and T_4 .

Figure 4–3.



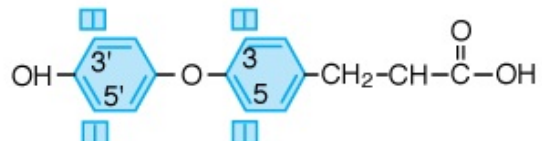
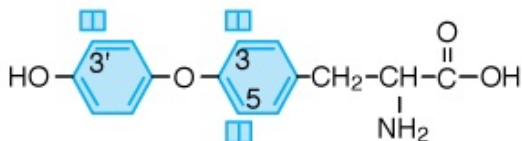


Peroxidase
I⁻+H₂O₂



MIT
+
DIT
} Peroxidase

Peroxidase {
DIT
+
DIT



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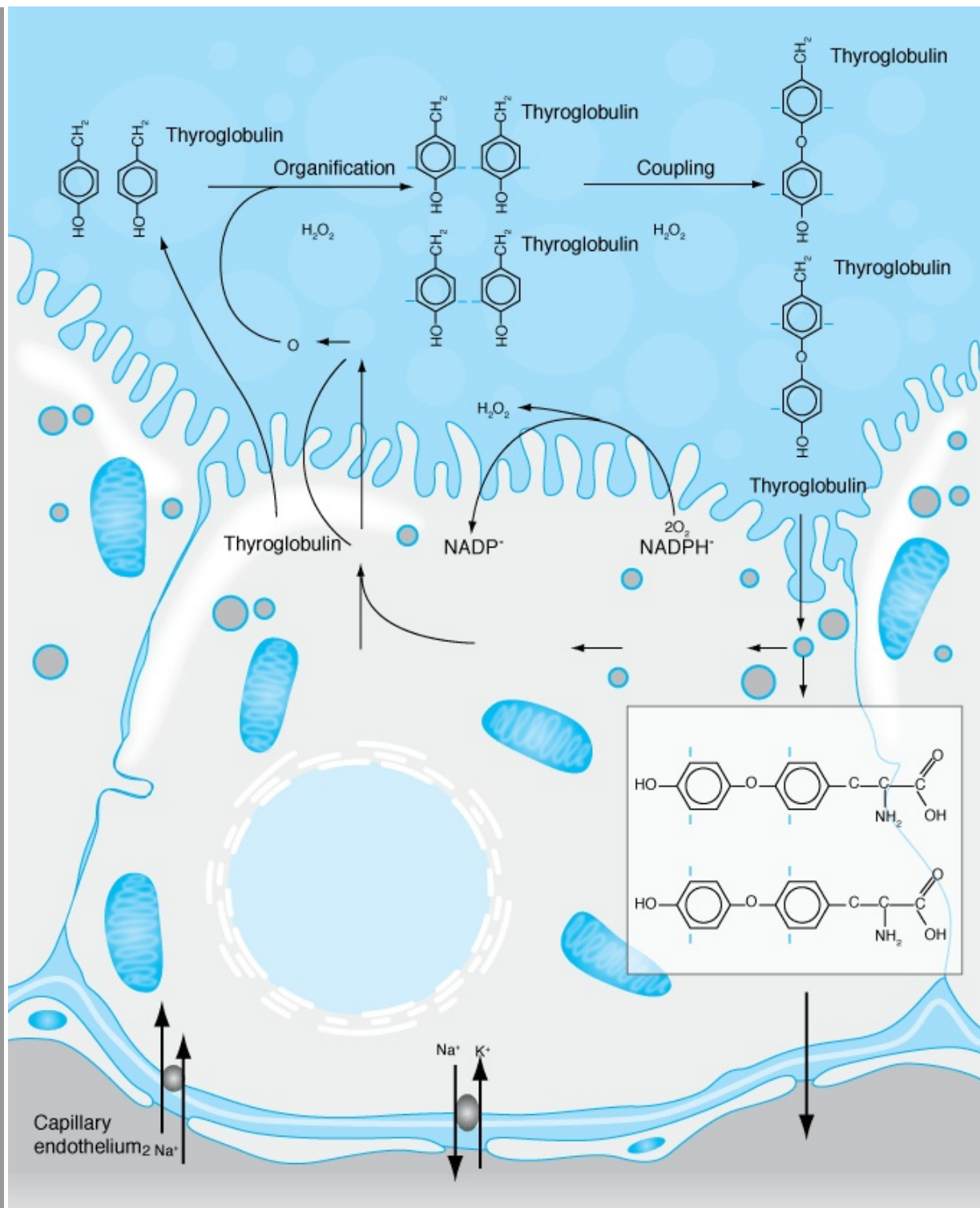
Biochemistry of thyroid hormone synthesis. In the follicular lumen, tyrosine residues within the thyroglobulin matrix are iodinated by iodine (I^+ ; formed by oxidation of I^- by thyroid peroxidase). Iodine bonds to carbon 3 or to carbon 5 of the tyrosine residues of thyroglobulin in a process referred to as the organification of iodine. This iodination of specific tyrosines located on thyroglobulin yields monoiodinated (MIT) and diiodinated (DIT) residues that are enzymatically coupled to form triiodothyronine (T_3) or tetraiodothyronine (T_4). The coupling of iodinated tyrosine residues, either of 2 diiodotyrosines or of 1 monoiodotyrosine and 1 diiodotyrosine residue, is catalyzed by the enzyme thyroid peroxidase.

As mentioned previously, TSH controls the energy-dependent uptake and concentration of iodide by the thyroid gland and its transcellular transport through the follicular epithelial cell. However, iodine metabolism within the thyroid can also be regulated independently of TSH. This mechanism is important when plasma iodide levels are elevated (15-to 20-fold above normal) because this elevation inhibits the organic binding of iodine within the thyroid. This autoregulatory phenomenon consisting of inhibition of the organification of iodine by elevated circulating levels of iodide is known as the **Wolff-Chaikoff effect**. This effect lasts for a few days and is followed by the so-called "escape" phenomenon, at which point the organification of intrathyroidal iodine resumes and the normal synthesis of T_4 and T_3 returns. The escape phenomenon results from a decrease in the inorganic iodine concentration inside the thyroid gland from downregulation of the sodium-iodide symporter. This relative decrease in intrathyroidal inorganic iodine allows the TPO- H_2O_2 system to resume normal activity. The mechanisms responsible for the acute Wolff-Chaikoff effect have not been elucidated but may be caused by the formation of organic iodocompounds within the thyroid.

Regulation of Thyroid Hormone Release

The preceding sections have described the steps involved in the synthesis of thyroid hormone, TSH regulation of the iodination of tyrosine residues on Tg, and coupling of the iodinated residues for the formation of thyroid hormones (Figure 4–4). TSH also regulates the release of thyroid hormones from the thyroid gland. The synthesis of thyroid hormone takes place in the colloid space. As mentioned previously, the apical surface of the follicular epithelial cell faces the colloid and not the interstitial space, and thus has no access to the bloodstream. Therefore, thyroid hormone release involves endocytosis of vesicles containing Tg from the apical surface of the follicular cell. The vesicles fuse with follicular epithelial phagolysosomes, leading to proteolytic digestion and cleavage of Tg. In addition to the thyroid hormones T_4 and T_3 , the products of this reaction include iodinated tyrosine residues (MIT and DIT). MIT and DIT are deiodinated intracellularly, and iodide is transported by apical efflux into the follicular colloid space, where it is reused in thyroid hormone synthesis. T_4 and T_3 are released from the basolateral membrane into the circulation. The thyroid gland releases greater amounts of T_4 than T_3 , so plasma concentrations of T_4 are 40-fold higher than those of T_3 (90 versus 2 nM). Most of the circulating T_3 is formed peripherally by deiodination of T_4 , a process that involves the removal of iodine from carbon 5 on the outer ring of T_4 . Thus, T_4 acts as a prohormone for T_3 . Although this deiodination occurs predominantly in the liver, some occurs in the thyroid follicular epithelial cell itself. This intrathyroidal deiodination of T_4 is the result of TSH stimulation of the intrathyroidal type I deiodinase.

Figure 4–4.



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Overview of thyroid hormone synthesis in the thyroid follicular epithelial cell. Thyroid hormone synthesis involves concentration of iodide by the

Na^+/I^- symporter and transport of iodide through the epithelial cell and into the extracellular compartment of the follicular cells, where it is oxidized to iodine by thyroid peroxidase (TPO) and is then used in the iodination of thyroglobulin (Tg). Iodine organification is an extracellular process that takes place inside the thyroid follicles at the apical membrane surface facing the follicular lumen. Iodine is bound to either carbon 3 or carbon 5 of the tyrosine residues of Tg to form moniodinated tyrosine (MIT) and diiodinated tyrosine (DIT). MIT and DIT, or DIT and DIT, are conjugated to form triiodothyronine (T_3) and tetraiodothyronine (T_4), respectively. Secretion of the hormone involves endocytosis of colloid containing Tg, followed by degradation of Tg and release of T_4 and T_3 . Some of the T_4 produced is deiodinated in the thyroid follicle to T_3 , which is then released into the bloodstream. In addition, intracellular deiodination provides a mechanism for recycling of iodide to participate in the synthesis of new thyroid hormone at the apical cell surface. A small fraction of Tg is released from the follicular epithelial cell into the circulation. TSHR, thyroid-stimulating hormone receptor. (Reproduced, with permission, from Kopp PA. Reduce, recycle, reuse-iodotyrosine deiodinase in thyroid iodide metabolism. *NEJM*. 2008;358(17):1856–1859. Copyright © Massachusetts Medical Society. All rights reserved.)



Two additional facts regarding thyroid hormone activity and storage should be noted. First, at physiological levels, T_4 is relatively inactive because it possesses 100-fold lower affinity than T_3 for binding to the thyroid receptor and does not enter the cell nucleus at high enough concentrations to occupy the ligand-binding site of the thyroid hormone receptor. Second, in contrast to most endocrine glands, which do not have storage capacity for their product, the thyroid gland is able to store 2-to 3-month supply of thyroid hormones in the Tg pool. Key features of thyroid regulation and function are listed in Table 4–1.

Table 4–1. Key Features of Thyroid Regulation and Function

	Key features
TSH	Binds to G _s protein–coupled receptor on thyroid cells
	Main second-messenger system is cAMP
	Stimulates all steps involved in thyroid hormone synthesis: iodine uptake and organification, production and release of thyroid hormone, and promotion of thyroid growth
Thyroid gland	Can store 2-to 3-months supply of thyroid hormones in the thyroglobulin pool (colloid)
	Produces more T ₄ than T ₃
Thyroid hormone	Synthesis and release are under negative feedback regulation by the hypothalamic-pituitary-thyroid axis T ₄ is converted to T ₃ in peripheral tissues
	Biologic activity of T ₃ is greater than that of T ₄
	Binds to nuclear receptors and modulates gene transcription

cAMP, cyclic adenosine monophosphate; TSH, thyroid-stimulating hormone; T₃, triiodothyronine; T₄, tetraiodothyronine.

Transport & Tissue Delivery of Thyroid Hormones

Once thyroid hormones are released into the circulation, most of them circulate bound to protein. Approximately 70% of T₄ and T₃ is bound to thyroid-binding globulin. Other proteins involved in thyroid binding include transthyretin, which binds 10% of T₄, and albumin, which binds 15% of T₄ and 25% of T₃. A small fraction of each hormone (0.03% of T₄ and 0.3% of T₃) circulates in its free form. This fraction of the circulating hormone pool is bioavailable and can enter the cell to bind to the thyroid receptor. Of the two thyroid hormones, T₄ binds more tightly to binding proteins than T₃ and thus has a lower metabolic clearance rate and a

longer half-life (7 days) than T_3 (1 day). The kidneys readily excrete free T_4 and T_3 . Binding of thyroid hormones to plasma proteins ensures a circulating reserve and delays their clearance.

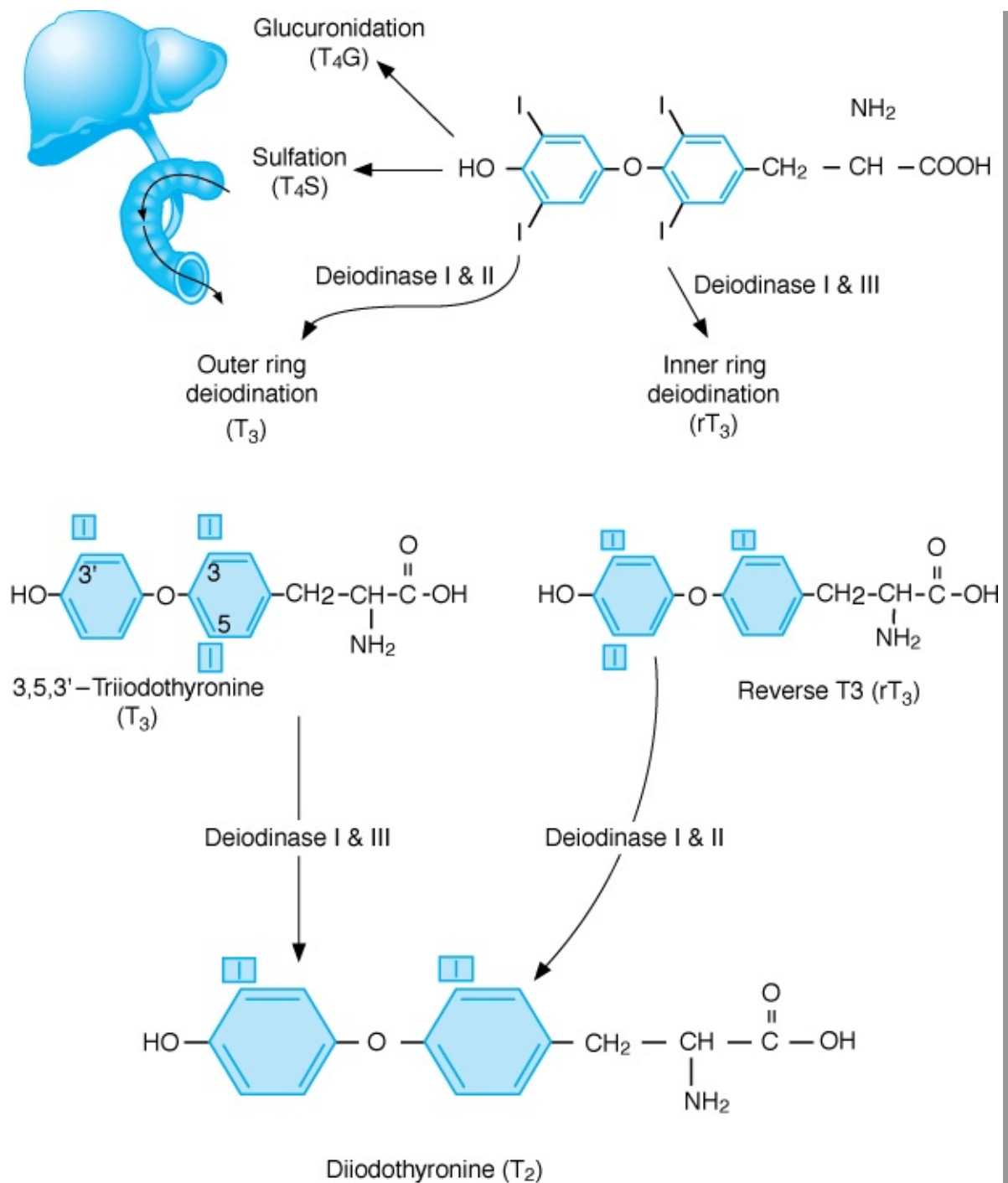
The release of hormone from its protein-bound form is in a dynamic equilibrium, although the role of binding proteins in delivery of hormone to specific tissues remains to be fully understood. It is known that drugs such as salicylate may affect thyroid hormone binding to plasma proteins. The binding-hormone capacity of the individual can also be altered by disease or hormonal changes. For example, liver disease is associated with a decrease in the synthesis of binding proteins, whereas high estrogen levels (eg, during pregnancy) increase binding-protein synthesis. These changes in the total amount of plasma proteins available to bind thyroid hormone will have an impact on the total amount of circulating thyroid hormone because of a constant homeostatic adjustment to changes in free hormone levels. As in a classic feedback system, the hypothalamic-pituitary-thyroid axis is controlled by the amount of free hormone available. Thus, a decrease in free thyroid hormone because of an increase in plasma binding-proteins will stimulate the release of TSH from the anterior pituitary, which will in turn stimulate the synthesis and release of thyroid hormone from the thyroid gland. In contrast, a decrease in binding-protein levels, with a resulting rise in free thyroid hormone levels, will suppress TSH release and decrease thyroid hormone synthesis and release. These dynamic changes occur throughout the life of the individual, whether in health or disease. Disruption in these feedback mechanisms will result in manifestations of excess or deficient thyroid hormone function.

Thyroid Hormone Metabolism



As already mentioned, the thyroid releases mostly T_4 and very small amounts of T_3 , yet T_3 has greater thyroid activity than T_4 . The main source of circulating T_3 is peripheral deiodination of T_4 by **deiodinases** (Figure 4–5). Approximately 80% of T_4 produced by the thyroid undergoes deiodination in the periphery. Approximately 40% of T_4 is deiodinated at carbon 5 in the outer ring to yield the more active T_3 , principally in liver and kidney. In about 33% of T_4 , iodine is removed from carbon 5 in the inner ring to yield **reverse T_3 (rT_3)**. Reverse T_3 has little or no biologic activity, has a higher metabolic clearance rate than T_3 , and has a lower serum concentration than T_3 . Following conversion of T_4 to T_3 or rT_3 , these are converted to T_2 , a biologically inactive hormone. Therefore, thyroid hormone peripheral metabolism is a sequential deiodination process, leading first to a more active form of thyroid hormone (T_3) and finally to complete inactivation of the hormone. Thus, loss of a single iodine from the outer ring of T_4 produces the active hormone T_3 , which may either exit the cell (in deiodinase type I–containing cells), enter the nucleus directly (in deiodinase type II–containing cells), or possibly even both (eg, in human skeletal muscle).

Figure 4–5.



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Thyroid hormone metabolism. Peripheral metabolism of thyroid hormones involves the sequential removal of iodine molecules, converting tetraiodothyronine (T_4) into the more active triiodothyronine (T_3) and inactivating thyroid hormones before their excretion. In

addition, thyroid hormones can undergo conjugation in the liver, which increases their solubility and facilitates their biliary excretion. The type I iodothyronine deiodinase is expressed predominantly in liver, kidney, and thyroid. It catalyzes both outer-and inner-ring deiodination of thyroid hormones. It is the primary site for clearance of plasma reverse T_3 (rT_3) and a major source of circulating T_3 . Type II deiodinase is expressed primarily in the human brain, anterior pituitary, and thyroid. It has only outer-ring deiodination activity and plays an important role in the local production of T_3 in tissues that express this enzyme. The type III deiodinase is located predominantly in human brain, placenta, and fetal tissues. It has only inner-ring activity and catalyzes the inactivation of T_3 more effectively than that of T_4 , thereby regulating intracellular T_3 levels.

This extrathyroidal progressive deiodination of thyroid hormones catalyzed by deiodinases plays a significant role in thyroid hormone metabolism and requires the trace element selenocysteine for optimal enzymatic activity. The 3 types of deiodinases that have been identified differ in their tissue distribution, catalytic profiles, substrate specificities, physiologic functions, and regulation.

TYPE I DEIODINASE

Type I deiodinase catalyzes outer-and inner-ring deiodination of T_4 and rT_3 . It is found predominantly in the liver, kidney, and thyroid. It is considered the primary deiodinase responsible for T_4 to T_3 conversion in hyperthyroid patients in the periphery. This enzyme also converts T_3 to T_2 . The activity of type I deiodinase expressed in the thyroid gland is increased by TSH-stimulated cAMP production and has a significant influence on the amount of T_3 secreted by the thyroid. Propylthiouracil and iodinated X-ray contrast agents such as iopanoic acid inhibit the activity of this enzyme and consequently the thyroïdal production of T_3 .

TYPE II DEIODINASE

Type II deiodinase is expressed in the brain, pituitary gland, brown adipose tissue, thyroid, placenta, and skeletal and cardiac muscle. Type II deiodinase has only outer-ring activity and converts T_4 to T_3 . This enzyme is thought to be the major source of T_3 in the euthyroid state. This enzyme plays an important role in tissues that produce a relatively high proportion of the receptor-bound T_3 themselves, rather than deriving T_3 from plasma. In these tissues, type II deiodinases are an important source of intracellular T_3 and provide >50% of the nuclear receptor-bound T_3 . The critical role of the type II deiodinases is underscored by the fact that T_3 formed in the anterior pituitary is necessary for negative feedback inhibition of TSH secretion.

TYPE III DEIODINASE

Type III deiodinase is expressed in the brain, placenta, and skin. Type III deiodinase has inner-ring activity and converts T_4 to rT_3 , and T_3 to T_2 , thus inactivating T_4 and T_3 . This process is an important feature in placental protection of the fetus. The placental conversion of T_4 to rT_3 and of T_3 to T_2 reduces the flow of T_3 (the most active thyroid hormone) from mother to fetus. Small amounts of maternal T_4 are transferred to the fetus and converted to T_3 , which increases the T_3 concentration in the fetal brain, preventing hypothyroidism. In the adult brain, the expression of type III deiodinases is enhanced by thyroid hormone excess, serving as a protective mechanism against high thyroid hormone concentrations.

As outlined in Figure 4–5, thyroid hormone is deactivated through deiodination by type I deiodinases (converting T_4 to T_3) and type III deiodinases (through the formation of rT_3). Thyroid hormones can be excreted following hepatic sulfo- and glucuronide conjugation and biliary excretion.

Biologic Effects of Thyroid Hormones

Thyroid hormone receptors are expressed in virtually all tissues and affect multiple cellular events. Their effects are mediated primarily by the transcriptional regulation of target genes, and are thus known as **genomic effects**. Recently, it has become evident that thyroid hormones also exert **nongenomic effects**, which do not require modification of gene transcription. Some of these effects include stimulation of activity of Ca^{2+} -ATPase at the plasma membrane and sarcoplasmic reticulum, rapid stimulation of the Na^+/H^+ antiporter, and increases in oxygen consumption (Chapter 10). The nature of the receptors that mediate these effects and the signaling pathways involved are not yet completely elucidated. However, T_3 exerts rapid effects on ion fluxes and electrophysiologic events, predominantly in the cardiovascular system.



Thyroid hormones enter cells by a carrier-mediated energy-, temperature-, and Na^+ -dependent process. Several transporters have been identified to be involved in their entry into the cell, including those belonging to the sodium taurocholate cotransporting polypeptide, the sodium independent organic anion transporting polypeptide (OATP), L- and T-type amino acid transporters, and members of the monocarboxylate transporter family. Two transporters have been demonstrated to have particular specificity for thyroid hormone transport, the OATP1C1, which shows preference for T_4 and the MCT8, which shows preference for T_3 . Mutations or deletions in the MCT8 gene have been linked to psychomotor retardation and thyroid hormone resistance, indicating their contribution to optimal thyroid hormone function.

The cellular actions of thyroid hormones are mediated by multiple thyroid hormone receptor isoforms derived from two distinct genes (α and β) encoding thyroid hormone receptors. The functional significance of the different isoforms has not yet been elucidated. However, mutations in the thyroid receptor gene result in a rare disorder of thyroid hormone resistance characterized by elevated circulating levels of free thyroid hormone because of reduced target tissue

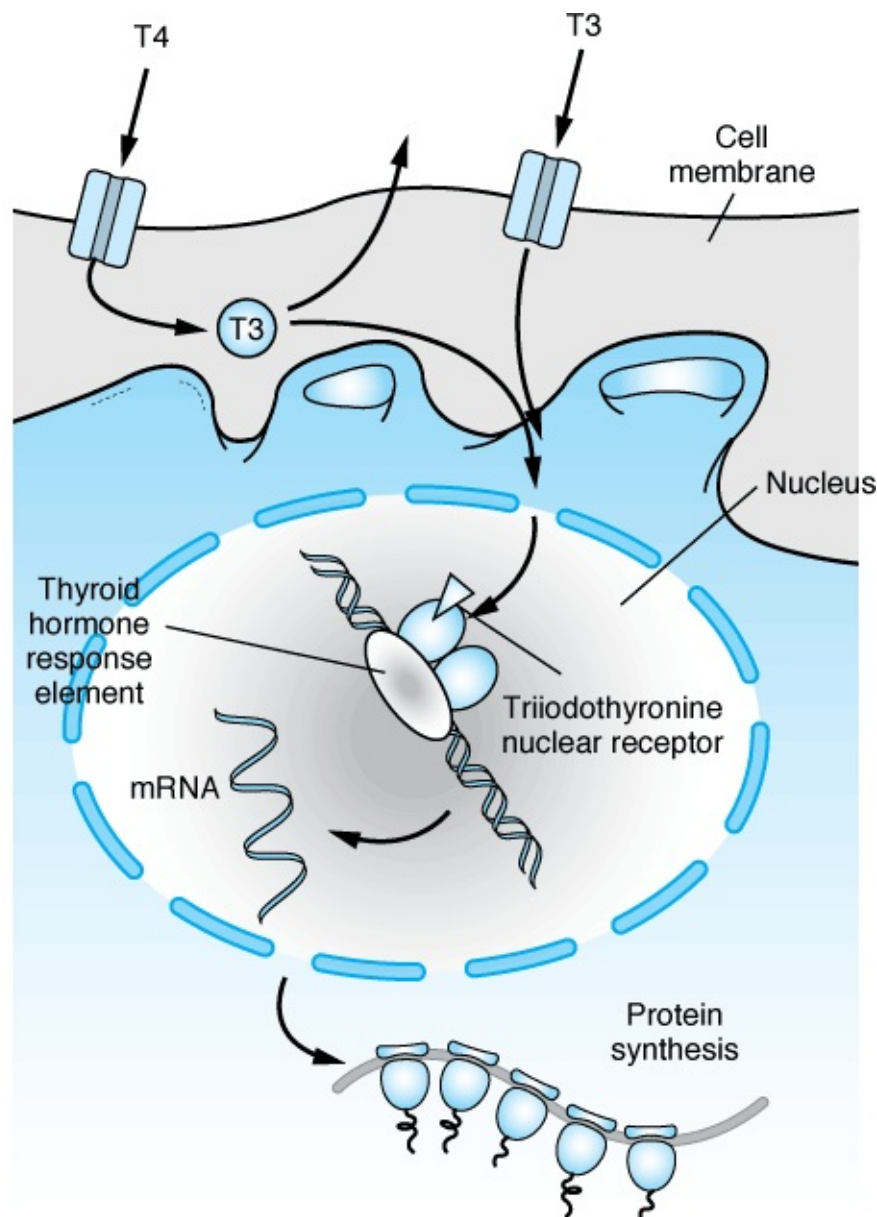
responsiveness and normal or elevated levels of TSH. Patients usually present with enlargement of the thyroid gland (**goiter**) and either normal (**euthyroid**) or mildly decreased (**hypothyroid**) metabolic state.

Thyroid Hormone Receptors

Thyroid hormone receptors are nuclear receptors intimately associated with chromatin (Figure 4–6). They bind thyroid hormones with high affinity and specificity. They have low capacity but high affinity for T_3 . The majority (85%) of nuclear-bound thyroid hormone is T_3 , and approximately 15% is T_4 . Thyroid hormone receptors are DNA-binding transcription factors that function as molecular switches in response to hormone binding. The hormone receptor can activate or repress gene transcription, depending on the promoter context and ligand-binding status. Unoccupied thyroid hormone receptors are bound to DNA thyroid hormone response elements and are associated with a complex of proteins containing corepressor proteins. Hormone binding to the receptor promotes corepressor dissociation and binding of a coactivator, leading to modulation of gene transcription.

Figure 4–6.





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Cellular effects of thyroid hormones. Thyroid hormones (triiodothyronine [T_3] and tetraiodothyronine [T_4]) enter the cell and bind to their receptors located in the nucleus. The affinity for T_3 is greater than for T_4 . The thyroid nuclear receptors function as ligand-activated transcription factors that influence transcription from target genes. Nuclear receptors bind enhancer elements on DNA called hormone response elements to regulate transcription from genes. Binding of thyroid hormone, leads to recruitment of specific

coactivators leading to gene activation and ultimately protein synthesis.

Because thyroid hormone receptors are present in virtually all tissues, thyroid hormones play a vital role in cellular metabolism. The cellular events that they mediate include the following:

- Transcription of cell membrane Na^+/K^+ -ATPase, leading to an increase in oxygen consumption
- Transcription of uncoupling protein, enhancing fatty acid oxidation and heat generation without production of ATP
- Protein synthesis and degradation, contributing to growth and differentiation
- Epinephrine-induced glycogenolysis and gluconeogenesis, affecting insulin-induced glycogen synthesis and glucose utilization
- Cholesterol synthesis and low-density lipoprotein receptor regulation.

Organ-Specific Effects of Thyroid Hormone



Thyroid hormones are essential for normal growth and development; they control the rate of metabolism and hence the function of practically every organ in the body. Their specific biologic effects vary from one tissue to another. The following are some examples of the specific effects of thyroid hormone.

Bone—Thyroid hormone is essential for bone growth and development through activation of osteoclast and osteoblast activities. Deficiency during childhood affects growth. In adults, excess thyroid hormone levels are associated with increased risk of osteoporosis.

Cardiovascular system—Thyroid hormone has cardiac inotropic and chronotropic effects, increases cardiac output and blood volume, and decreases systemic vascular resistance. These responses are mediated through thyroid hormone changes in gene transcription of several proteins including Ca^{2+} -ATPase, phospholamban, myosin, β -adrenergic receptors, adenylate cyclase, guanine-nucleotide-binding

proteins, $\text{Na}^+/\text{Ca}^{2+}$ exchanger, Na^+/K^+ -ATPase, and voltage-gated potassium channels.

Fat—Thyroid hormone induces white adipose tissue differentiation, lipogenic enzymes, and intracellular lipid accumulation; stimulates adipocyte cell proliferation; stimulates uncoupling proteins; and uncouples oxidative phosphorylation. Hyperthyroidism enhances and hypothyroidism decreases lipolysis through different mechanisms. The induction of catecholamine-mediated lipolysis by thyroid hormones results from an increased β -adrenoceptor number and a decrease in phosphodiesterase activity resulting in an increase in cAMP level and hormone-sensitive lipase activity.

Liver—Thyroid hormone regulates triglyceride and cholesterol metabolism, as well as lipoprotein homeostasis. Thyroid hormone also modulates cell proliferation and mitochondrial respiration.

Pituitary—Thyroid hormone regulates the synthesis of pituitary hormones, stimulates growth hormone production, and inhibits TSH.

Brain—Thyroid hormone controls expression of expression of genes involved in myelination, cell differentiation, migration, and signaling. Thyroid hormone is necessary for axonal growth and development.

DISEASES OF THYROID HORMONE OVERPRODUCTION & UNDERSECRETION

The widespread distribution of thyroid hormone receptors and the multitude of physiologic effects that they exert are highlighted in cases of abnormal thyroid function (Table 4–2). Dysfunction can result from 3 factors: (1) alterations in the circulating levels of thyroid hormones, (2) impaired metabolism of thyroid hormones in the periphery, and (3) resistance to thyroid hormone actions at the tissue level.

Table 4–2. Clinical and Laboratory Manifestations of Excess or Deficient Thyroid Hormone Function

Hypothyroidism		Hyperthyroidism	
Clinical presentation	Laboratory values	Clinical presentation	Laboratory values

In utero: Cretinism, mental and growth retardation, short limbs Adult onset: Tiredness, lethargy, constipation, decreased appetite, cold intolerance, abnormal menstrual flow, hair loss, brittle nails, dry coarse skin, hoarse voice Chronic: Myxedema, thickened features, periorbital edema, swelling of hands and feet without indentation, delayed muscle contraction and relaxation, delayed tendon reflexes, reduced stroke volume and heart rate, decreased cardiac output, enlarged heart, pericardial effusion, pleural and peritoneal fluid accumulation, slowing of mental function, impaired memory, slow speech, decreased initiative, somnolence,	Primary: Low free T_4; low or sometimes normal T_3; high TSH Secondary: Low T_4, T_3, and TSH	Palpitations, exercise impairment, widened pulse pressure, tachycardia at rest and during exercise, increase in blood volume, palpable enlarged thyroid gland, infiltrative ophthalmopathy, nervousness, irritability, hyperactivity, emotional instability, tenseness, pounding of the heart, heat intolerance, weight loss despite increased food intake, decreased or absent menstrual flow, increased number of bowel movements, warm moist skin or velvety texture, proximal muscle weakness, fine hair, fine tremor, excessive sweating, moist skin	Primary: Low TSH below limit of detection ($0.1 \mu\text{U/mL}$); high T_4 (2-fold) and T_3 (3- to 4-fold). In Graves disease, elevated anti-TSH receptor antibody titers. Secondary: High TSH, T_4, and T_3

hypothermia			

TSH, thyroid-stimulating hormone; T₃, triiodothyronine; T₄, thyroxine.

An individual whose thyroid function is normal is said to be in a euthyroid state. The clinical state resulting from an alteration in thyroid function is classified as either **hypothyroidism** (low thyroid function) or **hyperthyroidism** (excessive thyroid function). As with most endocrine abnormalities, altered thyroid function may be genetic or acquired, and its duration may be transient or permanent. Autoimmune diseases play an important role in thyroid disease. Abnormal immune responses directed to thyroid-related proteins result in 2 opposite pathogenic processes: thyroid enlargement (hyperplasia) in **Graves disease** and thyroid destruction in **Hashimoto thyroiditis**.

The most common presentations of thyroid hormone abnormalities are summarized as follows. This is not meant to be a comprehensive description of thyroid pathology, but an overview of some of the diseases that may produce alterations in thyroid hormone levels, thyroid hormone metabolism, or thyroid hormone responsiveness. The examples are intended to highlight different aspects of thyroid pathophysiology and the consequences of these derangements (Table 4–2).

Hypothyroidism

Hypothyroidism is the condition resulting from insufficient thyroid hormone action. It has an incidence of 2% in adult women and is less common in men. Two main types are distinguished, primary and secondary hypothyroidism, although the former is by far the more common.

PRIMARY HYPOTHYROIDISM

The most frequent cause (95% of patients) is disease of the thyroid gland. When the decrease in thyroid function occurs in utero, the result is severe mental retardation or cretinism, underscoring the vital role that thyroid hormone plays in development and growth.

Hypothyroidism in adult patients may be associated with a decrease in thyroid tissue. The decrease in thyroid tissue is usually due to autoimmune disease, leading to the destruction of thyroid parenchyma, but it can also be the result of surgery or radioactive iodine treatment. Not all cases of hypothyroidism are associated with a decrease in the size of the thyroid gland. Hypothyroidism may also be associated with thyroid enlargement, resulting from lymphocytic infiltration as in Hashimoto disease or dietary iodine deficiency.

SECONDARY HYPOTHYROIDISM

Secondary hypothyroidism is characterized by decreased TSH secretion and subsequently decreased thyroid hormone release. It is the result of disorders of the anterior pituitary or hypothalamus and can sometimes occur in association with other abnormalities of anterior pituitary hormones. Secondary hypothyroidism is not due to alterations at the level of the thyroid gland itself and is strictly due to a lack of stimulation of the TSH receptor because of impaired TSH release.

Clinically, patients may present with signs and symptoms of hypothyroidism but without a decrease in circulating thyroid hormone levels. This condition, known as **thyroid hormone resistance**, is a rare dominant inherited condition in which the responsiveness of target tissues to thyroid hormone is reduced. It occurs in 1 per 50,000 live births. The low incidence is probably due to the reduced survival of these embryos. In most cases, thyroid hormone resistance is due to a thyroid receptor mutation. The presentation of patients with thyroid hormone resistance can be variable. Most patients are euthyroid, although some present with hyperthyroidism or hypothyroidism. The T_3 , T_4 , and TSH levels are high in what appears to be a compensatory mechanism to ensure maintenance of euthyroid and eumetabolic states.

Hyperthyroidism

Hyperthyroidism is excessive functional activity of the thyroid gland, characterized by increased basal metabolism and disturbances in the autonomic nervous system as a result of excess thyroid hormone production. The incidence is higher in women (2%) than in men (0.02%). Several conditions can lead to hyperthyroidism: diffuse toxic goiter or Graves disease, toxic nodular goiter, toxic adenoma, therapy-induced hyperthyroidism (eg, excess T_4 or T_3 substitution), excess iodine intake, thyroiditis, follicular carcinoma, and TSH-producing tumor of the hypophysis. However, the most common cause of hyperthyroidism in adults is diffuse toxic goiter or Graves disease.

GRAVES DISEASE

Graves disease is an autoimmune condition leading to autonomous thyroid hormone secretion because of TSH receptor stimulation by immunoglobulin G. The continuous stimulation of TSH receptor by TSH-like antibodies results in excess T_4 and T_3 production. The incidence of Graves disease peaks in the third to fourth decades and is 8 times more common in women than in men.

Clinically, about 40–50% of patients with hyperthyroidism present with protruding eyes (exophthalmos) as a result of lymphocyte and fibroblast infiltration of the extraocular tissues and muscles, and accumulation of hyaluronate, a glycosaminoglycan produced by fibroblasts in the tissues and muscles. The majority (90%) of patients with thyroid ophthalmopathy have Graves hyperthyroidism.

TSH-SECRETING ADENOMAS

Secondary hyperthyroidism is due to increased thyroid hormone release by the thyroid gland in response to elevated TSH levels derived from TSH-secreting pituitary adenomas. TSH-secreting adenomas represent a small fraction (1–2%) of all pituitary adenomas and result in a syndrome of excess secretion of TSH. The hormonal profile is characterized by the inability to suppress TSH despite elevated levels of free thyroid hormones (T_3 and T_4).

Euthyroid Sick Syndrome

Euthyroid sick syndrome is a clinical condition in which patients suffering from nonthyroid diseases show biochemical evidence of altered thyroid function, but appear euthyroid when examined clinically. These patients present with normal or decreased T_4 concentration, decreased T_4 binding to thyroid-binding globulin, decreased T_3 concentration, increased rT_3 concentration, normal TSH, and normal scintigraphic imaging. This condition can be precipitated by stresses such as fasting, starvation, anorexia nervosa, protein malnutrition, surgical trauma, myocardial infarction, chronic renal failure, diabetic ketosis, cirrhosis, sepsis, and hyperthermia (Chapter 10). The alteration in thyroid hormone profile is due to an increase in type III deiodinase activity. Euthyroid sick syndrome may also be due to impaired conversion of T_4 to T_3 , as occurs with pharmacologic agents such as propranolol and amiodarone.

Abnormalities in Iodine Metabolism

Iodine is an essential component of thyroid hormone, but both low and high iodine intake may lead to disease. The daily requirement of iodine for thyroid hormone synthesis is 150 µg. As already described, the thyroid gland has intrinsic mechanisms that maintain normal thyroid function even in the presence of iodine excess. The thyroid also tries to compensate when dietary sources of iodine are limited. Several drugs can interfere with the ability of the thyroid gland to concentrate iodide. Perchlorate is a contaminant that can be found in drinking and ground water, and occasionally in cow's milk. Ingestion of high levels, as with consumption of well water can lead to inhibition of iodine transport into the follicular cells. Methimazole, the active form of carbimazole, inhibits iodide uptake and organification of iodine. Thiouracil and propylthiouracil inhibit iodine organification; the latter also inhibits the peripheral conversion of T_4 to T_3 .

Abnormalities in the metabolism or supply of iodine have particular importance in fetal development. Severe iodine deficiency of the mother may lead to insufficient thyroid hormone synthesis in both mother and fetus, resulting in developmental brain injury. Iodine deficiency is the leading cause of preventable mental retardation. On the other hand, excess iodine given to the mother (eg, use of amiodarone or excess iodine supplementation) may inhibit fetal thyroid function, leading to hypothyroidism and goiter, or may precipitate hyperthyroidism (iodine toxicity).

A rare familial disorder may lead to a defect in iodide transport. This defect is due to the genetic absence or derangement of the sodium/iodide symporter protein, which is involved in the active transport and concentration of iodide in the thyroid follicular cells. This condition can present in neonates as goiter with or without cretinism. In these individuals, T_3 and T_4 levels tend to be low and the TSH level is high. The diagnosis is confirmed by demonstrating poor thyroid uptake of radiolabeled iodine (^{131}I) or technetium ($^{99\text{m}}\text{TcO}_4$).

Thyroiditis

Thyroiditis, or inflammation of the thyroid gland, may lead to abnormalities in thyroid hormone state. This condition may be acute, subacute, or chronic.

ACUTE THYROIDITIS

Patients with **acute thyroiditis** present with a painful thyroid; chills and fever; a hot, enlarged, and palpable gland; and initially with hyperthyroidism. This condition is rare and infectious. Levels of T_3 , T_4 , and TSH are usually normal, and rT_3 is increased.

CHRONIC THYROIDITIS

Chronic thyroiditis (Hashimoto thyroiditis, chronic lymphocytic thyroiditis, autoimmune thyroiditis) is an autoimmune disease of the thyroid gland characterized by lymphocyte infiltration and circulating autoimmune antibodies. These antibodies inhibit the Na^+/I^- symporter, preventing iodide uptake and consequently thyroid hormone synthesis. Hashimoto thyroiditis is the most common cause of adult hypothyroidism. It is more prevalent in women than in men (ratio 8:1) and peaks at the age of 30–50 years. Individuals presenting with Hashimoto thyroiditis frequently have a family history of thyroid disorders. Hashimoto thyroiditis is also more common in persons with chromosomal disorders, such as Turner, Down, or Klinefelter syndromes, and in some cases is associated with autoimmune conditions such as Addison disease, hypoparathyroidism, and diabetes. Hashimoto thyroiditis may present early on with variable T_4 , T_3 , and TSH concentrations and antibody titers to TPO and Tg. As the disease progresses, some patients develop hypothyroidism with low T_4 and T_3 levels; high TSH levels and specific antibodies are usually no longer detectable.

EVALUATION OF THE HYPOTHALAMIC-PITUITARY-THYROID AXIS

The previous section provided a brief description of some of the diseases resulting from abnormalities in thyroid function. Tests of thyroid function allow the clinician to diagnose the disease and provide the follow-up necessary once therapy has been instituted. Although the most important keys to diagnosis are a thorough interview and physical examination of the patient, an understanding of the normal physiology of thyroid function allows proper interpretation of the diverse laboratory approaches for measuring function. Some of the most useful approaches are described as follows.

TSH & Thyroid Hormone Levels

Normal TSH values average 0.4–4.5 $\mu\text{U}/\text{mL}$ and can be measured in plasma by immunometric assays. TSH levels are useful in evaluation

of the patient because small changes in free thyroid hormone levels lead to greater changes in TSH levels. Serum TSH concentrations are considered the single most reliable test to diagnose all common forms of hypothyroidism and hyperthyroidism, particularly in the ambulatory setting. Overt hyperthyroidism is accompanied by low serum TSH concentrations, typically less than 0.1 mIU/L. Interpretation of TSH levels is best done with simultaneous measures of thyroid hormone levels.

Total T_4 and T_3 levels are measured by competitive immunoassay methods and normal ranges are 5–12 $\mu\text{g/dL}$ and 60–180 ng/dL, respectively. Total T_4 includes both bound and free hormone. As described earlier, only the free hormone (0.05% of the total) is biologically active; thus, changes in the levels of thyroid-binding globulin, albumin, or thyroid-binding prealbumin will affect total T_4 but not the free hormone. The same applies for measures of total T_3 . T_3 levels are increased in almost all cases of hyperthyroidism, usually earlier than T_4 , making it a more sensitive indicator of hyperthyroidism than total T_4 . In hypothyroidism, T_3 is often normal even when T_4 is low. T_3 is decreased during acute illness and starvation and is affected by several medications, including propranolol, steroids, and amiodarone.

Free T_3 & T_4

The amount of free hormone depends on how much is bound to proteins. Estrogens and acute liver disease increase thyroid binding, whereas androgens, steroids, chronic liver disease, and severe illness can decrease it. To rule out the contribution of alterations in hormone binding to the values of total thyroid hormone levels measured, free T_4 or T_3 levels can be measured after equilibrium dialysis or ultrafiltration of the serum sample to separate the free from the protein-bound hormones. New and more accurate assays for direct measurement of free T_4 and free T_3 have largely replaced the measurements of total T_4 and T_3 .

Reverse T_3

Critical illness is associated with reduced TSH and thyroid hormone secretion, and with changes in peripheral thyroid hormone metabolism, resulting in low serum T_3 and high rT_3 . As a result of altered peripheral thyroid hormone metabolism, decreased serum levels of T_3 are associated with increased concentrations of reverse T_3 (rT_3), an inactive form of thyroid hormone.

Antibody Levels

Determination of antithyroid antibodies is important in establishing the cause of thyroid disease. The antibodies of greatest clinical importance are the antithyroid microsomal (antiperoxidase) antibodies, the anti-Tg antibodies, and the thyroid-simulating immunoglobulins (TSI). In general, the antithyroid microsomal antibodies are usually elevated in patients with autoimmune thyroiditis (Hashimoto thyroiditis). Less frequently and to a lesser degree, the anti-Tg antibodies may be elevated in patients with autoimmune thyroiditis. Elevation of TSI is associated with Graves disease and is the likely cause of the hyperthyroidism seen in this condition.

Thyroid Nodule

The management of patients with thyroid masses or nodules involves utilization of several diagnostic techniques, which are briefly presented here.

Thyroid ultrasound—Ultrasonography of a thyroid mass allows determination of size, composition (solid, cystic, mixed, or complex), calcifications, and presence of additional nodules.

Fine needle aspiration—Performed by palpation or guided by an ultrasound, this technique allows for cytological analysis of the fluid and cells obtained through percutaneous needle aspiration of thyroid nodules.

Thyroid scan—The Na^+/I^- symporter of the thyroid follicular epithelial cell does not discriminate between dietary iodide and radioactive isotopes such as ^{123}I or ^{99}Tc . Therefore, these isotopes are used to visualize the functional anatomy of the thyroid. Regions of the thyroid that are functioning and actively incorporating the isotope are detected with a counter. The image of the thyroid reflects the ability of specific regions of the thyroid to take up the isotope and therefore to function normally (Figure 4–7). Areas that do not take up the radiolabeled iodine are referred to as nonfunctional or "cold." Because most malignant tumors do not express the Na^+/I^- symporter, they often present as cold nodules. Areas that accumulate iodide in excess of that of the surrounding tissues are referred to as "hot nodules."

Figure 4–7.

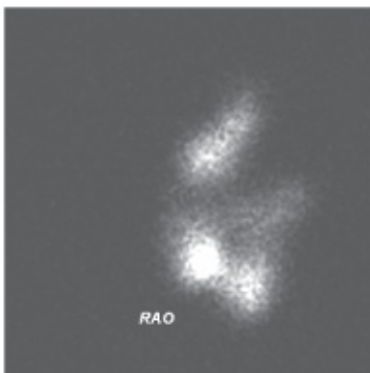


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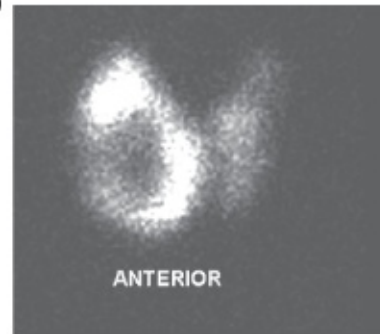
Homogeneous I^{125} uptake
Generalized thyroid enlargement

B

Increased I^{125} uptake
Diffuse thyroid enlargement

C

Increased I^{125} uptake
Enlarged thyroid
Hyperfunctioning nodules

D

Increased I^{125} uptake
Enlarged right lobe
Single-"cold" nodule

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Radioactive thyroid scans. **A.** Normal to increased thyroid uptake of ^{125}I . **B.** Thyroid with a marked decrease in ^{125}I uptake in a large palpable mass. **C.** Increased ^{125}I uptake isolated to a single nodule, the "hot nodule." **D.** Decreased thyroid ^{125}I uptake in an isolated region, the "cold nodule." (Images courtesy of Richard Kuebler, MD. LSUHSC University Hospital, New Orleans, LA.)

KEY CONCEPTS



The thyroid gland is regulated by the hypothalamic-pituitary-thyroid axis.



Dietary iodine is required for thyroid hormone synthesis.



The thyroid gland produces thyroid hormones by a process of concentration of iodine in the thyroid, iodination of tyrosine residues of thyroglobulin in the colloid space of the follicle, and endocytosis of colloid followed by proteolytic release of thyroid hormones (T_4 and T_3).



Thyroid hormones undergo metabolism in peripheral tissues, leading to the production of the more active T_3 and deactivation of thyroid hormones.



The presence of deiodinases and their substrate specificity play a central role in thyroid hormone function in target tissues.



Thyroid hormone receptors are located intracellularly, bound to DNA, and alter gene transcription upon binding by thyroid hormone.



Thyroid hormone actions are systemic and vital for development, growth, and metabolism.

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OBJECTIVES

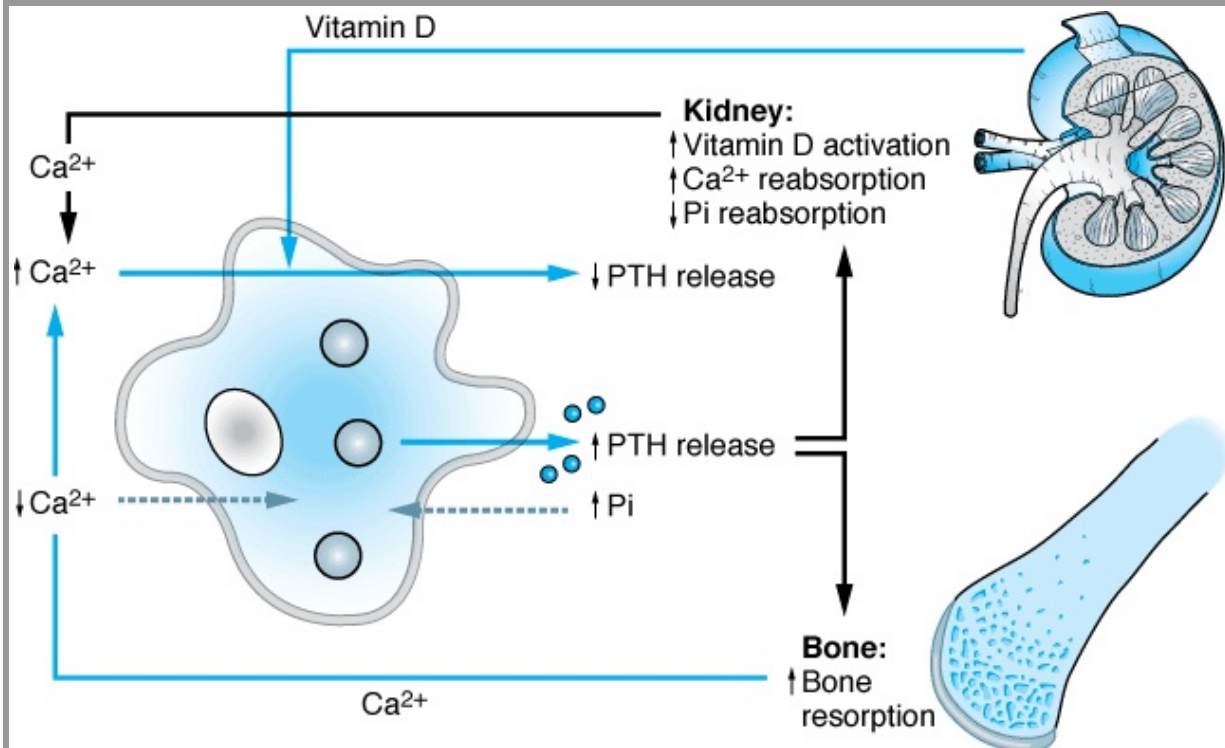
- ▶ *Identify the origin, target organs and cell types, and physiologic effects of parathyroid hormone.*
- ▶ *Describe the functions of osteoblasts and osteoclasts in bone remodeling and the factors that regulate their activities.*
- ▶ *Describe the regulation of parathyroid hormone secretion and the role of the calcium-sensing receptor.*
- ▶ *Identify the sources of vitamin D and describe the biosynthetic pathway involved in modifying it to its biologically active form.*
- ▶ *Identify the target organs and cellular mechanisms of action of vitamin D.*
- ▶ *Describe the negative feedback relationship between parathyroid hormone and the biologically active form of vitamin D.*
- ▶ *Describe the causes and consequences of excess or deficiency of parathyroid hormone and of vitamin D.*
- ▶ *Describe the regulation of calcitonin release and the cell of origin and target organs for calcitonin action.*

PARATHYROID GLAND & Ca^{2+} & PO_4^- REGULATION: INTRODUCTION

The regulation of plasma Ca^{2+} levels is critical for normal cell function, neural transmission, membrane stability, bone structure, blood coagulation, and intracellular signaling. This regulation relies on the interactions among **parathyroid hormone (PTH)** from the parathyroid glands, dietary **vitamin D** and **calcitonin** from the thyroid gland (Figure 5–1). PTH stimulates bone resorption and the release of Ca^{2+} into the circulation. In the kidney, PTH promotes Ca^{2+} reabsorption and inorganic phosphate (Pi) excretion in the urine. In addition, PTH stimulates the hydroxylation of 25-hydroxyvitamin D_3 at the 1-position, leading to the formation of the active form of vitamin D (calcitriol). Vitamin D increases intestinal absorption of dietary Ca^{2+} and renal reabsorption of filtered

Ca^{2+} . In bone, vitamin D increases bone resorption with a resulting increase in the release of Ca^{2+} into the circulation. Calcitonin counteracts the effects of PTH by inhibiting bone resorption and increasing renal Ca^{2+} excretion. The overall result of the interactions among PTH, vitamin D, and calcitonin is the maintenance of normal plasma Ca^{2+} concentrations.

Figure 5–1.



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Negative feedback regulation of parathyroid hormone (PTH) release. A sudden decrease in Ca^{2+} stimulates the release of PTH from the parathyroid gland. PTH increases the activity of 1α -hydroxylase in the kidney, leading to increased activation of vitamin D. In addition, PTH increases the reabsorption of Ca^{2+} and decreases the reabsorption of inorganic phosphate (Pi). In bone, PTH stimulates bone resorption, increasing the plasma Ca^{2+} levels. The elevations in vitamin D and plasma Ca^{2+} levels exert negative feedback inhibition of PTH release. Elevations in plasma Pi levels stimulate the release of PTH.



FUNCTIONAL ANATOMY

The parathyroid glands are pea-sized glands located at the top and bottom posterior borders of the lateral lobes of the thyroid gland. The glands are richly vascularized and consist primarily of chief cells and fat, with a thin capsule of connective tissue that divides the gland into lobules. The chief cells synthesize and secrete PTH, a polypeptide hormone that plays a major role in bone remodeling and calcium homeostasis. In addition to its central role in the regulation of Ca^{2+} levels and bone mass, PTH participates in the renal excretion of Pi and in the activation of vitamin D. Because of the central role of PTH in calcium homeostasis, its physiologic effects are described in the context of its interaction with the other major regulators of calcium: calcitonin, the hormone synthesized in the C cells of the thyroid gland; and calcitriol, the active form of vitamin D.

PTH BIOSYNTHESIS & TRANSPORT

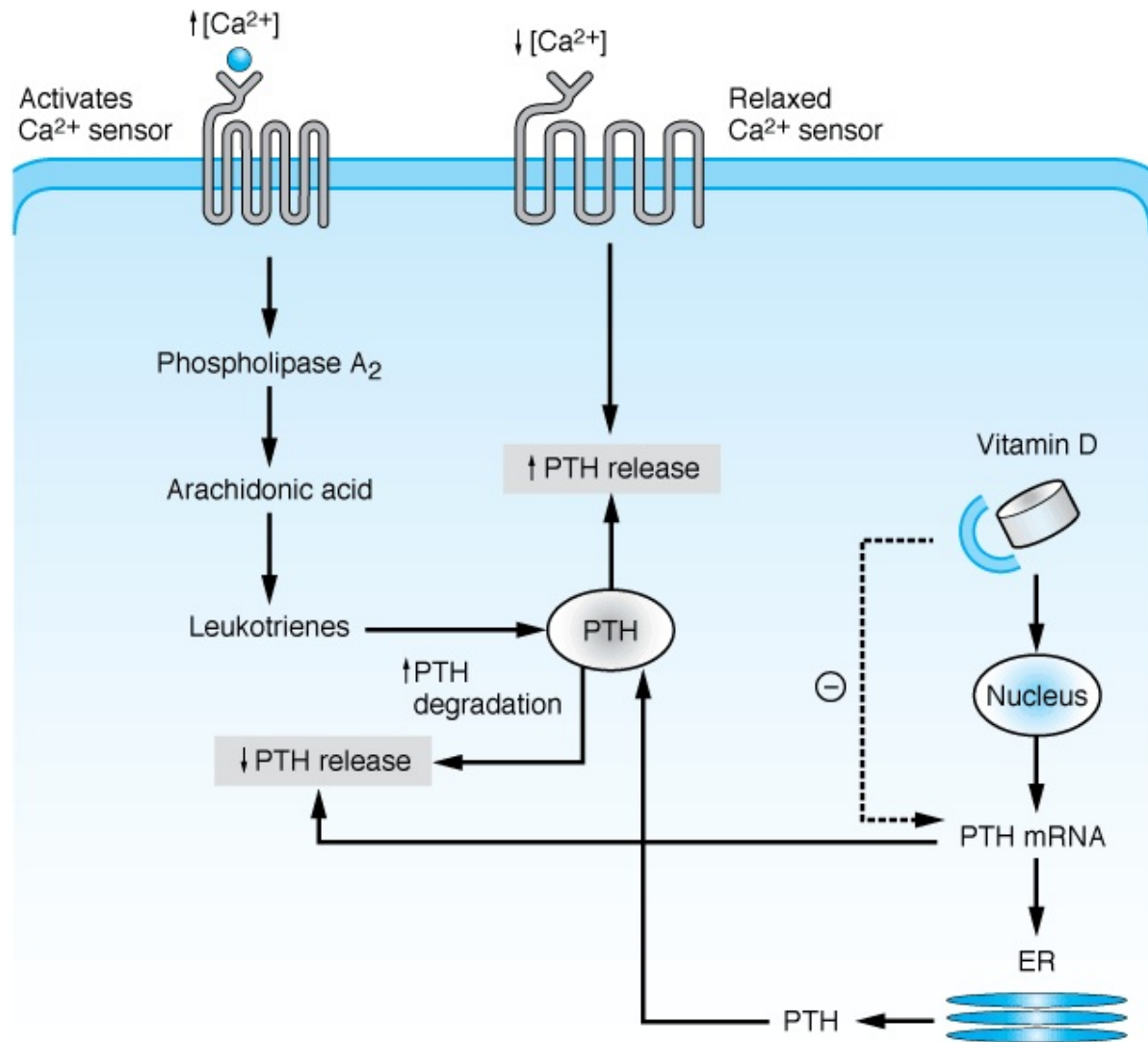
Like other peptide hormones, PTH is synthesized as a pre-propeptide. The propeptide is rapidly cleaved to yield pro-PTH and subsequently the mature form of PTH, which consists of 84 amino acids. This intact PTH is the major secretory product of the parathyroid gland. PTH synthesis is continuous. Its release is also continuous, with about 6–7 superimposed pulses each hour. Most PTH release (70%) occurs during the continuous or tonic phase, and approximately 30% can be attributed to pulsatile secretion. PTH is metabolized by the kidney and the liver to amino-terminal (PTH 1–34) and carboxy-terminal fragments. The amino-terminal fragments constitute about 10% of circulating PTH fragments. They are biologically active but have a short half-life (4–20 minutes). In contrast, the carboxy-terminal fragments, which constitute 80% of the circulating PTH fragments, have no biologic activity and have a longer half-life; they are therefore easier to measure in the plasma. Overall, the intact hormone accounts for 10% of the circulating PTH-related peptides. Because multiple peptide products of PTH breakdown are present in the circulation, determination of the intact molecule is the only reliable index of PTH levels.

Regulation of PTH Release



The main physiologic effect of PTH is to maintain plasma Ca^{2+} homeostasis. Its release is controlled in a tight feedback system by plasma Ca^{2+} concentrations. Small changes in plasma Ca^{2+} levels are detected by the parathyroid Ca^{2+} -sensing receptor (Figure 5–2). An acute decrease in circulating calcium levels (hypocalcemia) elicits a biphasic wave of PTH release. Preformed PTH is released within seconds, followed by an increase in PTH synthesis and release hours later. Thus, for a given Ca^{2+} level, there is an optimal PTH level in the circulation, and this relationship is altered during disease. The tight regulation of PTH release by circulating calcium levels is another example of the negative feedback regulation of hormone release. Table 5–1 lists the factors that regulate PTH release.

Figure 5–2.



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Parathyroid Ca^{2+} -sensing receptor and regulation of parathyroid hormone (PTH) release. Increased Ca^{2+} levels activate the Ca^{2+} sensor on parathyroid cells. The calcium sensor is a G protein–coupled receptor that activates phosphatidylinositol-phospholipase C (PI-PLC), leading to intracellular Ca^{2+} mobilization, protein kinase C (PKC) activation, and downstream activation of phospholipase A_2 , resulting in activation of the arachidonic acid cascade and the production of biologically active leukotrienes. The leukotrienes trigger the degradation of preformed PTH molecules and decrease the release of intact PTH. The inhibition of PTH release by elevated vitamin D levels

is mediated by decreasing the stability of PTH mRNA and consequently the synthesis of PTH. During hypocalcemia, the Ca^{2+} sensor is in a relaxed conformation and does not activate the second messengers involved in the breakdown of preformed PTH. Acute decreases in plasma calcium cause immediate release of preformed PTH and stimulation of new hormone synthesis. ER, endoplasmic reticulum.

Table 5–1. Regulation of Parathyroid Hormone Release

PTH release is increased by	PTH release is suppressed by
Hypocalcemia	Hypercalcemia
Hyperphosphatemia	Vitamin D
Catecholamines	Severe hypomagnesemia

PTH, parathyroid hormone.

Parathyroid Ca^{2+} Receptor

The Ca^{2+} sensor is a G protein ($\text{G}_{q/11}$ and G_i)–coupled receptor located on the plasma membrane of the parathyroid chief cells; it is also found in kidney tubule cells and thyroid C cells. The release of PTH is inhibited in response to elevations in plasma Ca^{2+} concentrations and activation of the Ca^{2+} receptor (Figure 5–2). Activation of the Ca^{2+} receptor initiates a signaling cascade involving phospholipases C, D, and A_2 . The phosphorylation and activation of phospholipase A_2 activate the arachidonic acid cascade and increase leukotriene synthesis. The active leukotriene metabolites inhibit PTH secretion. Interestingly, the inhibition of PTH secretion by elevated Ca^{2+} levels is not due to an alteration in the rate of PTH synthesis, but due to increased degradation of preformed hormone. The parathyroid has a limited number of preformed secretory granules containing mature PTH. The granules are under regulatory control by plasma Ca^{2+} levels; persistent hypercalcemia leads to rapid degradation of most (90%) of the mature PTH in the cell. This process results in the formation of inactive carboxy-terminal PTH fragments, which are either released into the circulation or further

degraded in the parathyroid. The amino acids released during degradation of the formed PTH inside the parathyroid cells are reused in the synthesis of other proteins. In contrast, inhibition of PTH release by the active form of vitamin D (1,25-dihydroxyvitamin D₃; 1,25(OH)₂D₃) is the result of a decrease in PTH gene expression.

During hypocalcemia, the parathyroid Ca²⁺ receptor is relaxed and PTH secretion is not restrained. The rapid secretion of preformed PTH elicited by acute hypocalcemia is rapidly followed by increased stability of PTH mRNA and the synthesis of new hormone.

Additional Factors that Regulate PTH Release

PTH release is also under regulatory control by phosphate and magnesium levels. Elevations in plasma phosphate levels increase PTH secretion by decreasing phospholipase A₂ activity and arachidonic acid formation, thus removing the inhibitory effect on PTH secretion.

Elevations in phosphate levels can also affect PTH release indirectly by decreasing plasma Ca²⁺ levels and vitamin D activation. In contrast, hypophosphatemia markedly decreases PTH mRNA and plasma PTH. The role of phosphate in PTH release is critical in patients with impaired renal function. If not controlled, the increasing levels of phosphate in these individuals result in abnormal elevations in PTH release. As the disease progresses, the negative feedback regulation of PTH release by Ca²⁺ and vitamin D is also impaired, contributing to the elevation in PTH release. As explained in the following section, chronic elevation of PTH results in excess bone resorption and loss of bone mass.

Plasma Mg²⁺ concentrations also regulate PTH secretion in a similar manner to that of calcium. PTH release can be stimulated by a decrease in plasma Mg²⁺. Plasma Mg levels reflect those of Ca most of the time. The balance of magnesium is closely linked to that of calcium; magnesium depletion or deficiency is frequently associated with hypocalcemia. This combined decrease in Mg²⁺ and Ca²⁺ leads to impairment in the individual's ability to secrete PTH. Moreover, severe hypomagnesemia impairs not only the release of PTH from the parathyroid gland in response to hypocalcemia, but it also prevents the responsiveness of bone to PTH-mediated bone resorption. Adrenergic

agonists have been shown to increase PTH release through β -adrenergic receptors on parathyroid cells.

PTH TARGET ORGANS & PHYSIOLOGIC EFFECTS



The primary target organs for the physiologic effects of PTH are kidney and bone. The main physiologic response elicited by PTH is to increase plasma calcium levels by increasing renal Ca^{2+} reabsorption, bone resorption, and intestinal absorption indirectly (via vitamin D_3 activation). PTH also increases 1α -hydroxylase activity and renal phosphate excretion. As with other peptide hormones, the effects of PTH are mediated by binding to a cell membrane receptor in target organs. Three types of PTH receptors (PTHRs) have been identified (PTHR1, PTHR2, PTHR3), all of which are G protein–coupled receptors. The important physiologic effects of PTH are mediated by PTHR1; the physiologic importance of PTHR2 and PTHR3 is not clear.

PTH Receptor 1



PTHR1 is expressed in bone osteoblasts and kidney, where it binds PTH and PTH-related protein (PTHrP). PTHrP differs from PTH in structure, sharing only 13 amino acids with the amino-terminal of the hormone, and is a product of a separate gene. PTHrP is important because it mimics the physiologic effects of PTH in the bone and kidney. The identification of PTHrP followed a long search for the factor responsible for the hypercalcemia of malignancy. Because PTHrP is expressed in multiple tissues, it does not mediate its effects in an endocrine fashion, but rather behaves in a paracrine and autocrine manner. PTHrP binds to the PTHR1 in bone and kidney, resulting in elevated plasma Ca^{2+} levels. Therefore, PTHR1 not only mediates the physiologic effects of PTH, but also plays an important role in the pathophysiologic effects of PTHrP.

PTH binding to the G protein–coupled PTHR1 initiates a cascade of intracellular processes primarily by signaling through the α -subunit of the

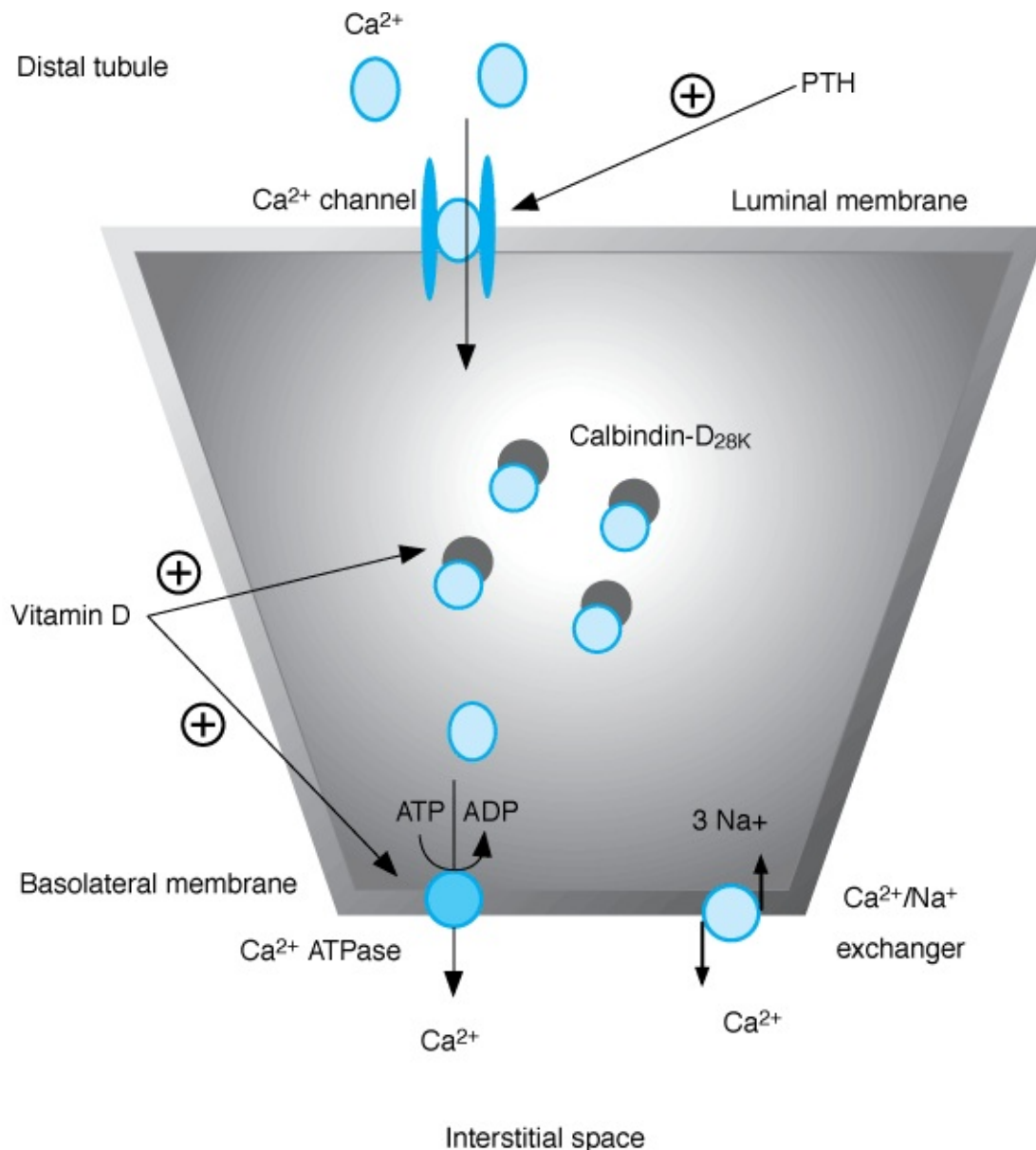
stimulatory G-protein, G_{α_s} leading to an increased synthesis of cAMP and activation of protein kinase A and phosphorylation of target proteins at serine residues. The result is the activation of preformed proteins, as well as the induction of gene transcription. In addition, activation of the PTHR1 can utilize additional signaling pathways through G_{α_q} leading to the activation of phospholipase C, increased intracellular inositol 1,4,5-trisphosphate and calcium concentrations.

Cellular Effects of PTH



As mentioned earlier, PTH directly stimulates Ca^{2+} reabsorption in the kidney, it decreases the reabsorption of phosphate, and it stimulates the activity of 1α -hydroxylase, the enzyme responsible for formation of the active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$). PTH regulation of Ca^{2+} reabsorption is mediated in the distal tubules. Ca^{2+} reabsorption by the proximal tubules occurs primarily through a paracellular pathway that is not regulated by hormones or drugs (Figure 5–3). In the thick ascending limbs, Ca^{2+} is absorbed through a combination of transcellular and paracellular routes. PTH regulates the active, transcellular component, whereas the passive, paracellular route is determined by the extent of concomitant sodium absorption. In the distal tubule, Ca^{2+} absorption is entirely transcellular and is regulated by PTH, vitamin D, and calcitonin; it can also be affected by Ca^{2+} -sparing drugs such as thiazide diuretics. PTH stimulates the insertion and opening of the apical Ca^{2+} channel, facilitating the entry of Ca^{2+} into the cell. These epithelial cells, like the thyroid follicular epithelial cells described in Chapter 4, are polarized; that is, they permit unidirectional flux of the ion from the apical to the basolateral membrane.

Figure 5–3.



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Parathyroid hormone (PTH) increases renal Ca^{2+} reabsorption. The transcellular reabsorption of Ca^{2+} by the distal tubule is regulated by PTH, $1,25(\text{OH})_2\text{D}_3$, and calcitonin, and by calcium-sparing drugs such as thiazide diuretics. PTH increases the insertion of calcium channels in the apical membrane and facilitates the entry of Ca^{2+} . Inside the cell, Ca^{2+} binds to calbindin- $\text{D}_{28\text{K}}$, a vitamin D–dependent calcium-binding protein that facilitates the cytosolic diffusion of Ca^{2+} from the apical influx to the basolateral efflux sites. Ca^{2+} transport out of the cell

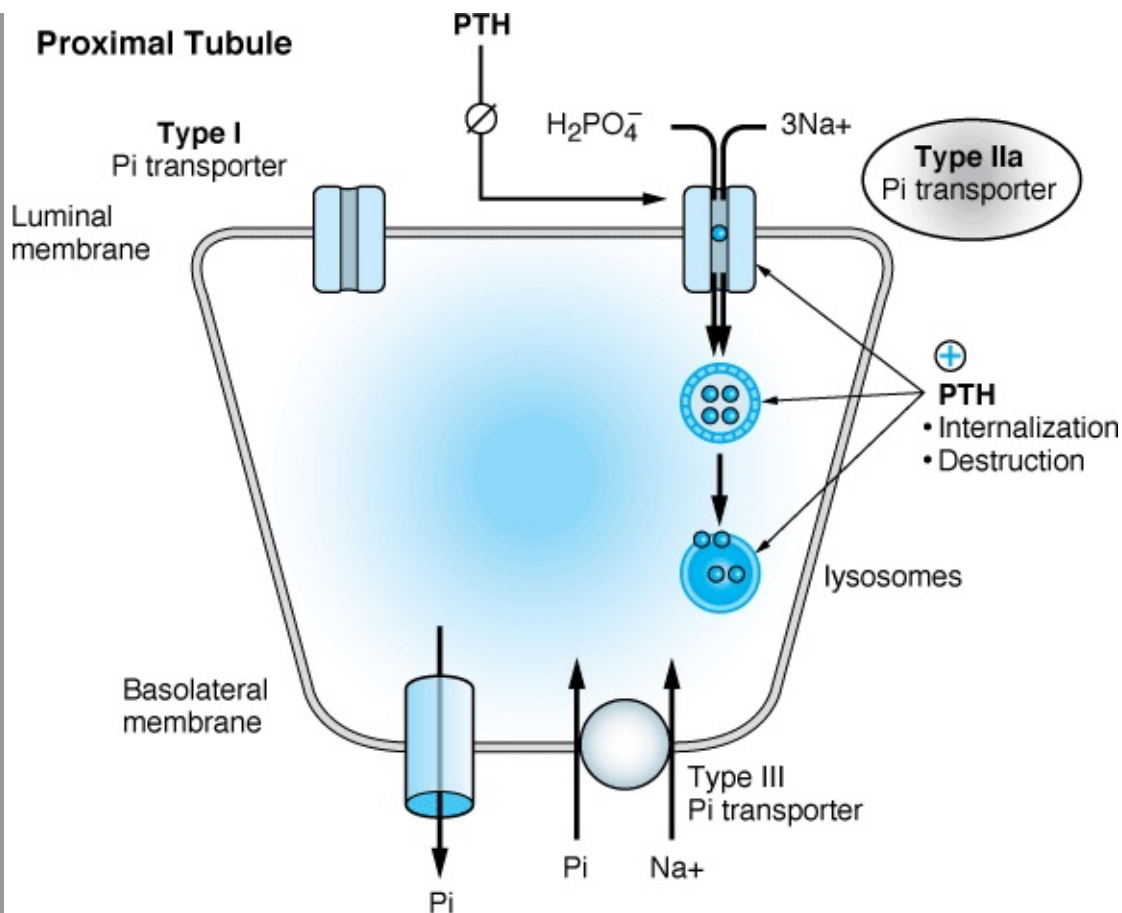
through the basolateral membrane into the interstitial space is mediated by a $\text{Na}^+/\text{Ca}^{2+}$ exchanger and a Ca^{2+} -ATPase. Vitamin D contributes to the enhanced calcium reabsorption by stimulating the synthesis of calbindin and the activity of Ca^{2+} -ATPase. ADP, adenosine diphosphate; ATP, adenosine triphosphate.

Inside the tubular epithelial cell, Ca^{2+} binds to calbindin- $\text{D}_{28\text{K}}$ and then diffuses out through the basolateral membrane (Figure 5–3). Calbindin- $\text{D}_{28\text{K}}$ is a vitamin D–dependent calcium-binding protein that is present in the cytosol of cells lining the distal part of the nephron. Calbindin is thought to act as either a transport protein or a buffer to prevent excess elevation of cytosolic Ca^{2+} levels; it facilitates the cytosolic diffusion of Ca^{2+} from the apical influx to the basolateral efflux sites. Transport of Ca^{2+} out of the cell into the interstitial space is mediated by a $\text{Na}^+/\text{Ca}^{2+}$ exchanger and a Ca^{2+} adenosine triphosphatase (ATPase). A similar mechanism to aid in Ca^{2+} absorption is found in intestinal epithelial cells.



PTH decreases the renal (and intestinal) reabsorption of Pi by decreasing the expression of the type II Na^+/Pi cotransporters (Figure 5–4). In the kidney, PTH acutely (in minutes to hours) decreases the expression of the Na^+/Pi IIa cotransporters by stimulating their internalization via coated vesicles. PTH binding to its receptor initiates signaling pathways (that are still not well understood) leading to membrane retrieval followed by lysosomal degradation of this transporter. The decreased expression of the transporter, results in decreased Pi reabsorption. Thus, unlike other transporters such as aquaporin 2 (discussed in Chapter 2), once the Na^+/Pi cotransporter is internalized, it is degraded; thus this is an irreversible internalization.

Figure 5–4.



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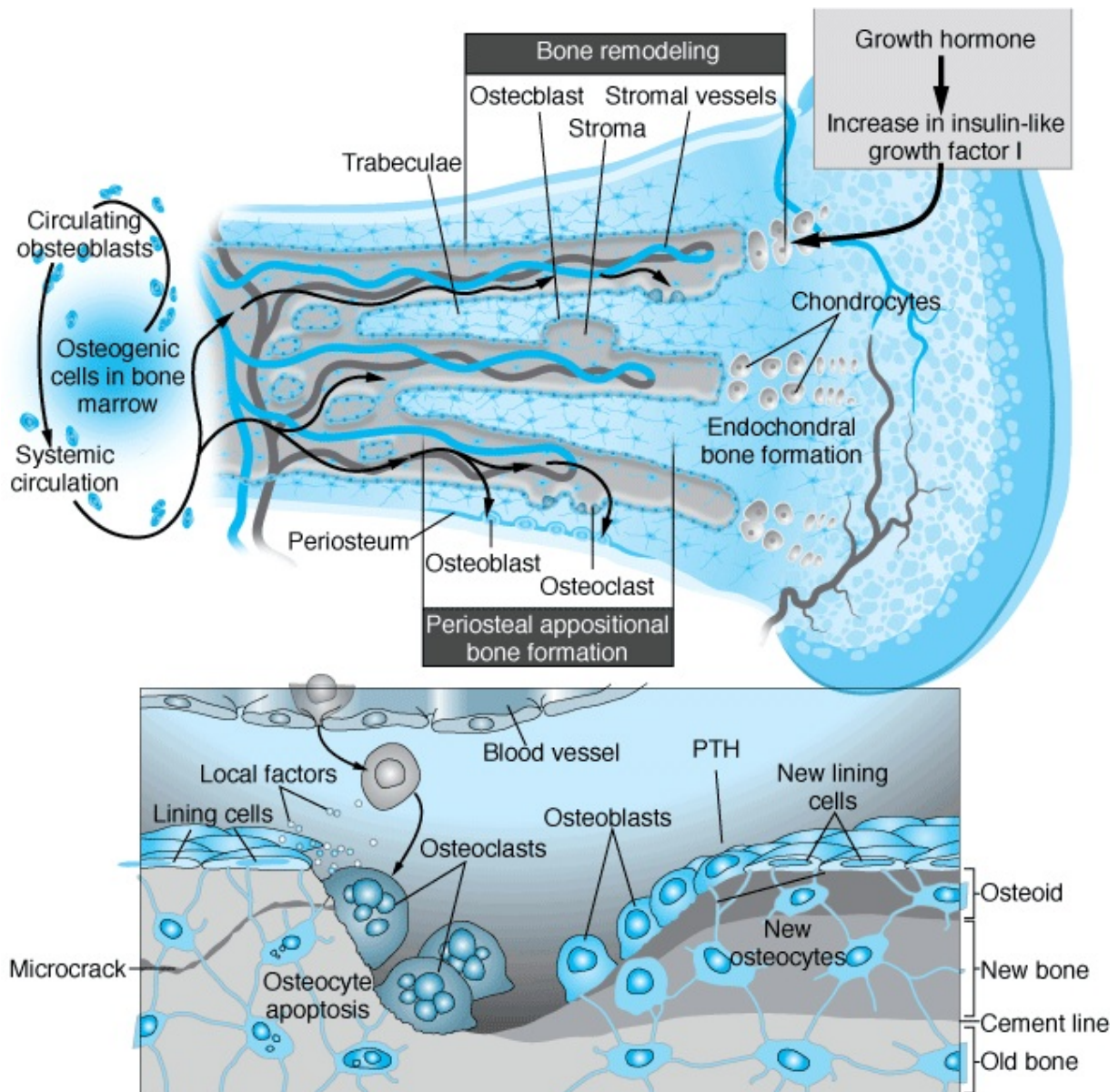
Parathyroid hormone (PTH) decreases renal inorganic phosphate (Pi) reabsorption. Renal reabsorption of phosphate occurs through apical sodium (Na^+)/ Pi cotransport. Three different Na/Pi cotransporters have been identified: types I, II, and III. Type I and type II cotransporters are located in the apical membrane. Type II cotransporters are expressed in the renal proximal tubule (type IIa) and in the small intestine (type IIb). Type IIa cotransporters are the major target for PTH regulation and contribute to most (up to 70%) of proximal tubular Pi reabsorption. PTH acutely stimulates internalization of the type IIa cotransporters, directing them to the lysosomes for destruction. Type III cotransporters are most likely located at the basolateral membrane and play a general "housekeeping" role in ensuring basolateral P_i influx if apical P_i entry is insufficient to satisfy cellular requirements. Basolateral exit, which is necessary to complete transcellular P_i reabsorption, is not well defined.

Several P_i transport pathways have been suggested including Na- P_i cotransport, anion exchange, and even an "unspecific" P_i channel.



In the skeletal system, PTH binds to receptors found in osteoblasts resulting in a cascade of events culminating in an increase in bone turnover, leading to a rapid release of Ca^{2+} from the bone matrix into the extracellular compartment, where it enters the systemic circulation. These receptor-mediated effects of PTH in osteoblasts are mediated through the synthesis or activity of several proteins, including **osteoclast-differentiating factor (ODF)**, also known as receptor activator of nuclear factor- κ B ligand (RANKL) or osteoprotegerin ligand (Figure 5–5). In bone, the overall effects of PTH are osteoblast activation and stimulation of genes vital to the processes of degradation of the extracellular matrix and bone remodeling (collagenase-3), production of growth factors (insulin-like growth factor-I), and stimulation and recruitment of osteoclasts (RANKL and interleukin-6).

Figure 5–5.



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Bone remodeling involves bone formation by osteoblasts and bone resorption by osteoclasts. Parathyroid hormone (PTH) stimulates both aspects of the process. Bone remodeling ensures bone repair and is necessary to maintain calcium homeostasis. (Reproduced, with permission, from Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. *NEJM*. 2007;357:905–916. Copyright © Massachusetts Medical Society. All rights reserved.)

PTH Mobilization of Bone Ca^{2+}



To understand how PTH mobilizes calcium from bone, it is important to have a basic idea of bone structure and of the cells involved in mediating the responses to PTH. Bone consists of an extracellular matrix, the organic phase that is composed of type I collagen, proteoglycans, and noncollagenous proteins. This extracellular bone matrix also contains growth factors and cytokines that have an important regulatory role in bone remodeling, or formation of new bone. The inorganic phase of bone matrix is composed mainly of calcium hydroxyapatite, which functions as a reservoir of calcium and phosphate ions and plays a major role in the homeostasis of these minerals. Most of the skeleton (80%) is composed of **cortical bone**, found mainly in the shafts of long bones and the surfaces of flat bones. Cortical bone consists of compact bone surrounding central canals (haversian systems) that contain blood vessels, lymphatic tissue, nerves, and connective tissue. **Trabecular bone**, found mainly at the ends of long bones and within flat bones, consists of interconnecting plates and bars, inside which lies hematopoietic or fatty bone marrow.

Three cell types are found in bone.

Osteoblasts—Osteoblasts are responsible for bone formation and mineralization and express PTHRs. They are derived from pluripotent mesenchymal stem cells, which can also differentiate into chondrocytes, adipocytes, myoblasts, and fibroblasts. Several hormonal and nonhormonal molecules stimulate the differentiation of osteoblasts from stem cell precursors.

Osteoclasts—Osteoclasts are large multinucleated bone-resorbing cells derived from hematopoietic precursors of the monocyte-macrophage lineage. They are formed by the fusion of mononuclear cells and are characterized by a ruffled border, which consists of an infolding of the plasma membrane, and a prominent cytoskeleton. Osteoclasts are rich in lysosomal enzymes.

Osteocytes—Osteocytes, the most numerous cells in bone, are small, flattened cells within the bone matrix. They are connected to one another

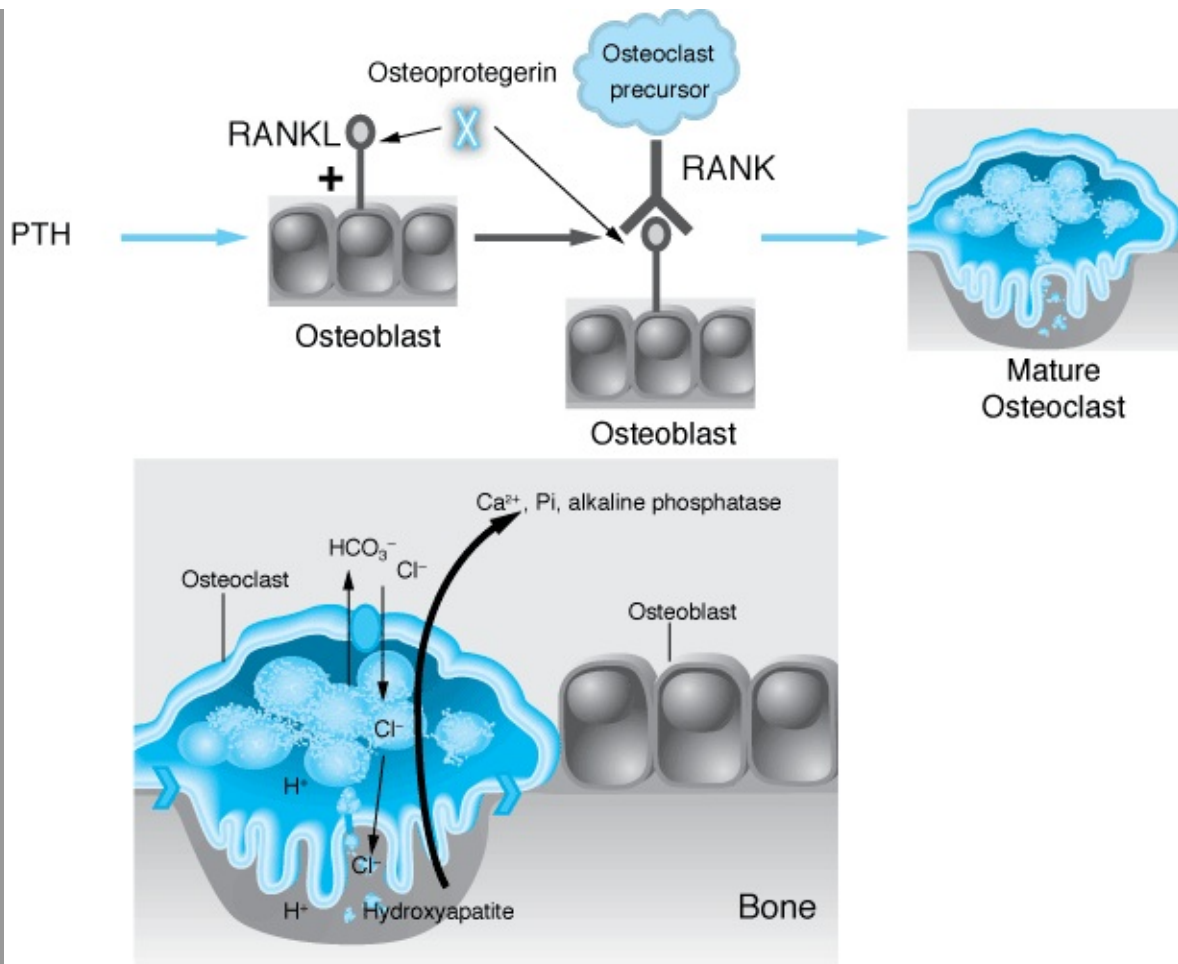
and to osteoblastic cells on the bone surface by an extensive canalicular network that contains the bone's extracellular fluid. These cells are terminally differentiated from osteoblasts and ultimately undergo apoptosis or phagocytosis during osteoclastic resorption. Their function and their contribution to osteoclastic bone resorption are not completely understood.

Mechanism of Bone Resorption

Bone remodeling involves the continuous removal of bone (bone resorption) followed by synthesis of new bone matrix and subsequent mineralization (bone formation). This coupling of the functions of osteoblasts and osteoclasts is central not only for the hormonal regulation of bone turnover, but also for the evaluation of their altered function, as discussed later. During skeletal development and throughout life, cells from the osteoblast lineage synthesize and secrete molecules that in turn initiate and control osteoclast differentiation. Osteoclasts are stimulated hormonally and locally by growth factors and cytokines. Osteoclastic activity induced by PTH is indirectly mediated through osteoblast activation. Osteoclastic bone resorption consists of multiple steps, including recruitment and differentiation of osteoclast precursors into mononuclear osteoclasts (preosteoclasts) and fusion of preosteoclasts to form multinucleated functional osteoclasts (Figure 5–6).

Figure 5–6.





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Parathyroid hormone (PTH)–mediated osteoclast differentiation. PTH binds to PTHR1 in osteoblasts and stimulates the expression of receptor activator of nuclear factor- κ B ligand (RANKL) expression on the cell surface. RANKL binds to receptor activator of nuclear factor- κ B (RANK), a cell-surface protein on osteoclast precursors. Binding of RANKL to RANK activates osteoclast gene transcription and the differentiation into a mature osteoclast, characterized by the ruffled membrane under which bone resorption occurs.

Osteoclastic bone resorption requires the recruitment and differentiation of osteoclast precursors from the granulocyte-macrophage colony-forming unit (CFU) into preosteoclasts followed by fusion of preosteoclasts to form multinucleated functional osteoclasts. PTH binding to PTH receptor 1 on osteoblasts triggers

the synthesis of RANKL also known as osteoclast differentiation factor (ODF) and as osteoprotegerin ligand. RANKL (expressed in the osteoblasts) binds to RANK in the osteoclast precursors and stimulates their differentiation into functional osteoclasts. The initial event in bone degradation is the attachment of osteoclasts to the bone surface. The mature and activated osteoclast is characterized by the formation of the ruffled membrane under which bone resorption occurs.

Osteoclasts attach to the bone surface through β -integrins generating an isolated extracellular microenvironment. Acidic intracellular vesicles fuse to the cell membrane facing the bone matrix forming the scalloped or ruffled border. Hydrogen ions generated by carbonic anhydrase II are delivered across the plasma membrane by H^+ -ATPases recruited into the cell's ruffled membrane. The acidification of this isolated extracellular microenvironment to a pH of about 4 favors the dissolving of hydroxyapatite and provides optimal conditions for the action of the lysosomal proteases including collagenase and cathepsins to dissolve bone mineral. The products of bone degradation are endocytosed by the osteoclast and transported to and released at the cell's antiresorptive surface providing ionized Ca^{2+} , inorganic phosphate, and alkaline phosphatases, into circulation. Osteoprotegerin is a soluble protein secreted by osteoblasts that serves as a decoy ligand for RANKL, preventing binding of RANKL to RANK, thereby inhibiting the process of osteoclastic bone resorption. As a result there is a decrease in the differentiation of precursor cells into osteoclasts and decreased bone resorption. Production of osteoprotegerin is increased by estrogen and decreased by glucocorticoids and PTH. PTH inhibits the production of osteoprotegerin.

The initial event in bone degradation is the attachment of osteoclasts to the bone surface following their recruitment. PTH binding to osteoblasts triggers the synthesis of ODF, also known as RANKL or osteoprotegerin ligand. PTH stimulates the expression of this cell membrane protein in osteoblastic cells. This ligand binds to the ODF receptor (**RANK**) expressed on the hemopoietic osteoclastic precursors and stimulates their differentiation into functional

osteoclasts. These 2 cell-surface proteins, RANK expressed on osteoclast precursor cells and its partner RANKL expressed on osteoblasts, are the key regulators of osteoclast formation and function (Figures 5–5 and 5–6).

Activation by RANKL increases the expression of specific genes leading to osteoclast maturation. When an osteoclast precursor encounters an osteoblast, the resulting interaction between RANK and RANKL stimulates the osteoclast precursor to mature into a fully differentiated, bone-resorbing osteoclast. Additional proteins play important roles in the regulation of this mechanism. **Osteoprotegerin** is a protein member of the TNF receptor superfamily, secreted by osteoblasts, which acts as a natural antagonist of RANKL and thus contributes to the regulation of bone resorption. Osteoprotegerin functions as an osteoclastogenesis inhibitory factor. It works as a soluble "decoy" protein that binds to RANKL, preventing it from binding to RANK and thereby effectively inhibiting RANKL-mediated osteoclast maturation. The regulation of osteoprotegerin production is not completely understood. However, it is known that PTH and glucocorticoids decrease the production of osteoprotegerin, whereas estrogens increase its expression.

Osteoclastic bone resorption involves the attachment of osteoclasts by proteins called β -integrins to the bone surface, generating an isolated extracellular microenvironment between osteoclasts and the bone surface that in effect functions as a lysosome. Acidic intracellular vesicles fuse to the scalloped border of the cell membrane facing the bone matrix—the hallmark of the resorbing surface—under which bone resorption takes place. Acidification of this extracellular microenvironment is mediated by H^+ -ATPases recruited into the cell's ruffled membrane. During bone resorption, hydrogen ions generated by carbonic anhydrase II are delivered across the plasma membrane by a proton pump to dissolve bone mineral. The decrease in pH (pH 4) in the sealed compartment favors the dissolving of hydroxyapatite bone mineral and provides optimal conditions for the action of the lysosomal proteases, including collagenase and cathepsins secreted by osteoclasts. The products of bone degradation (including Ca^{2+} and P_i), are endocytosed by the osteoclast and released at the cell's

antiresorptive surface by a process called transcytosis (transit across the cell), from where they then reach the systemic circulation. During bone degradation, intracellular enzymes such as alkaline phosphatase are also released into the interstitial space to enter the circulation. A rise in circulating levels of alkaline phosphatase can be used clinically as a marker of increased osteoclastic activity.

CALCIUM HOMEOSTASIS

The human body contains approximately 1100 g of calcium, 99% of which is deposited in bones and teeth. The small amount found in plasma is divided into 3 fractions: ionized calcium (50%), protein-bound calcium (40%), and calcium complexed to citrate and phosphate forming soluble complexes (10%). The complexed and ionized Ca^{2+} fractions (about 60% of plasma Ca^{2+}) can cross the plasma membrane. The majority (80–90%) of protein-bound Ca^{2+} is bound to albumin, and this interaction is sensitive to changes in blood pH. Acidosis leads to a decrease in protein binding of Ca^{2+} and an increase in "free" or ionized Ca^{2+} in the plasma. Alkalosis results in increased Ca^{2+} binding and a decrease in ionized Ca^{2+} in the plasma. A smaller fraction (10–20%) of protein-bound Ca^{2+} is bound to globulins.

The resting intracellular (cytosolic) calcium concentration is about 100 nM, but this can increase to 1 μM by the release of Ca^{2+} from intracellular stores or by uptake of extracellular Ca^{2+} in response to cellular activation. In contrast, the extracellular ionized calcium concentration is approximately 10,000-fold higher than the intracellular calcium concentration and remains virtually constant at approximately 1 mM. Multiple physiologic functions involve calcium ions. Ca^{2+} is a key intracellular messenger and cofactor for various enzymes. Ca^{2+} also has diverse extracellular functions (eg, in the clotting of blood, maintenance of skeletal integrity, and modulation of neuromuscular excitability). Therefore, stable Ca^{2+} levels are critical for normal physiologic function. For example, Na^+ channel voltage-gating is dependent on the extracellular Ca^{2+} concentration. Decreased plasma Ca^{2+} concentrations reduce the voltage threshold for the action potential firing, resulting in neuromuscular hyperexcitability. This can result in numbness and tingling of fingertips, toes, and the perioral region or muscle cramps. Clinically, neuromuscular irritability can be demonstrated by mechanical stimulation of the hyperexcitable nerve leading to tetanic-like muscle contraction by eliciting Chvostek's (ipsilateral contraction of facial muscles elicited by tapping the skin

over the facial nerve) or Trousseau's (carpal spasm induced by inflation of the blood pressure cuff to 20 mm Hg above the patient's systolic blood pressure for 3–5 minutes) sign.

Interaction of Bone, Kidney, and Intestine in Maintaining Calcium Homeostasis

Normal plasma concentrations of Ca^{2+} range between 8.5 and 10.5 mg/dL and are mainly regulated by the actions of PTH, vitamin D, and calcitonin on 3 tissues: bone, kidney, and intestine.

Bone—Calcium in bone is distributed in a readily exchangeable pool and a stable pool. The readily exchangeable pool is involved in maintaining Ca^{2+} plasma levels by the daily exchange of 550 mg of calcium between the bone and extracellular fluid. The stable Ca^{2+} pool is involved in bone remodeling. Bone is metabolically active throughout life. After skeletal growth is complete, remodeling of both cortical and trabecular bone continues with an annual turnover rate of about 10% of the adult skeleton.

Kidney—In the kidney, virtually all filtered Ca^{2+} is reabsorbed. About 40% of the Ca^{2+} that is reabsorbed is under hormonal regulation by PTH. Most filtered Ca^{2+} is reabsorbed in the proximal tubules, mainly by passive transport processes independent of hormonal regulation. Ca^{2+} absorption in the cortical thick ascending limbs is mediated by a combination of active and passive absorption. Ca^{2+} absorption in the distal convoluted tubules is mediated by active cellular absorption, which is stimulated by PTH binding to PTHR1. Transcellular transport of Ca^{2+} is facilitated by vitamin D through the increase in the Ca^{2+} -binding protein calbindin- $\text{D}_{28\text{K}}$ and in the expression of Ca^{2+} transporters in the basolateral membrane.

Intestine—The availability of dietary calcium is a critical determinant of calcium homeostasis. Dietary intake of calcium averages 1000 mg/d, of which only 30% is absorbed in the intestinal tract. This percentage of dietary Ca^{2+} that is absorbed is significantly enhanced by vitamin D during growth, pregnancy, and lactation. During growth, there is a net bone accretion. After completion of the growth phase in the young and

healthy individual, there is no net gain or loss of Ca^{2+} from bone despite a continuous turnover of bone mass; the amount of Ca^{2+} lost in urine is approximately equal to net Ca^{2+} absorption.

Intestinal absorption of Ca^{2+} occurs by a saturable, transcellular process and a nonsaturable, paracellular pathway. The paracellular pathway predominates when dietary Ca^{2+} is abundant. The active transcellular pathway is vitamin D–dependent and plays a major role in absorption when the Ca^{2+} supply is limited. Intestinal transepithelial Ca^{2+} transport, similar to that in the distal tubule, is a 3-step process consisting of passive entry across the apical membrane, cytosolic diffusion facilitated by vitamin D–dependent calcium-binding proteins (calbindins), and active extrusion of Ca^{2+} across the opposing basolateral membrane mediated by a high-affinity Ca^{2+} -ATPase and $\text{Na}^+/\text{Ca}^{2+}$ exchanger.

Hormonal Regulation of Calcium Homeostasis

As explained previously, a slight decrease in the free ionized Ca^{2+} level is sensed through the Ca^{2+} sensor in parathyroid chief cells, resulting in an increased release of PTH. PTH binds to receptors in osteoblasts leading to the recruitment of preosteoclasts and their maturation to active osteoclasts, which are responsible for increased bone resorption and release Ca^{2+} and Pi into the circulation. In the kidney, PTH promotes Ca^{2+} reabsorption and Pi excretion in urine. In addition, PTH stimulates the hydroxylation of 25-hydroxyvitamin D_3 at the 1-position, leading to the formation of the active form of vitamin D (calcitriol). Vitamin D increases intestinal absorption of dietary Ca^{2+} and renal reabsorption of filtered Ca^{2+} . In bone, vitamin D increases the number of osteoclasts and stimulates bone resorption, with a resulting increase in the release of Ca^{2+} into the circulation. An increase in free ionized Ca^{2+} levels decreases the release of PTH from the parathyroid gland, decreases the activation of vitamin D in the kidney, and stimulates parafollicular cells of the thyroid gland to release the hormone calcitonin.



Calcitonin counteracts the effects of PTH. Calcitonin inhibits osteoclast activity, decreasing bone resorption and increasing renal Ca^{2+} excretion; the result is a decrease in free ionized Ca^{2+} levels. Overall, PTH, calcitriol, and calcitonin work together to maintain plasma Ca^{2+} levels within a normal range. The previous section described in detail the physiologic effects of PTH. The following section discusses the contributions of vitamin D and calcitonin to the regulation of Ca^{2+} homeostasis.

Role of Vitamin D in Calcium Homeostasis

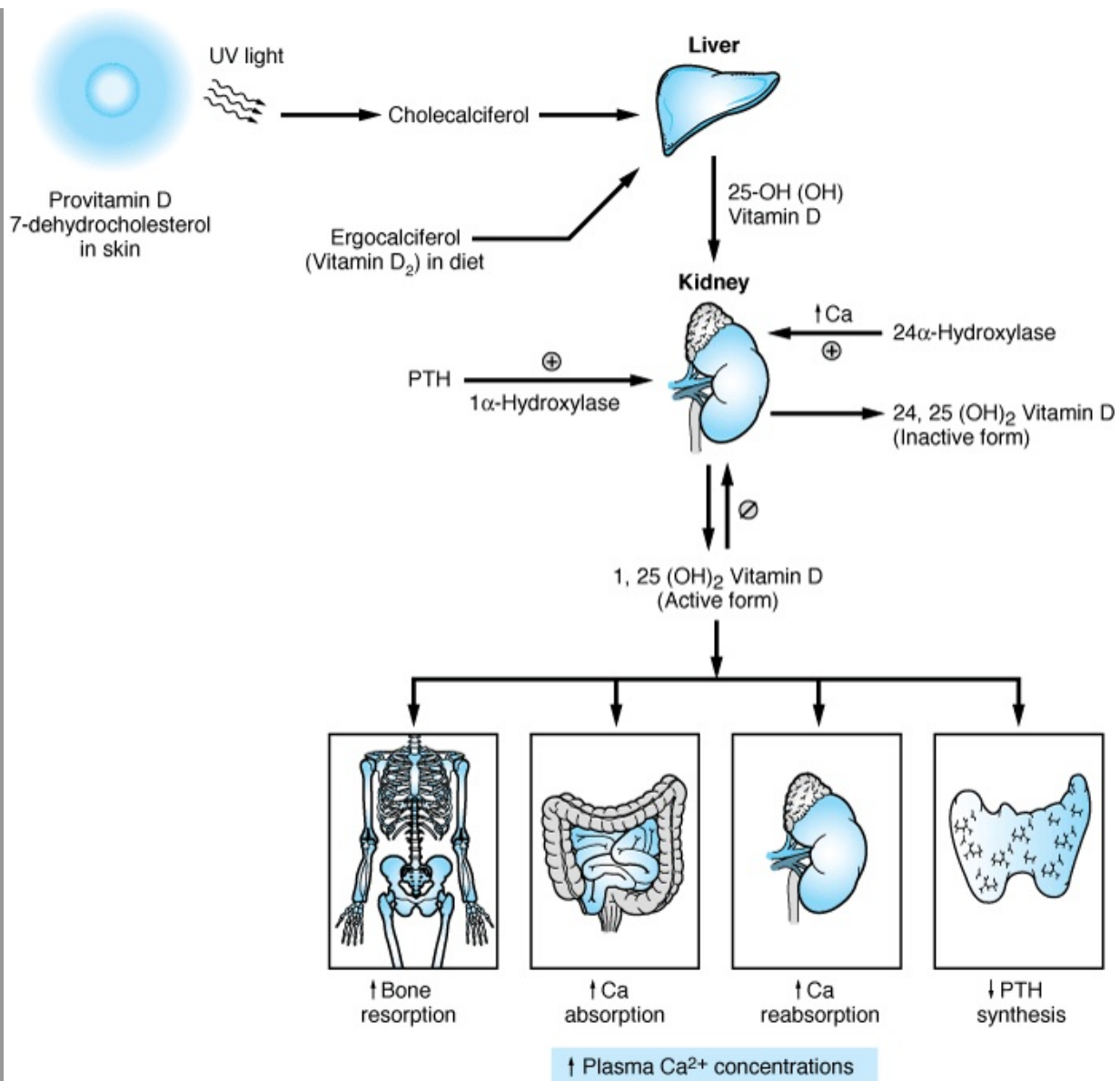
SYNTHESIS AND ACTIVATION OF VITAMIN D



Vitamin D is a lipid-soluble vitamin that can be synthesized from plant-derived precursors or through the action of sunlight from cholesterol-derived precursors found in the skin (Figure 5–7). Active vitamin D (calcitriol) is the product of 2 consecutive hydroxylation steps,

(first in the liver and then in the kidney) of its precursors cholecalciferol (derived from skin) and ergocalciferol (derived from diet). Cholecalciferol is produced in the skin by ultraviolet radiation from 7-dehydrocholesterol, an inert precursor. This previtamin D₃ (cholecalciferol) is isomerized to vitamin D₃ and is transported in the circulation bound to vitamin D-binding protein, the major plasma carrier protein of vitamin D and its metabolites. Cholecalciferol (vitamin D₃) and vitamin D₂ (ergocalciferol from plants) are transported to the liver, where they undergo the first step in bioactivation, the hydroxylation at C-25 to 25-hydroxyvitamin D₃ (25(OH)D₃). The resulting prehormone, 25-hydroxyvitamin D₃, is the major circulating form of vitamin D (15–60 ng/mL). 25-hydroxyvitamin D₃ is the principle storage form of vitamin D, has a half-life of 15 days, is in equilibrium with the storage pool in muscle and fat, and is the value measured by most clinical laboratories to assess levels of vitamin D in individuals. 25-Hydroxyvitamin D₃ circulates bound to vitamin D-binding protein. This protein can be filtered at the glomerulus and enter the proximal tubular facilitating the delivery of the precursor, 25-hydroxyvitamin D₃, to the 1 α -hydroxylase. Renal 1 α -hydroxylase is the enzyme responsible for the second step in the activation of the prehormone, the hydroxylation at C-1, resulting in the hormonally active vitamin D (1,25(OH)₂D₃), also known as **calcitriol**. Calcitriol is released into the circulation (20–60 pg/mL), where it functions as an endocrine hormone, regulating cellular processes in a host of target tissues. This second hydroxylation step, the production of 1,25(OH)₂D₃ by 1 α -hydroxylase in the kidney, is a tightly regulated process and is a central factor in the feedback regulation of calcium homeostasis. The production of the active form of vitamin D is under negative feedback regulation by plasma Ca²⁺ levels. A rise in plasma Ca²⁺ levels inhibits the hydroxylation at C-1 and favors hydroxylation at C-24, leading to the synthesis of an inactive metabolite of vitamin D (24,25(OH)₂D₃).

Figure 5–7.



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Vitamin D metabolism and physiologic effects at target organs. Provitamin D (7-dehydrocholesterol) in the skin is converted to cholecalciferol by ultraviolet (UV) light. Cholecalciferol and ergocalciferol (from plants) are transported to the liver, where they undergo the first step in bioactivation, the hydroxylation at C-25 to 25-hydroxyvitamin D₃ (25(OH)D₃), the major circulating form of vitamin D. The second hydroxylation step, at C-1, occurs in the kidney and results in the hormonally active vitamin D (1,25(OH)₂D₃), also known as calcitriol. This activation step, mediated by 1α-hydroxylase, is under

tight regulation by parathyroid hormone (PTH), Ca^{2+} levels, and vitamin D ($1,25(\text{OH})_2\text{D}_3$). The activity of 1α -hydroxylase is stimulated by PTH and inhibited by Ca^{2+} and $1,25(\text{OH})_2\text{D}_3$. Decreased activity of 1α -hydroxylase favors formation of the inactive form of vitamin D by C-24 hydroxylation. Vitamin D increases bone resorption, increases Ca^{2+} absorption from the intestine, increases renal Ca^{2+} reabsorption, and decreases the production of PTH by the parathyroid glands. The overall effect of vitamin D is to increase plasma Ca^{2+} concentrations.

In summary, PTH stimulates the activity of 1α -hydroxylase, favoring an increase in the synthesis of the active form of vitamin D. Vitamin D, as well as high Ca^{2+} levels, suppresses the activity of 1α -hydroxylase, decreasing its own synthesis and favoring the synthesis of $24,25(\text{OH})_2\text{D}_3$, the less active form of the hormone. Vitamin D increases intestinal Ca^{2+} absorption and suppresses the synthesis and release of PTH from the parathyroid gland (Figure 5–7).

CELLULAR EFFECTS OF VITAMIN D



The principal physiological effects mediated by vitamin D result from its binding to a vitamin D steroid receptor located in the principal target organs; intestine, bone, kidney, and parathyroid gland. The receptor has high affinity for calcitriol and very low affinity for the metabolites of the hormone. The effects of active vitamin D are to stimulate calcium absorption from the intestine, regulate bone resorption and formation, enhance calcium reabsorption in the distal renal tubules, and repress the transcription and secretion of PTH. Additional actions of calcitriol have been identified and these involve the immune response, reproduction, and cellular differentiation and proliferation.

Vitamin D is able to generate biologic effects both by genomic mechanisms (changes in gene transcription) and rapid nongenomic mechanisms. The genomic effects are dependent upon the interaction of $1,25(\text{OH})_2\text{D}_3$ with a cytosolic-nuclear receptor protein, followed by interaction of the steroid-receptor complex in the nucleus with selective regions of the promoters of genes that are either activated or repressed. The stimulation of rapid responses by $1,25(\text{OH})_2\text{D}_3$ may result from interaction of the vitamin with a cell membrane receptor for $1,25(\text{OH})_2\text{D}_3$ that activates a variety of signal transduction systems, including protein kinase C, phospholipase C, and adenylate cyclase, and modulates ion (ie, Ca^{2+} or Cl^-) channels.

ABNORMAL VITAMIN D LEVELS

The US-recommended daily allowance of vitamin D is 200 U for adults and 400 U for children, pregnant women, and lactating women. Vitamin D belongs to the class of vitamins that are lipid soluble (ie, A, D, E, and K), and can be stored in tissues. Excess vitamin D may lead to problems such as calcinosis (calcification of soft tissues), deposition of Ca^{2+} and PO_4 in the kidney, and increased plasma Ca^{2+} levels, resulting in cardiac arrhythmia.

Deficiency of vitamin D can be the result of decreased dietary intake or lack of sunlight and the resulting decreased conversion from the inactive precursor in skin to the active form of the vitamin. Vitamin D deficiency results in bone deformities (rickets) when it occurs in children and decreased bone mass (osteomalacia) in adults. Vitamin D deficiency is associated with weakness, bowing of the weight-bearing bones, dental defects, and hypocalcemia. Factors that may contribute to vitamin D deficiency include the use of sunscreen, particularly in the elderly population, lack of sunlight during November through March in certain latitudes (above 40 N and below 40 S), and use of clothing that covers most of the skin. Less frequently, people may have a mutation in 1α -hydroxylase, the enzyme that catalyzes the second and final step in vitamin D activation, or a resistance to vitamin D action in the tissues due to mutations in the vitamin D receptor.

Role of Calcitonin in Calcium Homeostasis

A third hormone involved in calcium homeostasis, although to a lesser extent than PTH and vitamin D, is calcitonin. Calcitonin is a 32–amino acid peptide hormone derived from pro-calcitonin, produced by cells of neural crest origin (parafollicular or C cells) in the thyroid gland. Calcitonin belongs to a family of peptides including amylin, calcitonin gene-related peptides (CGRPs) and adrenomedullin. These are distributed in various peripheral tissues as well as in the central nervous system and induce multiple biological effects including potent vasodilatation (CGRP and adrenomedullin), reduction in nutrient intake (amylin), and decreased bone resorption (calcitonin).

The release of calcitonin is regulated by plasma calcium levels through

a Ca^{2+} receptor on the parafollicular cells. Elevations in plasma Ca^{2+} higher than 9 mg/dL stimulate the release of calcitonin. Calcitonin has a half-life of approximately 5 minutes and is metabolized and cleared by the kidney and the liver. The release of calcitonin is also stimulated by gastrin, a gastrointestinal hormone.

CELLULAR EFFECTS OF CALCITONIN

The main physiologic function of calcitonin is to decrease plasma Ca^{2+} and phosphate concentrations, mainly by decreasing bone resorption. The 2 target organs for calcitonin's physiologic effects are bone and kidney. The overall effect of calcitonin in bone is to inhibit bone resorption, predominantly by altering osteoclast activity. Calcitonin directly and rapidly inhibits osteoclast motility, prevents osteoclast differentiation, causes a loss of the ruffled border of osteoclasts. Calcitonin inhibits osteoclast secretory activity (particularly of tartrate-resistant acid phosphatase), alters Na^+ - K^+ -ATPase activity, carbonic anhydrase localization, and inhibits H^+ -ATPase activity, reducing osteoclast acid secretion. In the kidney, calcitonin increases urinary Ca^{2+} excretion by inhibition of renal tubular calcium reabsorption. The mechanism involved is through opening of low affinity Ca^{2+} channels in the luminal membrane and the stimulation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger in the basolateral membrane, both actions depending on the activation of adenylate cyclase. In hypercalcemic patients with metastatic bone disease, the administration of calcitonin induces a rapid decrease in plasma calcium primarily through inhibition of renal tubular reabsorption.

CALCITONIN RECEPTORS

The cellular effects of calcitonin are mediated through G protein (G_s , G_q , or G_i)–coupled receptors from the same receptor family as the PTH, PTHrP, calcitonin, adrenomedullin, and secretin receptor superfamily. Several calcitonin receptor subtypes have been identified, and they all bind calcitonin with high affinity. Calcitonin binding to its receptor stimulates adenylate cyclase, increasing the formation of cAMP and activation of protein kinase A and phospholipase C, resulting in the release of Ca^{2+} from intracellular stores and influx of extracellular Ca^{2+} .

CALCITONIN AND DISEASE

Calcitonin does not appear to be critical for the regulation of calcium homeostasis; in fact, total removal of the thyroid does not produce major alterations in Ca^{2+} homeostasis. In addition, no significant clinical findings have been associated with calcitonin excess or deficiency.

However, calcitonin has been used therapeutically for the prevention of bone loss and for the short-term treatment of hypercalcemia of malignancy. Interestingly, salmon- or eel-derived calcitonin is more potent than the human hormone. Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration of bone tissue, resulting in bone fragility and susceptibility to fracture (discussed in Chapter 10). The ability of calcitonin to inhibit osteoclast-mediated bone resorption has made it a useful agent for the treatment of osteoporosis; it also relieves pain in osteoporotic patients with vertebral crush fractures. Calcitonin is also used in the treatment of Paget disease, which is characterized by an abnormality in bone remodeling, with increased bone resorption and hypercalcemia.

Additional Regulators of Ca^{2+} & Bone Metabolism

Although PTH and vitamin D play central roles in the regulation of bone metabolism, the contribution of other hormones cannot be ignored (Table 5–2). Sex steroids (androgens and estrogens) have been shown to increase 1α -hydroxylase activity, decrease bone resorption, and increase osteoprotegerin synthesis. Estrogen stimulates the proliferation of osteoblasts and the expression of type I collagen and alkaline phosphatase; influences the expression of receptors for vitamin D, growth hormone, and progesterone; and modulates responsiveness of bone to PTH. Estrogen decreases the number and activity of osteoclasts, as well as the synthesis of cytokines affecting bone resorption.

Table 5–2. Factors Involved in the Regulation of Ca^{2+} and Bone Metabolism

Regulator	Action
PTH	Increases bone resorption and plasma Ca^{2+}
Vitamin D	Increases intestinal Ca^{2+} absorption, bone resorption, and plasma Ca^{2+}
Calcitonin	Decreases bone resorption and plasma Ca^{2+}
Sex steroids (androgens and estrogens)	Increase 1α -hydroxylase activity

Increase osteoprotegerin synthesis

Net decrease in bone loss	
Growth hormone and insulin-like growth factor	Stimulate bone synthesis and growth
Thyroid hormone	Increases bone turnover
Prolactin	Increases renal Ca^{2+} reabsorption and 1α -hydroxylase activity
Glucocorticoids	Increase bone resorption, decrease bone synthesis
Inflammatory cytokines	Increase bone resorption

PTH, parathyroid hormone.

Growth hormone and insulin-like growth factor-I both exert effects on bone metabolism. Growth hormone stimulates the proliferation and differentiation of osteoblasts and bone protein synthesis and growth. Insulin-like growth factors, produced by the liver and by bone cells, stimulate bone formation by increasing the proliferation of osteoblast precursors and by enhancing the synthesis and inhibiting the degradation of type I collagen. Normal thyroid function is required for physiological bone remodeling. However, excess thyroid hormone levels result in increased bone resorption. Prolactin increases Ca^{2+} reabsorption and 1α -hydroxylase activity. Glucocorticoids play an overall catabolic role in bone metabolism by increasing bone resorption and decreasing bone synthesis, resulting in an increase in the risk of fractures. The mechanisms by which glucocorticoids exert their effects are not fully understood, but inhibition of osteoprotegerin may help stimulate osteoclastic bone resorption. The cytokines tumor necrosis factor- α and - β , interleukin- 1α and - 1β , and interleukin-6 increase the proliferation and differentiation of osteoclast precursors and their osteoclastic activity and are therefore potent stimulators of bone resorption in vitro and in vivo. The overall interaction of these various factors during health and disease plays an important role in maintaining bone mass. Their specific contributions may vary depending on the disease and on the prevailing hormone and cytokine

levels in bone.

HORMONAL REGULATION OF BONE METABOLISM

Bone remodeling results from the interactions of multiple elements, including osteoblasts, osteoclasts, hormones, growth factors, and cytokines, the result being a dynamic maintenance of the bone architecture and systemic preservation of calcium homeostasis. In early life, a careful balance exists between bone formation by osteoblasts and bone resorption by osteoclasts. With aging, the process of coupled bone formation-resorption (turnover) is affected by the reductions in osteoblast differentiation, activity, and life span, which are further potentiated in the perimenopausal years by hormone deprivation (estrogen and dehydroepiandrosterone) and an increase in osteoclast activity.

Decreased calcium intake below obligatory calcium loss (through the urine, feces, and skin) mobilizes calcium from the skeleton to maintain the ionized calcium concentration in the extracellular fluid (ECF), resulting in bone destruction. Vitamin D deficiency lowers the concentration of ionized calcium in the ECF (from loss of the calcemic action of vitamin D on bone), resulting in stimulation of PTH release (secondary hyperparathyroidism), increased phosphate excretion (hypophosphatemia), and failure to mineralize new bone as it is being formed. Simple calcium deficiency is associated with compensatory increases in PTH and calcitriol, which together cause loss of whole bone, whereas true vitamin D deficiency reduces the mineral content of the bony tissue itself, leading to abnormal bone composition. However, these 2 nutritional deficiencies cannot be completely separated because calcium malabsorption is the first manifestation of vitamin D deficiency.

Childhood and puberty—Bone mass increases throughout childhood and adolescence. In girls, the rate of increase in bone mass decreases rapidly after menarche, whereas in boys, gains in bone mass persist up to 17 years of age and are closely linked to pubertal stage and androgen status. By age 17–23, the majority of peak bone mass has already been achieved in both sexes. Skeletal growth is achieved primarily through bone modeling and only partially through bone remodeling. These mechanisms involve interaction between osteoblasts and osteoclasts, which work cooperatively under the

influence of the mechanical strain placed on bone by skeletal muscle force such as that exerted during exercise. The mechanical loading or strain oscillates within a given range in response to physical activity, leading to bone maintenance without loss or gain. Decreased mechanical strain (such as that associated with prolonged bed rest or immobilization) leads to bone loss, whereas increased mechanical strain (weight-bearing exercise) stimulates osteoblastic activity and bone formation. The loading on the bone cells is exerted primarily by muscles and to a lesser extent by body weight. Muscle force or tension applied on long bones increases the thickness of cortical bone through continuous subperiosteal accretion. This relationship between muscle tension exerted on bones and bone formation is positively affected during exercise.

Peak bone mass is attained in the third decade of life and is maintained until the fifth decade, when age-related bone loss begins both in men and women. Sex steroids play an important role in bone growth and the attainment of peak bone mass. They are also responsible for the sexual dimorphism of the skeleton, which emerges during adolescence and is characterized by larger bone size in males (even when corrected for body height and weight), with both a larger diameter and a greater cortical thickness in the long bones.

Pregnancy and lactation—The uptake and release of calcium from the skeleton are increased during pregnancy, and the rate of calcium mobilization continues to be increased during the early months of lactation, returning to prepregnancy rates during or after weaning. Calcium absorption, urinary calcium excretion, and bone resorption are higher during pregnancy than before conception or after delivery. The increases are evident in early to mid-pregnancy and precede the increased demand for calcium by the fetus for skeletal growth. The alterations in calcium and bone metabolism during pregnancy are accompanied by increases in vitamin D, but without significant alterations in either intact PTH or calcitonin concentrations. Changes in maternal bone mineral content during this period may influence bone mineral status in the long term. After delivery, calcium absorption and urinary calcium excretion return to prepregnancy rates. However, lactating mothers have decreased urinary calcium output and higher

bone turnover than at the end of pregnancy. During this period, approximately 5 mmol/d (200 mg/d) of calcium is provided to the infant through breast milk, and this can exceed 10 mmol/d (400 mg/d) in some women. Thus, requirements for calcium are significantly increased during pregnancy and lactation.

Menopause—The acute loss of bone that accompanies menopause involves most of the skeleton but particularly affects the trabecular component. The associated biochemical changes include increases in the complexed fraction of plasma calcium (bicarbonate), increases in plasma alkaline phosphatase and urinary hydroxyproline (representing increased bone resorption followed by a compensatory increase in bone formation), increased obligatory calcium loss in the urine, and a small but significant decline in calcium absorption (Table 5–3). These changes are ameliorated by hormone treatment, calcium supplementation, thiazide administration (which reduces calcium excretion), and restriction of salt intake, which reduces obligatory calcium loss. In some (50%) cases of osteoporosis, calcium absorption is low, and high bone resorption can be suppressed by treatment with vitamin D, which in turn leads to improvement in calcium absorption. In males, bone loss begins at about the age of 50, but it is not associated with a rise in bone resorption markers. Instead, bone loss in men is linked to an age-related decline in gonadal function and is due to a decrease in bone formation, not so much an increase in bone resorption.

Table 5–3. Parameters Used Frequently for the Clinical Evaluation of Abnormalities in Parathyroid Hormone Function or Ca²⁺ Homeostasis

Parameter	Normal range	Abnormality
Ionized calcium	8.5–10.5 mg/dL	Elevated with ↑ PTH, ↑ vitamin D, ↑ bone resorption
Plasma phosphate	3–4.5 mg/dL	Decreased in hyperparathyroidism, vitamin D deficiency

		Increased in renal failure, hypoparathyroidism, vitamin D intoxication
Intact plasma PTH levels	10–65 pg/mL	Elevated in hyperparathyroidism; decreased in hypoparathyroidism
Alkaline phosphatase	30–120 U/L	High levels indicate increased osteoblastic activity (bone turnover)
Bone-specific alkaline phosphatase	17–48 U/L	High bone turnover, useful marker of active bone formation
Osteocalcin (intact)	<1–23 ng/ml	Marker for skeletal bone turnover

PTH, parathyroid hormone.

Estrogen deficiency is a major pathogenic factor in the bone loss associated with menopause and the subsequent development of postmenopausal osteoporosis. Estrogen replacement at or after menopause, whether natural or induced, prevents menopausal bone loss and usually results in an increase in bone mineral density (BMD) during the first 12–18 months of treatment. Estrogen regulates osteoclast activity through effects on osteoclast number, resorptive activity, and life span of the cell. The process of bone loss is progressive, starting at about the age of 50 in men and at menopause in women, and loss proceeds at an average rate of 1% per year to the end of life. Bone loss is faster in women than in men and affects some bones more than others; the consequences include decreased BMD and increased risk of fractures.

BONE DENSITY

Bone density determines the degree of osteoporosis and the fracture risk. The main determinants of peak bone density are genetics, calcium intake, and exercise. The most common test for measuring bone density is dual-energy X-ray absorptiometry (DXA) scanning. Additional approaches include computed tomography, radiologic techniques (morphometry or densitometry), or bone biopsy. DXA uses X-rays to measure bone density and provides 2 measures of how dense bone is: the *T* score and the *Z* score. The *T* score compares the person's bone density with the average bone density of 25-to 30-year-olds of the same sex. This age group is used because it is when bone density is at its peak. A *T* score of 0 means that bone density is the same as the average bone density of 25-to 30-year-olds. A score above 0 (a positive score) means that bones are denser than the average. A score below 0 (a negative score) means that bones are less dense than the average. The *Z* score compares a person's bone density with that of people of the same age, sex, and weight, and is less valuable in making predictions of risk of fracture or in making decisions about treatment.

PREVENTION OF OSTEOPOROSIS

Understanding of the hormonal and nutritional regulation of calcium balance has led to the implementation of several measures to reduce bone loss. The principal current approaches are the following.

Estrogen replacement therapy—Estrogen decreases bone loss in postmenopausal women by inhibiting bone resorption, resulting in a 5–10% increase in BMD over 1–3 years. Estrogen treatment is approved for prevention of osteoporosis. Calcium supplements enhance the effect of estrogen on BMD.

Bisphosphonates—Bisphosphonates have a strong affinity for bone apatite and are potent inhibitors of bone resorption. These agents reduce the recruitment and activity of osteoclasts and increase their apoptosis.

Calcitonin—Calcitonin, reduces bone resorption by direct inhibition of osteoclast activity. Intranasal calcitonin (200 IU/d) produces significant effects on BMD. Calcitonin is less effective in prevention of cortical bone loss than cancellous bone loss in postmenopausal women. Calcitonin is approved for treatment of osteoporosis in women who have been postmenopausal for 5 or more years.

Parathyroid hormone—Human recombinant PTH is approved for the treatment of osteoporosis in postmenopausal women and men who are at high risk for fracture. Intermittent PTH administration restores bone strength by stimulating new bone formation at the periosteal (outer) and endosteal (inner) bone surfaces, thickening the cortices and existing trabeculae of the skeleton, and perhaps increasing trabecular numbers and their connectivity.

Selective estrogen receptor modulators—Selective estrogen receptor modulators are compounds that exert estrogenic effects in specific tissues and antiestrogenic effects in others. Raloxifene is a benzothiophene that competitively inhibits the action of estrogen in the breast and the endometrium and acts as an estrogen agonist on bone and lipid metabolism. In early postmenopausal women, raloxifene prevents postmenopausal bone loss at all skeletal sites, reduces markers of bone turnover to premenopausal concentrations, and reduces the serum cholesterol concentration and its low-density

lipoprotein fraction without stimulating the endometrium. Thus, it exerts the beneficial effects of estrogen in the skeleton and cardiovascular system without its adverse effects in the breast and endometrium. Because raloxifene does not have agonistic effects on the endometrium, unwanted vaginal bleeding and increased risk of endometrial cancer are avoided.

Vitamin D analogs—Vitamin D analogs induce a small increase in BMD that seems to be limited to the spine. Vitamin D deficiency is due to restricted skin exposure to light at wavelengths between 290 and 310 nm, reduced capacity for endogenous synthesis, or an increased requirement for vitamin D. The recommended daily intake of vitamin D is 400–800 IU if sunlight exposure is low. Adequate protein intake is also essential in frail elderly individuals.

Exercise—Physical activity early in life contributes to high peak bone mass. Various activities, including walking, weight training, and high-impact exercises, induce a small (1–2%) increase in BMD at some, but not all, skeletal sites. These effects disappear if the exercise program is stopped. Load-bearing exercise is more effective for increasing bone mass than are other types of exercise. Some of the benefits of exercise may be due to increases in muscle mass and strength, plus a reduction in the risk of falls by about 25% in frail elderly individuals.

DISEASES OF PTH PRODUCTION

Primary Hyperparathyroidism

Excess PTH production is often due to parathyroid gland hyperplasia, adenoma, or carcinoma. The clinical manifestations include elevated intact PTH levels, increased plasma calcium levels (hypercalcemia), increased urinary calcium excretion (hypercalciuria) with increased formation of kidney stones (urolithiasis), and decreased plasma phosphate levels. The elevation of PTH results in increased bone resorption and further increases in extracellular calcium concentrations.

Secondary Hyperparathyroidism

Secondary hyperparathyroidism and hyperplasia of the parathyroid glands are complications that occur in patients with chronic renal failure. In early renal failure, a reduction in plasma vitamin D and moderate decreases in ionized Ca^{2+} contribute to greater synthesis and secretion of PTH. As renal disease progresses, parathyroid expression of vitamin D and Ca^{2+} receptors is reduced, making the parathyroid gland more resistant to both the vitamin D and Ca^{2+} negative feedback regulation of PTH release. Thus, for any increase in plasma Ca^{2+} , the inhibition of PTH secretion is less efficient. As a result, for any particular plasma Ca^{2+} concentration, secretion of PTH is enhanced, resulting in a shift in the Ca^{2+} -PTH set point toward secondary hyperparathyroidism. Hyperphosphatemia ($\text{Pi} > 5 \text{ mg/dL}$ in adults), independent of Ca^{2+} and calcitriol levels, further enhances uremia-induced parathyroid gland hyperplasia and PTH synthesis and secretion, the latter by post-transcriptional mechanisms.

Hypoparathyroidism

Hypoparathyroidism, resulting from impaired production of PTH, can be associated with other endocrine disorders and neoplasias or may result from surgical removal of the parathyroid glands. Because of the important role of PTH in the acute regulation of plasma Ca^{2+} levels, an early manifestation of surgical removal of the parathyroid glands is hypocalcemic tetany. The classic clinical sign is known as the Chvostek sign, which is twitching or contraction of the facial muscles in response to tapping the facial nerve at a point anterior to the ear and above the zygomatic bone.

Pseudohypoparathyroidism

Pseudohypoparathyroidism is not due to decreased PTH levels, but to an abnormal response to PTH because of a congenital defect in the G-protein associated with the PTHR1. Pseudohypoparathyroidism type Ia is characterized by a generalized hormone resistance to PTH, thyroid-stimulating hormone, luteinizing hormone, and follicle-stimulating hormone and is associated with abnormal physical features including short stature and skeletal anomalies. Pseudohypoparathyroidism type Ib is characterized by renal resistance to PTH and normal appearance. In these patients, the PTHR1 has decreased G_{α_s} activity, and this can be tested by measuring the increase in urinary cAMP in response to PTH administration. Patients present with low plasma Ca^{2+} and high phosphate levels and elevated PTH levels.

Clinical Evaluation of PTH Abnormalities

The differential diagnosis for individuals presenting with abnormal calcium levels or with urolithiasis is made using several indices of hormone levels (PTH and vitamin D), the metabolites that the hormones regulate (Ca and Pi), and the target tissue effects of excess hormone production (bone densitometry). As seen in the brief overview of some of these measures (Table 5–3 and Appendix), the laboratory values indicate the physiologic regulation of PTH, vitamin D, phosphate, and calcium levels. Plasma PTH levels are determined using radioimmunoassay or more specific and sensitive radioimmunometric assays. Because PTH loses its activity once it is cleaved, only the intact PTH measurement is a reliable indicator of hormone status. Vitamin D levels can be measured, although this test is expensive and time consuming; therefore, the concentrations of the precursor 25-hydroxyvitamin D₃ are also used. Bone density studies using DXA are used to determine the extent of bone loss.

Measurements are usually obtained in the lumbar spine or femur. When there is a possibility of increased bone turnover, as would occur with increased PTHrP production in some malignancies, bone scans are used to assess the rate of incorporation of radiolabeled phosphate. The greater the rate of bone formation, the greater the deposition of labeled phosphate in bone, reflecting an increase in bone turnover. Generalized increases are observed in hyperparathyroidism, whereas focal increases are observed in malignant diseases.

KEY CONCEPTS



PTH release is under negative feedback regulation by Ca^{2+} and vitamin D.



The main physiologic effects of PTH are mediated by the PTHR1 expressed in bone and kidney.



PTHR1 binds PTH and PTHrP, a peptide responsible for the pathophysiologic elevation of Ca^{2+} in some malignancies.



In the kidney, PTH increases renal Ca^{2+} reabsorption, increases the activity of 1α -hydroxylase (which mediates the final activation step in the synthesis of vitamin D), and decreases Pi reabsorption.



In bone, PTH increases osteoclast-mediated bone resorption indirectly through stimulation of osteoblast activity.



Calcitonin decreases bone resorption and lowers plasma Ca^{2+} levels.



Synthesis of active vitamin D involves hydroxylation in the liver (C-25) and kidney (C-1).



Vitamin D increases bone resorption, renal Ca^{2+} reabsorption, and intestinal Ca^{2+} absorption.

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OBJECTIVES

- ▶ *Identify the functional anatomy and zones of the adrenal glands and the principal hormones secreted from each zone.*
- ▶ *Describe and contrast the regulation of synthesis and release of the adrenal steroid hormones (glucocorticoids, mineralocorticoids, and androgens) and the consequences of abnormalities in their biosynthetic pathways.*
- ▶ *Understand the cellular mechanism of action of adrenal cortical hormones and identify their major physiologic actions, particularly during injury and stress.*
- ▶ *Identify the major mineralocorticoids, their biologic actions, and their target organs or tissues.*
- ▶ *Describe the regulation of mineralocorticoid secretion and relate this to the regulation of sodium and potassium excretion.*
- ▶ *Identify the causes and consequences of oversecretion and undersecretion of glucocorticoids, mineralocorticoids, and adrenal androgens.*
- ▶ *Identify the chemical nature of catecholamines and their biosynthesis and metabolic fate.*
- ▶ *Describe the biologic consequences of sympatho-adrenal medulla activation and identify the target organs or tissues for catecholamine effects along with the receptor types that mediate their actions.*
- ▶ *Describe and integrate the interactions of adrenal medullary and cortical hormones in response to stress.*
- ▶ *Identify diseases caused by oversecretion of adrenal catecholamines.*

ADRENAL GLAND: INTRODUCTION

The adrenal glands are important components of the endocrine system. They contribute significantly to maintaining homeostasis particularly through their role in the regulation of the body's adaptive response to stress, in the maintenance of body water, sodium and

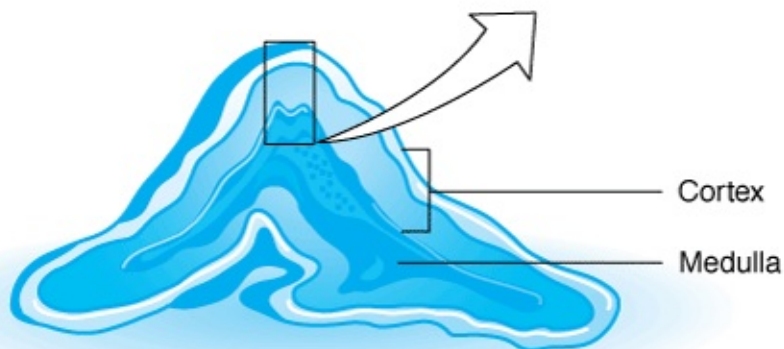
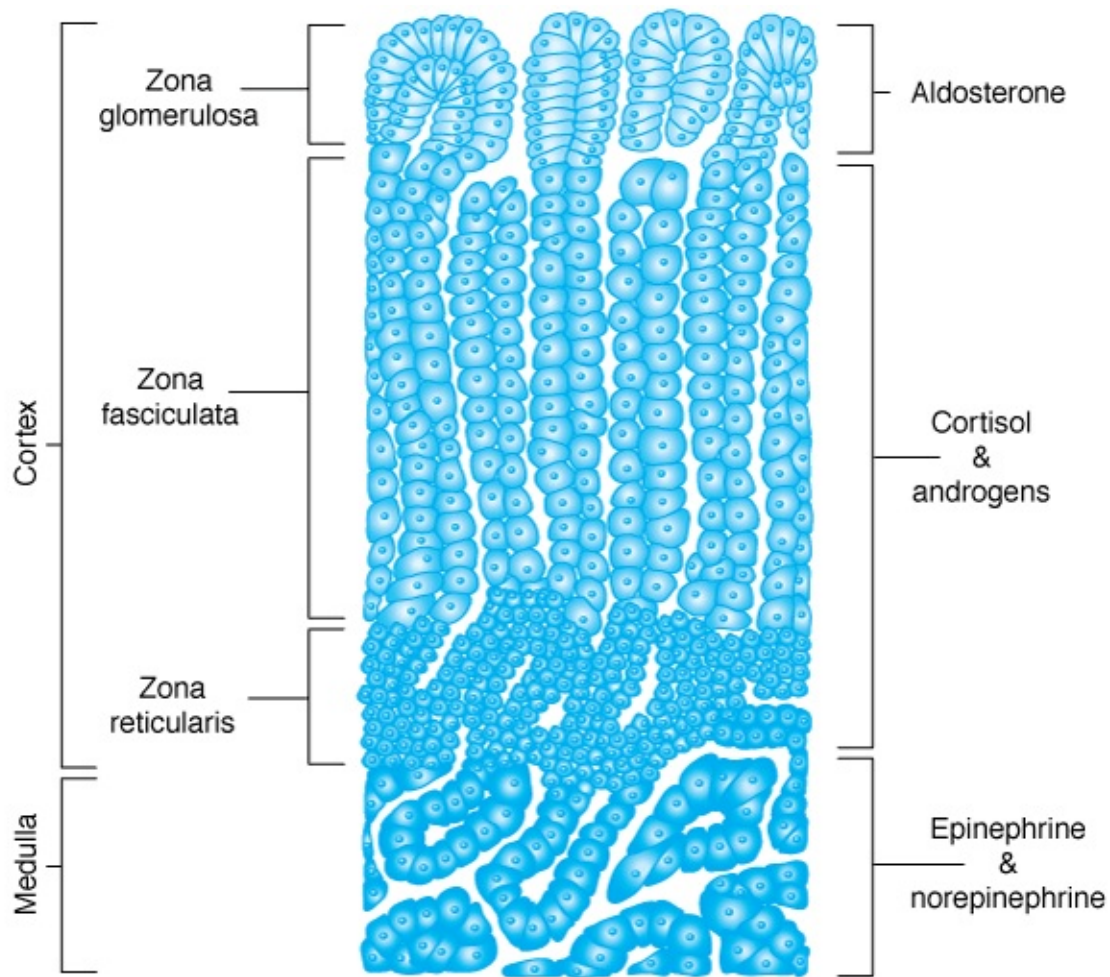
potassium balance, and in the control of blood pressure. The main hormones produced by the human adrenal glands belong to 2 different families based on their structure; these are the steroid hormones including the glucocorticoids, mineralocorticoids, and androgens, and the catecholamines, norepinephrine and epinephrine. The adrenal gland, like the pituitary, has 2 different embryologic origins, which as we will discuss, influence the mechanisms that control hormone production by each of the 2 components.

FUNCTIONAL ANATOMY & ZONATION

The adrenal glands are located above the kidneys. They are small, averaging 3–5 cm in length, and weigh 1.5–2.5 g and as mentioned above, consist of 2 different components: the cortex and the medulla (Figure 6–1), each with a specific embryologic origin. The outer adrenal cortex is derived from mesodermal tissue and accounts for about 90% of the weight of the adrenals. The cortex synthesizes the adrenal steroid hormones called glucocorticoids, mineralocorticoids, and androgens (eg, cortisol, aldosterone, and dehydroepiandrosterone [DHEA]) in response to hypothalamic-pituitary-adrenal hormone stimulation (Figure 6–2). The inner medulla is derived from a subpopulation of neural crest cells and makes up the remaining 10% of the mass of the adrenals. The medulla synthesizes catecholamines (eg, epinephrine and norepinephrine) in response to direct sympathetic (sympatho-adrenal) stimulation.

Figure 6–1.





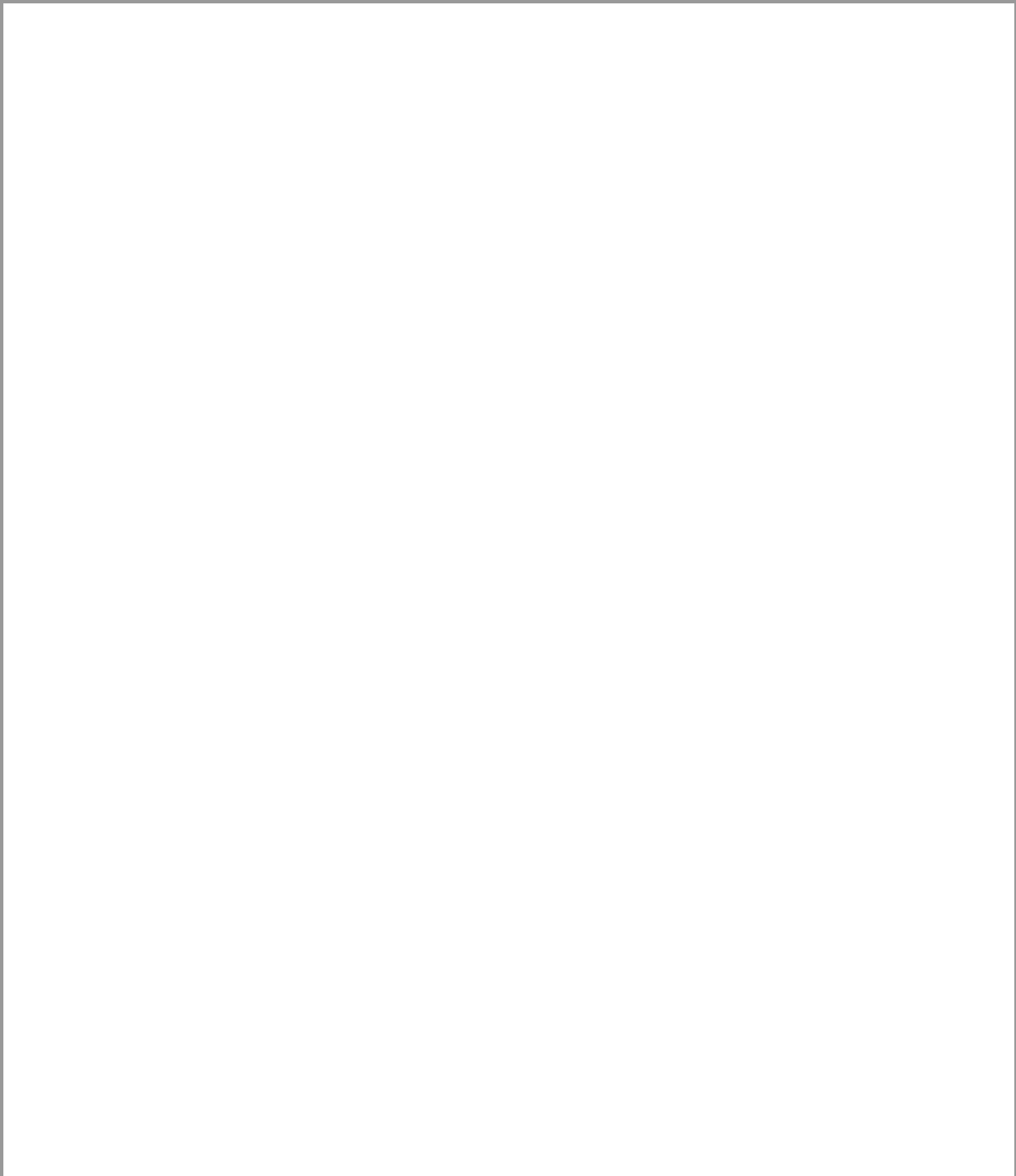
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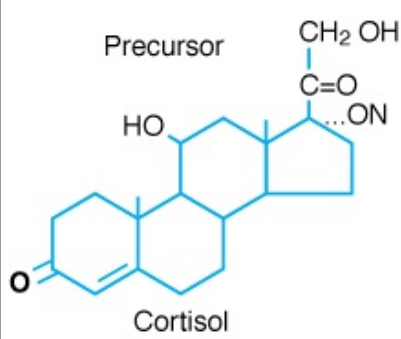
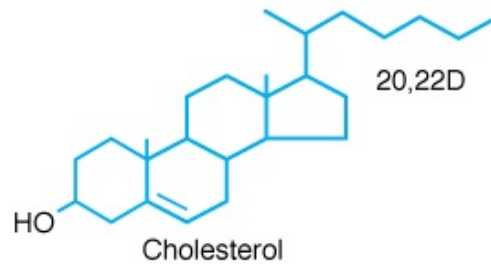
Adrenal glands. The adrenal glands are composed of a cortex and a medulla, each derived from a different embryologic origin. The cortex is divided into 3 zones: reticularis, fasciculata, and glomerulosa. The cells that make up the 3 zones have distinct enzymatic capacities,

leading to a relative specificity in the products of each of the adrenal cortex zones. The adrenal medulla is made of cells derived from the neural crest.

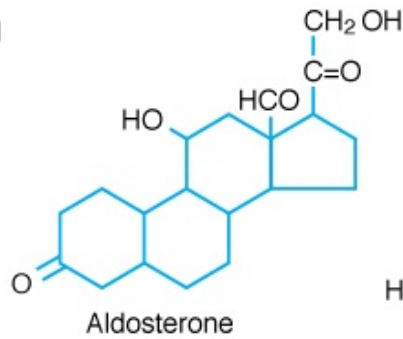
Figure 6–2.



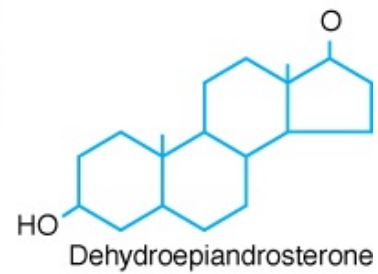
Adrenal cortex hormones



Glucocorticoid

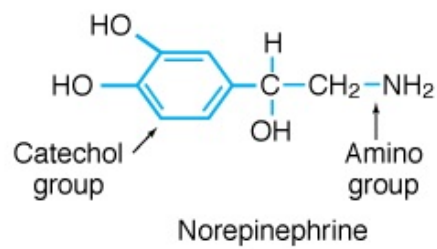
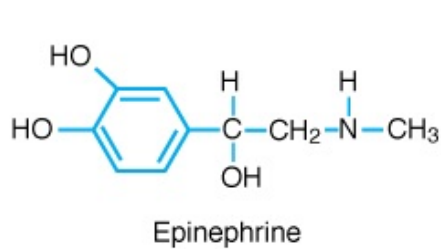
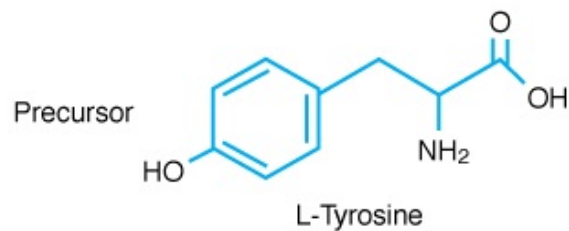


Mineralocorticoid



Androgen

Adrenal medulla hormones



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Adrenal gland hormones. The principal hormones synthesized and released by the adrenal cortex are the glucocorticoid cortisol, the mineralocorticoid aldosterone, and the androgen dehydroepiandrosterone. These steroid hormones are derived from cholesterol. The principal hormones synthesized and released by the adrenal medulla are the catecholamines epinephrine and norepinephrine. These catecholamines are derived from L-tyrosine.

Several features of the adrenal glands contribute to the regulation of steroid hormone and catecholamine synthesis, including the architecture, blood supply, and the enzymatic machinery of the individual cells. Blood supply to the adrenal glands is derived from the superior, middle, and inferior suprarenal arteries. Branches of these arteries form a capillary network arranged so that blood flows from the outer cortex toward the center area, following a radially oriented sinusoid system. This direction of blood flow controls the access of steroid hormones to the circulation and concentrates the steroid hormones at the core of the adrenals, thus modulating the activities of enzymes involved in catecholamine synthesis. The venous drainage of the adrenal glands involves a single renal vein on each side; the right vein drains into the inferior vena cava and the left vein drains into the left renal artery.

HORMONES OF THE ADRENAL CORTEX

The adrenal cortex consists of 3 zones that vary in both their morphologic and functional features and thus, the steroid hormones they produce (Figure 6–1).

- The **zona glomerulosa** contains abundant smooth endoplasmic reticulum and is the unique source of the mineralocorticoid aldosterone.
- The **zona fasciculata** contains abundant lipid droplets and produces the glucocorticoids, cortisol and corticosterone, and the androgens, DHEA and DHEA sulfate (DHEAS).
- The **zona reticularis** develops postnatally and is recognizable at about age 3; it also produces glucocorticoids and androgens.

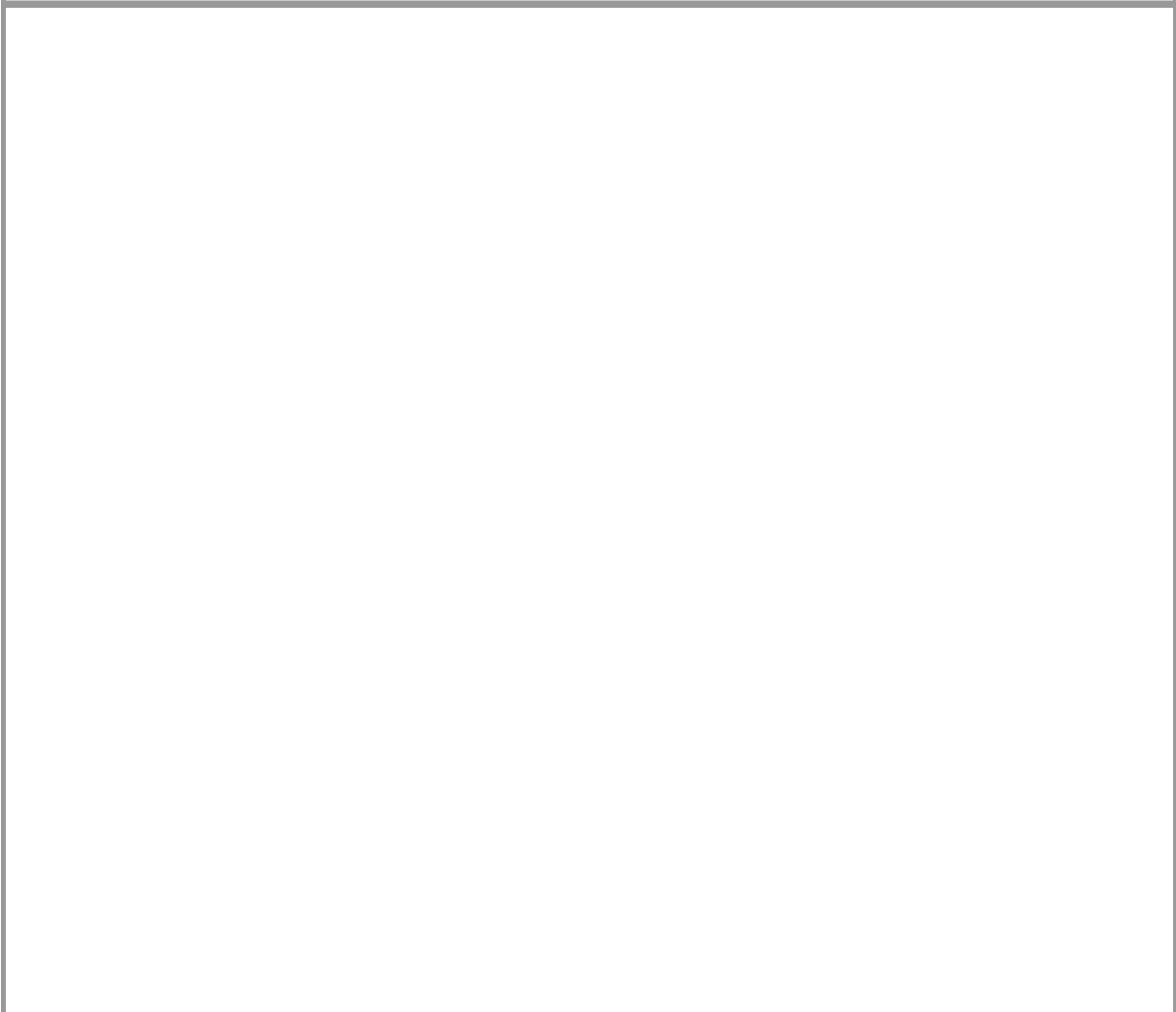
The products of the adrenal cortex are classified into 3 general categories: glucocorticoids, mineralocorticoids, and androgens (Figure 6–2), which reflect the primary effects mediated by these hormones. This will become clear when their individual target organ effects are discussed.

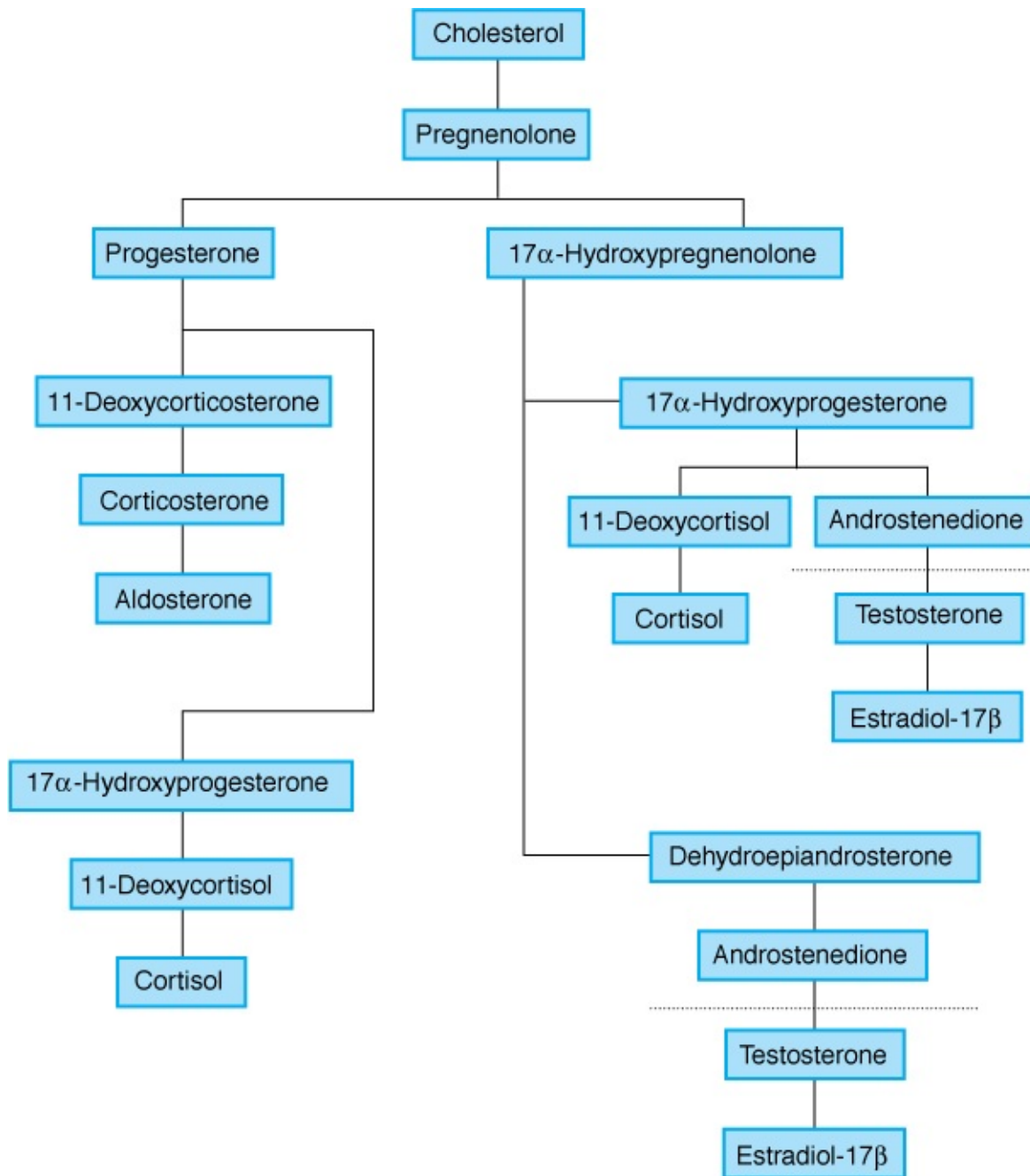
Chemistry & Biosynthesis

Steroid hormones share an initial step in their biosynthesis (steroidogenesis), which is the conversion of cholesterol to pregnenolone (Figure 6–3). Cholesterol used for steroid hormone synthesis can be derived from the plasma membrane or from the cytosolic pool known as the steroidogenic cytoplasmic pool. Cholesterol that enters steroid hormone synthesis is released by the action of the enzyme cholesterol esterase. The conversion of cholesterol to pregnenolone requires the action of cytochrome P450 side-chain cleavage enzyme (P450_{scc}), which is present in the inner mitochondrial membrane of all steroidogenic cells. In order for cholesterol to be a substrate for P450_{scc}, free cholesterol must be transferred from the outer mitochondrial membrane to the inner mitochondrial membrane. This transport step is mediated by STeroid Acute Regulatory (STAR) protein. This delivery of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane,

followed by the conversion to pregnenolone by P450scc enzyme, is considered the **rate-limiting step** in steroid hormone synthesis. This conversion of cholesterol to pregnenolone is the first step in a sequence of multiple enzymatic reactions involved in the synthesis of steroid hormones. Because the cells that constitute the different sections of the adrenal cortex have specific enzymatic features, the synthetic pathway of steroid hormones will result in preferential synthesis of glucocorticoids, mineralocorticoids, or androgens, depending on the region. Defects in the activity of specific enzymes involved in the pathway leading to production of these hormones can result in disease with various degrees of severity depending to the enzymatic step that is compromised as discussed below.

Figure 6–3.





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Adrenal steroid hormone synthetic pathway. Cholesterol is converted to pregnenolone by the cytochrome P450 side-chain cleavage enzyme. Pregnenolone is converted to progesterone by 3 β -hydroxysteroid dehydrogenase. Progesterone is converted to 11-deoxycorticosterone by 21-hydroxylase; 11-deoxycorticosterone is then converted to corticosterone by 11 β -hydroxylase. Pregnenolone

can also be converted to 17α -OH-pregnenolone by 17α -hydroxylase. Thereafter, 17α -OH-pregnenolone is converted to 17α -OH-progesterone by 3β -hydroxysteroid dehydrogenase, 17α -OH-progesterone is converted to 11-deoxycortisol by the enzyme 21 -hydroxylase, and 11-deoxycortisol is converted to cortisol by 11β -hydroxylase. In addition, 17α -OH-progesterone can be converted to androstenedione by $17,20$ -lyase. Both 17α -OH-pregnenolone and 17α -OH-progesterone can be converted to the androgens dehydroepiandrosterone (DHEA) and androstenedione, respectively, by $17,20$ -lyase. DHEA is converted to androstenedione by 3β -hydroxysteroid dehydrogenase. Cells in the zona glomerulosa do not have 17α -hydroxylase activity. Therefore, pregnenolone can be converted only into progesterone. Cells of the zona glomerulosa also lack 11β -hydroxylase activity; so deoxycorticosterone cannot be converted to corticosterone. However, the zona glomerulosa possesses aldosterone synthase activity, and this enzyme converts deoxycorticosterone to corticosterone, corticosterone to 18 -hydroxycorticosterone, and 18 -hydroxycorticosterone to aldosterone, the principal mineralocorticoid produced by the adrenal glands. The dotted line denotes which steps occur outside the adrenal glands.

GLUCOCORTICOID HORMONE SYNTHESIS

Cells of the adrenal zona fasciculata and zona reticularis synthesize and secrete the glucocorticoids cortisol or corticosterone through the following pathway (Figure 6–3). Pregnenolone exits the mitochondria and is converted to either progesterone or 17α -OH-pregnenolone. Conversion of pregnenolone to progesterone is mediated by **3β -hydroxysteroid dehydrogenase**. Progesterone is converted to 11-deoxycorticosterone by **21-hydroxylase**; then 11-deoxycorticosterone is converted to corticosterone by **11β -hydroxylase**. Conversion of pregnenolone to 17α -OH-pregnenolone is mediated by **17α -hydroxylase**; 17α -OH-pregnenolone is converted to 17α -OH-progesterone by 3β -hydroxysteroid dehydrogenase; 17α -OH-progesterone is converted to either 11-deoxycortisol or androstenedione. The enzyme 21-hydroxylase mediates the conversion of 17α -OH-progesterone to 11-deoxycortisol, which is then converted to cortisol by 11β -hydroxylase. The conversion of 17α -OH-progesterone to androstenedione is mediated by **17,20-lyase**. Both 17α -OH-pregnenolone and 17α -OH-progesterone can be converted to the androgens DHEA and androstenedione, respectively, by 17,20-lyase. DHEA is converted to androstenedione by 3β -hydroxysteroid dehydrogenase.

MINERALOCORTICOID HORMONE SYNTHESIS

The adrenal zona glomerulosa cells preferentially synthesize and secrete the mineralocorticoid aldosterone. The cells of the zona glomerulosa do not have 17α -hydroxylase activity. Therefore, pregnenolone can be converted only to progesterone. Cells of the zona glomerulosa also lack 11β -hydroxylase activity; so 11-deoxycorticosterone cannot be converted to corticosterone. However, the zona glomerulosa possesses **aldosterone synthase** activity, and this enzyme converts 11-deoxycorticosterone to corticosterone, corticosterone to 18-hydroxycorticosterone, and 18-hydroxycorticosterone to aldosterone, the principal mineralocorticoid produced by the adrenal glands.

ADRENAL ANDROGEN HORMONE SYNTHESIS

The initial steps in the biosynthesis of DHEA from cholesterol are similar to those involved in glucocorticoid and mineralocorticoid hormone synthesis. The product of these initial enzymatic conversions, pregnenolone, undergoes 17α -hydroxylation by microsomal P450c17 and conversion to DHEA by the 17,20-lyase activity of P450c17. The production rate is 6–8 mg/d, and circulating concentrations are approximately 1–10 ng/mL (3–35 nmol/L). As mentioned earlier, 17α -pregnenolone can also be converted to 17α -OH-progesterone, which in turn can be converted to androstenedione in the zona fasciculata. The production rate is 1.4–6.2 mg/d, and circulating concentrations are in the range of 0.5–2 ng/mL (2–8 nmol/L). DHEAS is a unique secretory product of the adrenal zona reticularis and is produced at a rate of 3.5–20 mg/d during reproductive life. Circulating concentrations are in the range of 1–4 μ g/mL (3–12 μ mol/L).

ABNORMALITIES IN STEROID HORMONE BIOSYNTHESIS

Any deficiency in the pathway of enzymatic events leading to the synthesis of glucocorticoids, mineralocorticoids, and androgens causes serious pathology. The key enzymes involved in steroid hormone synthesis and the consequences of their deficiency are described in Table 6–1. The severity of the manifestations ranges from death in utero as in the case of congenital deficiency of cholesterol side-chain cleavage enzyme (P450_{scc} also known as 20,22-desmolase), to abnormalities that become evident in adult life and that are not life threatening. An enzymatic defect of 21-hydroxylase accounts for 95% of the genetic abnormalities in adrenal steroid hormone synthesis (Figure 6–4). As mentioned earlier, this enzyme converts progesterone to 11-deoxycorticosterone and 17 α -hydroxyprogesterone to 11-deoxycortisol. The second most frequent abnormality in glucocorticoid synthesis is deficiency of the enzyme 11 β -hydroxylase, which converts 11-deoxycortisol to cortisol.

Table 6–1. Key Enzymes Involved in Steroid Hormone Synthesis and Metabolism

Enzyme and relevance	Physiologic function	Consequence of deficiency
21-Hydroxylase		
Accounts for 95% of genetic abnormalities in adrenal steroid hormone synthesis	Converts progesterone to 11-deoxycorticosterone and 17 α -hydroxyprogesterone to 11-deoxycortisol	

Decreased cortisol and aldosterone

Loss of sodium because of mineralocorticoid deficiency

Virilization because of excess androgen production

11 β -Hydroxylase

Second most frequent abnormality in adrenal steroid hormone synthesis

Converts 11-deoxycorticosterone to corticosterone; 11-deoxycortisol to cortisol

Excess 11-deoxycortisol and 11-deoxycorticosterone

Excess mineralocorticoid activity

Salt and
water
retention

11 β -Hydroxysteroid dehydrogenase type II

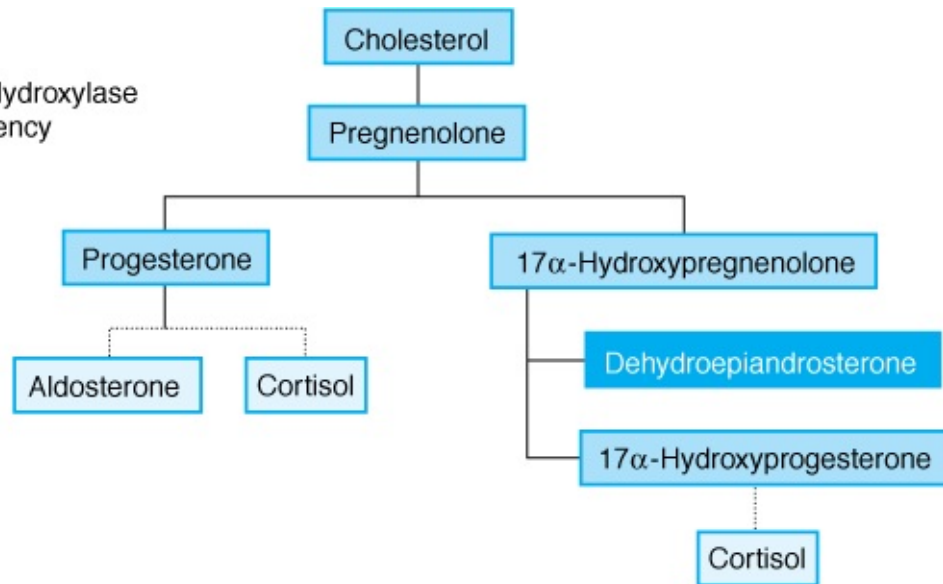
Inhibited by
glycyrrhetic acid, a
compound of
licorice

Converts cortisol into
corticosterone, which
has less affinity for the
mineralocorticoid
receptor

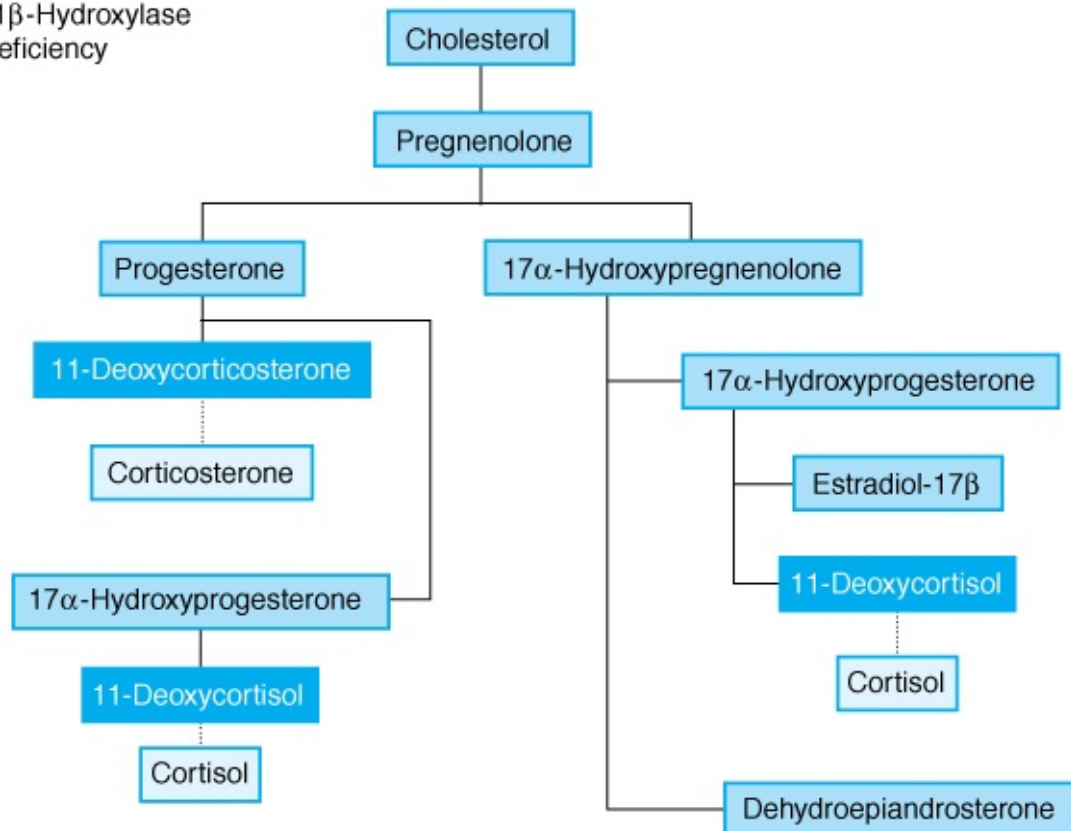
Decrease in glucocorticoid
inactivation in
mineralocorticoid-sensitive
cells leading to excess
mineralocorticoid activity

Figure 6–4.

A 21 β -Hydroxylase deficiency



B 11 β -Hydroxylase deficiency



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Alterations in steroid hormone synthesis in 21-hydroxylase and 11 β -hydroxylase enzymatic deficiency. **A.** Deficiency of 21-hydroxylase accounts for 95% of genetic abnormalities in adrenal steroid hormone

synthesis. This enzyme converts progesterone to deoxycorticosterone and 17-hydroxyprogesterone to 11-deoxycortisol. Thus, more pregnenolone is shunted to the DHEA-androstenedione pathway (more androgen synthesis), resulting in virilization (presence of masculine traits). In addition, aldosterone deficiency leads to sodium wasting. **B.** The second most frequent abnormality in glucocorticoid synthesis is 11 β -hydroxylase deficiency. This is the enzyme that converts deoxycortisol to cortisol and 11-deoxycorticosterone to corticosterone. Deficiency of 11 β -hydroxylase results in excess 11-deoxycortisol and 11-deoxycorticosterone production. Both metabolites have active mineralocorticoid activity. The resulting excess in mineralocorticoid-like activity leads to salt and water retention and may cause hypertension. Metabolites in dark blue boxes are produced in excess. Dotted lines indicate pathways affected by enzymatic abnormalities.

Deficiencies in these enzymes result in impaired cortisol synthesis, lack of negative feedback inhibition of the release of adrenocorticotrophic hormone (ACTH), high ACTH levels, and greater stimulation of cholesterol conversion to pregnenolone. Because of the lack of negative feedback inhibition of ACTH release (due to insufficient cortisol production) and the resulting high ACTH levels and greater stimulation of steroidogenesis (resulting from the higher ACTH-mediated stimulation of the initial steps in steroid hormone synthesis), the intermediate metabolites (before the enzymatic step that is deficient) continue to be synthesized, and their buildup leads to a shunting to the alternate enzymatic pathways.

Thus, more pregnenolone is shunted to the DHEA-androstenedione pathway and more intermediate metabolites are converted to androgens, resulting in virilization (presence of masculine traits). One consequence of 21-hydroxylase deficiency is the loss of sodium as a result of mineralocorticoid deficiency. In contrast, patients with 11 β -hydroxylase deficiency produce excess 11-deoxycortisol and 11-deoxycorticosterone, which have active mineralocorticoid activity. Because of the resulting excess in mineralocorticoid-like activity, patients with this deficiency retain salt and water and may present with hypertension.

Steroid Hormone Synthesis

As already mentioned, the initial steps in the biosynthetic pathways of steroid hormones are identical regardless of the steroid hormone synthesized. The production of the hormones can be regulated acutely and chronically. Acute regulation results in the rapid production of steroids in response to immediate need and occurs within minutes of the stimulus. The biosynthesis of glucocorticoids to combat stressful situations and the rapid synthesis of aldosterone to rapidly regulate blood pressure are examples of this type of regulation. Chronic stimulation, such as that which occurs during prolonged starvation and chronic disease, requires the synthesis of enzymes involved in steroidogenesis to enhance the synthetic capacity of the cells. Although both glucocorticoids and mineralocorticoids are released in response to stressful conditions, the conditions under which they are stimulated differ and the cellular mechanisms responsible for stimulating their release are different. Thus, the mechanisms involved in the regulation of their release differ and are specifically controlled as described below.

GLUCOCORTICOID SYNTHESIS AND RELEASE



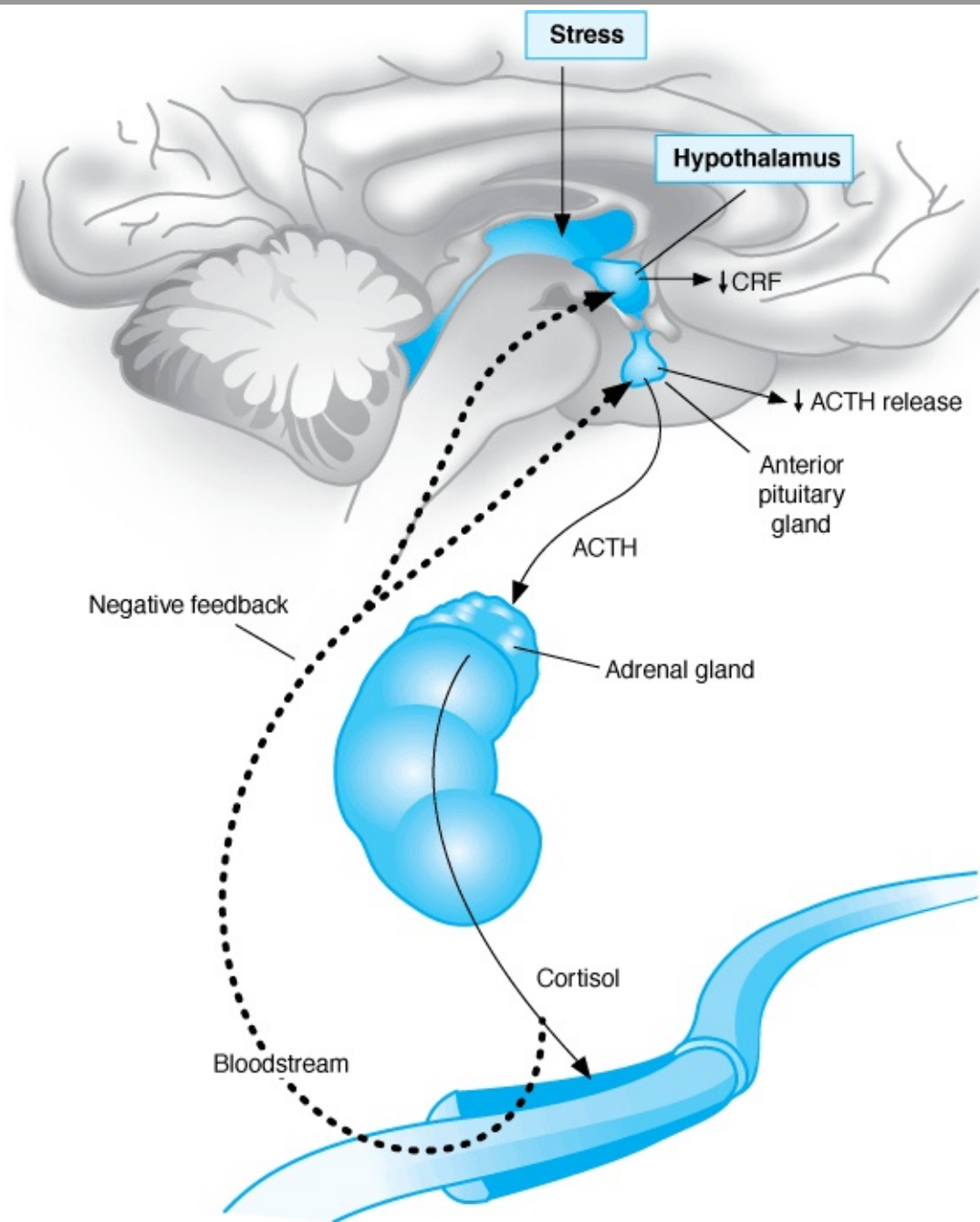
The release of cortisol is pulsatile and is under direct stimulation by ACTH released from the anterior pituitary. ACTH, or corticotropin, is synthesized in the anterior pituitary as a large precursor, proopiomelanocortin (POMC). POMC is processed post-translationally into several peptides, including corticotropin, β -lipotropin, and β -endorphin, as presented and discussed in Chapter 3 (Figure 3–4). The release of ACTH is pulsatile with about 7–15 episodes per day. The stimulation of cortisol release occurs within 15 minutes of the surge in ACTH. An important feature in the release of cortisol is that in addition to being pulsatile, it follows a circadian rhythm that is exquisitely sensitive to light, sleep, stress, and disease (Figure 1–8). Release of cortisol is greatest during the early waking hours, with levels declining as the afternoon progresses. This variability in the resulting circulating levels of the hormone has a direct impact on how values are interpreted based on the timing of blood sample collection for cortisol determinations.

ACTH stimulates cortisol release by binding to a G_s protein–coupled plasma membrane melanocortin 2 receptor on adrenocortical cells, resulting in activation of adenylate cyclase, an increase in cyclic adenosine monophosphate, and activation of protein kinase A (Figure 3–3). Protein kinase A phosphorylates the enzyme cholesterol ester hydrolase, increasing the enzymatic activity of this enzyme responsible for the release of cholesterol from cholesterol esters, providing the free cholesterol needed for steroid hormone synthesis. In addition, ACTH activates and increases the synthesis of STAR, the enzyme involved in the transport of cholesterol into the inner mitochondrial membrane, where cytochrome P450_{scc} converts cholesterol to pregnenolone. Therefore, ACTH stimulates the 2 initial and rate-limiting steps in steroid hormone synthesis.

The release of ACTH from the anterior pituitary is regulated by corticotropin-releasing hormone (CRH), produced by the hypothalamus and released in the median eminence. Cortisol inhibits the biosynthesis and secretion of CRH and ACTH in a classic example of negative

feedback regulation by hormones. This closely regulated circuit is referred to as the hypothalamic-pituitary-adrenal (HPA) axis (Figure 6–5).

Figure 6–5.



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Hypothalamic-pituitary-adrenal axis. Corticotropin-releasing factor (CRF), produced by the hypothalamus and released in the median eminence, stimulates the synthesis and processing of proopiomelanocortin, with resulting release of proopiomelanocortin peptides that include adrenocorticotrophic hormone (ACTH) from the anterior pituitary. ACTH binds to the melanocortin-2 receptor in the adrenal gland and stimulates the cholesterol-derived synthesis of adrenal steroid hormones. Glucocorticoids released into the systemic circulation exert negative feedback inhibition of CRF and ACTH release from the hypothalamus and pituitary, respectively, in a classic example of negative feedback hormone regulation. This closely regulated circuit is referred to as the HPA axis.

METABOLISM OF GLUCOCORTICOIDS

Because of their lipophilic nature, free cortisol molecules are mostly insoluble in water. Therefore, cortisol is usually found in biologic fluids either in a conjugated form (eg, as sulfate or glucuronide derivatives) or bound to proteins (noncovalent, reversible binding). In the plasma, unconjugated steroids are usually bound to carrier proteins.

The majority of cortisol is bound to glucocorticoid-binding α_2 -globulin (transcortin or cortisol-binding globulin), a specific carrier of cortisol. Normal levels of transcortin average 3–4 mg/dL and are saturated with cortisol levels of 28 μ g/dL. The hepatic synthesis of transcortin is stimulated by estrogen and decreased by hepatic disease (cirrhosis). About 20–50% of bound cortisol is bound nonspecifically to plasma albumin. Approximately 1–10% of total plasma cortisol circulates unbound and is referred to as the free fraction. This is considered to represent the biologically active fraction of the hormone that is directly available for action.

The major role of plasma-binding proteins is to act as a "buffer" or reservoir for active hormones. Because of the noncovalent nature of the binding, protein-bound steroids are released into the plasma in free form as soon as the free hormone concentration decreases. Plasma-binding proteins also protect the hormone from peripheral metabolism (notably by liver enzymes) and increase the half-life of biologically active forms. The half-life of cortisol is 70–90 minutes.

Because of their lipophilic nature, steroid hormones diffuse easily through cell membranes and therefore have a large volume of distribution. In their target tissues, steroid hormones are concentrated by an uptake mechanism that relies on their binding to intracellular receptors. High concentrations of steroid hormones are found in adipose tissue. Adipose tissue contains enzymatic activity in the form of aromatase (5 α -reductase), which converts circulating androgens to estrogens participating in the metabolism of adrenal androgens (discussed in Chapter 9).

The liver and kidney are the 2 major sites of hormone inactivation and elimination, or catabolism. Inactive hormones are mainly eliminated as urinary (mostly conjugated) metabolites. Usually, steroids are

eliminated once they have been inactivated. Urinary excretion of metabolic products requires conversion from lipophilic to hydrophilic compounds to ensure their solubility in biologic fluids at rather high concentrations. Several pathways are involved in this process, including reduction, oxidation, hydroxylation, and conjugation, to form the sulfate and glucuronide derivatives of the steroid hormones. These processes occur in the liver through phase I and phase II biotransformation reactions, and their function is to produce a water-soluble compound that can be easily excreted. Inactivation of cortisol to cortisone and to tetrahydrocortisol and tetrahydrocortisone is followed by conjugation and renal excretion. These metabolites are referred to as 17-hydroxycorticosteroids, and their determination in 24-hour urine collections is used to assess the status of adrenal steroid production.

Localized tissue metabolism contributes to modulation of the physiological effects of glucocorticoids by the isoforms of the enzyme **11 β -hydroxysteroid dehydrogenase**. Corticosteroid **11 β -hydroxysteroid dehydrogenase type I** is a low-affinity NADPH-dependent reductase that converts cortisone back to its active form cortisol. This enzyme is expressed in liver, adipose tissue, lung, skeletal muscle, vascular smooth muscle, gonads, and the central nervous system. The high expression of this enzyme, particularly in adipose tissue has gained recent attention, as it is thought to contribute to the development of metabolic syndrome (Chapter 10).

The conversion of cortisol to cortisone, its less active metabolite, is mediated by the enzyme **11 β -hydroxysteroid dehydrogenase type II**. This high-affinity NAD-dependent dehydrogenase is expressed primarily in the kidney, where it converts cortisol to cortisone. As discussed below, this conversion is critical in preventing excess mineralocorticoid activity resulting from cortisol binding to the mineralocorticoid receptor. Increased expression and activity of **11 β -hydroxysteroid dehydrogenase type I** amplifies glucocorticoid action within the cell, whereas increased **11 β -hydroxysteroid dehydrogenase type II** activity decreases glucocorticoid action.

MINERALOCORTICOID SYNTHESIS AND RELEASE

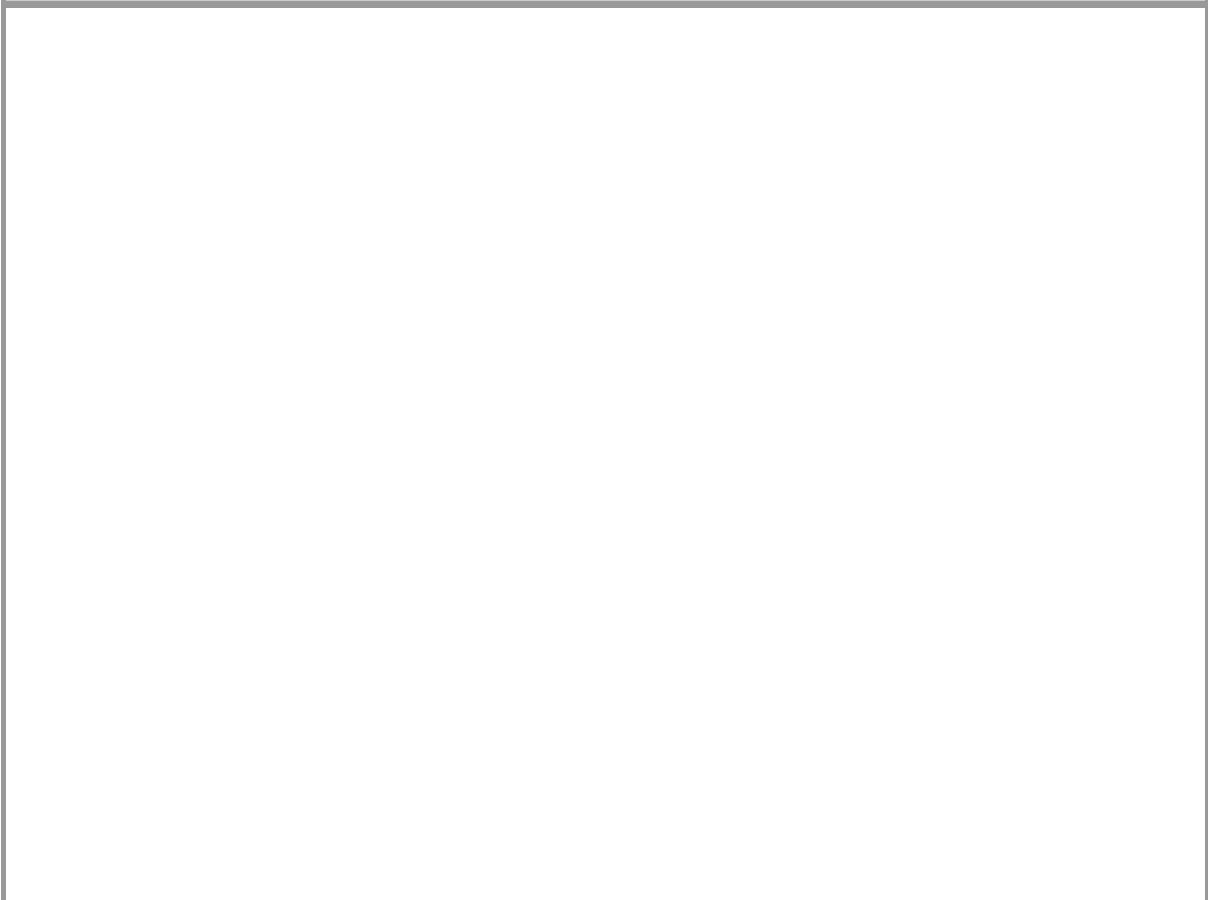


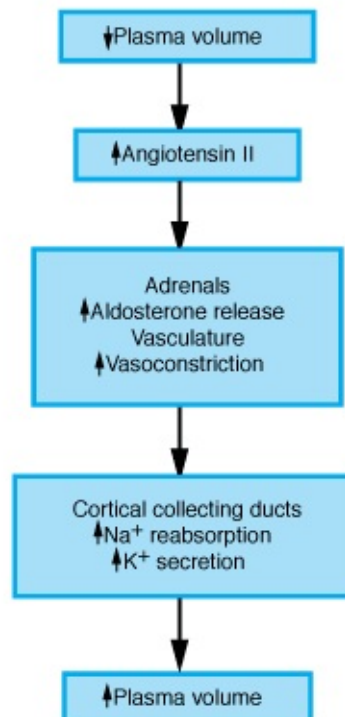
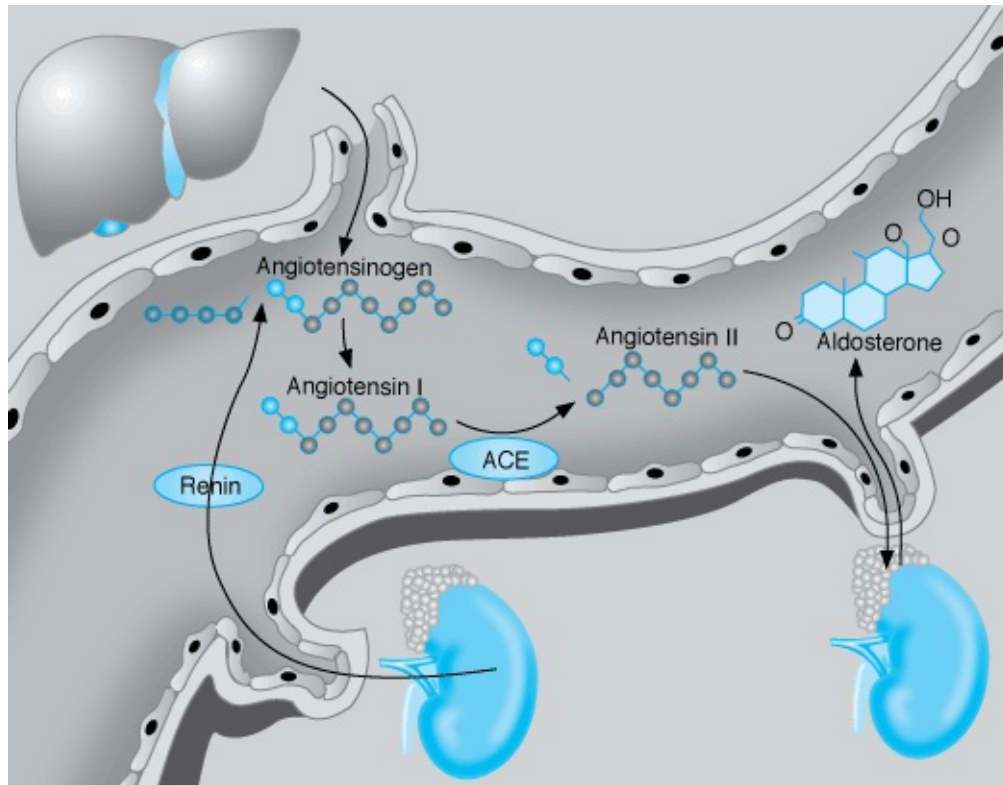
In contrast to glucocorticoids, which are under exclusive neuroendocrine regulation by the hypothalamic-pituitary axis, aldosterone synthesis and release in the adrenal zona glomerulosa are predominantly regulated by angiotensin II and extracellular K^+ and, to a lesser extent, by ACTH. Aldosterone is part of the **renin-angiotensin-aldosterone** system, which is responsible for preserving circulatory homeostasis in response to a loss of salt and water (eg, with intense and prolonged sweating, vomiting, or diarrhea). The components of the renin-angiotensin-aldosterone system respond quickly to reductions in intravascular volume and renal perfusion. Angiotensin II is the principal stimulator of aldosterone production when intravascular volume is reduced.

Although both angiotensin II and K^+ stimulate aldosterone release by increasing intracellular Ca^{2+} concentrations, they achieve this result through different mechanisms. Angiotensin II binds to G protein–coupled cell surface receptors. Two types of receptors, AT_1 and AT_2 , have been identified that interact with various heterotrimeric G proteins, including $G_{q/11}$, G_i , $G_{\alpha_{12}}$, and $G_{\alpha_{13}}$. AT_1 receptors have been shown to mediate most of the physiologic actions of angiotensin II, particularly vascular functions. However, AT_2 receptors are expressed in several tissues, including the adrenal gland, and may contribute to angiotensin-induced aldosterone release. The different G-protein isoforms couple to distinct signaling cascades, resulting in activation of phospholipase C, which results in the release of diacylglycerol and inositol 1,4,5-trisphosphate from phosphatidylinositol-4,5-bisphosphate. Diacylglycerol stimulates protein kinase C, whereas inositol 1,4,5-trisphosphate increases intracellular calcium levels, by stimulating the release of Ca^{2+} from internal stores. Angiotensin II also mediates an influx of external Ca^{2+} through dihydropyridine-insensitive Ca^{2+} channels in the plasma membrane. K^+ , on the other hand, mediates an influx of extracellular Ca^{2+} via voltage-gated L- and T-type Ca^{2+} channels.

The main physiologic stimulus for aldosterone release is a decrease in the effective intravascular blood volume (Figure 6–6). A decline in blood volume leads to decreased renal perfusion pressure, which is sensed by the juxtaglomerular apparatus (baroreceptor) and triggers the release of renin. Renin release is also regulated by NaCl concentration in the macula densa, plasma electrolyte concentrations, angiotensin II levels, and sympathetic tone. Renin catalyzes the conversion of angiotensinogen, a liver-derived protein, to angiotensin I. Circulating angiotensin I is converted to angiotensin II by angiotensin-converting enzyme (ACE), highly expressed in vascular endothelial cells. The increase in circulating angiotensin II produces direct arteriolar vasoconstriction, but it also binds to the angiotensin II receptor in the adrenocortical cells of the zona glomerulosa, stimulating phospholipase C. This results in an increase in intracellular Ca^{2+} , leading to stimulation of aldosterone synthesis and release.

Figure 6–6.





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Regulation of aldosterone release by the renin-angiotensin-

aldosterone system. A decrease in the effective circulating blood volume triggers the release of renin from the juxtaglomerular apparatus in the kidney. Renin cleaves angiotensinogen, the hepatic precursor of angiotensin peptides, to form angiotensin I. Angiotensin I is converted to angiotensin II by angiotensin-converting enzyme (ACE), which is bound to the membrane of endothelial cells, predominantly in the pulmonary circulation. Angiotensin II is a potent vasoconstrictor and stimulates the production of aldosterone in the zona glomerulosa of the adrenal cortex. Aldosterone production is also stimulated by potassium, ACTH, norepinephrine, and endothelins. (Modified, with permission, from Weber KT. Mechanisms of disease: Aldosterone in congestive heart failure. *NEJM*. 2001;345:1689. Copyright © Massachusetts Medical Society. All rights reserved.)

Potassium is also a major physiologic stimulus for aldosterone production, illustrating a classic example of hormone regulation by the ion it controls. Aldosterone is critical in maintaining potassium homeostasis by increasing potassium excretion in urine, feces, sweat, and saliva, preventing hyperkalemia during periods of high potassium intake or after potassium release from skeletal muscle during strenuous exercise. In turn, elevations in circulating potassium concentrations stimulate the release of aldosterone from the adrenal cortex.

METABOLISM OF MINERALOCORTICIDS

The total amount of aldosterone released is markedly less than that of glucocorticoids. Plasma aldosterone levels average 0.006–0.010 $\mu\text{g/dL}$ (in contrast to cortisol levels of 13.5 $\mu\text{g/dL}$). Secretion can be increased 2- to 6-fold by sodium depletion or by a decrease in the effective blood volume, such as occurs with ascites. Binding of aldosterone to plasma proteins is minimal, resulting in a short plasma half-life of approximately 15–20 minutes. This fact is relevant to mineralocorticoid and glucocorticoid receptor-mediated effects and their specificity as will be discussed below.

Aldosterone is metabolized in the liver to tetrahydroglucuronide derivative and excreted in the urine. A fraction of aldosterone is metabolized to aldosterone 18-glucuronide, which can be hydrolyzed back to free aldosterone under low pH conditions; thus it is an "acid-labile conjugate." Approximately 5% of aldosterone is excreted in the acid-labile form; a small fraction of aldosterone appears intact in the urine (1%) and up to 40% is excreted as tetraglucuronide.

ADRENAL ANDROGEN SYNTHESIS AND RELEASE

The third class of steroid hormones produced by the zona reticularis of the adrenal glands is the adrenal androgens, including DHEA and DHEAS (Figure 6–3). DHEA is the most abundant circulating hormone in the body and is readily conjugated to its sulfate ester DHEAS. Its production is controlled by ACTH. Although much of the physiology of DHEA and DHEAS is still not completely understood, in females these adrenal androgens may contribute to libido. Their importance is evident in congenital adrenal hyperplasia, in which pregnenolone is shunted to the androgen biosynthetic pathway.

METABOLISM OF ADRENAL ANDROGENS

The adrenal androgens are converted into androstenedione and then into potent androgens or estrogens in the peripheral tissues. The synthesis of dihydrotestosterone and 17α -estradiol, the most potent androgen and estrogen from DHEA, respectively, involves several enzymes, including 3β -hydroxysteroid dehydrogenase/D5-D4 isomerase, 17α -hydroxysteroid dehydrogenase, and 5α -reductase or aromatase (Chapters 8 and 9). The importance of the adrenal-derived androgens to the overall production of sex steroid hormones is highlighted by the fact that approximately 50% of total androgens in the prostate of adult men are derived from adrenal steroid precursors.

The control and regulation of the release of adrenal sex steroids are not completely understood. However, it is known that adrenal secretion of DHEA and DHEAS increases in children at the age of 6–8 years, and values of circulating DHEAS peak between the ages of 20 and 30 years. Thereafter, serum levels of DHEA and DHEAS decrease markedly. In fact, at 70 years of age, serum DHEAS levels are at approximately 20% of their peak values and continue to decrease with age. This 70–95% reduction in the formation of DHEAS by the adrenal glands during the aging process results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissues. Despite the marked decrease in the release of DHEA as the individual ages, this is not paralleled by a similar decrease in ACTH or cortisol release. The clinical impact of this age-related deficiency in DHEA production is not fully understood but may play an important role in the regulation of immune function and intermediary metabolism, among other aspects of physiology of the aging process.

Steroid Hormone Target Organ Cellular Effects



The physiologic effects of steroid hormones can be divided into genomic and nongenomic effects. Genomic effects (lasting from hours to days) usually involve interaction of the hormone with a specific intracellular steroid hormone receptor. These receptors are DNA-binding proteins of the steroid and thyroid hormone receptor superfamily. Hormone binding promotes conformational changes in the

receptor, which can then act as a ligand-dependent transcription factor. The steroid-receptor complex binds to hormone-responsive elements on the chromatin and thereby regulates gene transcription, resulting in the synthesis or repression of proteins, which are ultimately responsible for the physiologic effects of the hormones. Steroid receptor genes are expressed only in target tissues.

The physiologic effects of steroid hormones can also occur through nongenomic action. A nongenomic action is any mechanism that does not directly involve gene transcription, such as the rapid steroid effects on the electrical activity of nerve cells or the interaction of steroid hormones with the receptor for γ -aminobutyric acid. In contrast to the genomic effects, nongenomic effects require the continued presence of the hormone and occur more quickly because they do not require the synthesis of proteins. Some of the nongenomic effects may be mediated by specific receptors located on the cell membrane. The nature of these receptors and the signal transduction mechanisms involved are not completely understood and are still under investigation.

Steroid Hormone Receptors



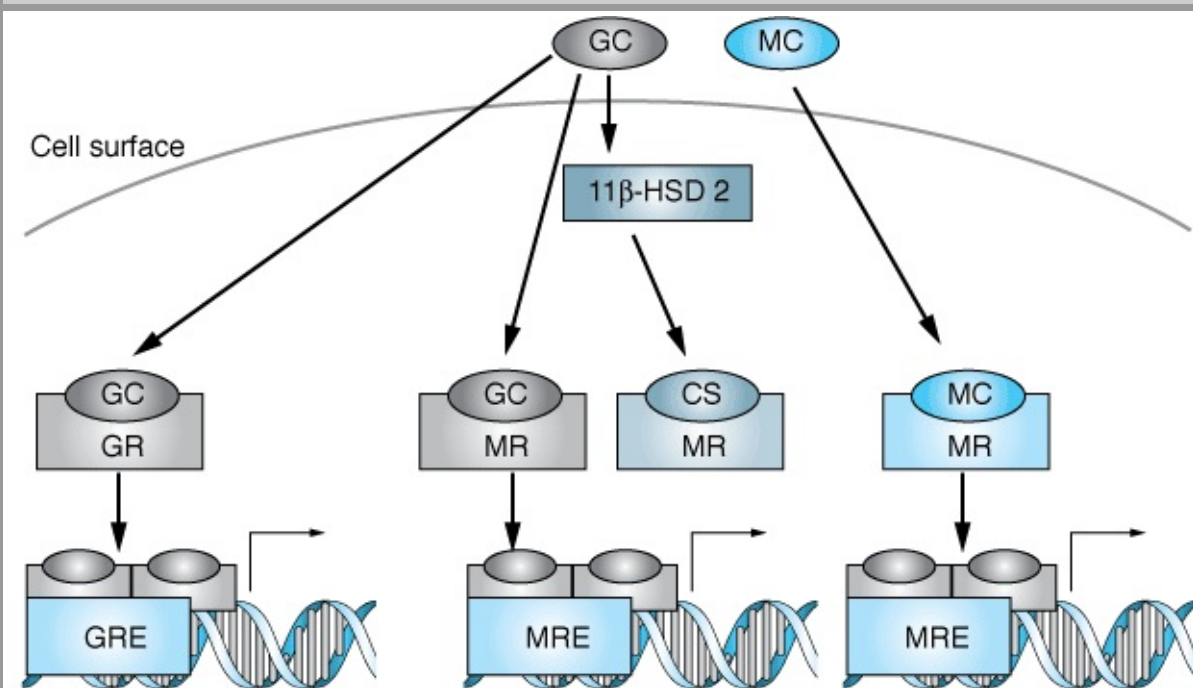
Most of the physiological effects of glucocorticoid and mineralocorticoid hormones are mediated through binding to intracellular receptors that belong to a superfamily of steroid, thyroid, retinoid, and orphan receptors and that operate as ligand-activated transcription factors to regulate gene expression. Mineralocorticoid and glucocorticoid receptors are closely related; they share 57% homology in the ligand-binding domain and 94% homology in the DNA-binding domain, and are classified in 2 types of receptors; Type I and Type II. Type I receptors are specific for mineralocorticoids but have a high affinity for glucocorticoids. Type II receptors are specific for glucocorticoids and are expressed in virtually all cells.



As already mentioned, plasma concentrations of glucocorticoid hormones are much higher (100-to 1000-fold) than those of aldosterone (0.1–1 nM). The higher concentration of glucocorticoids and the high affinity of the mineralocorticoid receptor for glucocorticoids raise the issue of ligand-receptor specificity and resulting physiologic action. Given the high levels of circulating glucocorticoids (cortisol), one might predict permanent maximal occupancy of the mineralocorticoid receptor by cortisol, leading to sustained maximal sodium reabsorption and precluding any regulatory role of aldosterone. However, several factors are in place to enhance the specificity of the mineralocorticoid receptor for aldosterone (Figure 6–7). First, plasma glucocorticoids bind to glucocorticoid-binding protein and albumin. This hormone binding allows only a small amount (approximately 10%) of the unbound hormone to freely cross cell membranes. Second, aldosterone target cells possess enzymatic activity of **11 β -hydroxysteroid dehydrogenase type II**. This enzyme metabolizes the active forms of glucocorticoids (cortisol) into inactive forms (cortisone) that have significantly less affinity for the mineralocorticoid receptor. Third, the mineralocorticoid receptor discriminates between aldosterone and glucocorticoids. Aldosterone dissociates from the mineralocorticoid receptor 5 times more slowly

than do the glucocorticoids, despite their similar affinity constants. In other words, aldosterone is less easily displaced from the mineralocorticoid receptor than is cortisol. Together, these mechanisms ensure that under normal conditions, mineralocorticoid action is restricted to aldosterone. However, it is important to keep in mind that when production and release of glucocorticoids is excessive, or when the conversion of cortisol to its inactive metabolite cortisone is impaired; the higher circulating and tissue cortisol levels may lead to binding and stimulation of mineralocorticoid receptors.

Figure 6–7.



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Steroid hormone receptors and mineralocorticoid (MC) specificity. MCs (aldosterone) and glucocorticoid (GC) (cortisol) hormones bind to intracellular receptors that share 57% homology in the ligand-binding domain and 94% homology in the DNA-binding domain. Cortisol binds the MC receptor (MR) with high affinity. Because more cortisol is produced than aldosterone, this could complicate the regulation of aldosterone specific effects. Nonspecificity is prevented by the presence of an enzyme; 11-hydroxysteroid dehydrogenase

type 2 (11β -HSD2) in mineralocorticoid target cells. This enzyme converts cortisol into its less active form cortisone (CS), which has less affinity for the MR. Another factor that contributes to ensure that MC effects are kept under regulation is the fact that aldosterone dissociates from the MR at a rate that is 5 times slower than that of GCs, despite their similar affinity constants. In other words, aldosterone is less easily displaced from the MR than cortisol. Thus GCs and MCs bind to intracellular receptors, which dimerize before binding to GC or MC responsive elements in the nucleus modulating (increasing or suppressing) transcription of specific genes. Cortisol because of its high-affinity binding to the MR can produce MC-like effects (sodium retention). Conversion to cortisone (CS) decreases the affinity for the receptor shown by the ill-fit of CS with the MR. Decreased activity of the 11β -HSD2 leads to decreased conversion of cortisol to cortisone and increased mineralocorticoid activity. GRE, glucocorticoid response element; MRE, mineralocorticoid response element.

Specific Effects of Adrenal Cortex Hormones

GLUCOCORTICIDS



Cortisol, the main glucocorticoid in humans, enters the cell by passive diffusion and binds to the glucocorticoid receptor (type II glucocorticoid receptor) (Figure 6–7). The hormone-receptor complex translocates to the nucleus, where it binds to specific DNA sequences (glucocorticoid response elements) and exerts its physiologic effects by altering transcription of genes. Because virtually all cells express glucocorticoid receptors, the physiologic effects are multisystemic. Glucocorticoids affect intermediary metabolism, stimulate proteolysis and gluconeogenesis, inhibit muscle protein synthesis, and increase fatty acid mobilization. Their hallmark effect is to increase blood glucose concentrations, hence the name "glucocorticoids." In the liver, glucocorticoids increase the expression of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase, tyrosine aminotransferase, and glucose-6-phosphatase. In muscle, glucocorticoids interfere with GLUT 4 translocation to the plasma membrane (Chapter 7). In bone and cartilage, glucocorticoids decrease the insulin-like growth factor I, insulin-like growth factor–binding protein 1, and growth hormone expression and action, and affect thyroid hormone interactions. Excessive glucocorticoid levels result in osteoporosis and impair skeletal growth and bone formation by inhibiting osteoblasts and collagen synthesis. Particularly at high circulating levels, glucocorticoids are catabolic and result in loss of lean body mass including bone and skeletal muscle. Glucocorticoids modulate the immune response by increasing anti-inflammatory cytokine synthesis and decreasing proinflammatory cytokine synthesis, exerting an overall anti-inflammatory effect. Their anti-inflammatory effects have been exploited by the use of synthetic analogs of glucocorticoids, such as prednisone, for the treatment of chronic inflammatory diseases. In the vasculature, glucocorticoids interact with reactivity to vasoactive substances, like angiotensin II and norepinephrine. This interaction becomes evident in patients with glucocorticoid deficiency and manifests as hypotension and decreased sensitivity to vasoconstrictor administration. In the central nervous system, they modulate perception and emotion and may produce

marked changes in behavior. This should be kept in mind when administering synthetic analogs, particularly in elderly patients. Some of the main physiologic effects of glucocorticoids are listed in Table 6–2.

Table 6–2. Physiologic Effects of Glucocorticoids

System	Effects
Metabolism	Degrades muscle protein and increases nitrogen excretion Increases gluconeogenesis and plasma glucose levels

Increases hepatic glycogen synthesis

Decreases glucose utilization (anti-insulin action)

Decreases amino acid utilization

Increases fat mobilization

Redistributes fat
Permissive effects on glucagon and catecholamine effects
Hemodynamic

Maintains vascular integrity and reactivity

Maintains responsiveness to catecholamine pressor effects

Maintains fluid volume	
Immune function	Increases anti-inflammatory cytokine production

Decreases proinflammatory cytokine production

Decreases inflammation by inhibiting prostaglandin and leukotriene production

Inhibits bradykinin and serotonin inflammatory effects

Decreases circulating eosinophil, basophil, and lymphocyte counts (redistribution effect)

Impairs cell-mediated immunity

Increases neutrophil, platelet, and red blood cell counts

Central nervous system

Modulates perception and emotion

Decreases CRH and ACTH release

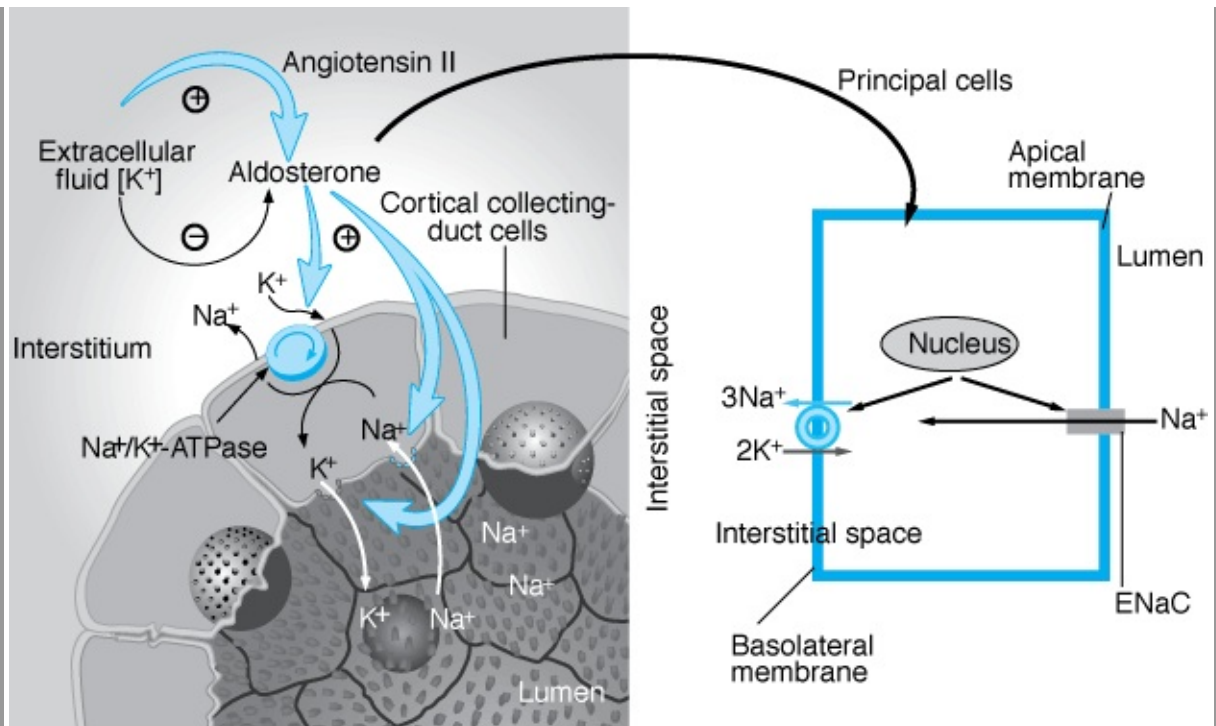
ACTH, adrenocorticotrophic hormone; CRH, corticotropin-releasing hormone.

MINERALOCORTICOIDS



The principal physiologic function of aldosterone is to regulate renal sodium reabsorption and potassium excretion, hence the name "mineralocorticoid." Aldosterone binds to the mineralocorticoid receptor in the principal cells of the distal tubule and the collecting duct of the nephron, producing an increase in sodium reabsorption and potassium excretion (Figure 6–8). Aldosterone also diffuses across the plasma membrane and binds to its cytosolic receptor. The receptor-hormone complex is translocated to the nucleus, where it interacts with the promoter region of target genes, activating or repressing their transcriptional activity. Aldosterone-induced activation of preexisting proteins and stimulation of new proteins mediate an increase in transepithelial sodium transport. Aldosterone increases sodium entry at the apical membrane of the cells of the distal nephron through the amiloride-sensitive epithelial Na^+ channel. The Na^+/K^+ -adenosine triphosphatase (ATPase), located in the basolateral membrane of the cells, maintains the intracellular sodium concentration by extruding the reabsorbed sodium toward the extracellular and blood compartments. The increase in Na^+ reabsorption leads to increased water reabsorption, creating an electrochemical gradient that facilitates the transfer of intracellular K^+ from tubular cells into the urine. When most of the filtered Na^+ is reabsorbed in the proximal tubule, only a small amount of sodium reaches the distal tubule (the site of aldosterone regulation). In this case, no net Na^+ reabsorption occurs even in the presence of elevated levels of aldosterone. As a result, potassium excretion is minimal. In fact, only 2% of filtered sodium is under regulation by aldosterone.

Figure 6–8.



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Renal physiologic effects of aldosterone. Aldosterone diffuses across the plasma membrane and binds to its cytosolic receptor. The receptor-hormone complex is translocated to the nucleus, where it interacts with the promoter region of target genes, activating or repressing their transcriptional activity and thereby increasing transepithelial Na⁺ transport. Aldosterone increases Na⁺ entry at the apical membrane of the cells of the distal nephron through the amiloride-sensitive epithelial Na⁺ channel (ENaC). Aldosterone promotes potassium excretion through its effects on Na⁺/K⁺-adenosine triphosphatase (Na⁺/K⁺-ATPase) and epithelial Na⁺ and K⁺ channels in collecting-duct cells. An increase in the extracellular fluid K⁺ concentration stimulates the secretion of aldosterone, and a decrease in K⁺ inhibits aldosterone secretion. Angiotensin II has a synergistic effect on the stimulation of aldosterone production induced by hyperkalemia. (Modified, with permission, from Gennari JF. Current concepts: Hypokalemia. *NEJM*. 1998;339:451. Copyright © Massachusetts Medical Society. All rights reserved.)

The specific effects of aldosterone are to increase the synthesis of Na⁺

channels in the apical membrane, increase the synthesis and activity of Na^+/K^+ -ATPase in the basolateral membrane (which pulls cytosolic Na^+ to the interstitium in exchange for K^+ transport into the cell), and increase the expression of H^+ -ATPase in the apical membrane and the $\text{Cl}^-/\text{HCO}_3^-$ exchanger in the basolateral membrane of intercalated cells. These cells express carbonic anhydrase and contribute to the acidification of urine and alkalization of plasma.

Mineralocorticoids act on fewer cell types than do glucocorticoids. Their receptors are not as widely expressed as those for glucocorticoids. Classic aldosterone-sensitive tissues include epithelia with high electrical resistance, such as the distal parts of the nephron, the surface epithelium of the distal colon, and the salivary and sweat gland ducts. More recently, other cells that express mineralocorticoid receptor have been identified, such as epidermal keratinocytes, neurons of the central nervous system, cardiac myocytes, and endothelial and smooth muscle cells of the vasculature (large vessels). Therefore, additional effects of aldosterone include increased sodium reabsorption in salivary and sweat glands, increased K^+ excretion from the colon, and a positive inotropic effect on the heart.

Although it was once believed that aldosterone is derived only from the zona glomerulosa of the adrenal glands, it now appears that aldosterone may be synthesized in other tissues, including the heart, and thus may have a paracrine effect. Recent studies have demonstrated aldosterone synthase activity, messenger RNA, and aldosterone production by endothelial and vascular smooth muscle cells in the heart and blood vessels. The physiologic importance of locally produced aldosterone is not yet clear, but some studies have proposed that it may contribute to tissue repair after myocardial infarction. Aldosterone is thought to promote cardiac hypertrophy and fibrosis. These localized effects of aldosterone are unrelated to hemodynamic effects and have been implicated in the development of myocardial fibrosis in ventricular hypertrophy. In the brain, aldosterone affects neural regulation of blood pressure, salt appetite, volume regulation, and sympathetic outflow. Extraadrenal sites of aldosterone production, release, and action may become prevalent areas of pharmacologic manipulation in the near future.

The role of aldosterone in regulation of blood pressure is considered central. By regulating sodium transport across the tight epithelia of Na^+ -reabsorbing tissues such as the distal nephron and colon, aldosterone is a major factor determining total-body Na^+ levels and thus long-term blood pressure regulation (Chapter 10).

ANDROGENS

The physiologic effects of DHEA and DHEAS are not completely understood. Current knowledge indicates that low levels of DHEA are associated with cardiovascular disease in men and with an increased risk of premenopausal breast and ovarian cancer in women. In contrast, high levels of DHEA might increase the risk of postmenopausal breast cancer. Exogenous administration of DHEA to the elderly increases several hormone levels, including insulin-like growth factor-1, testosterone, dihydrotestosterone, and estradiol. However, the clinical benefit of these changes and the side effects of long-term use remain to be clearly defined. Furthermore, the specific mechanisms through which DHEA exerts its actions are not completely understood.

Diseases of Overproduction & Undersecretion of Glucocorticoids

GLUCOCORTICOID EXCESS

Glucocorticoid excess can be due to overproduction by an adrenal tumor, overstimulation of adrenal glucocorticoid synthesis and release by ACTH produced by a pituitary tumor or an ectopic tumor, or the iatrogenic administration of excess synthetic glucocorticoids. The clinical manifestation of glucocorticoid excess, known as Cushing syndrome, can be separated into 2 categories depending on its etiology.

Corticotropin-dependent Cushing syndrome is characterized by elevated glucocorticoid levels due to excess stimulation by ACTH. The chronic elevation of ACTH causes bilateral enlargement (hyperplasia) of the adrenal cortex. Corticotropin production may also be ectopic (derived from extrapituitary tissue), most frequently because of small cell lung carcinoma. Tumor secretion of corticotropin is usually not suppressed by glucocorticoids, and this feature is helpful in distinguishing the source of corticotropin. The name "Cushing disease" is reserved for Cushing syndrome caused by excess secretion of corticotropin by pituitary corticotroph tumors and is the most common form of the syndrome.

In corticotropin-independent Cushing syndrome, excess cortisol production is due to abnormal adrenocortical glucocorticoid production regardless of ACTH stimulation. In fact, the elevated circulating cortisol levels suppress CRH and ACTH levels in plasma.

Clinically, the most common initial presentation of glucocorticoid excess is the relatively sudden onset of weight gain, which is usually central but may be general in distribution; thickening of the facial features, giving the typical round face or "moon face," an enlarged dorsocervical fat pad or "buffalo hump," and increased fat that bulges above the supraclavicular fossae. Hypertension, glucose intolerance, decreased or absent menstrual flow in premenopausal women, decreased libido in men, and spontaneous bruising are frequent concomitant findings. Muscle wasting and weakness are manifested by difficulty in climbing stairs or rising from a low chair. In children and young adolescents, glucocorticoid excess causes stunted linear growth and excessive weight gain. Depression and insomnia often accompany the other symptoms. Older patients and those with chronic Cushing

syndrome tend to have thinning of the skin and osteoporosis, with low back pain and vertebral collapse due to increased bone turnover leading to osteoporosis. In addition to endogenous causes, chronic pharmacologic administration of synthetic analogs of glucocorticoids can also produce clinical manifestations indistinguishable from those of endogenous overproduction of glucocorticoids.

GLUCOCORTICOID DEFICIENCY

Glucocorticoid deficiency is less common than diseases due to excess production of glucocorticoids. Glucocorticoid deficiency can result from lack of ACTH stimulation of adrenal glucocorticoid production (secondary deficiency) or from adrenal dysfunction (primary deficiency). Exogenous administration of synthetic analogs of glucocorticoids in the chronic treatment of some diseases will also suppress CRH and ACTH. Therefore, the sudden discontinuation of treatment may be manifested as an acute case of adrenal insufficiency, a medical emergency. Thus, it is important to carefully taper the withdrawal of glucocorticoid treatment allowing CRH and ACTH production rhythms to be restored and the endogenous synthesis of cortisol to be normalized.

Most cases of ACTH deficiency involve deficiencies of other pituitary hormones. Because aldosterone is mainly under the regulation of angiotensin II and K^+ , individuals may not necessarily manifest with simultaneous mineralocorticoid deficiency when impaired ACTH release is the causative factor. Glucocorticoid deficiency due to adrenal hypofunction is known as Addison disease, which can be the result of autoimmune destruction of the adrenal gland or inborn errors of steroid hormone synthesis (described earlier).

Diseases of Overproduction & Undersecretion of Mineralocorticoids

ALDOSTERONE EXCESS

Excess production of aldosterone can be classified as primary, secondary, tertiary, or pseudohyperaldosteronism.

Primary hyperaldosteronism, also known as Conn syndrome, is a condition in which autonomous benign tumors of the adrenal glands hypersecrete aldosterone. The excess aldosterone leads to hypertension because of Na^+ and H_2O retention and hypokalemia because of excess K^+ secretion. The release of renin is suppressed.

Secondary hyperaldosteronism is a renin-dependent phenomenon. A decrease in the effective arterial blood volume resulting from other pathologic states, such as ascites or heart failure, leads to continuous stimulation of the renin-angiotensin II-aldosterone system. The increase in angiotensin II leads to stimulation of aldosterone release.

Tertiary hyperaldosteronism, known as Bartter syndrome, is due to reactive hyperreninism. This NaCl -wasting renal genetic disorder leads to increased renin release to compensate for the excess sodium loss. The excess renin stimulates the angiotensin II-aldosterone system, resulting in excess aldosterone release.

Pseudohyperaldosteronism is the permanent activation of mineralocorticoid receptors by substances other than aldosterone. This condition is known as the syndrome of apparent mineralocorticoid excess. Several factors have been associated with this syndrome:

- congenital adrenal hyperplasia (11β -hydroxylase deficiency and 17α -hydroxylase deficiency) leading to excess production of 11-deoxycortisone (an active mineralocorticoid)
- deficiency of 11β -hydroxysteroid dehydrogenase, which leads to insufficient conversion of cortisol to its inactive metabolite cortisone in the principal cells of the distal tubule. An example of this alteration occurs with excess consumption of licorice. Glycyrrhetic acid, a compound of licorice, inhibits the activity of 11β -hydroxysteroid dehydrogenase. Inhibition of this enzyme results in a decrease in the inactivation of glucocorticoids in mineralocorticoid-sensitive cells.
- primary glucocorticoid resistance, characterized by hypertension,

excess androgens, and increased plasma cortisol concentrations

- Liddle syndrome, due to activating mutations of the renal epithelial sodium channel (ENaC), leading to salt-sensitive hypertension
- mutations in the mineralocorticoid receptor resulting in constitutive mineralocorticoid receptor activity and altered receptor specificity. In this condition, progesterone and other steroids lacking 21-hydroxyl groups become potent agonists of the mineralocorticoid receptor.

In summary, excess mineralocorticoid-like activity can result not only from excess production of aldosterone, but also from other mechanisms, including overproduction of 11-deoxycorticosterone, inadequate conversion of cortisol to cortisone by 11 β -hydroxysteroid dehydrogenase type II in target tissues, glucocorticoid receptor deficiency, and constitutive activation of renal sodium channels.

Chronic excess of mineralocorticoids can result in what is known as an **escape phenomenon**. Although sodium retention increases during the initial phase of mineralocorticoid excess, compensatory mechanisms involved in sodium excretion subsequently go into effect, resulting in a new sodium equilibrium in the body maintained by higher sodium excretion. The importance of this escape mechanism is that it limits the volume expansion related to Na⁺ retention.

ALDOSTERONE DEFICIENCY

Deficient production of aldosterone can be classified as primary, secondary, or pseudohypoaldosteronism.

Primary hypoaldosteronism, or Addison disease, results from destruction of the adrenal gland due to infection, injury, or autoimmune processes; from genetic disorders affecting the entire gland, such as lipoid hyperplasia, adrenoleukodystrophy, or adrenal hypoplasia congenita; or from genetic disorders affecting specific enzymatic conversions required for aldosterone biosynthesis. Two of these genetic diseases, the salt-wasting forms of 21-hydroxylase and 3 β -hydroxysteroid dehydrogenase deficiencies, also affect cortisol biosynthesis. In primary aldosterone deficiency, plasma renin activity is elevated; so this condition is also referred to as hyperreninemic hypoaldosteronism.

Secondary hypoaldosteronism may be due to inadequate stimulation of aldosterone secretion (hyporeninemic hypoaldosteronism) despite normal adrenal function. This condition is usually associated with renal insufficiency.

Pseudohypoaldosteronism is an inherited disease characterized by severe neonatal salt wasting, hyperkalemia, metabolic acidosis, and unresponsiveness to mineralocorticoid hormone action. The disease can be due to a loss-of-function mutation in the mineralocorticoid receptor or, in the more severe recessive form, to a loss-of-function mutation in the ENaC subunits.

Diseases of Overproduction & Undersecretion of Adrenal Androgens

ADRENAL ANDROGEN EXCESS

The most likely cause of excessive androgen secretion is dysregulation of the 17-hydroxylase and 17,20-lyase activities of P450c17, the rate-limiting step in androgen biosynthesis. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is one of the most common autosomal recessive disorders. In this condition, impaired cortisol production leads to a lack of negative glucocorticoid feedback on the pituitary, hypothalamic, and suprahypothalamic centers, resulting in an increase in corticotropin, a buildup of cortisol precursors, and androgen excess. The classic form of congenital adrenal hyperplasia presents in infancy and early childhood as signs and symptoms of virilization with or without adrenal insufficiency.

ADRENAL ANDROGEN DEFICIENCY

Similar to the deficiencies of glucocorticoids and mineralocorticoids, adrenal androgen deficiency can be primary or secondary to hypopituitarism. Of greater importance is the continuous decrease in adrenal androgen production that is associated with aging and menopause (discussed in Chapters 8 and 9). Pharmacologic treatment with oral glucocorticosteroids results in ACTH suppression, which in turn results in reduced adrenal androgen production.

HORMONES OF THE ADRENAL MEDULLA

All of the previous discussion focused on the hormones produced and released from the adrenal cortex. As mentioned at the beginning of this chapter, the adrenal gland is composed of 2 embryologically distinct regions. The medulla can be considered a sympathetic nervous system ganglion, which, in response to sympathetic neuronal stimulation and the resulting release of acetylcholine, stimulates the production and release of catecholamines. The medulla is the central part of the adrenal gland (Figure 6–1). It is extremely vascular and consists of large chromophil cells arranged in a network. It is made of 2 cell types called pheochromocytes—epinephrine-producing (more numerous) and norepinephrine-producing cells. These cells synthesize and secrete the catecholamines **epinephrine** (in greater amounts), **norepinephrine** and, to a lesser extent, **dopamine**.

Chemistry & Biosynthesis

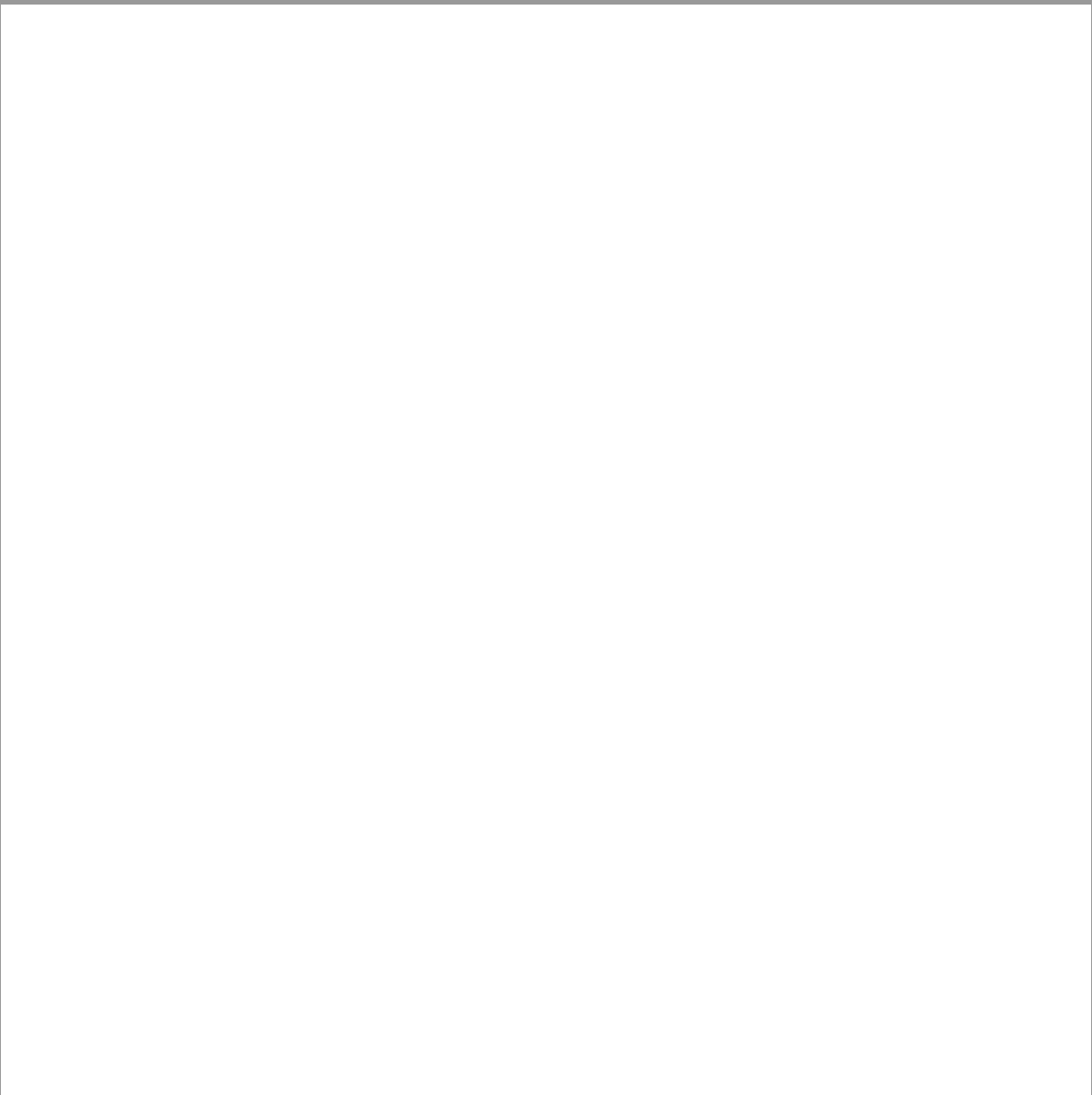
In addition to the principal products released by the adrenal medulla, epinephrine and norepinephrine; additional products that are coreleased include chromogranins, ATP, endogenous opioid peptides, and adrenomedullin (Figure 6–2). Catecholamines are amino acid–derived hormones, synthesized from the amino acid tyrosine (Figure 6–9). Tyrosine is actively transported into the cells, where it undergoes 4 enzymatic cytosolic reactions for its conversion to epinephrine. These are as follows:

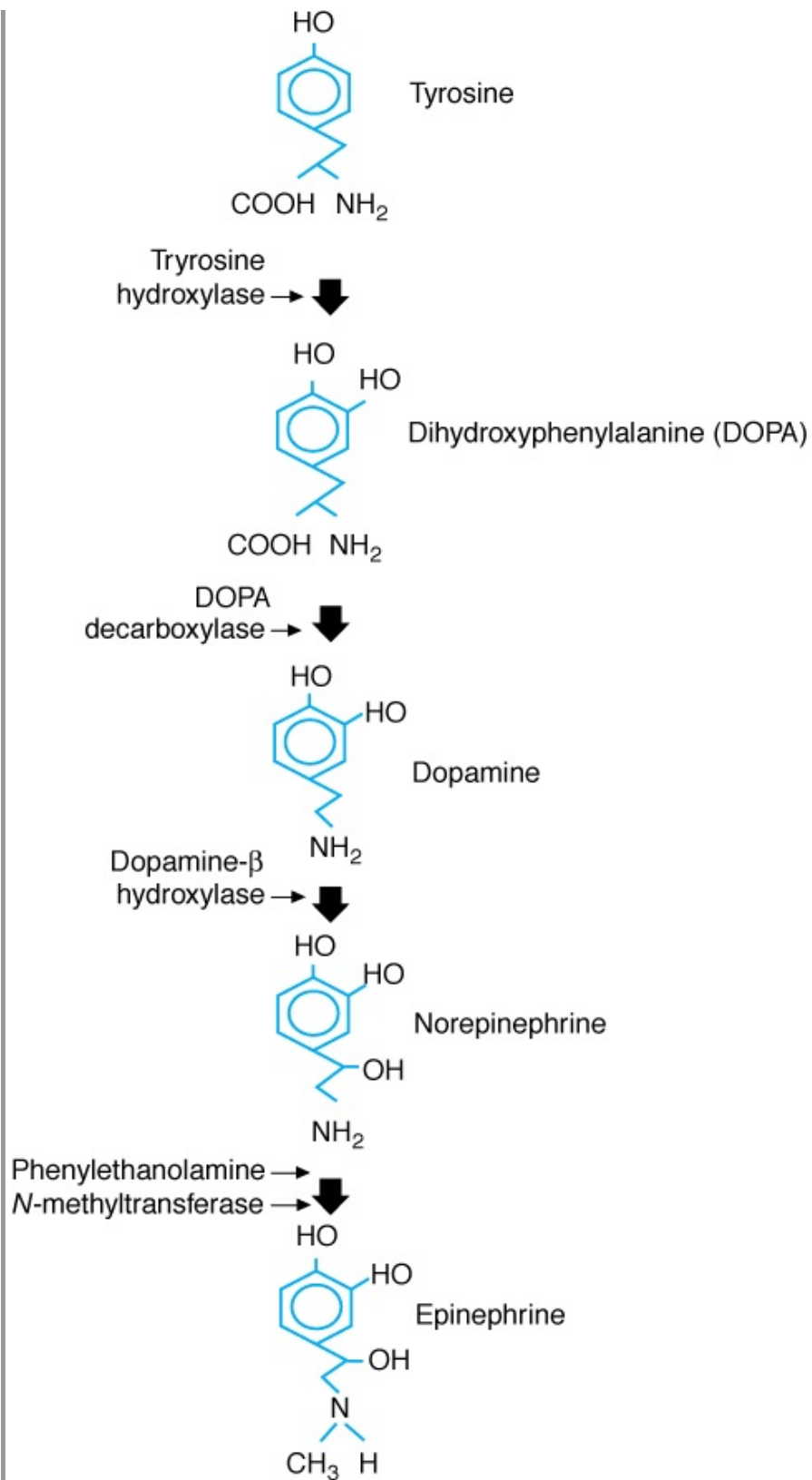
- Hydroxylation of tyrosine to L-dihydrophenylalanine (L-Dopa) by the enzyme **tyrosine hydroxylase**. This enzyme is found in the cytosol of catecholamine-producing cells and is the main control point for catecholamine synthesis. The activity of this enzyme is inhibited by noradrenaline, providing feedback control of catecholamine synthesis.
- Decarboxylation of L-Dopa to dopamine by the enzyme **dopa decarboxylase** in a reaction that requires pyridoxal phosphate as a cofactor. This end product is packaged into secretory vesicles.
- Hydroxylation of dopamine to norepinephrine by the enzyme **dopamine β -hydroxylase**, a membrane-bound enzyme found in

synaptic vesicles that uses vitamin C as a cofactor. This reaction occurs inside the secretory vesicles.

- Methylation of norepinephrine to epinephrine by the enzyme **phenylethanolamine N-methyltransferase**. The activity of this adrenal medullary enzyme, found in the cytosol of the chromaffin cell, is modulated by adjacent adrenal steroid production, underscoring the importance of radial arterial flow from the cortex to the medulla.

Figure 6–9.





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Catecholamine synthetic pathway. Catecholamine synthesis from the precursor L-tyrosine involves 4 enzymatic reactions that take place in the cytosol of chromaffin cells. These are the following: (1) hydroxylation of tyrosine to L-dihydrophenylalanine (L-Dopa) by tyrosine hydroxylase, (2) decarboxylation of L-Dopa to dopamine by dopa decarboxylase, (3) hydroxylation of dopamine to norepinephrine by dopamine β -hydroxylase, and (4) methylation of norepinephrine to epinephrine by phenylethanolamine *N*-methyltransferase.

The latter enzymatic reaction occurs in the cytoplasm and thus requires that norepinephrine leave the secretory granules by a passive transport mechanism. The epinephrine produced in the cytoplasm must reenter the secretory vesicles through ATP-driven active transport. The transporters involved are the vesicular monoamine transporters, which are expressed exclusively in neuroendocrine cells. Because of the expression of these transporters in sympathomedullary tissues, their function can be used diagnostically (like that of the iodide transporter) for radioimaging and localization of catecholamine-producing tumors (pheochromocytomas). The synthesis of catecholamines can be regulated by changes in the activity of tyrosine hydroxylase by release from end-product inhibition (acute) or by an increase in enzyme synthesis (chronic).

Release of Catecholamines



The release of catecholamines is a direct response to sympathetic nerve stimulation of the adrenal medulla. Acetylcholine released from the preganglionic sympathetic nerve terminals binds to nicotinic cholinergic receptors in the plasma membrane of the chromaffin cells, depolarizing the cells. Depolarization of the cells leads to activation of voltage-gated Ca^{2+} channels, producing an influx of Ca^{2+} . The synaptic vesicles containing the preformed catecholamines are docked beneath the synaptic membrane and are closely associated with voltage-gated Ca^{2+} channels. The influx of Ca^{2+} triggers the exocytosis of secretory granules, which release their contents (catecholamines, chromogranins, ATP, adrenomedullin, POMC products, and other peptides) into the interstitial space, from where they are transported in the circulation to their target organs. The physiologic role of the peptides coreleased with the catecholamines has not been fully established and will not be discussed.

Catecholamine Transport & Metabolism

The half-life of circulating catecholamines is short and is estimated to range from 10 seconds to 1.7 minutes. Most of the catecholamines released (50–60%) circulate bound to albumin with low affinity.

Circulating catecholamines can undergo reuptake by extraneuronal sites, degradation at target cells by **catechol-O-methyltransferase (COMT)** or **monoamino oxidase (MAO)**, or direct filtration into the urine. MAO catalyzes the first step of oxidative deamination of catecholamines. COMT catalyzes conversion of epinephrine and norepinephrine to metanephrine and normetanephrine. The joint action of MAO and COMT on norepinephrine and epinephrine; especially in the liver (>94%), produces the metabolite **vanillylmandelic acid (VMA)**, which is then excreted in the urine; dopamine metabolized through this pathway yields homovanillic acid. Because these metabolites are water soluble and have high levels of urinary excretion, they play an important role in the clinical detection of tumors that produce excess catecholamines. In humans, VMA is the major end

product of norepinephrine and epinephrine metabolism.

Target Organ Cellular Effects

The physiological effects of catecholamines are mediated by binding to cell membrane G protein–coupled adrenergic receptors distributed widely throughout the body. These G protein–coupled receptors have differential effects depending on the subtype of G protein to which they are associated and the signal transduction mechanism linked to that specific G protein (Table 6–3). Catecholamines released from the adrenal medulla exert their effects almost exclusively in peripheral tissues and not in the brain, because catecholamines do not cross the blood-brain barrier easily.

Table 6–3. Adrenergic Receptors and Signaling Pathways

Adrenergic receptor	G protein	Second messenger
β -Adrenergic receptors β_1 , β_2 , β_3	G_{α_s} G protein	Activate adenylate cyclase
α_1 -Adrenergic receptors α_{1A} , α_{1B} , α_{1D}	Mostly $G_{q/11}$ family of G proteins	Usually activate PLC_{α} (thereby activating PKC via DAG and increasing intracellular Ca^{2+} via IP_3) or PLA_2
α_2 -Adrenergic receptors α_{2A} , α_{2B} , α_{2C}	Mostly varied G_{α_i} and G_o proteins	May decrease the activity of adenylate cyclase (opposing the effects of β -adrenergic receptors)

Activate K channels

Inhibit Ca^{2+} channels and activate $\text{PLC}\beta$ or PLA_2 (an effect similar to that of α_1 -adrenergic receptors)

DAG, diacylglycerol; IP_3 , inositol 1,4,5-trisphosphate; PKC, protein kinase C; PL, phospholipase.

The adrenergic receptors are classified as predominantly stimulatory receptors (α) or predominantly inhibitory receptors (β). Understanding the selectivity of the receptors and their tissue distribution is therefore key to predicting the individual's response to their therapeutic use.

ALPHA-ADRENERGIC RECEPTORS

Alpha-adrenergic receptors have greater affinity for epinephrine than for norepinephrine or for isoproterenol, a synthetic agonist. They are subdivided into α_1 - and α_2 -receptors.

Alpha₁-adrenergic receptors are further subdivided into α_{1A} , α_{1B} , and α_{1D} . These are G protein–coupled receptors ($\text{G}_{\alpha_q/11}$) that activate phospholipase C, resulting in activation of protein kinase C via diacylglycerol (Table 6–3). This reaction produces an increase in intracellular Ca^{2+} (via inositol 1,4,5-trisphosphate) and phospholipase A_2 . The increase in intracellular Ca^{2+} calmodulin kinase–mediated phosphorylation of myosin light-chain kinase in smooth muscle produces contraction in vascular, bronchial, and uterine smooth muscle. Alpha₁-adrenergic receptors play important roles in the regulation of several physiologic processes, including myocardial contractility and chronotropy and hepatic glucose metabolism (Table 6–4).

Table 6–4. Catecholamine Physiologic Effects

α -Adrenergic mediated	β -Adrenergic mediated
Vasoconstriction	Vasodilation
Iris dilation	Cardioacceleration

Intestinal relaxation	Increased myocardial strength
Intestinal sphincter contraction	Intestinal and bladder wall relaxation
Pilomotor contraction	Uterus relaxation
Bladder sphincter contraction	Bronchodilation
Bronchoconstriction	Calorigenesis
Uterine smooth muscle contraction	Glycogenolysis
Cardiac contractility	Lipolysis
Hepatic glucose production	

Alpha₂-adrenergic receptors are also subdivided into 3 groups, including α_{2A} , α_{2B} , and α_{2C} , and have varied second-messenger systems. They may be associated with G_{α_i} and G_0 proteins and may decrease adenylate cyclase activity, activate K^+ channels, inhibit Ca^{2+} channels, and activate phospholipase C_{β} or phospholipase A_2 (Table 6–3). Alpha₂-adrenergic receptors were initially characterized as presynaptic receptors involved in a negative feedback loop to regulate the release of norepinephrine. However, they are also involved in postsynaptic functions and play a role in blood pressure homeostasis (Table 6–4). Some of the physiologic effects mediated by this subtype of receptor involve actions at 2 counteracting α_2 -receptor subtypes. For example, stimulation of α_{2A} receptors decreases sympathetic outflow and blood pressure, whereas stimulation of α_{2B} receptors increases blood pressure by direct vasoconstriction. Alpha₂-adrenergic receptors are implicated in diverse physiologic functions, particularly in the cardiovascular system and the central nervous system. Alpha₂-adrenergic receptor agonists are used clinically in the treatment of hypertension, glaucoma, and attention deficit disorder, in the suppression of opiate withdrawal, and as adjuncts to general anesthesia.

BETA-ADRENERGIC RECEPTORS

Beta-adrenergic receptors have been subclassified as β_1 , β_2 , and β_3

receptors. They have greater affinity for isoproterenol than for epinephrine or norepinephrine. All 3 receptor subtypes are associated with G_{α_s} proteins, and their stimulation leads to an increase in cyclic adenosine monophosphate (Table 6–3).

The β_1 -adrenergic receptor plays an important role in regulating contraction and relaxation of cardiac myocytes (Table 6–4). Several mechanisms are thought to be involved, including the phosphorylation of L-type Ca^{2+} channels in the sarcolemma, ryanodine-sensitive Ca^{2+} channels in the sarcoplasmic reticulum, troponin I, and phospholamban. The overall physiologic effect is an increase in contractility. The β_1 -receptor antagonists are first-line medication for patients with hypertension, coronary heart disease, or chronic heart failure.

The β_2 -adrenergic receptor mediates several physiologic responses, including vasodilatation, bronchial smooth muscle relaxation, and lipolysis, in various tissues. Abnormalities in the function of this adrenergic receptor may lead to hypertension. Selective agonists for the β_2 -adrenergic receptor are used in the treatment of asthma.

The β_3 -adrenergic receptor plays an important role in mediating catecholamine-stimulated thermogenesis and lipolysis.

Catecholamine Physiologic Effects



Catecholamines are released in response to sympathetic stimulation and are central to the stress response to a physical or psychological insult such as severe blood loss, decrease in blood glucose concentration, traumatic injury, surgical intervention, or a fearful experience. Because catecholamines are part of the "fight or flight" response, their physiologic effects include arousal, alerting, papillary dilation, piloerection, sweating, bronchial dilation, tachycardia, inhibition of smooth muscle activity in the gastrointestinal tract, constriction of the sphincters, and relaxation of the uterine muscles (Table 6–4). Most of the events involved in coping with a stressful situation require the expenditure of energy. Catecholamines ensure substrate mobilization from the liver, muscle, and fat by stimulating the breakdown of glycogen (glycogenolysis) and fat (lipolysis). Thus, an increase in circulating catecholamines is associated with elevations in plasma glucose and free fatty acid levels. Some of the most important effects of catecholamines are exerted in the cardiovascular system, where they increase heart rate (tachycardia), produce peripheral vasoconstriction, and elevate vascular resistance.

Regulation of Adrenergic Receptors

The release of catecholamines and their effects are of short duration under normal physiologic conditions. However, when their release is chronically stimulated leading to continued elevations in circulating levels, the sustained stimulation of adrenergic receptors can lead to alterations in tissue responsiveness to catecholamines. Similar alterations in responsiveness can be elicited by either endogenously produced agonists or exogenously administered pharmacologic agonists. Examples include β -agonist-promoted desensitization in asthma and α -agonist-stimulated tachyphylaxis in patients receiving sympathomimetic nasal decongestants. Persistent exposure to an agonist of the adrenergic receptor can also result in an actual loss of receptors because of degradation or receptor desensitization. Several mechanisms of desensitization have been described. For example, after only a few minutes of exposure to a β_2 -adrenergic agonist, the receptor is phosphorylated. This phosphorylation interferes with receptor coupling to the G protein. Following a more prolonged exposure to adrenergic-receptor agonists, the receptors are internalized below the cell surface. Finally, with chronic exposure to receptor agonists, the number of receptors in the plasma membrane can be reduced because of decreased synthesis of the receptor (downregulation).

Adrenergic receptors can also undergo upregulation because of increased transcription of the gene for the receptor. Two hormones are known to produce this effect: glucocorticoids and thyroid hormone. In addition, glucocorticoids and thyroid hormone can regulate the expression of several types of adrenergic receptors through post-transcriptional events. The various subtypes of adrenergic receptors differ in their susceptibility to these agonist-promoted events. Receptor upregulation by thyroid hormone is critical in hyperthyroid patients because the combined effects of thyroid hormone and catecholamines can exacerbate cardiovascular manifestations of disease.

Diseases of Overproduction & Undersecretion of Adrenal Catecholamines

As mentioned at the beginning of this chapter, the adrenal medulla and ganglia of the sympathetic nervous system are derived from the embryonic neural crest. On the basis of histochemical staining, the endocrine cells of this sympatho-adrenal system are named chromaffin cells, and the tumors arising from these cells are called pheochromocytomas. Pheochromocytomas produce catecholamines, and patients present with signs of excess catecholamine effects, such as sustained or paroxysmal hypertension associated with headache, sweating, or palpitations.

Biochemical Evaluation of Adrenal Function

Several approaches to the evaluation of adrenal function are available for clinical use. All of them involve the physiologic basis of metabolism and regulation of adrenal hormone production. Some of the most prevalent are mentioned here. The simplest approach is the measurement of urinary hormone or degradation products of hormone metabolism. The 24-hour urine collection for these measurements has the advantage of providing an integrated measure of hormone production throughout the 24 hours.

Understanding the mechanisms that regulate glucocorticoid synthesis and release are important in medical diagnosis of the underlying causes of glucocorticoid excess or deficiency. Because of the variability in plasma concentrations of cortisol as a result of its pulsatile release and circadian rhythm, measures of plasma cortisol levels are difficult to interpret. Cortisol hypersecretion is confirmed by measuring urinary cortisol excretion over a 24-hour period. The total 24-hour urinary excretion of cortisol integrates the plasma free cortisol concentrations during the entire day and is a more reliable measure of total cortisol production. The mean ($\pm$ SD) rate of cortisol secretion in normal adults is 9.9 ± 2.7 mg per 24 hours (27 ± 7.5 μ mol per 24 hours), about 12–40 μ g (33–110 nmol) of which is filtered by the kidney and excreted in urine as unmetabolized cortisol (usually called urinary free cortisol).

DEXAMETHASONE SUPPRESSION TEST

A functional test of the responsiveness of the system involves the administration of low-dose dexamethasone, a synthetic glucocorticoid analog. In this test, dexamethasone is administered over 2 days (at a low dose) or overnight (when given at a higher dose), and the concentration of cortisol in urine is measured over 24 hours. The effect of the exogenous glucocorticoid analog is to suppress CRH and ACTH release and thus cortisol production, if the HPA axis is functional. The basis of this test is that, in most situations, the corticotroph tumor cells in Cushing disease retain some responsiveness to the negative feedback effects of glucocorticoids. In contrast, ectopic ACTH-producing tumors do not. The standard test is performed on 24-hour collections of urine for the measurement of cortisol or its metabolite. The degree of suppression is calculated from day 1 to day 3 after the administration of oral dexamethasone at a dose of 2 mg every 6 hours for 48 hours. Urinary cortisol values greater than 10 μg ($>28 \mu\text{mol}$) per 24 hours or urinary 17-hydroxycorticosteroid values greater than 2.5 mg ($>6.9 \mu\text{mol}$) per 24 hours indicate the presence of Cushing syndrome in the 2-day low-dose suppression test, as do 8:00 AM plasma cortisol values greater than 5 $\mu\text{g/dL}$ ($>138 \text{ nmol/L}$) in the overnight test.

METYRAPONE STIMULATION TEST

Metyrapone inhibits the synthesis of cortisol by inhibiting the cleavage of cholesterol to form pregnenolone and, through the inhibition of 11 β -hydroxylase, preventing the hydroxylation of 11-deoxycortisol to form cortisol. In patients with primary adrenal pathology, administration of metyrapone should decrease excretion of the metabolite 17-hydroxycorticosteroid following the decreased glucocorticoid synthesis. In Cushing disease, the decrease in plasma cortisol (by metyrapone inhibition of synthesis) and hence the decrease in negative feedback at both the hypothalamic and pituitary levels results in a compensatory increase in plasma ACTH. The rise in ACTH overcomes the early step of metyrapone cortisol synthesis inhibition and is followed by a rise in urinary 17-hydroxycorticosteroid secretion.

CRH STIMULATION TEST

Intravenous administration of CRH produces an excessive rise in plasma ACTH and cortisol in patients with ACTH-dependent Cushing syndrome. In contrast, patients with ectopic ACTH-producing tumors rarely respond with an exacerbation of cortisol levels following CRH administration.

KEY CONCEPTS



Glucocorticoid production and release is under ACTH regulation.



Mineralocorticoid release is under angiotensin II and K⁺ regulation.



Steroid hormone receptors undergo conformational changes upon hormone binding that allow them to bind to DNA and stimulate gene transcription.



Specificity of the mineralocorticoid receptor is conferred by conversion of glucocorticoids to cortisone by 11 β -hydroxysteroid dehydrogenase.



Glucocorticoids facilitate fuel mobilization, decrease glucose utilization, and produce immunosuppression.



Aldosterone regulates body sodium balance and stimulates excretion of potassium.



Catecholamine release is under sympathetic neural control.



The response to stress by the host relies on close interaction between the steroid hormones and catecholamines to ensure adequate fuel mobilization and hemodynamic control.

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OBJECTIVES

- ▶ *Identify the principal hormones secreted from the endocrine pancreas, their cells of origin, and their chemical nature.*
- ▶ *Understand the nutrient, neural, and hormonal mechanisms that regulate pancreatic hormone release.*
- ▶ *List the principal target organs for insulin and glucagon action and their major physiologic effects.*
- ▶ *Identify the time course for the onset and duration of the biologic actions of insulin and glucagon.*
- ▶ *Identify the disease states caused by oversecretion, undersecretion, or decreased sensitivity to insulin, and describe the principal manifestations of each.*

ENDOCRINE PANCREAS: INTRODUCTION

The pancreas is a mixed exocrine and endocrine gland that plays a central role in digestion and in the metabolism, utilization, and storage of energy substrates. Normal pancreatic function involving the production and release of the hormones insulin and glucagon is essential for the physiologic control of glucose homeostasis. This chapter focuses on the endocrine function of the pancreas through the release of insulin and glucagon and the mechanisms by which these hormones regulate events central to maintaining glucose homeostasis. Maintenance of glucose homeostasis is similar to the maintenance of calcium balance discussed in Chapter 6, in which several tissues and hormones interact in the regulatory process. In the case of glucose, the process involves a regulated balance among hepatic glucose release (from glycogen breakdown and gluconeogenesis), dietary glucose absorption, and glucose uptake and disposal from skeletal muscle and adipose tissue. The pancreatic hormones insulin and glucagon play central roles in regulating each of these processes and their overall effects are in part modified by other hormones such as growth hormone, cortisol, and epinephrine. In addition to secreting insulin and glucagon, the endocrine pancreas also secretes somatostatin, amylin, and pancreatic polypeptide.

FUNCTIONAL ANATOMY

The pancreas is a retroperitoneal gland divided into a head, body, and tail and is located near the duodenum. Most of the pancreatic mass is composed of exocrine cells that are clustered in lobules (acini) divided by connective tissue and connected to a duct that drains into the pancreatic duct and into the duodenum. The product of the pancreatic exocrine cells is an alkaline fluid rich with digestive enzymes, which is secreted into the small intestine to aid in the digestive process.

Embedded within the acini are richly vascularized, small clusters of endocrine cells called the **islets of Langerhans**, in which 2 endocrine cell types (β and α) predominate. The β -cells constitute about 73–75% of the total mass of endocrine cells, and their principal secretory product is **insulin**. The α -cells account for about 18–20% of the endocrine cells and are responsible for **glucagon** secretion. A small number of δ -cells (4–6%) secrete **somatostatin**, and an even smaller number of cells (1%) secrete **pancreatic polypeptide**. The localization of these cell types within the islets has a particular pattern, with the β -cells located centrally, surrounded by α - and δ -cells. This arrangement plays a role in the cell-to-cell paracrine regulation of hormone release.

The arterial blood supply to the pancreas is derived from the splenic artery and the superior and inferior pancreaticoduodenal arteries. Although islets represent only 1–2% of the mass of the pancreas, they receive about 10–15% of the pancreatic blood flow. The rich vascularization by fenestrated capillaries allows ready access to the circulation for the hormones secreted by the islet cells. The direction of blood flow is preferentially from the center of the islet to the periphery. Therefore, α - and δ -cells are exposed to high concentrations of hormones produced by the β -cells (ie, insulin), contributing to the inhibition of glucagon release by high local insulin concentrations. Venous blood from the pancreas drains into the hepatic portal vein. Therefore, the liver, a principal target organ for the physiologic effects of pancreatic hormones, is exposed to the highest concentrations of pancreatic hormones. Following first-pass hepatic metabolism, the pancreatic endocrine hormones are distributed to the systemic circulation.

Parasympathetic, sympathetic, and sensory nerves richly innervate the pancreatic islets, and the respective neurotransmitters and neuropeptides released from their nerve terminals exert important regulatory effects on pancreatic endocrine hormone release.

Acetylcholine, vasoactive intestinal polypeptide, pituitary adenylate cyclase-activating polypeptide, and gastrin-releasing peptide are released from the parasympathetic nerve terminals. Norepinephrine, galanin, and neuropeptide Y are released from sympathetic nerve terminals. Vagal nerve activation stimulates the secretion of insulin, glucagon, somatostatin, and pancreatic polypeptide. Sympathetic nerve stimulation inhibits basal and glucose-stimulated insulin secretion and somatostatin release and stimulates glucagon and pancreatic polypeptide secretion.

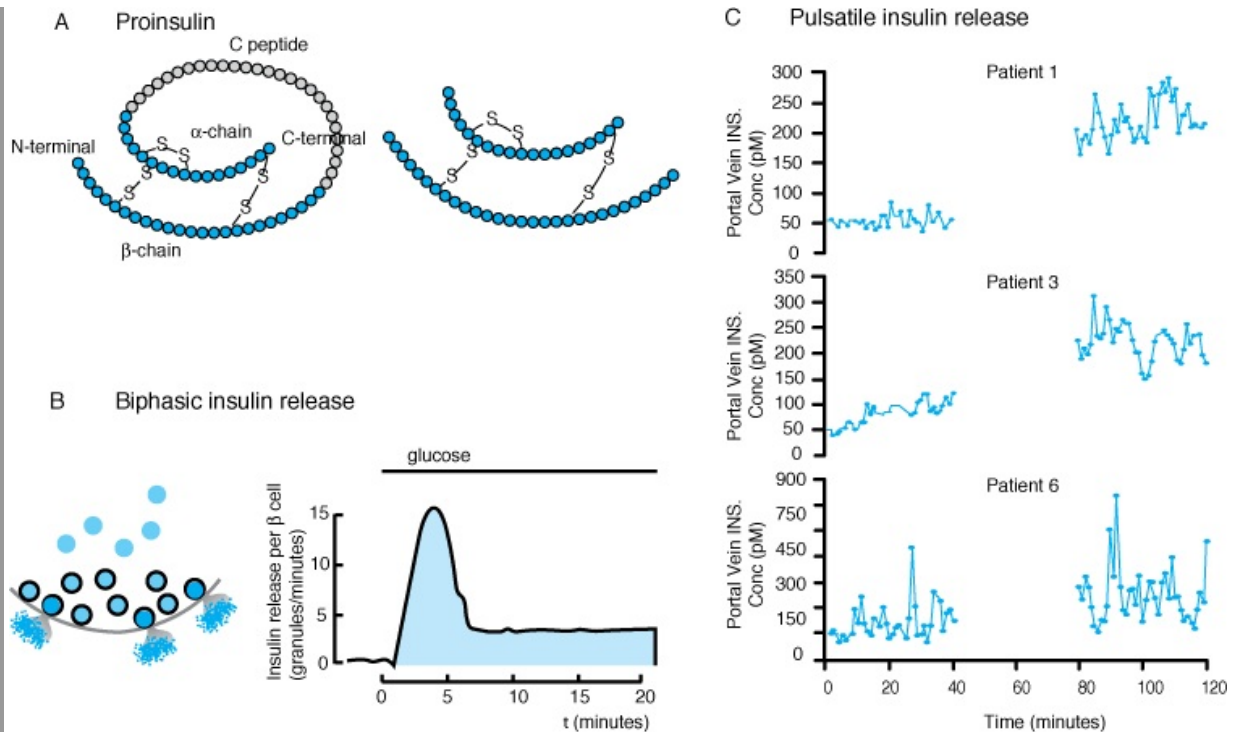
PANCREATIC HORMONES

Insulin

INSULIN SYNTHESIS

The process involved in the synthesis and release of insulin, a polypeptide hormone, by the β -cells of the pancreas is similar to that of other peptide hormones, as discussed in Chapter 1 (Figure 1–5). Preproinsulin undergoes cleavage of its signal peptide during insertion into the endoplasmic reticulum, generating proinsulin (Figure 7–1). Proinsulin consists of an amino-terminal β -chain, a carboxy-terminal α -chain, and a connecting peptide in the middle known as the **C-peptide**. C-peptide links the α - and β -chains, allowing proper folding of the molecule and the formation of disulfide bonds between the 2 chains. In the endoplasmic reticulum, proinsulin is processed by specific endopeptidases known as prohormone convertases, which cleave the C-peptide to generate the mature form of insulin. Removal of the C-peptide exposes the end of the insulin chain that interacts with the insulin receptor. Insulin and the free C-peptide are packaged into secretory granules in the Golgi apparatus and are released together. These secretory granules accumulate in the cytoplasm. About 5% of the granules are stored in a readily releasable pool. Most of the granules (more than 95%) belong to a reserve pool and need to be chemically modified, or even physically translocated, to become immediately available for release. This release of insulin granules from different pools leads to a biphasic pattern of insulin release in response to stimulation of the β -cell by glucose. Only a small proportion of the cellular stores of insulin are released even under maximal stimulatory conditions.

Figure 7–1.



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A. Insulin synthesis starts with the translation of insulin mRNA into an inactive protein called preproinsulin. Preproinsulin undergoes post-translational modification in the endoplasmic reticulum (ER) to form proinsulin. The active form of insulin is produced by modification of proinsulin by cleavage of the C-peptide structure linking the α and β chains. Insulin is composed of 2 chains; an α chain of 21 amino acids and a β chain of 30 amino acids. The chains are held together by 2 disulfide (S-S) bonds. A third disulfide bond is present within the α chain. Both insulin and the cleaved C-peptide are packaged in secretory granules, which accumulate in the β -cell cytosol and are coreleased in response to glucose stimulation. **B.** Insulin release occurs in a biphasic mode and is derived from secretory granules that are immediately available for release (<5%), from granules that must undergo a series of preparatory reactions including mobilization to the plasma membrane (>95%). These granule preparatory or maturation processes are modulated by intracellular levels of ATP, ADP and, Ca^{++} . **C.** Insulin release in response to a meal is characterized by increased frequency and amplitude of pulsatile release. Shown are portal insulin concentrations during basal state (left) and after ingestion of a mixed

meal (right) in normal patients. (Reproduced, with permission, from: Porksen N, Grofte T, Greisen J, Mengel A, Juhl C, Veldhuis JD, Schmitz O, Rossle M, Vilstrup H. Human insulin release processes measured by intraportal sampling. *Am J Physiol Endocrinol Metab*. 2002;282(3):E695–E702. Figure 4 of article.)

Stimulation of the pancreatic β -cell leads to exocytosis of the contents of the secretory granules, with the resulting release of equal amounts of insulin and C-peptide into the portal circulation. Insulin circulates in its free form and has a half-life of 3–8 minutes. Insulin levels in the circulation average 43–186 pmol/L (6–26 μ U/mL) in the fasting state. Insulin is degraded predominantly by the liver during its first pass, which extracts about 40–80% of insulin delivered. Additional degradation of insulin occurs in the kidneys as well as at target tissues by insulin proteases following endocytosis of the receptor-bound hormone. C-peptide, which was previously thought to be biologically inert, appears to have some biological action as recent evidence indicates that replacement of C-peptide improves renal function and nerve dysfunction in patients with type 1 diabetes. The receptor and signaling mechanisms involved in mediating these responses are still under investigation. The importance of C-peptide is that unlike insulin, it is not readily degraded in the liver. Thus, the relatively long half-life of the peptide (35 minutes) allows its release to be used as an index of the secretory capacity of the endocrine pancreas.

The amino acid sequence of insulin is highly conserved among species. In the past, porcine and bovine insulin were used to treat patients with diabetes. Currently, human recombinant insulin is available and has replaced animal-derived insulin, avoiding problems such as the development of antibodies to nonhuman insulin.

REGULATION OF INSULIN RELEASE

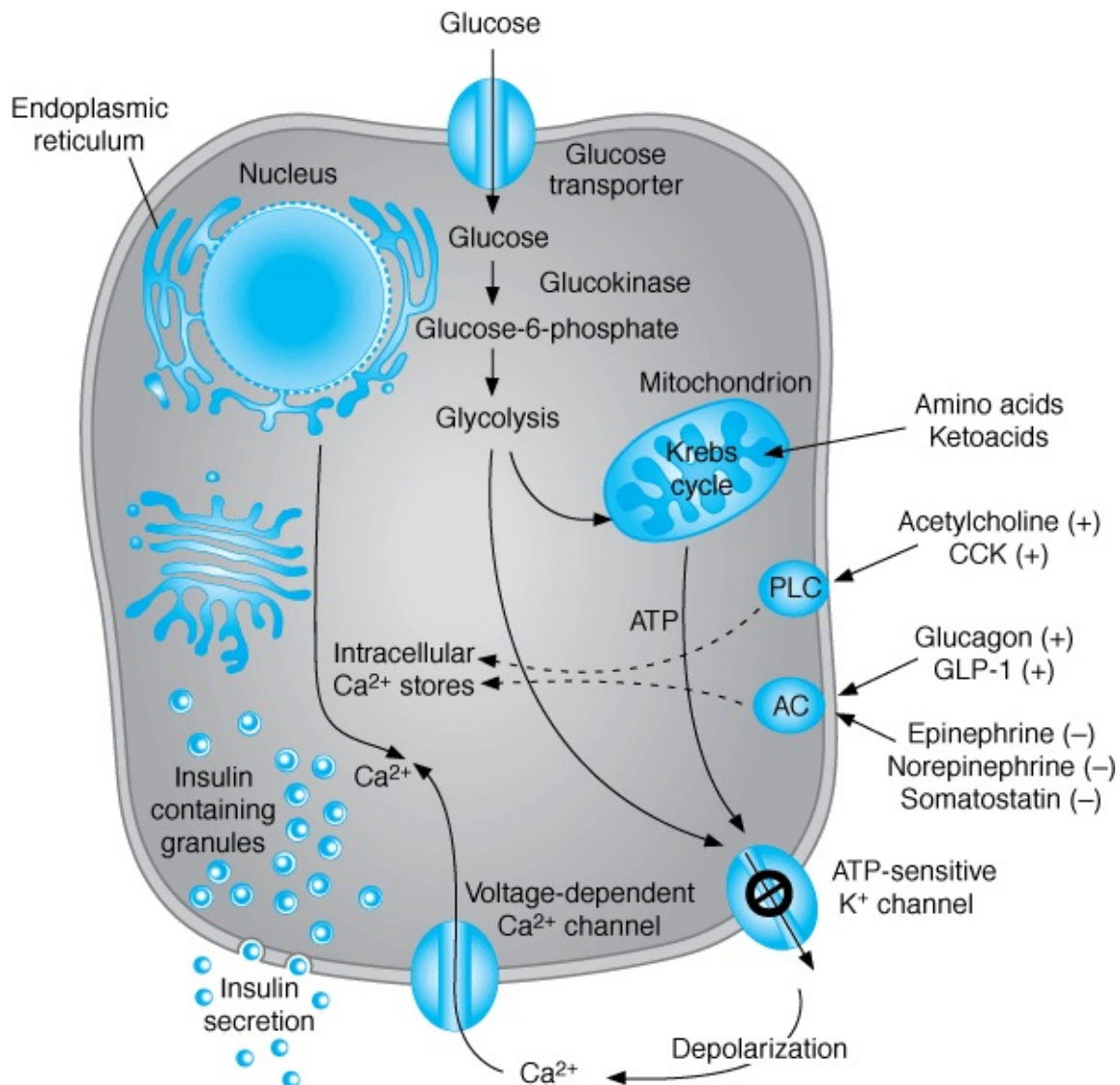


The release of insulin throughout the day is pulsatile and rhythmic in nature; 2 identifiable rhythms occur with periods of 5–10 and 60–120 minutes (Figure 7–1). The pulsatile release of insulin is important for achieving maximal physiologic effects. In particular, it appears to be critical in the suppression of liver glucose production and in insulin-mediated glucose disposal by adipose tissue. Insulin release rises after a meal in response to the increases in plasma levels of glucose and amino acids. Secretion is the result of a combination of an increase in the total amount of insulin released in each secretory burst (by about 50%) and an increased pulse frequency of a similar magnitude (Figure 7–1). The synchronized increase in insulin release is thought to be the result of recruitment of β -cells to release insulin. Although it is not clear how the β -cells communicate with each other to synchronize the release of insulin, some of the proposed mechanisms include gap junctions between the pancreatic β -cells, allowing the passage of ions and small molecules; membrane depolarization, aiding the propagation of the synchronization between the cells; and glucose-induced changes in the extracellular potassium concentration and in nitric oxide. In addition, intrapancreatic neural, hormonal, and substrate factors have been shown to play an important role in the pulsatile pattern of insulin release.



The pancreatic β -cell functions as a fuel sensor that responds to changes in plasma levels of energy substrates releasing insulin in response to integrated signals from nutrients (glucose and amino acids), hormones (insulin, glucagon-like peptide-1 (GLP-1), somatostatin, and epinephrine), and neurotransmitters (norepinephrine and acetylcholine) (Figure 7–2). Glucose is the principal stimulus for insulin release from the pancreatic β -cells, and in addition exerts a permissive effect for the other modulators of insulin secretion.

Figure 7–2.



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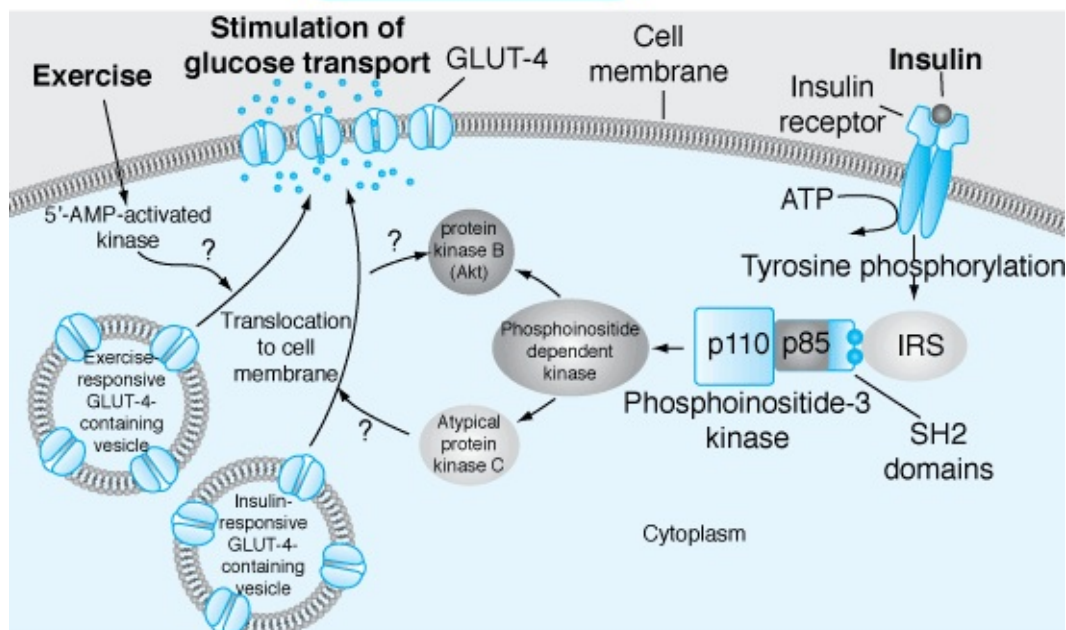
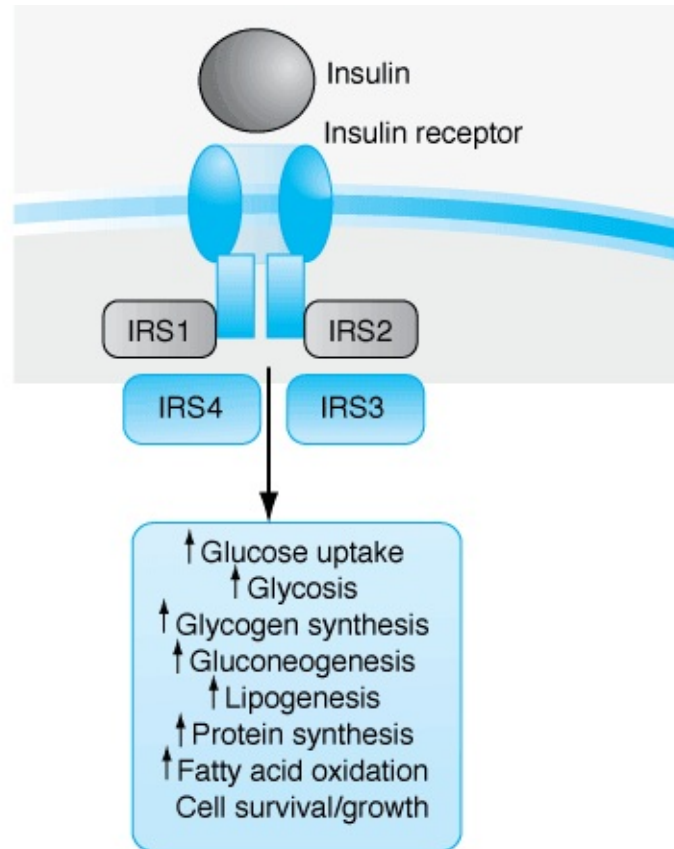
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Regulation of insulin release. Glucose is the principal stimulus for insulin release from the pancreatic β -cell. Glucose enters the β -cell by a specific glucose-transporter protein (GLUT-2) and is immediately phosphorylated by glucokinase. Formation of glucose-6-phosphate in the first step of glycolysis initiates the glycolytic metabolism of glucose followed by the Krebs cycle leading to generation of adenosine triphosphate (ATP). The increased concentrations of ATP and resulting greater ATP/adenosine diphosphate (ADP) ratio leads to inhibition and closure of the ATP-sensitive K^+ channels (the target of sulfonylurea drugs), resulting in depolarization of the plasma

membrane and opening of the voltage-dependent Ca^{2+} channels. As a result, there is an increased influx of extracellular Ca^{2+} as well as mobilization of Ca^{2+} from intracellular stores leading to the fusion of insulin-containing secretory granules with the plasma membrane and the release of insulin (and C-peptide) into the circulation. Addition factors can also stimulate insulin release from the β -cell, including hormones (glucagon-like peptide-1) and neurotransmitters (acetylcholine). Glucose synergizes with these mediators and enhances the secretory response of the β -cell to these factors. AC, adenylate cyclase; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; PLC, phospholipase C. (Modified, with permission, from Fajans SS *et al.* Mechanisms of disease: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *NEJM*. 2001;345:971. Copyright © Massachusetts Medical Society. All rights reserved.)

The glucose-induced stimulation of insulin release is the result of glucose metabolism by the β -cell, the generation of metabolic intermediates, an increase in the ATP/ADP ratio in the cytosol, and a rise in intracellular Ca^{2+} (Figure 7–3). Glucose enters the β -cell through a membrane-bound glucose transporter (GLUT-2). Once inside the β -cell, the enzyme glucokinase phosphorylates glucose in the initial step of glycolysis, leading eventually to the generation of acetyl-coenzyme A (CoA) and adenosine triphosphate (ATP) by the Krebs cycle. The resulting increase in intracellular ATP levels inhibits (closes) the ATP-sensitive K^+ channels (K_{ATP}) in the β -cell, reducing the efflux of K^+ . This process results in membrane depolarization, activation (opening) of voltage-dependent Ca^{2+} channels, and increased Ca^{2+} influx. The rise in intracellular Ca^{2+} concentrations triggers the exocytosis of insulin secretory granules and the release of insulin into the extracellular space and into the circulation. It is important to note that the regulation of K^+ channels by ATP is mediated by the sulfonylurea receptor. This is the basis for the therapeutic use of sulfonylurea drugs in the treatment of diabetes.

Figure 7–3.



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Insulin-receptor signaling. Insulin binding to the receptor activates the

intrinsic kinase activity of the intracellular domain of the receptor. This results in downstream activation of cellular events mediated through the phosphorylation of insulin receptor substrates. Signaling pathways including the phosphatidylinositol-3-kinase (PI₃-K) and the mitogen-activated protein kinase (MAPK) cascades contribute to the overall effects of insulin. As shown in the insert, one of the immediate effects of insulin is the active recruitment of GLUT-4, stored in intracellular vesicles to the cell surface. Insulin binds to its receptor in the plasma membrane, resulting in phosphorylation of the receptor and insulin-receptor substrates such as the IRS molecules. These substrates form complexes with docking proteins such as phosphoinositide-3-kinase at its 85-kDa subunit (p85) by means of SH2 (Src homology region 2) domains. Then p85 is constitutively bound to the catalytic subunit (p110). Activation of phosphoinositide-3-kinase is a major pathway in the mediation of insulin-stimulated glucose transport and metabolism. Exercise can also stimulate glucose transport by pathways that are independent of phosphoinositide-3-kinase and thought to involve 5'-AMP-activated kinase. (Reproduced, with permission, from Shepherd PR, Kahn BB. Glucose transporters and insulin action: Implications for insulin resistance and diabetes mellitus. *NEJM*. 1999;341(4):248–257. Copyright © Massachusetts Medical Society. All rights reserved.)

The β -cell Ca^{2+} concentrations can also be elevated by amino acids through their metabolism and ATP generation, or by direct depolarization of the plasma membrane. Other factors (shown in Figure 7–2) that amplify the glucose-induced release of insulin from the β -cell include acetylcholine, cholecystokinin, gastrointestinal peptide, and GLP-1. These substances all bind to cell surface receptors and activate adenylate cyclase and phospholipase C. Acetylcholine and cholecystokinin promote phosphoinositide breakdown, with a consequent mobilization of Ca^{2+} from intracellular stores, Ca^{2+} influx across the cell membrane, and activation of protein kinase C. GLP-1 raises levels of cyclic adenosine monophosphate (cAMP) and activates cAMP-dependent protein kinase A. The subsequent generation of cAMP, inositol 1,4,5-trisphosphate, diacylglycerol, and arachidonic acid and the activation of protein kinase C amplify the Ca^{2+} signal by

decreasing Ca^{2+} uptake by cellular stores and promoting both the phosphorylation and activation of proteins that trigger the exocytosis of insulin. Catecholamines and somatostatin inhibit insulin secretion through G protein–coupled receptor (GPCR) mechanisms, inhibition of adenylate cyclase, and modification of Ca^{2+} and K^{+} channel gating.

The short-term regulation of insulin release is mediated through modification of proinsulin mRNA translation. Over longer periods, glucose also increases proinsulin mRNA content by both stimulating proinsulin gene transcription and stabilizing the mRNA. As mentioned above, the release of insulin in response to glucose is biphasic, with an initial rapid release of preformed insulin followed by a more sustained release of newly synthesized insulin. An elevation in plasma glucose concentrations is followed by a transient stimulation of insulin secretion known as "**first-phase secretion**," which consists of a rapid burst of release to a high peak and then a steep decline to a low secretion rate. This is followed by "**second-phase secretion**," which consists of a gradually increasing rate of secretion to a plateau level. This biphasic response to glucose is a major characteristic of glucose-stimulated insulin secretion. The first phase occurs over a period of minutes, the second over an hour or more. Several hypotheses have been proposed to explain the biphasic nature of insulin secretion. The mechanism may involve insulin release from 2 separate pools of granules, with the first phase representing the readily releasable pool of β -granules already docked at the β -cell plasma membrane that does not require additional movement along microtubules. The second phase would require mobilization of β -granules from a storage pool to replenish the readily releasable pool of β -granules before β -granule docking and exocytosis.

PHYSIOLOGIC EFFECTS OF INSULIN

Insulin produces a wide variety of effects that range from immediate (within seconds), such as the modulation of ion (K^+) and glucose transport into the cell; early (within minutes), such as the regulation of metabolic enzyme activity; moderate (within minutes to hours), such as the modulation of enzyme synthesis; to delayed (within hours to days), such as the effects on growth and cellular differentiation. Overall, the actions of insulin at target organs are anabolic and promote the synthesis of carbohydrate, fat, and protein, and these effects are mediated through binding to the insulin receptor (Table 7–1).

Table 7–1. Insulin Effects on Carbohydrate, Fat, and Protein Metabolism

Metabolic effects	Insulin stimulates	Insulin inhibits
Carbohydrate metabolism	Glucose transport in adipose tissue and muscle Rate of glycolysis in muscle and adipose tissue Glycogen synthesis in adipose tissue, muscle, and liver	Glycogen breakdown in muscle and liver Rate of glycogenolysis gluconeogenesis in the liver
Lipid metabolism	Fatty acid and triacylglycerol synthesis in tissues Uptake of triglycerides from the blood into adipose tissue and muscle Rate of cholesterol synthesis in the liver	Lipolysis in adipose tissue, lowering the plasma fatty acid level Fatty acid oxidation in muscle and liver

Ketogenesis
Protein metabolism

Amino acid transport into tissues

Protein synthesis in muscle, adipose tissue, liver, and other tissues

Protein degradation in muscle

Urea formation

INSULIN RECEPTOR

The insulin receptor is part of the insulin-receptor family, which includes the insulin receptor, the insulin-like growth-factor receptor, and the insulin-related receptor, all of which are involved in cell division, metabolism, and development (Figure 7–3). The insulin receptor is a heterotetrameric glycoprotein membrane receptor composed of 2 α - and 2 β -subunits, linked by disulfide bonds. The α -chain is the extracellular portion and is the site for insulin binding. The β -chain consists of a short extracellular region, a transmembrane segment, and an intracellular segment that has intrinsic tyrosine kinase activity controlled by insulin binding to the extracellular α -subunit.

Insulin binding to the receptor triggers receptor autophosphorylation on tyrosine residues in the cytoplasmic domain (β -chain). The activated receptor phosphorylates tyrosine residues of several proteins known as **insulin-receptor substrates** (IRS-1, -2, -3, -4). These IRS proteins facilitate the interaction of the insulin receptor with intracellular substrates by serving as a scaffold for recruitment of proteins involved in signal transduction to downstream pathways. The result is coupling of insulin receptor activation to signaling pathways, mainly the phosphatidylinositol-3-kinase (PI₃-kinase) and the mitogen-activated protein kinase (MAPK) pathways (Figure 7–3).



The PI₃-kinase pathway involves phosphorylation of inositol phospholipids and the generation of phosphatidylinositol-3,4,5-trisphosphate and phosphatidylinositol-3,4-bisphosphate. These products, in turn, attract serine kinases to the plasma membrane, including the phosphoinositide-dependent kinase and different isoforms of protein kinase B, which, when activated, catalyze some of the cellular effects of insulin. The PI₃-kinase pathway is involved predominantly in mediating the metabolic effects of the hormone, including glucose transport, glycolysis, and glycogen synthesis, and plays a crucial role in the regulation of protein synthesis by insulin. Moreover, this pathway is involved in cell growth and transmits a strong antiapoptotic signal, promoting cell survival. The other main signaling pathway that is activated by insulin binding to its receptor is

the MAPK pathway. Although signaling cascades in this pathway do not appear to play a significant role in the metabolic effects of insulin, they are involved in mediating the proliferative and differentiation effects elicited by insulin.

Signal transduction by the insulin receptor is not limited to its activation at the cell surface. The activated ligand-receptor complex is internalized into endosomes. Endocytosis of activated receptors is thought to enhance the insulin receptor tyrosine kinase activity on substrates that are distant from those readily accessible at the plasma membrane. Following acidification of the endosomal lumen, insulin dissociates from its receptor, ending the insulin receptor-mediated phosphorylation events and promoting the degradation of insulin by proteases such as the acidic insulinase. The insulin receptor can then be recycled into the cell surface, where it becomes available for insulin binding again.

The number of available insulin receptors is modulated by exercise, diet, insulin, and other hormones. Chronic exposure to high insulin levels, obesity, and excess growth hormone all lead to a downregulation of insulin receptors. In contrast, exercise and starvation upregulate the number of receptors. The affinity of the receptor for insulin is increased following a period of decreased insulin levels and during adrenal insufficiency.

INSULIN EFFECTS AT TARGET ORGANS



Early effects—Although the expression of insulin receptors is widespread, the specific effects of insulin on skeletal muscle glucose utilization dominate insulin action. Insulin mediates about 40% of glucose disposal by the body, the great majority (80–90%) of which occurs in skeletal muscle. The movement of glucose into the cell is mediated by a family of carrier proteins, or glucose transporters, with their own unique tissue distribution. The main transporters and their predominant tissue distributions are summarized in Table 7–2.

Table 7–2. Main Features of Glucose Transporters

Transporter	Expression	Function
GLUT-1	Ubiquitous, with particularly high levels in human erythrocytes and in the endothelial cells lining the blood vessels of the brain. Expressed in skeletal muscle and fat.	Glucose uptake by skeletal muscle and fat under basal conditions
GLUT-2	Low-affinity glucose transporter present in pancreatic β -cells, liver, intestine, and kidney	Functions in the glucose sensor system and ensures that glucose uptake by pancreatic β -cells and hepatocytes occurs only when circulating glucose levels are high
GLUT-3	Primarily in neurons	Together, GLUT-1 and GLUT-3 are crucial in allowing glucose to cross the blood-brain barrier and enter neurons

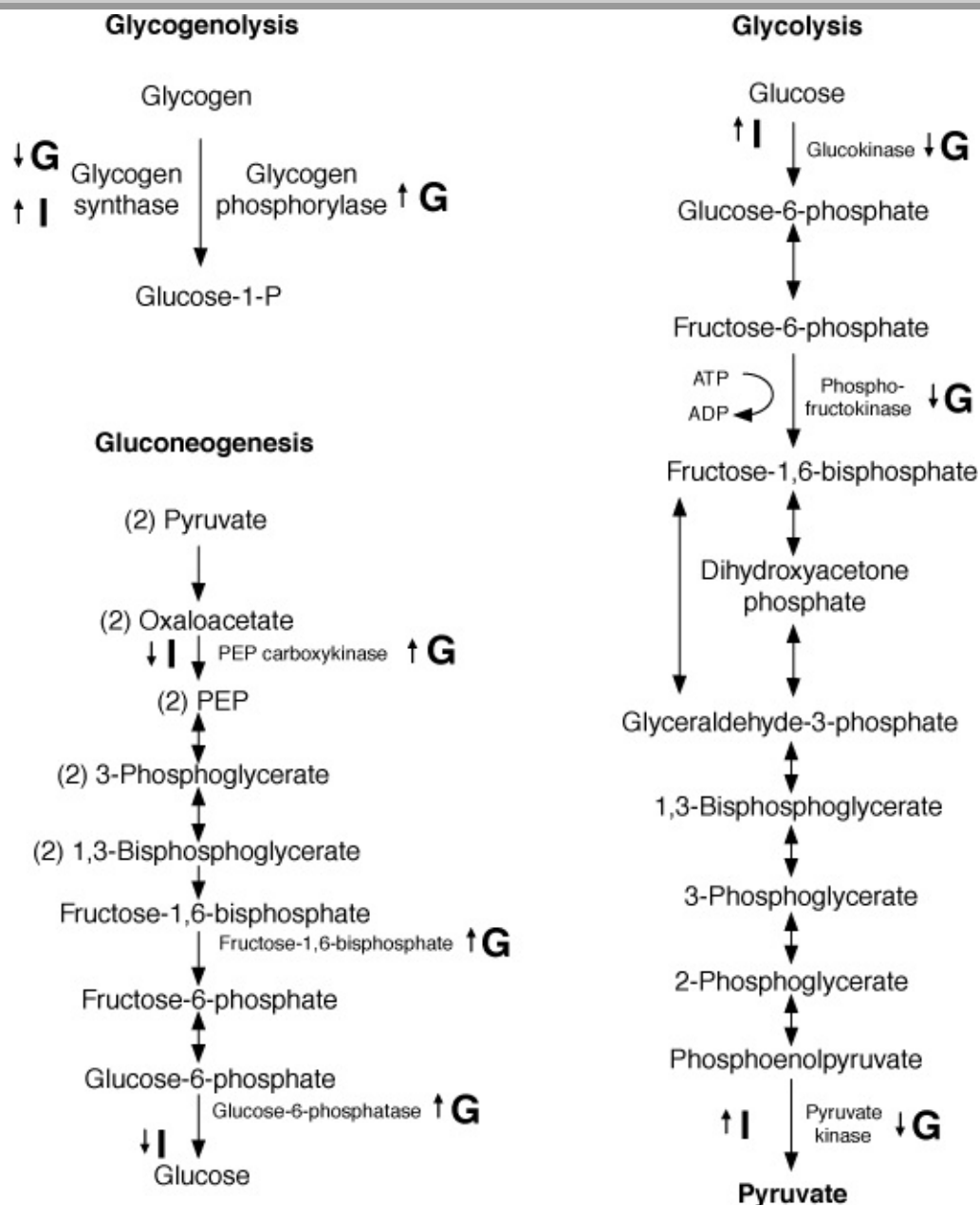
GLUT-4	Predominantly in striated muscle and adipose tissue. In contrast to the other GLUT isoforms, which are primarily localized on the cell membrane, GLUT-4 transporter proteins are sequestered in specialized storage vesicles that remain within the cell's interior under basal conditions.	The major insulin-responsive transporter
GLUT-5	Spermatozoa and small intestine	Predominantly a fructose transporter

Insulin-stimulated glucose transport is mediated through **GLUT-4**. Approximately 90% of GLUT-4 is sequestered intracellularly in the absence of insulin or other stimuli such as exercise. Insulin binding to its receptor results in recruitment of GLUT-4 from cytosolic vesicular compartments to the plasma membrane. Accumulation of GLUT-4 at the membrane is the net result of increased translocation through targeted exocytosis and decreased rate of GLUT-4 endocytosis. This is the underlying mechanism by which insulin stimulates glucose transport into fat and muscle cells.



Intermediate effects—The intermediate effects of insulin are mediated by modulation of protein phosphorylation of enzymes involved in metabolic processes in muscle, fat, and liver (Figure 7–4). In fat, insulin inhibits lipolysis and ketogenesis by triggering the dephosphorylation of hormone-sensitive lipase and stimulates lipogenesis by activating acetyl-CoA carboxylase. In the adipocytes, dephosphorylation of hormone-sensitive lipase inhibits the breakdown of triglycerides to fatty acids and glycerol, the rate-limiting step in the release of free fatty acids mediated by lipolysis. This process thereby reduces the amount of substrate that is available for ketogenesis. Insulin antagonizes catecholamine-induced lipolysis through the phosphorylation and activation of phosphodiesterase, leading to a decrease in intracellular cAMP levels and a concomitant decrease in protein kinase A activity.

Figure 7-4.



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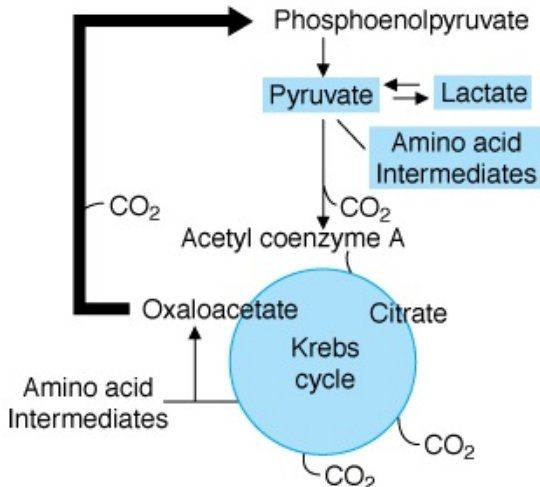
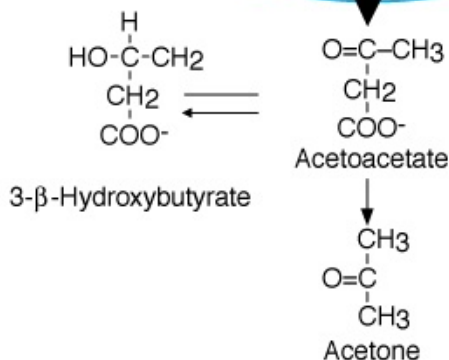
Glucagon and insulin effects on hepatic glucose metabolism. Binding of glucagon and insulin to their respective receptors stimulates a cascade of protein phosphorylation steps that activate (or inhibit) key enzymes involved in the regulation of glycogenolysis, gluconeogenesis, and glycolysis. The principal target enzymes for

insulin-and glucagon-mediated effects are presented. The overall result is an increase in hepatic glucose output. ADP, adenosine diphosphate; ATP, adenosine triphosphate; G, glucagon; I, insulin; PEP, phosphoenolpyruvate.

In the liver, insulin stimulates the gene expression of enzymes involved in glucose utilization (eg, glucokinase, pyruvate kinase, and lipogenic enzymes) and inhibits the gene expression of enzymes involved in glucose production (eg, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) (Figure 7–5). Insulin stimulates glycogen synthesis by increasing phosphatase activity, leading to the dephosphorylation of glycogen phosphorylase and glycogen synthase. In addition, insulin-mediated dephosphorylation of inhibitory sites on hepatic acetyl-CoA carboxylase increases the production of malonyl-CoA and simultaneously reduces the rate at which fatty acids can enter hepatic mitochondria for oxidation and ketone body production.

Figure 7–5.





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Process of ketogenesis in insulin deficiency. Insulin deficiency and high levels of counterregulatory hormones glucagon, epinephrine, and cortisol combine to increase the activity of hormone-sensitive lipase, increase the release of free fatty acids, and decrease the activity of acetyl-coenzyme A (CoA) carboxylase, thereby impairing the reesterification of free fatty acids and promoting fatty acid conversion into ketone bodies. The excess supply of fatty acetyl-CoA and the deficiency in oxaloacetate increase the oxidation to ketone bodies, with the resulting release of ketone bodies into the blood. The plus sign (+) denotes steps that are favored by insulin deficiency. FA, fatty acid; HMG, 3-hydroxy-3-methylglutaryl; HSL, hormone-sensitive lipase.

In muscle, insulin stimulates glucose uptake and favors protein synthesis through phosphorylation of a serine/threonine protein kinase known as mammalian target of rapamycin. In addition, insulin favors lipid storage in muscle as well as in adipose tissue. As discussed later, insulin deficiency leads to glucose accumulation in blood, a decrease in lipid storage, and protein loss, resulting in negative nitrogen balance and muscle wasting.

Long-term effects—Sustained insulin stimulation enhances the synthesis of lipogenic enzymes and the repression of gluconeogenic enzymes. The growth-promoting and mitogenic effects of insulin are long-term responses mediated through the **MAPK pathway**. Both MAPK and particularly the chronic activation of extracellular receptor kinase by insulin receptor binding lead to excessive cell growth. Although this pathway of insulin action is not as well elucidated as the effects that are mediated through the activation of IRS, evidence is now surfacing on the pathophysiologic effects of chronic elevations of insulin on specific cell populations.

Insulin levels are high during the development and early stages of type 2 diabetes. Chronic hyperinsulinemia has been linked to increased risk of cancers including endometrium, post-menopausal breast, colon, and kidney. Conditions that cause elevated insulin levels include high waist circumference, excess visceral fat, high waist-to-hip ratio, high body mass index, sedentary lifestyle, and high energy intake. In addition, the proliferative effects of chronic hyperinsulinemia influence vascular

smooth muscle cells, which are responsible for the maintenance of vascular tone. These cells play an important role in the pathogenesis of several diseases, including hypertension, atherosclerosis, cardiovascular disease, and dyslipidemia, all of which are closely associated with insulin resistance and hyperinsulinemia. The molecular basis of insulin's effect on vascular smooth muscle cell growth and its association with hypertension are currently unclear.

Glucagon

GLUCAGON SYNTHESIS

Glucagon, is a 29–amino acid polypeptide hormone secreted by the α -cells of the islets of Langerhans, which plays an important role in the regulation of glucose homeostasis by producing antagonistic effects on insulin action. The primary sequence of glucagon is almost perfectly conserved among vertebrates, and it is structurally related to the secretin family of peptide hormones. Glucagon is synthesized as proglucagon and then proteolytically processed to yield glucagon. The prohormone proglucagon is expressed in the pancreas, and also in other tissues, such as enteroendocrine cells in the intestinal tract and in the brain. However, the processing of the prohormone differs among tissues. The 2 main products of proglucagon processing are glucagon in the α -cells of the pancreas and GLP-1 in the intestinal cells. GLP-1 is produced in the intestine in response to a high concentration of glucose in the intestinal lumen. GLP-1 is known as an incretin, a mediator that amplifies insulin release from the β -cell in response to a glucose load. Glucagon has a short half-life (5–10 minutes) and is degraded mostly in the liver. Plasma glucagon concentrations average 50–100 ng/L (50–100 pg/mL).

REGULATION OF GLUCAGON RELEASE

The mechanisms involved in the regulation and stimulus-secretion coupling of glucagon release are not as well understood as those for insulin. Glucagon release is inhibited by hyperglycemia (high blood glucose levels) and stimulated by hypoglycemia (low blood glucose levels). A meal rich in carbohydrates suppresses glucagon release and stimulates insulin release from the β -cells through intestinal release of GLP-1. Somatostatin also inhibits glucagon release. High amino acid levels following an amino acid-rich meal stimulate glucagon release. Epinephrine stimulates release of glucagon through a β_2 -adrenergic mechanism (while it suppresses insulin release from β -cells through an α_2 -adrenergic mechanism). Vagal (parasympathetic) stimulation increases glucagon release.

PHYSIOLOGIC EFFECTS OF GLUCAGON

The principal target tissues for glucagon are the liver and adipose tissue. The role of glucagon receptors in the rest of the tissues is still unclear. Glucagon's main physiologic effect is to increase plasma glucose concentrations by stimulating de novo hepatic glucose production through gluconeogenesis and glycogen breakdown; overall, these actions counteract the effects of insulin (Figure 7–5). Glucagon mediates its effects by binding to the glucagon G_s protein–coupled receptor.

GLUCAGON RECEPTOR

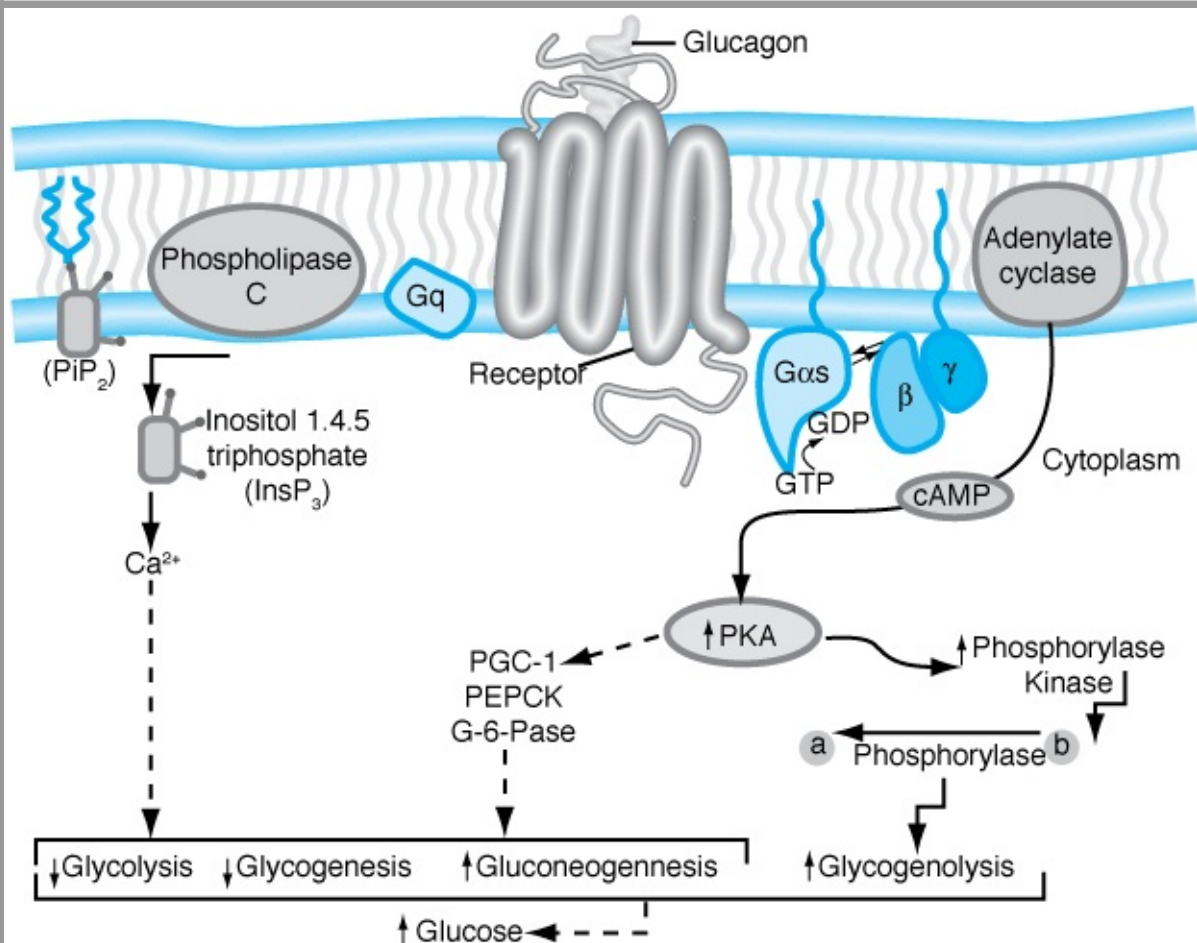
The glucagon and GLP-1 peptide receptors belong to a family of GPCRs that include those for secretin, calcitonin, vasoactive intestinal polypeptide, parathyroid hormone, and growth hormone–releasing factor. The glucagon receptor is expressed in liver, pancreatic β -cells, kidney, adipose tissue, heart, and vascular tissues, as well as in some regions of the brain, stomach, and adrenal glands. Glucagon binding activates adenylate cyclase and results in intracellular accumulation of cAMP, mobilization of intracellular Ca^{2+} , protein kinase A activation, and phosphorylation of effector proteins. The glucagon-receptor complex undergoes endocytosis into intracellular vesicles, where glucagon is degraded.

GLUCAGON EFFECTS AT TARGET ORGANS



The principal physiologic effects of glucagon are mediated in the liver. Glucagon stimulates hepatic glucose output by stimulating glycogen breakdown and gluconeogenesis and decreasing glycolysis (Figure 7–6). The key enzymatic steps regulated by glucagon that mediate the stimulation of hepatic glucose output are summarized in Table 7–3. The effects of glucagon on adipose tissue are relevant primarily during periods of stress or food deprivation, particularly when insulin release is suppressed.

Figure 7–6.



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Glucagon receptor–mediated cellular effects. Glucagon binds to G protein–coupled receptor (GPCR) on target cells. The principal effects of glucagon are mediated in hepatocytes where glucagon through activation of adenylate cyclase, elevation in cAMP leads to increased protein kinase A activity leading to phosphorylation of enzymes responsible for control of glucose metabolism. The ultimate result is an increase in hepatic glucose production through increased gluconeogenesis and glycogenolysis. G-6-Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; PGC-1, peroxisome proliferator–activated receptor-coactivator-1; PIP2, phosphatidylinositol 4,5-biphosphate. (Reproduced, with permission, from Guoqiang Jiang, Bei B. Zhang. Glucagon and regulation of glucose metabolism. *Am J Physiol Endocrinol Metab*. 2003;284:E671–E678. Figure 1 of article.)

Table 7–3. Effects of Glucagon on Hepatic Glucose Metabolism

Effect on target enzyme	Metabolic response
Increased expression of glucose-6-phosphatase	Frees glucose to enter the circulation
Suppression of glucokinase	Decreases glucose entry into the glycolytic cascade
Phosphorylation (activation) of glycogen phosphorylase	Stimulates glycogenolysis
Inhibition of glycogen synthase	Inhibits glycogen synthesis
Stimulation of phosphoenolpyruvate carboxykinase expression	Stimulates gluconeogenesis
Inactivation of phosphofructokinase-2 (PFK-2) and	

activation of fructose-6-phosphatase. PFK-2 is the kinase activity and fructose-2,6-bisphosphatase (F-2,6-BPase) is the phosphatase activity of the bifunctional regulatory enzyme, phosphofructokinase-2/fructose-2,6-bisphosphatase (PFK-2/F-2,6-BPase).



Inhibits glycolysis

Stimulates gluconeogenesis	
Suppression of activity of the pyruvate kinase	Decreases glycolysis

In the adipocyte, glucagon stimulates protein kinase A–mediated phosphorylation (activation) of hormone-sensitive lipase, the enzyme that breaks down triglycerides (stored fat) into diacylglycerol and free fatty acids, releasing them into the circulation. Glycerol released into the circulation can be utilized in the liver for gluconeogenesis or for reesterification. Free fatty acids are used as fuel by most tissues, predominantly skeletal muscle and liver. In the liver, free fatty acids are used for reesterification or they can undergo β -oxidation and conversion into ketone bodies. Thus, ketogenesis is regulated by the balance between the effects of glucagon and insulin at their target organs. The importance of this balance is evident during insulin deficiency and glucagon excess, as seen in uncontrolled diabetes (discussed later).

Somatostatin

Somatostatin is a 14–amino acid peptide hormone produced by the δ -cells of the pancreas. Its release is stimulated by high-fat, high-carbohydrate, and particularly protein-rich meals, and is inhibited by insulin. Somatostatin has a generalized inhibitory effect on virtually all gastrointestinal and pancreatic exocrine and endocrine functions. The regulation of its release is not well studied because of the difficulty in analyzing the small number of islet cells that produce this hormone. Further, the importance of endogenous paracrine inhibition of insulin and glucagon release is not well established. Because δ -cells are located in the periphery of the β -cells and because blood flows from the center of the islets of Langerhans toward the periphery, pancreatic somatostatin may have a limited contribution toward physiologic control of insulin and glucagon release. However, exogenous administration of somatostatin does suppress the release of both insulin and glucose and is used in the clinical setting for the management of insulin-or glucagon-producing tumors.

Pancreatic Polypeptide

Pancreatic polypeptide is a 36–amino acid peptide hormone that belongs to a peptide family including neuropeptide Y and peptide YY. It is produced in the endocrine type F cells located in the periphery of pancreatic islets and is released into the circulation after ingestion of food, exercise, and vagal stimulation. The effects of pancreatic polypeptide include inhibition of pancreatic exocrine secretion, gallbladder contraction, modulation of gastric acid secretion, and gastrointestinal motility. Pancreatic polypeptide crosses the blood-brain barrier and has been postulated to play a role in regulating feeding behavior.

Amylin

Amylin, or islet amyloid polypeptide, is a 37–amino acid peptide hormone that belongs to the calcitonin family, which includes calcitonin, calcitonin gene-related peptide, and adrenomedullin. Amylin is synthesized as a small precursor, undergoes post-translational modification (amidation), is stored in β -granules, and is released along with insulin and C-peptide. Plasma amylin concentrations increase after a meal or glucose infusion. Amylin appears to work with insulin to regulate plasma glucose concentrations in the bloodstream, suppressing the postprandial secretion of glucagon and slowing gastric emptying. In muscle, amylin opposes glycogen synthesis and activates glycogenolysis and glycolysis, thereby increasing lactate production. Circulating amylin is increased in obesity, hypertension, and gestational diabetes; it is low or absent in type 1 diabetes mellitus. In type 2 diabetes, the secretion of amylin is impaired before that of insulin. Amylin is the main component of pancreatic islet amyloid, found in the vast majority of patients with noninsulin-dependent (type 2) diabetes mellitus, and is thought to contribute to destruction of the pancreatic β -cell. Amylin binds to a variant of the calcitonin GPCR. The modified calcitonin receptor has higher affinity for amylin, an effect mediated by transmembrane proteins known as receptor activity-modifying proteins (RAMPs).

DISEASES ASSOCIATED WITH PANCREATIC HORMONES

Hormone-Producing Tumors

Excess pancreatic hormone production and release are usually due to hormone-producing tumors, with insulinoma being the most frequent. Insulinomas produce excessive amounts of insulin, and patients present with episodes of hypoglycemia, confusion, aggressiveness, palpitations, sweating, convulsions, and even unconsciousness. These symptoms are mostly observed before breakfast and following physical exercise. The compensatory or counterregulatory response of the body includes the release of catecholamines, glucagon, cortisol, and growth hormone.

Glucagonomas are unusual tumors that may produce symptoms of diabetes. Excessive glucagon production by the tumor may also result in an overall catabolic effect on fat and muscle, leading to severe weight loss and anorexia. Somatostatinoma is a rare tumor that may cause moderate diabetes.

Diabetes Mellitus

The most common disease resulting from impaired pancreatic hormone release is diabetes mellitus. The 2 forms of diabetes, type 1 and type 2, are characterized by impaired insulin release. Type 1 diabetes, also known as insulin-dependent diabetes mellitus, is the result of β -cell destruction. It accounts for 2–5% of cases and it occurs more frequently in younger people, hence its other name, juvenile-onset diabetes. Type 1 diabetes is characterized by the development of ketoacidosis in the absence of insulin therapy. Type 2 diabetes results from a loss of normal regulation of insulin secretion and accounts for more than 90% of diabetes cases. It is usually associated with obesity in adults and is characterized by mild hyperglycemia. It rarely leads to ketoacidosis. Type 2 diabetes is often part of "syndrome X" or "insulin resistance syndrome," a metabolic syndrome characterized by hypertension, atherosclerosis, and central obesity.

The diagnosis of diabetes is based on plasma glucose levels obtained in the fasting state (greater than 126 mg/dL). Diabetes can also present as random plasma glucose levels higher than 200 mg/dL in association with symptoms of diabetes (polyuria, polydipsia, and polyphagia) or as persistent elevations in plasma glucose levels following an oral glucose load (greater than 200 mg/dL, 2 hours after glucose ingestion). The pathophysiology of the disease involves impaired entry of glucose into the cells and accumulation of glucose in the blood. This process results in increased plasma osmolarity and urinary loss of glucose, accompanied by excess loss of water and sodium (**polyuria**). The resulting dehydration triggers compensatory mechanisms such as thirst (**polydipsia**). The inability of the cells to utilize glucose resembles a state of cellular starvation, stimulating hunger (**polyphagia**) and triggering the activation of compensatory responses to increase the release and availability of fuel substrates through activation of lipolysis and proteolysis. Lack of insulin results in increased circulating levels of free fatty acids and gluconeogenic amino acids. These exceed the liver's capacity for their metabolic utilization, leading to the buildup of ketone bodies in the blood (diabetic ketoacidosis) and their urinary excretion.

Diabetic Ketoacidosis



Diabetic ketoacidosis is an acute pathologic event characterized by elevated blood glucose levels and ketone bodies and metabolic acidosis, resulting directly from decreased insulin availability and simultaneous elevations of the counterregulatory hormones glucagon, catecholamines, cortisol, and growth hormone. Diabetic ketoacidosis is precipitated by infections, discontinuation of or inadequate insulin use, new-onset (untreated) diabetes, and other events such as the stress associated with surgery.

The insulin deficiency causes glucose to accumulate in the blood. As mentioned earlier, insulin mediates the majority of the body's glucose disposal through its effects on skeletal muscle. In diabetic ketoacidosis, gluconeogenesis in the liver proceeds unopposed by the physiologic presence of insulin. The excess blood glucose increases osmolarity, which, if severe, can result in diabetic coma. The lack of insulin and the high levels of counterregulatory hormones glucagon, epinephrine, and cortisol combine to increase the activity of hormone-sensitive lipase, increase the release of free fatty acids, and decrease the activity of acetyl-CoA carboxylase, thereby impairing the reesterification of free fatty acids and promoting fatty acid conversion into ketone bodies (Figure 7–6). Fatty acids released into the circulation undergo β -oxidation to acetyl-CoA in the liver. Acetyl-CoA condenses with oxaloacetate to form citrate in the entry step to the Krebs cycle (citric acid cycle or tricarboxylic cycle). During insulin deficiency and excess glucagon, oxaloacetate is preferentially used for gluconeogenesis, thus decreasing its availability for condensation with acetyl-CoA. As a result, acetyl-CoA is diverted from entering the Krebs cycle and is used preferentially for ketone body formation or ketogenesis, the process by which fatty acids are transformed into acetoacetate and 3-hydroxybutyrate in hepatocyte mitochondria. The steps involved in ketogenesis are β -oxidation of fatty acids to acetyl-CoA, formation of acetoacetyl-CoA, and conversion of acetoacetyl-CoA to 3-hydroxy-3-methylglutaryl-CoA and then to acetoacetate, which is then reduced to 3-hydroxybutyrate. The enzymes involved in

ketogenesis are summarized in Table 7–4. Acetoacetate can be spontaneously decarboxylated to acetone, a highly fat-soluble compound that is excreted slowly by the lungs and is responsible for the fruity odor of the breath of individuals with diabetic ketoacidosis.

Table 7–4. Three Principal Enzymes Involved in Ketogenesis

Enzyme	Tissue	Function
Hormone-sensitive lipase	Adipocytes	Breaks down triglycerides, releasing fatty acids into the circulation
Acetyl-CoA carboxylase	Liver	Catalyzes the conversion of acetyl-CoA to malonyl-CoA, the primary substrate of fatty acid biosynthesis
HMG-CoA synthase	Liver	Involved in the conversion of acetyl-CoA to acetoacetate

CoA, coenzyme A, HMG, 3-hydroxy-3-methylglutaryl.

During diabetic ketoacidosis, high amounts of ketone bodies are released into the blood, and a high ratio (3:1 or higher) of 3-hydroxybutyrate to acetoacetate is generated because of the highly reduced state of hepatic mitochondria. These ketone bodies can freely diffuse across cell membranes and serve as an energy source for extrahepatic tissues including the brain, skeletal muscle, and kidneys. Ketone bodies are filtered and reabsorbed in the kidney. At physiologic pH, ketone bodies, with the exception of acetone, dissociate completely. The resulting liberation of H^+ from ketone body metabolism exceeds the blood's buffering capacity, leading to metabolic acidosis with an increased anion gap. If severe, this condition can lead to coma.

Type 2 Diabetes

Type 2 diabetes is the result of inadequate responsiveness of the β -cells to glucose, which is later followed by a net reduction in β -cell mass and a decreased responsiveness of peripheral tissues to insulin

action. Patients with type 2 diabetes secrete normal amounts of insulin during fasting, but in response to a glucose load (or a meal), they secrete considerably less insulin (70%) than nondiabetic patients. In addition to a reduction in insulin release, the pattern of insulin release is also altered following a meal, with pulses that are significantly smaller, sluggish, and erratic, particularly after dinner. This abnormality results in significantly higher levels of fasting glucose in these patients.

Regardless of the etiology (eg, abnormalities in glucose transport; abnormal insulin synthesis, processing, storage, or secretion), the earliest physiologic indication of β -cell dysfunction is a delay in the acute insulin response to glucose. The defect in the initial response to a glucose load leads to an excessive rise in plasma glucose, which in turn produces a compensatory and exaggerated second-phase hyperinsulinemic response. This initial period of sustained hyperinsulinemia downregulates the insulin receptors, decreasing the sensitivity of tissues to insulin action and producing a state of insulin resistance. The main pathologic defects in diabetes are excessive hepatic glucose production, defective β -cell secretory function, and peripheral insulin resistance.

Insulin Resistance

Insulin resistance is the inability of peripheral target tissues to respond properly to normal circulating concentrations of insulin. To maintain euglycemia, the pancreas compensates by secreting increased amounts of insulin. In patients with type 2 diabetes, insulin resistance precedes the onset of the disease by several years. Compensating for insulin resistance by an increase in insulin release is effective only temporarily. As insulin resistance increases, impaired glucose tolerance develops. Ultimately, failure or exhaustion of the pancreatic β -cell results in decreased insulin secretion. The combination of insulin resistance and impaired β -cell function characterizes clinical type 2 diabetes. Exercise has been demonstrated to increase glucose transport in skeletal muscle and to decrease insulin resistance in patients with type 2 diabetes. The increase in glucose transport resulting from exercise is not mediated through the insulin receptor. The signaling pathways involve increased cytosolic Ca^{2+} and the enzyme AMP-activated protein kinase. AMP-activated protein kinase is activated during exercise and has been termed a master metabolic switch because it phosphorylates key target proteins that control flux through metabolic pathways.

Clinical Evaluation of Diabetes

According to the American Diabetes Association, hyperglycemia (fasting glucose at least 126 mg/dL or random glucose at least 200 mg/dL) is the diagnostic criterion and a main prognostic parameter in diabetes. The slow post-translational and nonenzymatic glycation of hemoglobin provides a measure of chronic glycemia. Values of glycosylated hemoglobin are strongly correlated with the average blood glucose level over the preceding 1–3 months and can be obtained in the nonfasted state. The measurement of glycosylated hemoglobin is used to monitor glycemic control in patients with known diabetes.

Treatment of the Diabetic Patient

The goal of therapy is tight glycemic control, which has been shown to delay the development of microvascular complications associated with diabetes. Glucose homeostasis results from the regulated balance among hepatic glucose release, dietary glucose absorption, and skeletal muscle and adipose tissue glucose uptake and disposal. All 3 of these components have been targeted for pharmacologic treatment of diabetic patients. Some of the approaches used, in addition to conventional insulin, warrant mention because they affect the physiologic mechanisms of pancreatic hormone release and target organ effects on the control of glucose.

Sulfonylureas—Sulfonylureas increase insulin release by closing K^+ ATP channels in the pancreatic β -cell membrane. This action is mediated by binding of the drug to the sulfonylurea receptor subunit of the channel. Because type 1 diabetes is characterized by β -cell destruction, this approach is ineffective in those patients.

Biguanides—Biguanides such as metformin reduce hepatic glucose output (primarily through inhibition of gluconeogenesis and, to a lesser extent, glycogenolysis) and increase insulin-stimulated glucose uptake in skeletal muscle and adipocytes. In insulin-sensitive tissues (such as skeletal muscle), metformin facilitates glucose transport by increasing tyrosine kinase activity in insulin receptors and enhancing glucose transporter trafficking to the cell membrane.

Alpha-glucosidase inhibitors—Alpha-glucosidase inhibitors delay the intestinal absorption of carbohydrates through inhibition of the brush-border enzymes that hydrolyze polysaccharides to glucose.

Thiazolidinediones—Thiazolidinediones reduce insulin resistance in skeletal muscle by activation of the γ -isoform of the peroxisome proliferator-activated receptor in the nucleus, thereby affecting the transcription of several genes involved in glucose and lipid metabolism and energy balance. Among the genes that are affected are those that code for lipoprotein lipase, fatty acid transporter protein, adipocyte fatty acid-binding protein, fatty acetyl-CoA synthase, malic enzyme, glucokinase, and the GLUT-4 glucose transporter.

Glucagon-like peptide—GLP-1 amplifies glucose-induced insulin release. In addition, GLP-1 increases insulin biosynthesis and insulin gene expression and has tropic and antiapoptotic effects on the pancreatic β -cells.

KEY CONCEPTS



Insulin release is under nutrient, neural, and hormonal regulation.



The pancreatic β -cell functions as a glucose sensor in the process of insulin release.



The PI_3 -kinase pathway mediates most of insulin's metabolic effects, and the MAPK pathway is mostly involved in mediating the proliferative responses.



The principal metabolic effects of insulin are to increase glucose utilization in skeletal muscle, suppress hepatic glucose production, and inhibit lipolysis.



Glucagon antagonizes insulin's effects by stimulating hepatic glucose release.



A disruption in the balance of insulin and glucagon leads to ketogenesis and hyperosmolar coma.

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OBJECTIVES

- ▶ *Describe the physiologic functions of the principal components of the male reproductive system.*
- ▶ *Describe the endocrine regulation of testicular function by gonadotropin-releasing hormone, follicle-stimulating hormone, luteinizing hormone, testosterone, and inhibin.*
- ▶ *Identify the cell of origin for testosterone, its biosynthesis, mechanism of transport within the blood, metabolism, and clearance. List other physiologically produced androgens.*
- ▶ *List the target organs or cell types, the cellular mechanisms of action, and the physiologic effects of testosterone.*
- ▶ *Describe spermatogenesis and the role of different cell types in this process.*
- ▶ *Understand the neural, vascular, and endocrine factors involved in the erection and ejaculation response.*
- ▶ *Compare and contrast the actions of testosterone, dihydrotestosterone, estradiol, and Müllerian inhibitory factor in the process of sexual differentiation.*
- ▶ *Identify the causes and consequences of androgen oversecretion and undersecretion in prepubertal and postpubescent adult males.*

MALE REPRODUCTIVE SYSTEM: INTRODUCTION

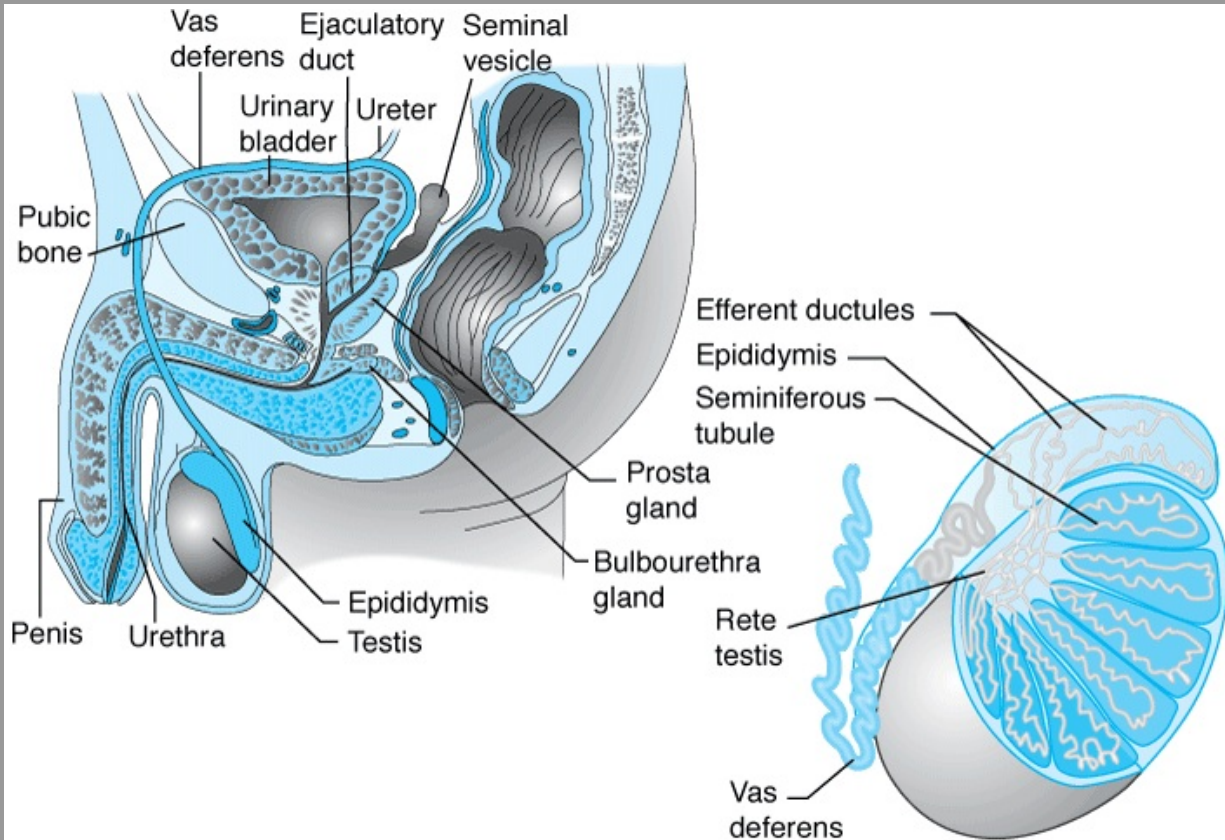
In utero sexual differentiation, maturation, spermatogenesis, and ultimately reproduction are all functions of the male reproductive system that are under endocrine regulation. The 2 principal functions of the testicles, the adult male sex organs, are the production of sperm and the synthesis of testosterone. These processes ensure fertility and maintain male sexual characteristics, or virility. Testicular function is under central nervous system control in a classic neuroendocrine feedback loop with the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) as the key hormonal signals. These gonadotropins, as discussed in Chapter 2, are under the

influence of hypothalamic gonadotropin-releasing hormone (GnRH) stimulation. Additional paracrine, neural, and endocrine factors contribute to the complex regulation of the hypothalamic-pituitary-gonadal axis. This chapter discusses the basic principles of endocrine regulation of the male reproductive system.

FUNCTIONAL ANATOMY

The male reproductive organs include the testes (the central male sex organs), the vas deferens, the ejaculatory ducts, the penis, and the accessory glands, which include the prostate and bulbourethral glands (Figure 8–1). The testes consist of numerous lobules made of convoluted tubes (tubuli seminiferi) supported by loose connective tissue. The seminiferous tubules represent approximately 80–85% of the testicular mass. Therefore, a small testicular volume (<15 mL) suggests a significant impairment of the seminiferous tubules.

Figure 8–1.



Source: Molina PE: *Endocrine Physiology, 3rd Edition*:
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Functional anatomy of the male reproductive system. The male reproductive organs include the testes, the ductus deferentes, the ejaculatory ducts, the penis, and the accessory glands, which include the prostate and bulbourethral glands. The testes consist of numerous

lobules made of tubuli seminiferi supported by loose connective tissue. The tubuli seminiferi are united to form larger ducts called tubuli recti. These larger tubules form a close anastomosing network of tubes called the rete testis, terminating in the ductuli efferentes. The tubular network carries the seminal fluid from the testis to the epididymis, from where spermatozoa enter the vas deferens and through the ejaculatory ducts enter the urethra. The penis is composed of two functional compartments: the paired corpora cavernosa and the corpus spongiosum. The corpora cavernosa forms the greater part of the substance of the penis and consists of bundles of smooth muscle fibers intertwined forming trabeculae, and containing numerous arteries and nerves. (Reproduced, with permission, from Widmaier EP, Raff H, Strang KT (Eds). *Vander's Human Physiology: The Mechanisms of Body Function*. 11 ed. Figures 17–5 and 17–6. McGraw-Hill; 2007.)

The **Leydig cells**, embedded in the connective tissue are the endocrine cells responsible for the production of the most important circulating androgen, **testosterone**. The tubules consist of a basement layer lined with epithelial cells forming the walls of the seminiferous tubules. These walls are lined with primitive germ cells (spermatogonia) and with **Sertoli cells**.



Sertoli cells form tight junctions that functionally divide the seminiferous tubules into 2 compartments or environments for the development of spermatozoa. The basal compartment below the tight junctions has contact with the circulatory system and is the space in which spermatogonia develop to primary spermatocytes. The tight junctions open at specific times and allow progression of spermatocytes to the adluminal compartment, where meiosis is completed. In the adluminal compartment, spermatocytes are sequestered or protected by a "blood-testis" barrier formed by tight junctions between the Sertoli cells. The principal functions of Sertoli cells are as follows:

- Provide support for germ cells, forming an environment in which they develop and mature
- Provide the signals that initiate spermatogenesis and sustain

spermatid development

- Regulate pituitary gland function and, in turn, control of spermatogenesis.

Together, the Sertoli and Leydig cells are the 2 principal cell types responsible for testicular function.

The tubuli seminiferi are united to form larger ducts called tubuli recti. These larger tubules form a close anastomosing network of tubes called the rete testis, terminating in the ductuli efferentes (Figure 8–1). This tubular network carries the seminal fluid containing sperm from the testis to the epididymis; from here, spermatozoa enter the vas deferens and then the ejaculatory ducts. The ejaculatory ducts move semen (sperm-containing fluid) into the urethra. In addition to serving as a route of transport, the tubular network or excretory system and the accessory organs play important roles in the production of sperm-containing semen through absorptive and secretory processes. As summarized in Table 8–1, absorptive and secretory processes contribute to the final composition of sperm-containing semen. Sperm account for approximately 10% of the volume of the ejaculated semen, which is composed of testicular and epididymal fluid together with the secretory products of male accessory glands. Most of the volume of the ejaculate is formed by the seminal vesicles with the remainder consisting of epididymal fluids and secretions from the prostate gland and bulbourethral glands.

Table 8–1. Contribution of the Excretory System and Accessory Organs to Sperm Production

Organ	Function
Excretory system	
Efferent ductules, vas deferens, ejaculatory duct, urethra	

Movement of spermatozoa

Fluid reabsorption	
Epididymis	H⁺ secretion and acidification of luminal fluid Incapacitation of spermatozoa; glycoconjugation

Reservoir for mature spermatozoa

Phagocytosis
of aging
spermatozoa

Accessory glands

Seminal vesicle	Secretion and storage of fructose-rich product (preferred energy substrate for sperm), prostaglandins, ascorbic acid, fibrinogen-like and thrombin-like proteins
Prostate	Secretion and storage of fluid rich in acid phosphatase and protease (prostate-specific antigen)
Cowper glands	Secretion of mucus into the urethra upon arousal

The penis is composed of 2 functional compartments: the paired corpora cavernosa and the corpus spongiosum. The corpora cavernosa form the greater part of the substance of the penis and consist of bundles of smooth muscle fibers intertwined in a collagenous extracellular matrix. Interspersed within this parenchyma is a complex network of endothelial cell-lined sinuses, or lacunae, arteries, and nerve terminals. The penis is innervated by somatic and autonomic (both sympathetic and parasympathetic) nerve fibers. The somatic innervation supplies the penis with sensory fibers and supplies the perineal skeletal muscles with motor fibers. Autonomic nerves mediate vascular dilation leading to penile erection, stimulate prostatic secretions, and control smooth muscle contraction of the vas deferens during ejaculation.

The arterial blood supply to the male reproductive organs is predominantly derived from the superficial and deep external pudendal branches of the femoral artery, the superficial perineal branch of the internal pudendal artery, and the cremasteric branch from the inferior epigastric artery. Venous drainage follows the course of the corresponding arteries. Lymphatics drain into the inguinal lymph glands.

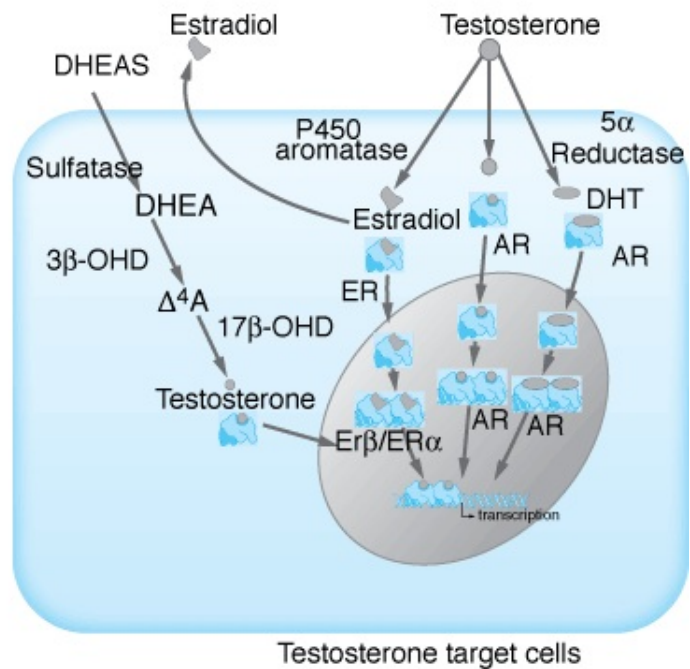
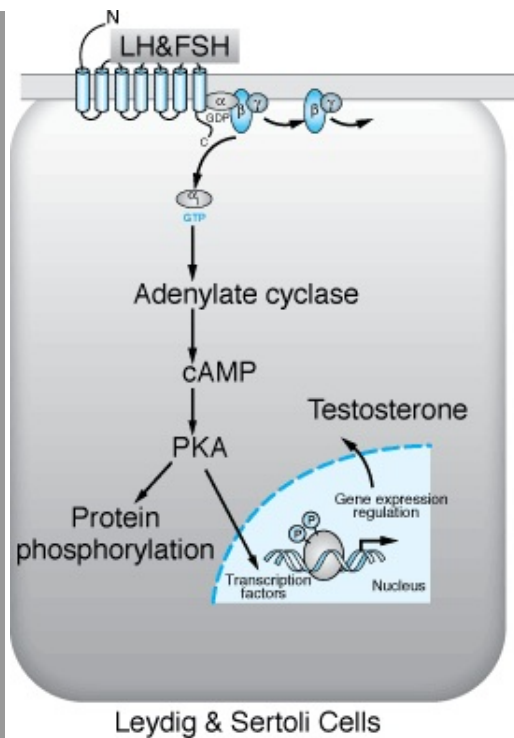
GONADOTROPIN REGULATION OF GONADAL FUNCTION



The primary functions of the Leydig and Sertoli cells are to produce the hormones involved in the regulation of reproductive function and virilization and to produce spermatozoa. These functions are regulated by the pituitary gonadotropins FSH and LH. LH and FSH circulate unbound in the plasma and have a half-life of 30 minutes (LH) and 1–3 hours (FSH). LH has higher amplitude fluctuations in plasma than FSH; FSH levels are more stable and show less variability.

The gonadotropins produce their physiologic responses by binding to cell membrane G_{α_s} protein–coupled receptors located in the Leydig and Sertoli cells. Gonadotropin binding to the G protein–coupled receptor activates adenylate cyclase and leads to an increase in the formation of cyclic adenosine monophosphate (cAMP) (Figure 1–5; Figure 8–2). The rise in intracellular cAMP results in the activation of protein kinase A and subsequent kinase-mediated protein phosphorylation, which mediates the cellular effects of the gonadotropins. This process is similar to that used for adrenocorticotrophic hormone (ACTH)–mediated stimulation of adrenal steroid hormone production (discussed below).

Figure 8–2.



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Receptor-mediated effects of gonadotropins and testosterone at target tissues. **A.** Model of signal transductional pathways of the gonadotropin receptor. Upon binding of FSH to the FSH receptor, the G_{α_s} subunit dissociates. Together with guanosine triphosphate (GTP), this complex directly activates adenylate cyclase, thereby leading to cyclic adenosine monophosphate (cAMP) synthesis. cAMP activates protein kinase A (PKA) producing the dissociation of the catalytic subunit from the regulatory subunit. The active catalytic site of PKA can activate proteins by phosphorylation and in the nucleus it can phosphorylate transcription factors such as cAMP response element-binding protein (CREB) leading to gene transcription. **B.** Testosterone (a steroid hormone) enters the cell by passive diffusion. Testosterone can be converted to dihydrotestosterone (DHT) by 5α -reductase and bind to the androgen receptor or it can be converted to 17β -estradiol by aromatase. 17β -estradiol can be released to act on a neighboring cell's estrogen receptors (paracrine mechanism), it can enter the circulation (endocrine effects) or it can bind to either the estrogen receptor α - or β and subsequently bind to the nucleus and affect transcription. Intracellular testosterone can arise from androstenedione (Δ^4A),

dehydroepiandrosterone (DHEA), or DHEA-sulfate (DHEAS). The desulfated DHEA is converted to androstenedione by 3β -hydroxysteroid dehydrogenase (3β -OHD) and androstenedione transformed into testosterone by 17β -hydroxysteroid dehydrogenase (17β -OHD). Testosterone, DHT, and estradiol all bind to cytosolic steroid receptors. The cytosolic androgen (and estrogen) receptor is complexed to regulatory proteins (heat-shock proteins). Hormone binding results in the dissociation of the heat-shock protein complex, dimerization of the receptor, nuclear translocation and DNA binding to regulatory elements. The final result is the activation of gene transcription.

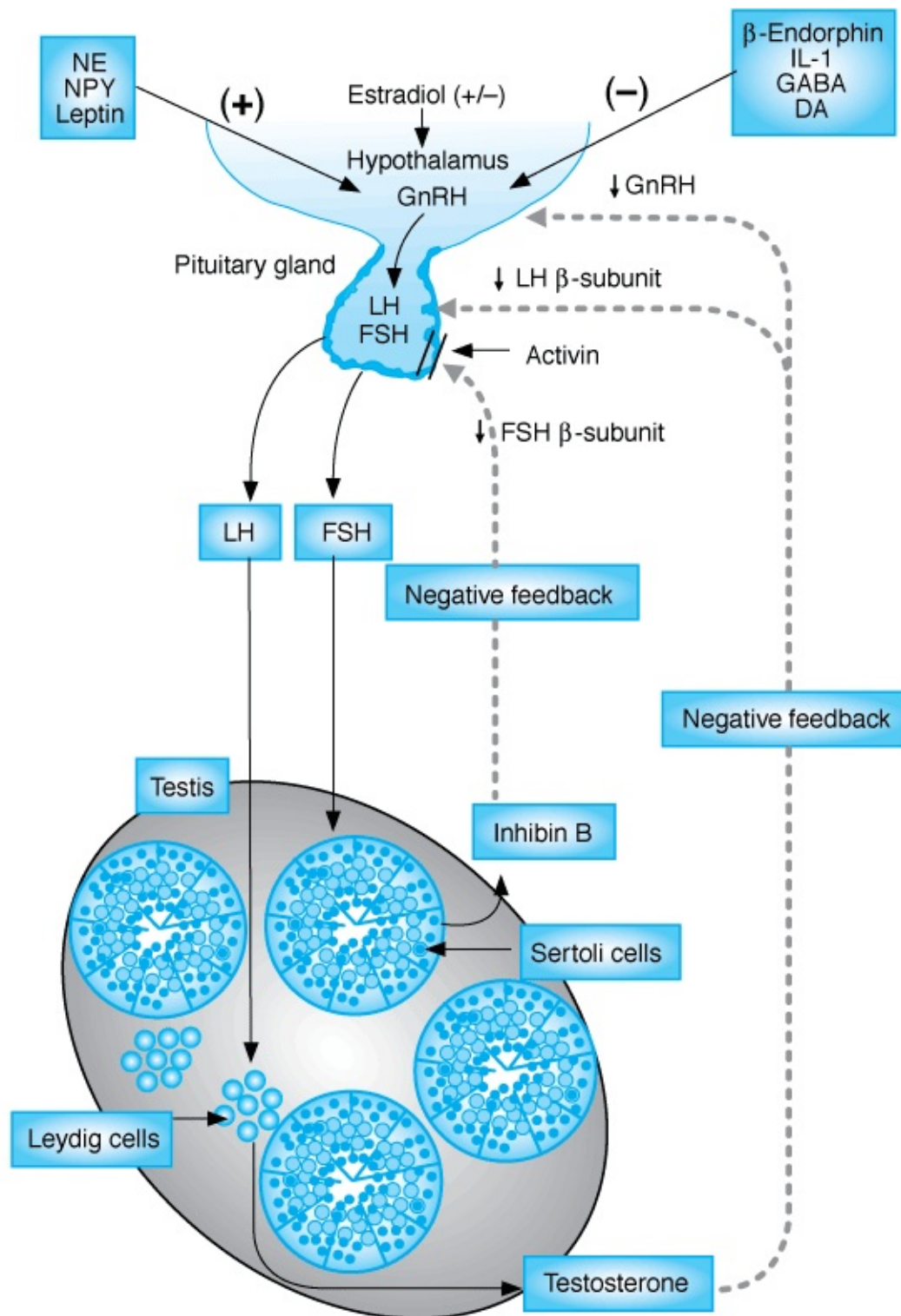
LH is the principal regulator of testosterone production by the Leydig cells. FSH plays an important role in the development of the immature testis, particularly by controlling Sertoli cell proliferation and seminiferous tube growth. Because the tubules account for about 80% of the volume of the testis, FSH is of major importance in determining testicular size, normally 4.1–5.2 cm in length and 2.5–3.3 cm in width in the adult male. FSH is important in the initiation of spermatogenesis during puberty. It is necessary for the production of androgen-binding protein by Sertoli cells and for the development of the blood-testis barrier.

Control of Gonadotropin Synthesis & Release



The overall regulation of FSH and LH secretion from the anterior pituitary was discussed in Chapter 3. These anterior pituitary glycoproteins consist of a common α -subunit and a unique β -subunit. The α -subunit is required for receptor binding, but the β -subunit confers the biologic specificity of LH and FSH. The synthesis and release of the gonadotropins are regulated by neuroendocrine signals from the central nervous system, particularly the hypothalamus through the pulsatile release of gonadotropin-releasing hormone (GnRH), as well as by circulating hormones or their metabolites, as illustrated in Figure 8–3.

Figure 8–3.



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Negative feedback regulation of gonadotropin synthesis and release.

Gonadotropin release from the anterior pituitary gland is controlled by the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator. Factors that stimulate GnRH release include norepinephrine (NE), neuropeptide Y (NPY), and leptin. Factors that inhibit GnRH release include β -endorphin, interleukin-1 (IL-1), γ -aminobutyric acid (GABA), and dopamine (DA) neurons. The activity of the pulse generator and the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are regulated by the gonadal hormones testosterone and inhibin B and by locally produced factors such as activin. Activin interacts with inhibin B, thus increasing FSH β -subunit synthesis. The negative feedback regulation exerted by testosterone is mediated by local conversion to 17β -estradiol.

Pulsatile release of GnRH is determined by a pulse generator. The neuronal structures and chemical interactions that result in pulsatile GnRH release have not been fully elucidated. However, several central and peripheral signals modulate the activity of GnRH-releasing neurons. Some of these signals are stimulatory to GnRH release, such as norepinephrine and neuropeptide Y; others are inhibitory, such as β -endorphin and interleukin-1; others are both stimulatory and inhibitory, such as the estrogen 17β -estradiol. GnRH binds to a G protein-coupled receptor (G_q and G_{11}) in the anterior pituitary gonadotrophs, activating phospholipase C and leading to the stimulation of inositol trisphosphate and diacylglycerol-protein kinase C. Activation of inositol trisphosphate leads to a rise in intracellular Ca^{2+} concentrations. GnRH also indirectly stimulates cAMP, contributing to control of LH and FSH release. The ratio between LH and FSH production is determined by the frequency of GnRH pulses. Synthesis of FSH β -subunit is highest in response to low-frequency pulses of GnRH and is suppressed at higher-frequency pulses of GnRH. Higher frequency and amplitude of GnRH stimulation increase LH β -subunit synthesis.

LH stimulates testosterone production by the Leydig cell. Testosterone released into the circulation inhibits LH release in a negative feedback loop. At the level of the hypothalamus testosterone inhibits GnRH release and at the anterior pituitary it decreases the gonadotropin-specific β -subunit synthesized (Figure 8–3). Testosterone reduces LH levels and the LH pulse amplitude. It is important to mention that most of

the inhibitory effect of testosterone on LH release is mediated by 17β -estradiol, a locally produced metabolite of testosterone aromatization (discussed later). The negative feedback inhibition of FSH release occurs at the level of the pituitary and is mediated mainly by inhibin B, a Sertoli cell–derived peptide (discussed later).

Inhibin-Activin

In addition to the traditional feedback inhibition of gonadotropin hormone release described above (Figure 8–3), locally produced factors (inhibin and activin) are also involved in regulation of gonadotropin release.

Inhibins are peptide hormones that belong to the transforming growth factor- β (TGF- β) superfamily of growth factors. Inhibin B is produced by Sertoli cells in response to FSH stimulation (discussed later), and it produces feedback inhibition of FSH β -subunit production (and thus, FSH release). An additional factor involved in the regulation of FSH release is activin. **Activin** is expressed in several tissues including the pituitary. Its role is to antagonize inhibin B action, resulting in stimulation of FSH release. Thus, in addition to the negative feedback inhibition produced by gonadal androgens, the interaction between inhibin and activin contributes to the regulation of gonadotropin release.

GONADAL FUNCTION



The 2 principal physiologic functions of the testes—the production of hormones involved in sexual differentiation, maturation, and virilization and spermatogenesis—are closely related to each other. This chapter first discusses hormone production in the testes and then describes the process of spermatogenesis.

Gonadal Hormone Synthesis



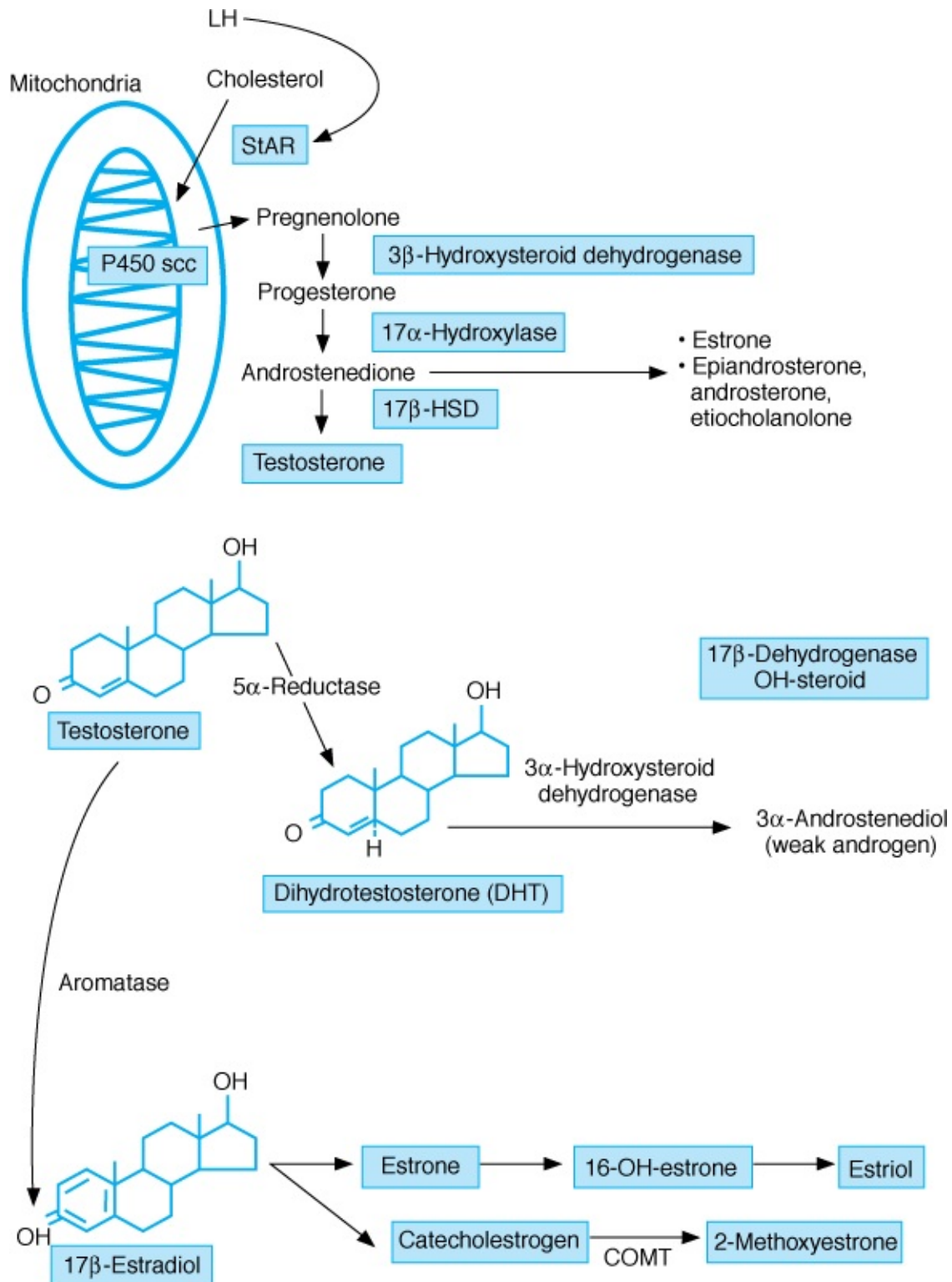
The 3 principal hormones produced by the testis are testosterone, estradiol, and inhibin.

TESTOSTERONE

Testosterone, produced by the Leydig cells, is the principal and most important testicular and circulating androgen. LH stimulates testosterone biosynthesis by increasing mobilization and transport of cholesterol into the steroidogenic pathway, an action that takes place within minutes; as well as by stimulating gene expression and activity of the steroidogenic enzymes (steroidogenic acute regulatory protein and P450 scc), a slower process that requires several hours (Figure 8–4). As discussed in Chapter 6, steroidogenic acute regulatory protein (also found in the adrenal cortex cells) has a key role in the transfer of cholesterol from the outer to the inner mitochondrial membrane, the first step in steroid hormone biosynthesis, for the conversion of cholesterol to pregnenolone. Pregnenolone diffuses out of the mitochondria to the smooth endoplasmic reticulum, where it is further metabolized by the action of 3β -hydroxysteroid dehydrogenase to progesterone. Progesterone in turn is converted by a 2-step process to androstenedione by the action of 17β -hydroxylase. The conversion of androstenedione to testosterone is catalyzed by 17β -hydroxysteroid dehydrogenase. Note that up until this last enzymatic reaction, the enzymatic steps involved in testosterone synthesis are similar to those involved in androstenedione synthesis by the adrenal glands (Figure 6–3). It is the activity of 17β -hydroxysteroid dehydrogenase and the enzymatic conversion of androstenedione to testosterone that are specific to the gonads.

Figure 8–4.





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Key steps in testosterone biosynthesis and metabolism.

Diagrammatic representation of the typical biochemical pathway and key enzymes involved in Leydig cell steroidogenesis that facilitate the biosynthesis of testosterone from the precursor cholesterol.

Testosterone finally diffuses out of the Leydig cell and reaches the interstitial space and the peripheral circulation. In target cells, testosterone can be converted to the most potent androgen, dihydrotestosterone (DHT), by 5α -reductase or to 17β -estradiol by aromatase. Testosterone, dehydroepiandrosterone (DHEA), androstenedione, and 17β -estradiol are degraded in the liver to 17-ketosteroids or polar metabolites that are excreted in the urine.

COMT, catechol-O-methyltransferase; HSD, hydroxysteroid dehydrogenase; scc, side chain cleavage; StAR, Steroidogenic Acute Regulatory protein.

INHIBIN

Inhibin is produced and released from the Sertoli cells in response to FSH stimulation and exerts both paracrine and endocrine responses. Inhibin belongs to the family of glycoprotein hormones and growth factors including TGF- β , Müllerian-inhibiting substance (MIS), and activin. Inhibins are heterodimer glycoproteins consisting of an α - and a β -subunit (β_A or β_B). Of the 2 forms of inhibin (α - β_A and α - β_B), inhibin B is the physiologically important form in males. Its main function is to suppress the secretion of FSH from the pituitary in a classic negative feedback endocrine mechanism through binding to a membrane-spanning serine/threonine kinase receptor. Inhibin B secretion appears to be dependent on Sertoli cell proliferation, maintenance, and spermatogenesis, all of these functions regulated by FSH. Inhibin B levels correlate with total sperm count and testicular volume and can be used as an index of spermatogenesis.

Activins, members of the same family of peptides as the inhibins, are homodimers or heterodimers of the β -subunit of the inhibins. They are synthesized in many adult tissues and cell types, and their receptors have been identified in those same tissues, a pattern more consistent with autocrine or paracrine mechanisms of action. At the pituitary, locally produced activin stimulates the synthesis of β -FSH (Figure 8–3).

ESTRADIOL

The conversion of testosterone to 17 β -estradiol is mediated by the enzyme **aromatase** (Figure 8–4). Aromatase is expressed in Leydig cells as well as in extragonadal tissues, particularly adipose tissue and placenta. The contribution of 17 β -estradiol produced by the Leydig cells to the total circulating estrogens in males is approximately 20%. The plasma concentration of estradiol in males is 2–3 ng/dL, and its production rate in blood is 25–40 μ g/24 hours.

Gonadal Hormone Metabolism

TESTOSTERONE METABOLISM

Most of the testosterone released into the circulation is bound to plasma proteins, primarily sex hormone-binding globulin (SHBG) and albumin (44 and 54%, respectively). In the testes, testosterone is bound to androgen-binding protein, a protein with great similarity to SHBG and a product of the same gene. Although SHBG is synthesized predominantly in the liver and released into the systemic circulation, androgen-binding protein is synthesized by Sertoli cells and is released into the lumen of the seminiferous tubules. SHBG is expressed in several tissues, including brain, placenta, and testes. SHBG also appears to function as part of a newly identified steroid-signaling system that is independent of the cytosolic androgen receptor. Unlike the effects mediated through binding to the intracellular steroid receptor produced by testosterone, SHBG interacts with a membrane form of the receptor and triggers cAMP signaling pathways. The physiologic relevance of the effects mediated through this pathway has not been elucidated.



At its target cells, testosterone can either have a direct androgen receptor–mediated effect or it can be metabolized to either 17β -estradiol through the action of aromatase or to 5α -**dihydrotestosterone (DHT)** through the action of 5α -**reductase** (Figure 8–4).

TESTOSTERONE TO ESTRADIOL CONVERSION

The majority of estradiol in males is produced in adipose tissue through the aromatization of testosterone and, to a smaller extent, of adrenal-derived androstenedione. Aromatase expression is directly related to the degree of adiposity; it is dependent on cytokine stimulation and requires the presence of glucocorticoids. Although some of the 17β -estradiol produced in peripheral tissues is released into the circulation, not all estrogens produced from testosterone are involved in mediating endocrine responses. Some are involved in intracrine regulation of physiologic responses through estrogen receptor stimulation (Figure 8–2). An example is the negative feedback regulation of GnRH in the hypothalamus and of gonadotropins in the anterior pituitary by testosterone. Another important example is the effect of testosterone on bones, where epiphysial closure is mediated through osteoblast and chondroblast aromatase conversion of testosterone to estradiol. In addition, the production of estrogens in the brain plays an important role in the masculinization of the brain during development and the maintenance of sexual behavior in the adult.

In the liver, testosterone is converted to androstenedione, which is then reduced and conjugated (glucuronidation) to form 17-ketosteroids (Figure 8–4). A similar degradation pathway is used in the metabolism of dehydroepiandrosterone (DHEA) and androstenedione. After 17β -estradiol is produced by the testes or by the peripheral metabolism of testosterone, it is initially converted to estrone, which is converted into either catecholestrogens or 16α -hydroxyestrone. Catecholestrogens are degraded by catechol-O-methyltransferase (involved in catecholamine degradation; discussed in Chapter 6), while 16α -hydroxyestrone is converted to estriol before being conjugated in the liver and excreted by the kidney. Thus, the excretion of testosterone and its metabolites in the urine is about 50% in the form of 17-ketosteroids and 50% in the form of polar metabolites such as -diols, -triols, and conjugated forms.

TESTOSTERONE TO DHT CONVERSION

The conversion of testosterone to DHT in peripheral tissues, particularly in the skin, produces the most potent natural androgen (Figure 8–4). Two isoenzymes (type 1 and type 2) of 5α -reductase are involved in the conversion of testosterone to DHT. The type 2 isoenzyme generates 3 times as much DHT as the type 1 isoenzyme and plays a critical role during sexual differentiation. During puberty, the contribution of the type 2 isoenzyme decreases and the type 1 reductase plays a more important role in adult males. A small amount of DHT can enter the circulation (about 10% of the total testosterone in blood) and can therefore exert effects at target cells that do not express 5α -reductase activity (Figure 8–2). DHT is inactivated to the weak androgen 3α -androstenediol by the action of 3α -hydroxysteroid dehydrogenase. The enzymatic conversion of testosterone to DHT is irreversible. Because DHT is involved in some pathologic processes, the enzymatic conversion of testosterone to DHT has been used effectively as a target for pharmacologic interventions. Androgens stimulate the growth of prostate cancer and are also involved in benign prostatic hypertrophy. Finasteride, a 5α -reductase inhibitor, is currently used for the treatment of benign prostatic hyperplasia and prostate cancer.

ANDROGEN RECEPTOR–MEDIATED PHYSIOLOGIC EFFECTS



Both testosterone and DHT bind to identical androgen receptors on their target cells (Figure 8–2). The androgen receptor is a member of the nuclear receptor superfamily and, like all other nuclear receptors; it consists of 3 functional domains involved in transcriptional regulation, DNA binding, and ligand binding. The ligand-free inactive cytosolic receptor is an inactive oligomer complexed to heat-shock proteins. Upon hormone binding, the oligomeric complex dissociates, undergoes a conformational change, and translocates into the nucleus. In the nucleus, it binds as a homodimer to DNA androgen response elements in the promotor region of target genes and functions as a nuclear transcription factor influencing the transcription of target genes.

and mediating androgen action.

The physiologic effects that are mediated by testosterone and DHT are related. DHT is the most potent activator of the androgen receptor, and the DHT-activated androgen receptor has a longer half-life, prolonging androgen action, and amplifying the androgen signal. However, distinct physiologic responses can be attributed to each hormone (Table 8–2), in part determined by the localized conversion of testosterone to DHT. Testosterone controls sexual differentiation (development of the Wolffian ducts), libido (the biologic need for sexual activity and sexual function), pubertal growth of the larynx, anabolic effects in muscle, and stimulation of spermatogenesis. In contrast, DHT plays a major role in embryonic and pubertal external virilization (eg, development of male external genitalia, urethra, and prostate, and growth of facial and body hair) and contributes to male-pattern balding in individuals with a genetic predisposition for baldness. Most of the effects of DHT are intracrine; they are mediated at target cells expressing 5 α -reductase. However, as already mentioned a small amount enters the circulation (10% of testosterone levels) and may have some endocrine effects on cells that do not express 5 α -reductase.

Table 8–2. Specific Actions of Testosterone, Dihydrotestosterone, and Estradiol

Testosterone	DHT (5α-reductase activity)	17β-estradiol (aromatase activity)
Embryonic development of Wolffian duct–derived structures	Embryonic development of the prostate	Epiphyseal closure
Postpubertal secretory activity	Descent of the testes	Prevention of osteoporosis
Pubertal growth of larynx and deepening of voice	Phallic growth	Feedback regulation of GnRH secretion
Anabolic effects on muscle and erythropoiesis	Male-pattern balding	

Inhibition of breast development	Development of pubic and underarm hair	

Stimulation of spermatogenesis

Libido	Activity of sebaceous glands	

DHT, dihydrotestosterone; GnRH, gonadotropin-releasing hormone.

PHYSIOLOGIC EFFECTS OF ANDROGENS AT TARGET ORGANS

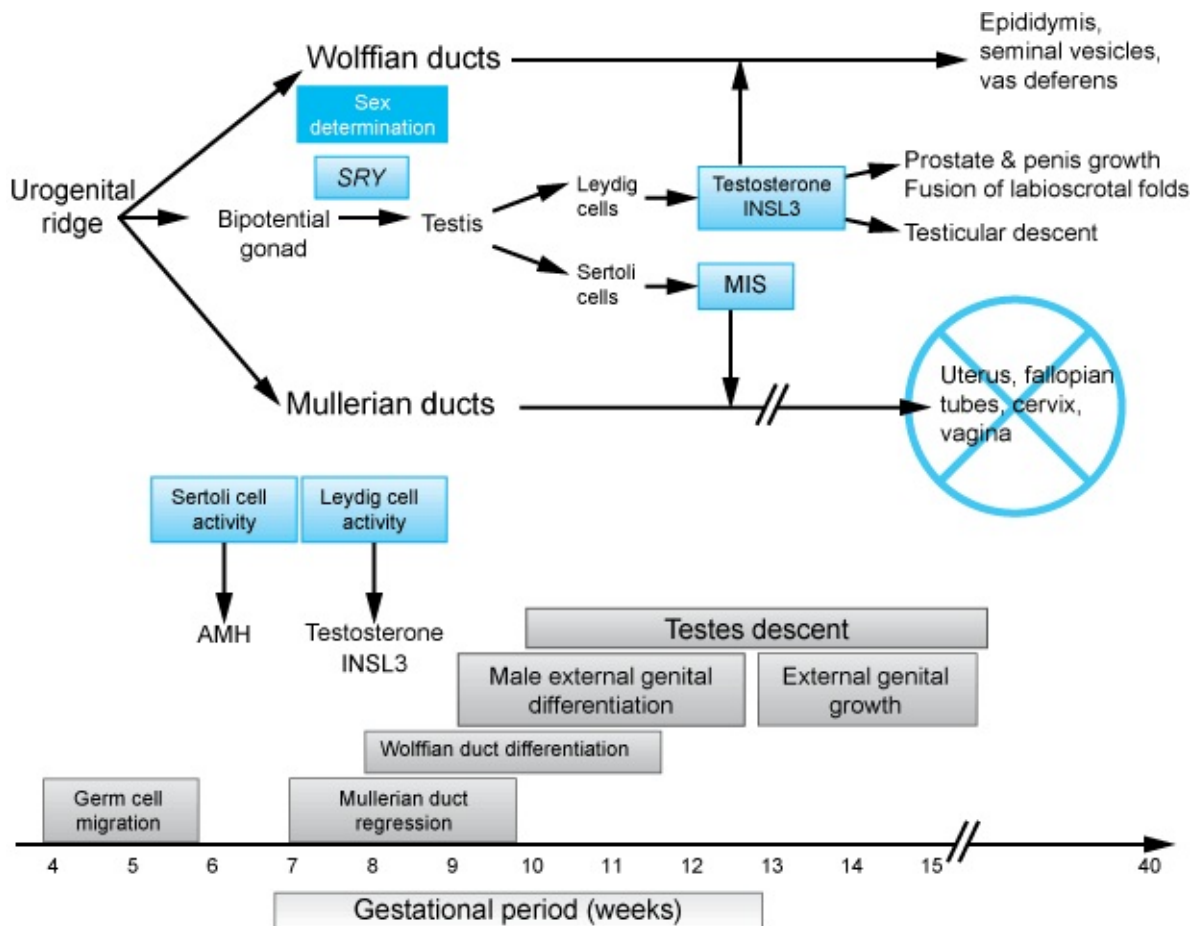
In addition to the regulation of LH release from the anterior pituitary, testosterone affects sexual development, maturation, and function, and contributes to the maintenance of fertility and secondary sexual characteristics in the adult male. In addition, testosterone exerts overall anabolic effects in muscle and bone.

Sexual Development & Differentiation

Sexual differentiation in humans is genetically and hormonally controlled. Genes on the Y chromosome signal primordial cells in the embryonic gonad ridge to differentiate into Sertoli cells and stimulate newly migrated germ cells to differentiate as spermatogonia, developing into a testis (Figure 8–5). The cells of the embryonic testis secrete hormones that lead to the development of male secondary sexual characteristics. The Sertoli cells secrete **Müllerian inhibitory factor** or substance (MIF or MIS), causing regression of the **Müllerian ducts**. The Leydig cells secrete testosterone, causing differentiation and growth of the **Wolffian duct** structures. DHT causes growth of the prostate and penis and fusion of the labioscrotal folds.

Figure 8–5.





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The bipotential gonad is differentiated into testes by the sex-determining region gene on the Y chromosome (*Sry*). This period of sex determination is followed by gonad differentiation of the different cell types of the testis. The Sertoli cells of the testis secrete Müllerian-inhibiting substance (MIS or AMH). The Leydig cells produce testosterone and insulin-like peptide 3 (INSL3). AMH (MIS) produces regression of the Mullerian ducts. Testosterone stimulates the growth and differentiation of the Wolffian ducts, growth of the penis and prostate. INSL3 participates in testicular descent; the final step in male sexual development. DHT produced from testosterone also participates in testis descent and development of the prostate.

The processes and factors involved in fetal development and sexual differentiation are complex and involve genetic, embryologic, histologic, and anatomic details not covered in this monograph. This

section aims to summarize the key events in sexual differentiation as they pertain to endocrine regulation and function and to highlight the role of androgens in the determination of male sexual development.

SEX DETERMINATION

Mammalian sex determination leading to the development of male or female phenotype involves 3 sequential processes:

- Determination of the **genetic sex** of the embryo when an X-or a Y-bearing sperm fertilizes the oocyte
- Determination of the fate of the bipotential or nondifferentiated gonad and thus of **gonadal sex**
- Differentiation of male or female internal and external genitalia, or determination of **phenotypic sex**.

The determination of genetic sex is mediated through the chromosomal set, which in the normal male is 46,XY. The subsequent sexual differentiation is determined by genetic factors. Several sex-specific genes have been found to regulate gonadal differentiation and subsequent male or female reproductive tract development. One of the first genes involved in sexual differentiation is a gene on the Y chromosome called *SRY* (sex-regulating region of the Y). The product of the *SRY* gene is a protein that stimulates the neutral gonadal tissues to differentiate into testes, thereby determining gonadal sex. *SRY* is necessary and sufficient to initiate the male development cascade. If the *SRY* gene is mutant or missing on the Y chromosome, the embryo develops into a female.

SEXUAL DIFFERENTIATION

The process of differentiation of the human male gonads begins in the sixth week of gestation. The first morphologically identifiable event in testicular differentiation is the development of precursor Sertoli cells, which aggregate to form the seminiferous cords, which are then infiltrated by primordial germ cells. By the end of the ninth week, the mesenchyme that separates the seminiferous cords gives rise to the interstitial cells, which differentiate as steroid-secreting Leydig cells. It is thought that the placenta-derived hormone, human chorionic gonadotropin (hCG), might be responsible for the initial development of Leydig cells because the onset of testosterone production precedes LH secretion. Hence, gonadotropic control of fetal testicular steroidogenesis is mediated initially by placenta-derived hCG and later by LH. The resulting increase in fetal testosterone production stimulates Leydig cell proliferation, increases the expression of steroidogenic enzymes (particularly 3 β -hydroxysteroid dehydrogenase and 17 β -hydroxylase), and increases the expression of the androgen receptor in the target tissues.



The postgonadal phase of sexual differentiation or external genitalia differentiation is almost exclusively hormone dependent (Figure 8–5). Once the gonads have differentiated into testes, the secretion of testicular hormones is sufficient to promote masculinization of the embryo. The production of testosterone and anti-Müllerian hormone (AMH, MIF, or MIS) during a critical time in early gestation ensures male development. Initially, both the male (mesonephric or Wolffian) and female (paramesonephric or Müllerian) internal genital ducts are present. In females, the mesonephric ducts regress and the paramesonephric ducts develop into the uterine tubes, uterus, and upper vagina. In males, starting at the eighth week of gestation, AMH (MIS, MIF) mediates the regression of the paramesonephric or Müllerian ducts. AMH is a member of the TGF- β family that is expressed in the Sertoli cell from the beginning of testicular differentiation up to puberty. AMH binds to the type 2 AMH serine/threonine kinase receptors expressed in the surrounding mesenchyme of the Müllerian ducts. The downstream signal

transduction pathway involved is not well defined, but the physiologic response includes apoptosis and regression of the Müllerian ducts. The mesonephric duct system (Wolffian ducts) remains and forms the vas deferens, epididymis, and seminal vesicle. This process is dependent primarily on testosterone. In the female, in the absence of androgens, the Wolffian ducts regress and the Müllerian ducts are spared from apoptosis, developing into the uterus, fallopian tubes, and vagina. Estrogens do not appear to be essential for normal sexual differentiation of either sex, as shown by normal genital development in males with a mutant estrogen receptor gene or with aromatase deficiency. The differentiation of external male genitalia is regulated particularly through the actions of DHT. During the developmental period, as already mentioned, the expression of the type 2 testosterone reductase is higher than that for the type 1 reductase. The capacity for converting testosterone to DHT is greater for the former isoenzyme.

Following Müllerian duct regression and androgen-dependent virilization of the urogenital system, the testes migrate from their site of origin next to the kidney into the scrotum. This is the final critical event in male sexual differentiation. The 2-step process of transabdominal migration followed by descent into the extra-abdominal scrotal sac finalizes sexual differentiation in the male and is completed during the late gestational period. The descent of the testis results from the regression of the cranial suspensory ligament, which connects them to the abdominal wall through the gubernaculum. Regression of the cranial suspensory ligament, transabdominal migration, and the final descent of the testis into the scrotum are mediated by testosterone and insulin-like growth factor 3. This hormone, also known as Leydig insulin-like hormone or relaxin-like factor, is a member of the insulin and insulin-like growth factor peptide hormone family. In humans, failure of complete testicular descent into the scrotum (cryptorchidism) is one of the most common congenital abnormalities, involving approximately 3% of male births. Cryptorchidism is important because spermatogenesis requires lower temperatures (as in the scrotum) than those found intra-abdominally. If untreated, cryptorchidism can lead to infertility, and it has been associated with an increased risk of testicular tumors.

SEXUAL MATURATION AND FUNCTION

Puberty—Puberty is the physiologic transition between childhood and adulthood and involves the development of secondary sexual characteristics and the pubertal growth spurt. The process takes place over a period of about 4 years. Puberty is triggered by increased pulsatile secretion of GnRH by the hypothalamus, leading to increases in serum gonadotropins and, thus, to increases in gonadal secretion of sex steroids. The hypothalamic-pituitary-gonadal system is active during the neonatal period but enters a dormant state in the juvenile, prepubertal period. During the initial phase of puberty, plasma levels of LH increase primarily during sleep. These sleep-associated surges are later present throughout the day and mediate or result in an increase in circulating testosterone levels.

Puberty is preceded by adrenarche, a period characterized by increased adrenal production of DHEA and androstenedione at around 6–8 years of age that is not associated with increased production of ACTH or cortisol. The peak concentrations of DHEA and androstenedione are reached during late puberty and early adulthood. During this stage, there is some conversion of adrenal-derived androgens to testosterone, resulting in a small rise in circulating testosterone levels. The signal that triggers upregulation of the synthesis of DHEA and androstenedione is not known.

The increase in pulsatile release of GnRH is essential for the onset of puberty. However, the mechanism controlling the pubertal increase in GnRH release is still unclear. Leptin, a hormone secreted from adipose tissue (Chapter 10), has been shown to have a permissive role in the timing of the activation of the GnRH pulse generator. The increase in the amplitude of GnRH pulses triggers a cascade of events including increases in the amplitude of FSH and LH pulses, followed by marked increases in gonadal sex hormone production. Accompanying the increase in gonadal steroid production during puberty is an increase in the amplitude of growth hormone secretory bursts. Together, growth hormone and gonadal steroids are responsible for producing normal pubertal growth. During the adolescent growth spurt, growth velocity increases from the prepubertal rate of 4–6 cm per year to as much as 10–15 cm per year. The physiologic changes associated with puberty

are summarized as follows:

- Leydig cells maturation and initiation of spermatogenesis
- Testis enlargement; reddening and wrinkling of scrotal skin
- Pubic hair growth from base of the penis
- Penis enlargement
- Prostate, seminal vesicle, and epididymis growth
- Facial (mustache and beard) and extremities hair growth, regression of scalp line
- Larynx enlargement, thickening of the vocal cords and deepening of the voice
- Enhanced linear growth
- Increased muscle mass and hematocrit
- Increased libido and sexual potency.

Maturity and senescence—Sexual maturity is achieved at approximately age 16–18 years, when plasma levels of testosterone range between 300 and 1000 ng/dL. During this stage, sperm production is optimal, plasma gonadotropins are normal (1–16 mIU/mL), and most sexual anatomic changes have been completed.

Beginning at age 40, there is a gradual decline in circulating testosterone levels, followed at age 50 by a reduction in sperm production. Testosterone levels in healthy, aging men decline on the order of 100 ng/dL per decade, accompanied by increases in SHBG. Together, the decreased testosterone levels and increased SHBG result in a decrease in free and bioavailable testosterone levels. Aging is associated with decreased testosterone clearance, decreased testosterone to estradiol ratio, decreased LH pulse frequency, loss of the diurnal rhythm of testosterone secretion, and diminished accumulation of 5 α -reduced steroids in reproductive tissues. These hormonal changes are well established by the age of 50 years. This period of androgen deficiency is called andropause and is characterized by diminished sexual desire and erectile capacity, decreased intellectual activity, fatigue, depression, decreased lean body mass, skin alterations, decreased body hair, decreased bone

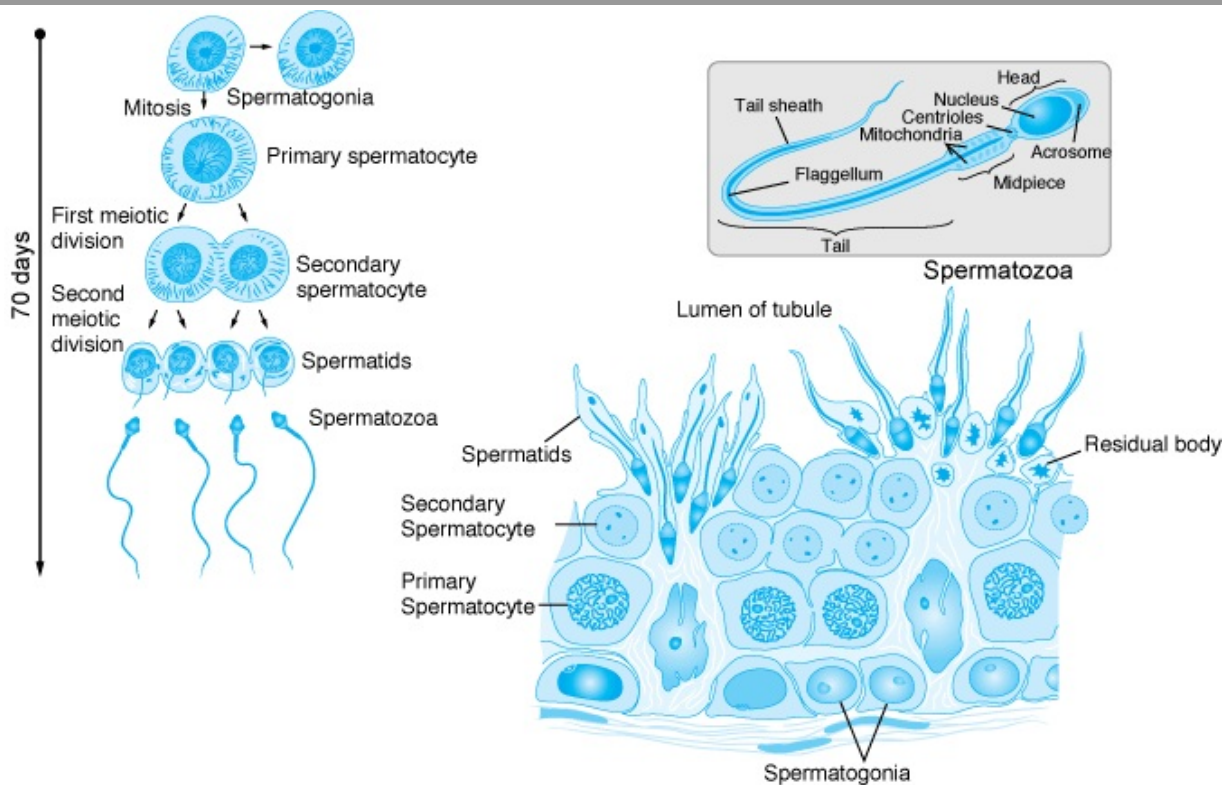
mineral density resulting in osteoporosis, and increased visceral fat and obesity. These physiologic changes that occur during aging are not solely due to decreased testosterone production. Circulating levels of DHEA and androstenedione also decrease progressively in the adult male. Furthermore, growth hormone production can be diminished during the aging process.

Fertility & Secondary Sexual Characteristics

SPERMATOGENESIS

Spermatogenesis is the process of continuous germ cell differentiation to produce spermatozoa (Figure 8–6). Spermatogenesis is initiated at the time of puberty and is associated with the transition from a relatively hypogonadotropic state in the prepubertal phase of development to the eugonadotropic state in adulthood. Spermatogenesis is compartmentalized within the blood-testis barrier. It is mainly under FSH regulation, in addition to paracrine regulation by locally produced factors, and involves close cell-to-cell interaction between Sertoli and germ cells at all stages of spermatogenic maturation.

Figure 8–6.



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Schematic representation of key events in spermatogenesis. The process of spermatogenesis involves proliferation (mitosis) of spermatogonia, producing primary spermatocytes (diploid cells; 46 chromosomes). Spermatocytes undergo 2 meiotic divisions to yield

spermatids, or haploid cells (23 chromosomes). Spermatids undergo a process of maturation (spermiogenesis) and development into spermatozoa. During this last phase, spermatozoa acquire the key elements for their function (Table 8–3). This continuous process takes about 70 days. At any given time, cells from all steps of spermatogenesis can be identified in the testes.

Spermatogenesis involves 4 basic processes:

Proliferation of spermatogonia (stem cells) giving rise to spermatocytes (diploid cells)—Spermatogonia line the seminiferous tubule near the basement membrane. They originate at puberty by proliferation of the gonocytes and are derived from primordial germ cells. One or 2 divisions of spermatogonia occur to maintain their population in a stem cell pool (Figure 8–6). Of the cells resulting from these mitotic divisions, some spermatogonia stay in the "resting" pool, whereas the remaining spermatogonia proliferate several times and undergo 1–5 stages of division and differentiation. After the last division, the resulting cells are termed primary spermatocytes. The "resting" or stem cell spermatogonia remain dormant for a time and then join a new proliferation cycle of spermatogonia. These cycles of spermatogonial divisions occur before the previous generation of cells has completed spermatogenesis, so that multiple stages of the process are occurring simultaneously in the seminiferous tubules. This overlap ensures a residual population of spermatogonia that maintain the ability of the testis to continuously produce sperm.

Meiosis of spermatocytes to yield spermatids (haploid cells; 23 chromosomes)—The primary spermatocytes undergo 2 divisions; the first meiotic division produces 2 secondary spermatocytes. Division of the secondary spermatocytes completes meiosis and produces the spermatids.

Spermiogenesis, or maturation and development of spermatids into spermatozoa (sperm)—This phase is characterized by nuclear and cytoplasmic changes that provide spermatozoa with key elements for their function. The main events during this phase involve spermatid condensation of nuclear material, formation of the acrosome, repositioning of the spermatid to allow formation and elongation of tail

structures, mitochondrial spiral formation, and removal of extraneous cytoplasm, resulting in spermatozoa. Each of the features acquired during this step plays an important role in sperm function, as summarized in Table 8–3.

Table 8–3. Key Events in Spermiogenesis and Their Functional Importance in Sperm Function

Key event	Functional importance
Nuclear chromatin condensation	Haploid chromatin carries either X or Y chromosome
Acrosome development	The acrosome is a large secretory vesicle that overlies the nucleus in the apical region of the sperm head and contains enzymes needed for mucus penetration and fertilization
Repositioning of spermatids; development and growth of flagellum	Microtubular structure provides motility, allowing sperm movement (3 mm/minute) through the genital tract
Formation of mitochondrial sheath around flagellum	Provides energy (fructose-derived ATP) for flagellar movement

ATP, adenosine triphosphate.

Spermiation—This is the final process of release of mature sperm from the Sertoli cells into the tubule lumen.

Throughout this process, the germ cells move from the basal to the adluminal region of the seminiferous tubule into the compartment protected by the blood-testis barrier. The mitotic phase occurs in the basal compartment, whereas the meiotic and postmeiotic phases occur in the luminal compartment. The overall results of spermatogenesis are the following: cell proliferation and maintenance of a reserve germ cell population, reduction in chromosome number and genetic variation through meiosis, and production of spermatozoa.

REGULATION OF SPERMATOGENESIS

Spermatogenesis is dependent on gonadotropin stimulation and testosterone production. FSH stimulates proliferation and secretory activity of the Sertoli cells, whereas LH stimulates the production of testosterone. Testosterone in turn stimulates spermatogenesis through receptor-mediated events in the Sertoli cells. The LH-induced rise in intratesticular testosterone plays an essential role in the initiation and maintenance of spermatogenesis by the Sertoli cells. Androgens produced by the Leydig cells are transported to the developing germ cells bound to androgen-binding protein. Androgen-binding protein is produced by the Sertoli cell in response to FSH stimulation and is released into the adluminal compartment. The synthesis of androgen-binding protein requires that the Sertoli cell be under androgen influence, underscoring the importance of testosterone in Sertoli cell function and the reliance on paracrine mechanisms of hormone action.

The complete maturation of sperm and the development of its capacity to fertilize the ovum are a function of additional processes occurring after sperm is released from the Sertoli cells. These processes are a function of the accessory glands and the excretory system, summarized in Table 8–1. The development and function of these organs are androgen dependent. These organs play an important role in the production of sperm-containing ejaculate and in its transport, storage, and ejaculation.

Anabolic & Metabolic Effects of Androgens

In bone, the main physiologic effect of testosterone is to reduce bone resorption. However, much of this action is indirect via aromatization of testosterone to estrogen. In bone, androgen receptors are found predominantly in osteoblasts at the site of bone formation as well as in osteoclasts and osteocytes. Testosterone increases osteoblast lifespan and proliferation. Androgen regulation of osteoclast function is controlled primarily by 17α -estradiol and the estrogen receptor. Testosterone-derived estrogen is the critical sex hormone in the pubertal growth spurt, skeletal maturation, accrual of peak bone mass, and maintenance of bone mass in the adult. It stimulates chondrogenesis in the epiphyseal growth plate, increasing pubertal

linear growth. At puberty, estrogen promotes skeletal maturation and the gradual, progressive closure of the epiphysial growth plate and the termination of chondrogenesis. In the adult, estrogen is important in maintaining the constancy of bone mass through its effects on remodeling and bone turnover. Testosterone reduces bone resorption, and this effect is primarily mediated through the aromatization of testosterone to estrogen. Testosterone decreases osteoblast and osteoclast apoptosis, stimulates osteoblast proliferation, enhancing bone formation, and increases periosteal apposition of bone. Testosterone increases protein synthesis and decreases protein breakdown, having an overall anabolic effect in muscle.

Testosterone inhibits lipid uptake and lipoprotein lipase activity in adipocytes, stimulates lipolysis by increasing the number of lipolytic β -adrenergic receptors, and inhibits differentiation of adipocyte precursor cells. DHEA stimulates resting metabolic rate and lipid oxidation and enhances glucose disposal by increasing the expression of glucose transporters on the plasma membrane of adipocytes.

NEUROENDOCRINE & VASCULAR CONTROL OF ERECTION & EJACULATION

The physiologic process of human reproduction involves the fertilization of a mature ovum through the deposition of sperm-containing semen in the vagina of the female. This event involves penile erection and ejaculation of the sperm-containing semen at the time of copulation. Penile erection results from smooth muscle relaxation mediated by a spinal reflex involving central nervous processing and integration of tactile, olfactory, auditory, and mental stimuli. Corporeal vasodilatation and corporeal smooth muscle relaxation allow increased blood flow into the corpus cavernosum. Corporeal vasodilatation is mediated by the parasympathetic nervous system. The concordant contraction of the perineal skeletal muscles leads to a temporary increase in corpus cavernosum blood pressure above mean systolic arterial pressure, helping to increase penile firmness.

Parasympathetic fibers directly innervating the corporeal smooth muscle and sinusoidal endothelial cells release acetylcholine,

stimulating the production of constitutive endothelial nitric oxide. Nitric oxide produced locally in the smooth muscle cell, or reaching it by diffusion from the adjacent endothelial cells, is the major mediator of smooth muscle relaxation through activation of guanylate cyclase and increased production of cyclic guanosine monophosphate (cGMP). The inactivation of cGMP phosphodiesterase, the enzyme that degrades cGMP with the drug sildenafil, preserves smooth muscle relaxation and prolongs the erection period. This is used commercially to treat erectile dysfunction.

The ejaculation phase of the sexual response consists of 2 sequential processes: emission and ejaculation. Emission is the deposition of seminal fluid into the posterior urethra and is mediated by simultaneous contractions of the ampulla of the vas deferens, the seminal vesicles, and the smooth muscles of the prostate. The second process is ejaculation, which results in expulsion of the seminal fluid from the posterior urethra through the penile meatus. This process is controlled by sympathetic innervation of the genital organs and occurs as a result of a spinal cord reflex arc. Detumescence of the penis following ejaculation and maintenance of the flaccid penis in the absence of sexual arousal, are produced by sympathetic corporeal vasoconstriction and corporeal smooth muscle contraction by noradrenergic, neuropeptide Y, and endothelin-1 fibers.

DISEASES OF TESTOSTERONE EXCESS OR DEFICIENCY

Excess androgen activity in childhood leads to precocious puberty, defined as the appearance of male secondary sex characteristics before age 9 (Table 8–2). Hypothalamic tumors, activating mutations of the LH receptor, congenital adrenal hyperplasia, and androgen-producing tumors are all causes of premature virilization. Two types of precocious puberty can be identified: gonadotropin dependent and independent. Decreased testosterone production, or hypogonadism, can be caused by disorders at the hypothalamic/pituitary level (hypogonadotropic or secondary hypogonadism) or by testicular dysfunction (hypergonadotropic or primary hypogonadism).

Hypogonadotropic hypogonadism can be due to abnormalities in

hypothalamic GnRH secretion or action, associated with impaired gonadotropin secretion by the anterior pituitary. This condition may result from genetic defects including Kallmann syndrome; adrenal hypoplasia; mutations of the GnRH receptor, LH, or FSH β -subunits; pituitary tumors (including prolactinoma); trauma; or surgery.

Abnormal testicular function in the presence of elevated gonadotropin levels (hypergonadotropic or primary hypogonadism) is caused by testicular damage or impaired testicular development, which can be either congenital or acquired following chemotherapy or radiation. Causes include Leydig cell tumors, cryptorchidism, gonadal dysgenesis, varicocele, enzyme defects in testosterone biosynthesis, or LH receptor defects. Klinefelter syndrome is the most common sex chromosome disorder, in which affected males carry an additional X chromosome. This genetic abnormality results in male hypogonadism, androgen deficiency, and impaired spermatogenesis. Klinefelter's syndrome is the most common genetic cause of human male infertility. Hyperprolactinemia from any cause results in both reproductive and sexual dysfunction because of prolactin inhibition of GnRH release, resulting in hypogonadotropic hypogonadism.

Clinical Presentation

Excess testosterone in the prepubescent male is associated with the appearance of all the changes of puberty at a very early age. These changes include enlargement of the penis and testicles; appearance of pubic, underarm, and facial hair; spontaneous erections; production of sperm; development of acne; and deepening of the voice.

Low levels of testosterone lead to severe symptoms, which differ according to the time of onset. Androgen deficiency during early childhood results in short stature, lack of deepening of the voice, female distribution of secondary hair, anemia, underdeveloped muscles, and genitalia with delayed or absent onset of spermatogenesis and sexual function. Androgen deficiency in the adult after normal virilization has been completed leads to a decrease in bone mineral density (bone mass), decreased bone marrow activity resulting in anemia, alterations in body composition associated with muscle weakness and atrophy, changes in mood and cognitive function, and regression of sexual function and spermatogenesis. In the adult male, androgen deficiency decreases nocturnal erections and libido.

Evaluation of Hypogonadism

Sparse facial, axillary, and pubic hair and small penis and testicles characterize the clinical presentation of the prepubescent male patient with hypogonadism. Low plasma levels of testosterone should be interpreted in conjunction with LH levels to distinguish between hypo- and hypergonadotropic forms of hypogonadism. Similar to the functional tests described for other endocrine organs, a challenge test with intravenously injected GnRH to determine the gonadotropin and testosterone response 25 and 40 minutes later is used to assess the gonadotropin reserve capacity of the pituitary. When the response is absent, a prolonged period (7 days) of GnRH can be used to further stimulate the anterior pituitary gonadotrophs. In adults, fertility assessment by semen evaluation is also indicated. Semen analysis is interpreted according to normal values shown in Table 8–4.

Table 8–4. Normal Values of Semen Parameters According to World Health Organization (1992)

Standard tests	
Volume	>2 mL
pH	7.2–8.0
Sperm concentration	>20 × 10 ⁶ spermatozoa/mL
Total sperm count	>40 × 10 ⁶ spermatozoa/ejaculate
Motility	>50% with forward progression (categories a and b) or >25% with rapid progression (category a) within 60 minutes of ejaculation
Morphology	>30% with normal forms
Vitality	>75% or more live, ie, excluding dye
White blood cells	<1 × 10 ⁶ /mL
Immunobead test	<20% spermatozoa with adherent particles

Mixed agglutination reaction test	<10% spermatozoa with adherent particles
Optional tests	
α-Glucosidase (neutral)	>20 mU/ejaculate
Zinc (total)	>2.4 μmol/ejaculate
Citric acid (total)	>52 μmol/ejaculate
Acid phosphatase (total)	>200 U/ejaculate
Fructose (total)	>13 μmol/ejaculate

KEY CONCEPTS



The Sertoli cells support spermatogenesis and form the blood-testis barrier.



Testicular function is under LH and FSH regulation.



LH and FSH production and release are under hypothalamic GnRH stimulation and feedback inhibition by gonadal hormones.



The main functions of the testes are testosterone production and spermatogenesis.



The main hormones produced by the testis are testosterone, estradiol, and inhibin.



Testosterone can be metabolized to DHT, a more potent androgen, or to 17α -estradiol, an estrogen.



Androgens exert their physiologic effects through modulation of gene transcription.



Three essential testis-derived hormones regulate male sexual development: androgens, AMH, and insulin-like growth factor 3.

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OBJECTIVES

- ▶ *Describe oogenesis, its relationship to follicular maturation, and the roles of pituitary and ovarian factors in their regulation.*
- ▶ *Describe the roles of pituitary hormones in regulation of ovarian function including ovulation, formation and decline of the corpus luteum, estrogen and progesterone biosynthesis and secretion.*
- ▶ *List the target organs and principal physiologic actions of estrogen and progesterone and how they interact with each other.*
- ▶ *Identify the transport mechanisms and degradation pathways for estrogen and progesterone.*
- ▶ *Describe the cellular mechanisms of action for estrogen and progesterone.*
- ▶ *Describe the endometrial changes (proliferative and secretory phases) that occur throughout the menstrual cycle and correlate them with the changes in blood levels of pituitary and ovarian hormones.*
- ▶ *Identify the pathways of sperm and egg transport required for fertilization and for movement of the embryo to the uterus.*
- ▶ *Describe the endocrine functions of the placenta, particularly the role of placental hormones in rescue of the corpus luteum and maintenance of pregnancy, and the fetal adrenal-placental interactions involved in estrogen production.*
- ▶ *Understand the roles of oxytocin, relaxin, and prostaglandins in the initiation and maintenance of parturition.*
- ▶ *Explain the hormonal regulation of mammary gland development during puberty, pregnancy, and lactation, and explain the mechanisms that control milk production and secretion.*
- ▶ *Explain the physiologic basis for the effects of steroid hormone contraceptive methods.*
- ▶ *Describe the age-related changes in the female reproductive system, including the mechanisms responsible for these changes, throughout life from fetal development to senescence.*

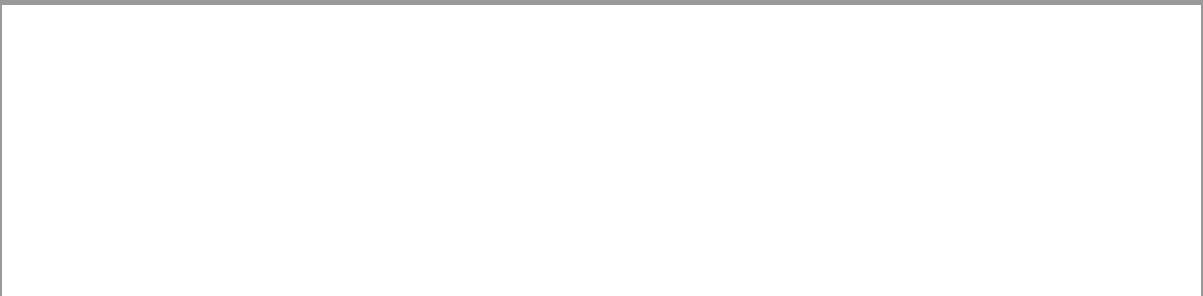
FEMALE REPRODUCTIVE SYSTEM: INTRODUCTION

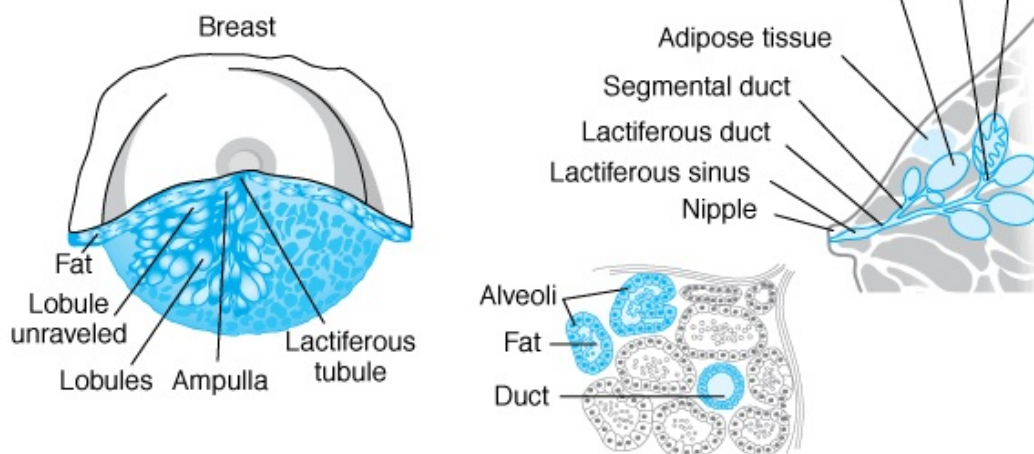
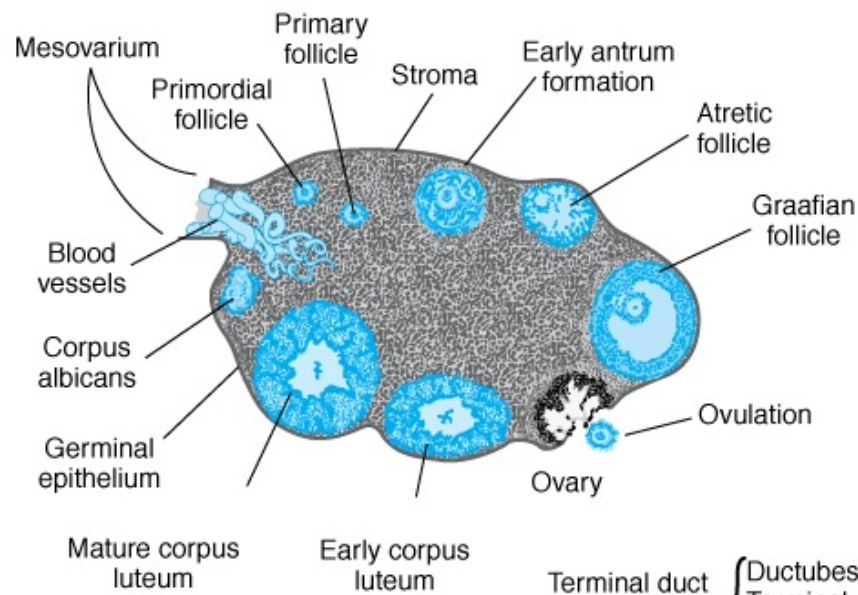
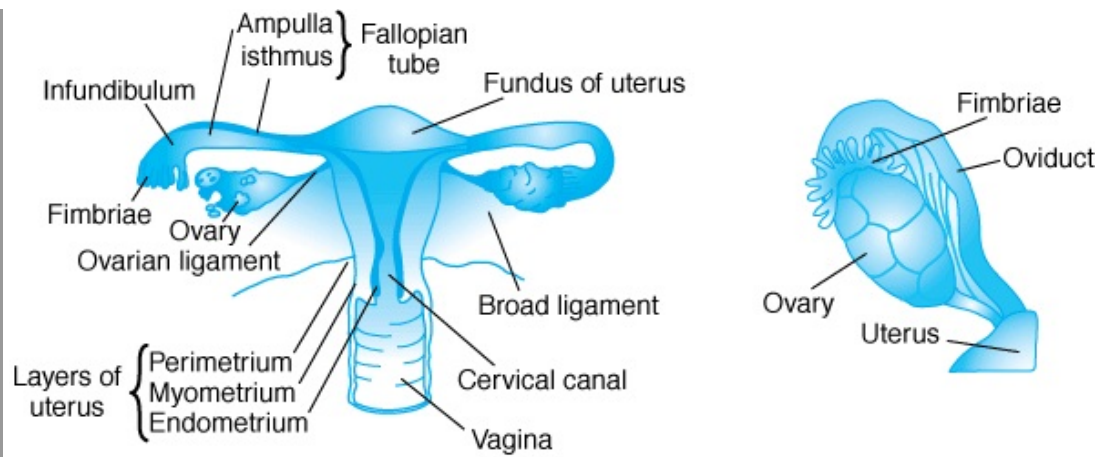
The principal functions of the female reproductive system are to produce the ova for sperm fertilization and to provide the appropriate conditions for embryo implantation, fetal growth and development, and birth. Endocrine regulation of the reproductive system is directed by the hypothalamic-pituitary-ovarian axis. The ovarian cycle, which involves changing patterns of hormone production and secretion, regulates the hypothalamic-pituitary-gonadal axis in a classical negative feedback pattern. In addition, the ovarian cycle mediates the maturation and development of the reproductive system throughout life. Throughout the cycle, a selected ovarian follicle is rescued from its apoptotic fate to undergo growth and development, culminating in ovulation. The remnant of the follicle undergoes transformation into the corpus luteum, a temporary endocrine organ that plays a central role in preparation and maintenance of the initial stages of pregnancy. Parallel changes in endometrial morphology and function occur throughout the ovarian cycle in preparation for implantation of a fertilized ovum. Ovarian and placental hormones maintain pregnancy and prepare the breast for lactation. This chapter describes the basic principles of the complex neuroendocrine regulation of this hypothalamic-pituitary-ovarian axis.

FUNCTIONAL ANATOMY

The female reproductive organs include the ovaries, the uterus and fallopian tubes, and the breasts or mammary glands (Figure 9–1). Their growth, development, and function are under hormonal regulation. The ovaries are considered the principal female reproductive organs. Their functions are the storage and release of the ovum and the production of the 2 principal female sex hormones, estrogen and progesterone. Functionally, the ovaries consist of an outer cortex layer that contains different-size follicles and their apoptotic remains embedded in connective tissue. The inner medulla contains vascular connective tissue and hilar cells, which contribute to testosterone production. The primordial follicle contains a primary oocyte surrounded by epithelial pre-granulosa cells separated from the ovarian stroma by a basement membrane. During follicular development, the epithelial cells differentiate into granulosa cells, and a layer of cells from the ovarian stroma is transformed into theca cells. The larger, more mature follicles are filled with a transparent albuminous fluid and consist of an external fibrovascular coat, connected with the surrounding stroma of the ovary by a network of blood vessels; and an internal coat, consisting of several layers of nucleated cells (granulosa cells) anchored in the zona pellucida, a glycoprotein-rich eosinophilic material surrounding the oocyte. The zona pellucida forms the corona radiata, which close to the time of ovulation is separated from the granulosa cells and expelled with the oocyte. Formation of the follicles begins before birth, and their development and maturation continue uninterrupted from puberty until the end of a woman's reproductive life, as discussed later.

Figure 9–1.





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Functional anatomy of the female reproductive tract. The female reproductive organs include the ovaries, the uterus and fallopian tubes, and the breasts or mammary glands. The ovaries consist of an outer cortex layer that contains different-sized follicles and their remains undergoing apoptosis, embedded in connective tissue. The fallopian tubes extend from each of the superior angles of the uterus and consist of the isthmus, the ampulla, and the infundibulum, which opens into the abdominal cavity and is surrounded by ovarian fimbriae and attached to the ovary. The cilia of the epithelial lining of the fallopian tubes contribute to sperm movement, aiding in fertilization, and facilitate movement of the zygote (fertilized ovum) to the uterus for implantation and fetal development. The breast is organized into lobes made of lobules, connected by connective tissue, blood vessels, and ducts. The lobules consist of a cluster of rounded alveoli, which open into excretory lactiferous ducts and unite to form larger ducts made of longitudinal and transverse elastic fibers. These ducts converge toward the areola, beneath which they form ampullar dilatations, which serve as reservoirs for the milk.

The female genital tract is derived from the Müllerian ducts (Chapter 8) and consists of the uterus, fallopian tubes, and vagina. The fallopian tubes extend from each of the superior angles of the uterus and consist of the isthmus, the ampulla, and the infundibulum, which opens into the abdominal cavity and is surrounded by ovarian fimbriae and attached to the ovary (Figure 9–1). The epithelial lining of the fallopian tubes has secretory and ciliated cells that contribute to sperm movement, aiding in fertilization and facilitating the movement of the zygote (fertilized ovum) to the uterus for implantation and fetal development. These functions are also aided by the rhythmic contraction of the smooth muscle walls.

The uterus is composed of 3 layers; serous, muscular, and mucous. The muscular coat accounts for the bulk of the uterus and consists of bundles of smooth muscle fibers, organized in layers and intermixed with loose connective tissue, blood vessels, lymphatic vessels, and nerves. The mucous membrane, or endometrium, is lined by columnar ciliated secretory epithelium that undergoes cycles of proliferation, differentiation, and breakdown every 28 days in preparation for embryo

implantation. It is composed of 2 layers, the stratum basale and the stratum functionale, which undergo cycles of regeneration (proliferative phase) and shedding (menstrual phase). The arterial blood supply to the female reproductive tract is provided by branches of the hypogastric and ovarian arteries from the abdominal aorta. The veins correspond with the arteries and end in the uterine plexuses. The nerves are derived from the hypogastric and ovarian plexuses and from the third and fourth sacral nerves.

The breast consists of glandular tissue organized in lobes connected by fibrous tissue, with fat deposits interspersed between the lobes (Figure 9–1). The mammary lobes themselves are made of lobules connected by connective tissue, blood vessels, and ducts. The lobules consist of a cluster of rounded alveoli, which open into excretory lactiferous ducts and unite to form larger ducts made of longitudinal and transverse elastic fibers. These ducts converge toward the areola, beneath which they form ampullar dilatations, which serve as reservoirs for the milk. The arterial blood supply to the breast is derived from the thoracic branches of the axillary, the intercostal, and the internal mammary arteries. The veins drain into the axillary and internal mammary veins.

GONADOTROPIN REGULATION OF OVARIAN FUNCTION



Pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates pulsatile pituitary release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). As already mentioned in Chapter 8, GnRH secretion is regulated by dopamine, serotonin, β -endorphin, and norepinephrine. Both FSH and LH stimulate ovarian hormone production through binding to a transmembrane G protein–coupled receptor, causing activation of the G_{α} -stimulatory (G_{α_s}) subunit, which interacts with adenylate cyclase to generate an increase in cyclic adenosine monophosphate (cAMP), leading to the activation of protein kinase A. cAMP also serves as a second messenger mediating the upregulation of steroidogenic acute regulatory protein and the cytochrome P450_{scc} enzyme system, leading to the increase in ovarian production of **estradiol** and **progesterone**. These are the 2 principal ovarian hormones involved in the regulation of ovarian function and control of the reproductive cycle. The variations in pulsatile release of the gonadotropins result in a cyclic response of ovarian function. Each cycle lasts 28 days and can be divided into 2 phases (follicular and luteal) of 14 days each.

Follicular phase—FSH is responsible for follicular recruitment and growth and for estrogen synthesis during the **follicular phase** of the ovarian cycle. Before the selection of the follicle for ovulation, granulosa cells are responsive only to FSH. As follicular maturation progresses, the coupling between FSH receptor stimulation and the activation of adenylate cyclase becomes more and more efficient, leading to a steady increase in cAMP production. The accumulation of FSH-induced cAMP coincides with the expression and subsequent upregulation of LH receptors, allowing LH to act as a surrogate for FSH in **granulosa cells**. When gonadotropin, especially FSH, plasma levels are too low to meet the follicle's tropic requirements, uncoupling of receptor stimulation from cAMP production is one of the earliest events during granulosa cell death and follicular atresia.

Luteal phase—LH is responsible for ovulation and **corpus luteum**

formation and for progesterone and estrogen production by the corpus luteum during the early and mid-luteal phases of the **menstrual cycle**. Activation of the LH receptors in theca cells stimulates androstenedione production, providing the substrate for the enzymatic conversion to 17 β -estradiol that is mediated by the enzyme aromatase in granulosa cells.

OVARIAN HORMONE SYNTHESIS

Ovarian production of steroid hormones (progesterone, estrogen, and testosterone) and peptide hormones (inhibins) varies throughout the ovarian cycle. The production and secretion rates of the principal ovarian steroid hormones are summarized in Table 9–1.

Table 9–1. Production and Secretion Rates of Principal Female Sex Steroid Hormones

Hormone	Production/secretion rate (mg/d)	
	Follicular	Luteal
Progesterone	2/1.7	25/24
Estradiol	0.09/0.08	0.25/0.24
Estrone	0.11/0.08	0.26/0.15
Androstenedione	3.2/2.8	NC
Testosterone	0.19/0.06	NC

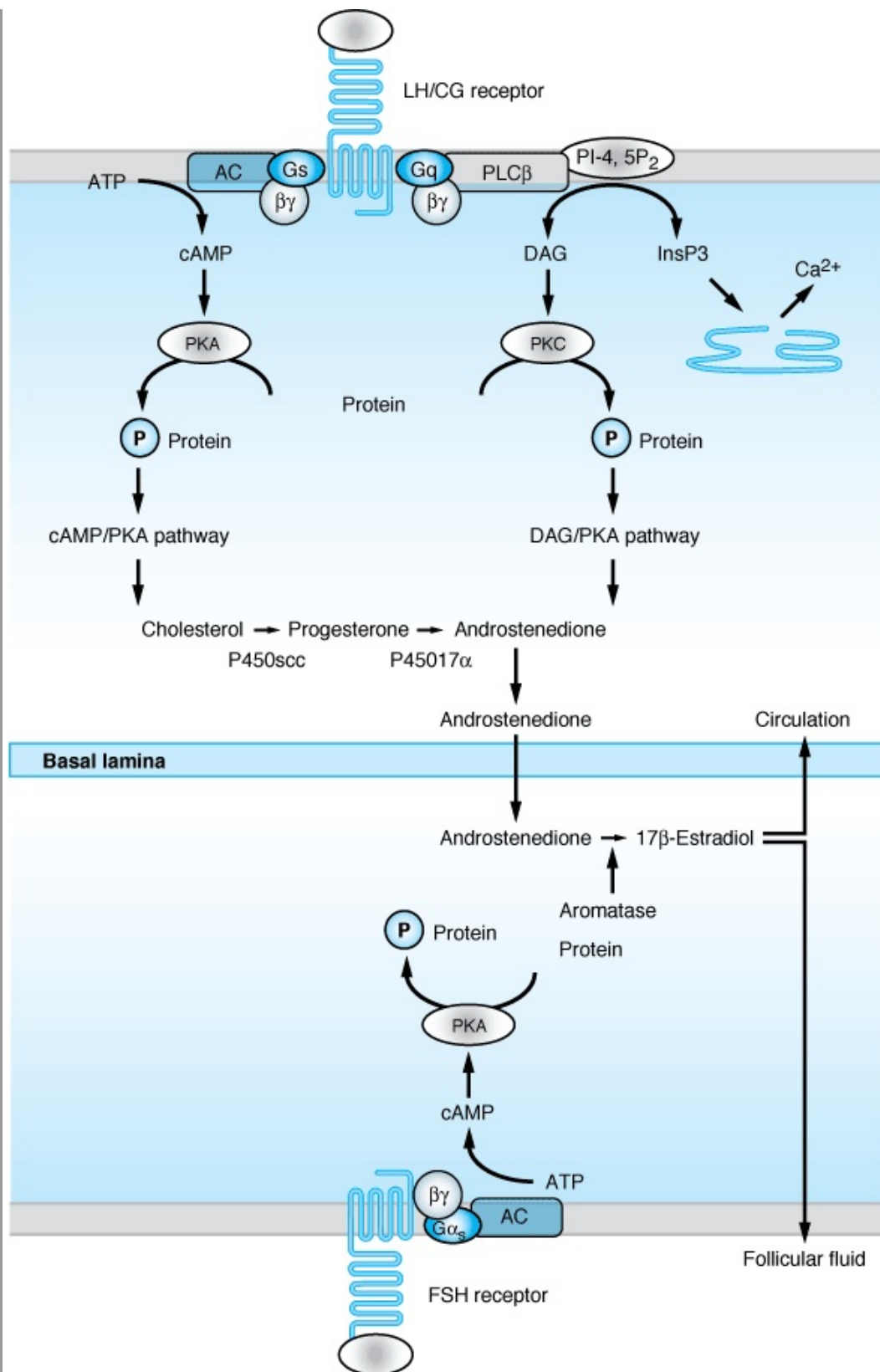
NC, no change.

Estrogen



The production of estrogen involves coordinated enzymatic activities between the **theca** and **granulosa cells** of the **ovarian follicle** (Figure 9–2). During follicle growth, theca cells become associated with the growing follicle. Theca cells express the enzymes necessary to convert cholesterol to androgens (mainly androstenedione, a weak androgen) but lack the enzymes necessary to convert androgens to estradiol. Granulosa cells can convert androgens to estradiol and they can produce progesterone, but they are unable to convert pregnenolone or progesterone to androgens. Thus, theca cell–produced androgens are aromatized to estradiol by granulosa cells (Figure 9–2). More than 95% of circulating estradiol is directly secreted, with a smaller contribution from peripheral conversion of estrone to estradiol in premenopausal women.

Figure 9–2.



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Theca and granulosa cells coordinate the production of estrogen. The secretion of estradiol by the dominant follicle requires cooperation between theca cells, which synthesize androstenedione and testosterone, and granulosa cells of mature follicles, which convert androgens to estradiol and estrone. Androgen synthesis in theca cells results from the activity of 3 enzymes: cholesterol side-chain cleavage (P450_{scc}), 17 α -hydroxylase-lyase (P450C17), and 3 β -hydroxysteroid dehydrogenase (3 β -HSD). Luteinizing hormone (LH) induces steroidogenic acute regulatory protein (StAR), which allows the entry of cholesterol into mitochondria. In granulosa cells, the enzyme 17 β -hydroxysteroid dehydrogenase transforms androstenedione into testosterone in follicles from the primary stage. In mature follicles, follicle-stimulating hormone (FSH) stimulates the activity of aromatase, which transforms testosterone into 17 β -estradiol. AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; InsP₃, inositol 1,4,5-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC β , phospholipase C β ; PI-4,5P₂, phosphatidylinositol 4,5-bisphosphate.

Androgens

Female androgens are derived from the adrenal glands (dehydroepiandrosterone and androstenedione), from the ovaries (androstenedione and testosterone), and from peripheral conversion of androstenedione and dehydroepiandrosterone to testosterone. Ovarian androgen secretion parallels that of estrogen throughout the menstrual cycle, whereas adrenal androgen production does not fluctuate throughout the menstrual cycle. Most of the circulating testosterone in females is derived from the peripheral conversion of androstenedione by 17 β -hydroxysteroid dehydrogenase; 30–40% of testosterone is directly secreted. The conversion of testosterone to dihydrotestosterone in peripheral tissues is limited in females because of their higher levels of sex hormone-binding globulin than in males, as well as by the peripheral conversion to estrogen by aromatase, protecting females from virilization by dihydrotestosterone.

Progesterone

The preovulatory LH surge results in luteinization of granulosa and theca cells, altering the steroidogenic pathway so that progesterone is the primary steroid hormone produced by each of these cell types after luteinization. The changes leading to the ability to produce progesterone include increased expression of the enzymes involved in the conversion of cholesterol to progesterone (cholesterol side-chain cleavage cytochrome P450 complex and 3 β -hydroxysteroid dehydrogenase) and decreased expression of the enzymes that convert progesterone to estrogens (17 β -hydroxylase cytochrome P450 and aromatase cytochrome P450).

Inhibins, Activins, & Follistatin

Inhibin production by granulosa cells of mature follicles is regulated by FSH and LH, and by local factors like growth factors (epidermal, transforming, and insulin-like) and hormones (androstenedione, activin, and follistatin) in an autocrine and paracrine way. Preantral follicles secrete inhibin B exclusively, whereas small antral follicles secrete both inhibin B and inhibin A. In clinical practice, inhibin B is a good marker of granulosa cell function under the control of FSH, whereas inhibin A is a marker of corpus luteum function under the control of LH. Inhibins contribute to the regulation of LH and FSH release through endocrine feedback regulation at the anterior pituitary.

Activin production by the granulosa cells changes during **folliculogenesis**, and its effects are probably limited to paracrine action on granulosa cells. Activin promotes granulosa cell proliferation, upregulates FSH receptor expression on granulosa cells, and modulates steroidogenesis in both granulosa and theca cells. In the pituitary-ovarian axis, activin is a physiologic antagonist to inhibin and specifically stimulates pituitary FSH synthesis and secretion.

Activin-binding protein, follistatin, is a product of granulosa cells. Its basal expression increases with differentiation of granulosa cells. Its function is to neutralize the effect of activin on steroid production. The physiologic endocrine role of follistatin is not completely understood, but its effects are most likely autocrine or paracrine on ovarian

steroidogenesis.

OVARIAN CYCLE

During the ovarian cycle, throughout the reproductive life span of the woman (from puberty to menopause), a single mature oocyte is produced each month. Most of the human oocytes (germ cells) present during uterine development are destined to undergo atresia through apoptosis, or programmed cell death. Only follicles that are responsive to FSH stimulation (about 350) will enter the final stage of development and progress to ovulation. The ovarian cycle is divided into a follicular and a luteal phase.

The follicular phase begins on day 1 of the cycle, the first day of menses, and corresponds to the growth and development of a dominant follicle. At mid-cycle (day 14), the rising levels of estrogen stimulate a surge in LH release, which results in ovulation 36 hours later.

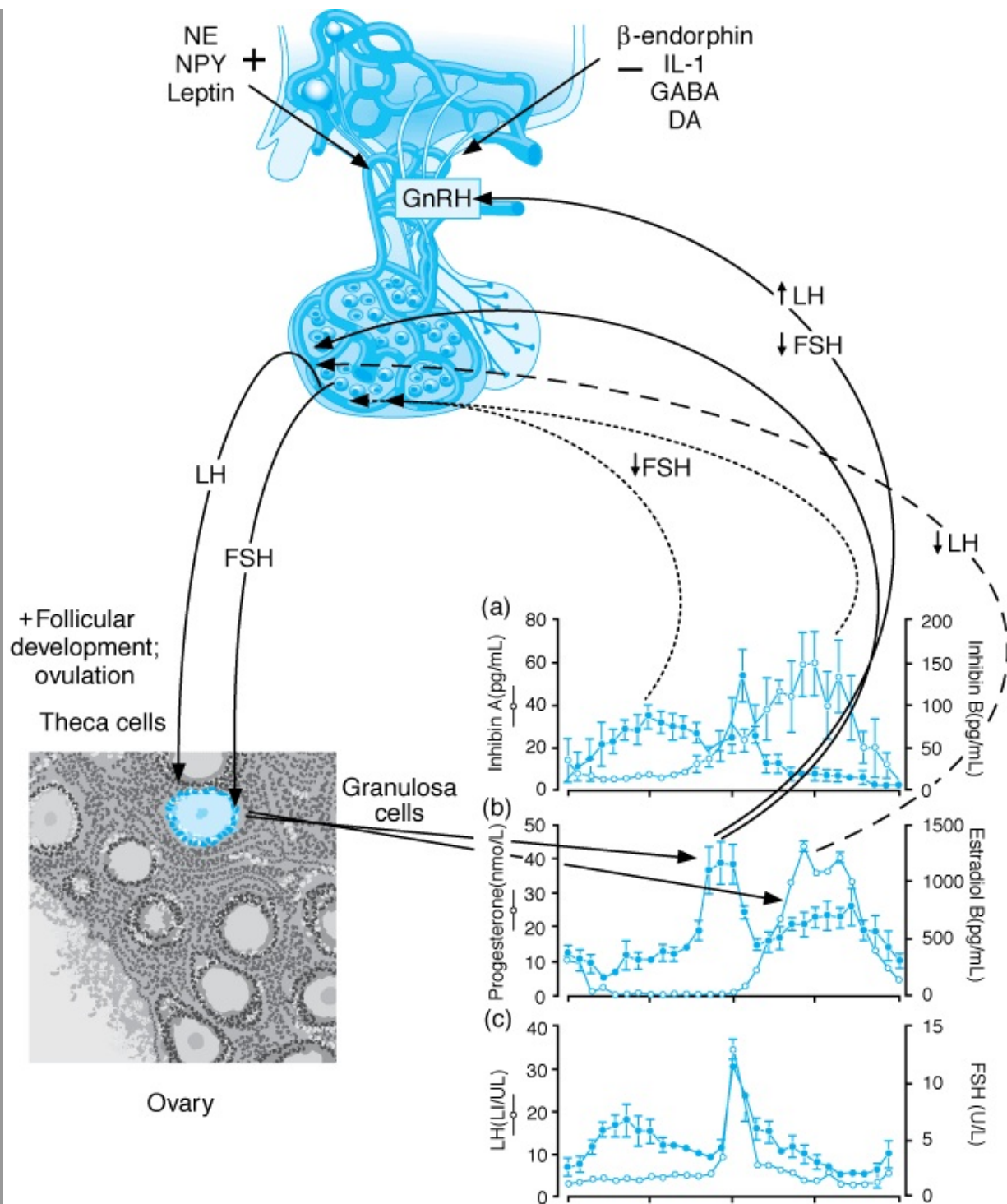
After ovulation, during the luteal phase, the remnants of the ovulatory follicle form the corpus luteum, a transient endocrine organ that produces estradiol, progesterone, and inhibin A and is under LH regulation. The end of the luteal phase is marked by regression of the corpus luteum and associated decreases in estradiol, inhibin A, and progesterone production. The hormonal changes that occur during the ovarian cycle produce differential regulation of gonadotropin release.

Ovarian Regulation of Gonadotropin Release

Gonadotropin release is under negative and positive feedback regulation by estradiol, progesterone, and inhibins A and B. Progesterone and 17 β -estradiol act both in the hypothalamus and the pituitary, and inhibins act at the level of the pituitary (Figure 9–3). The contributions of these ovarian hormones vary according to the stage of the ovarian cycle.

Figure 9–3.





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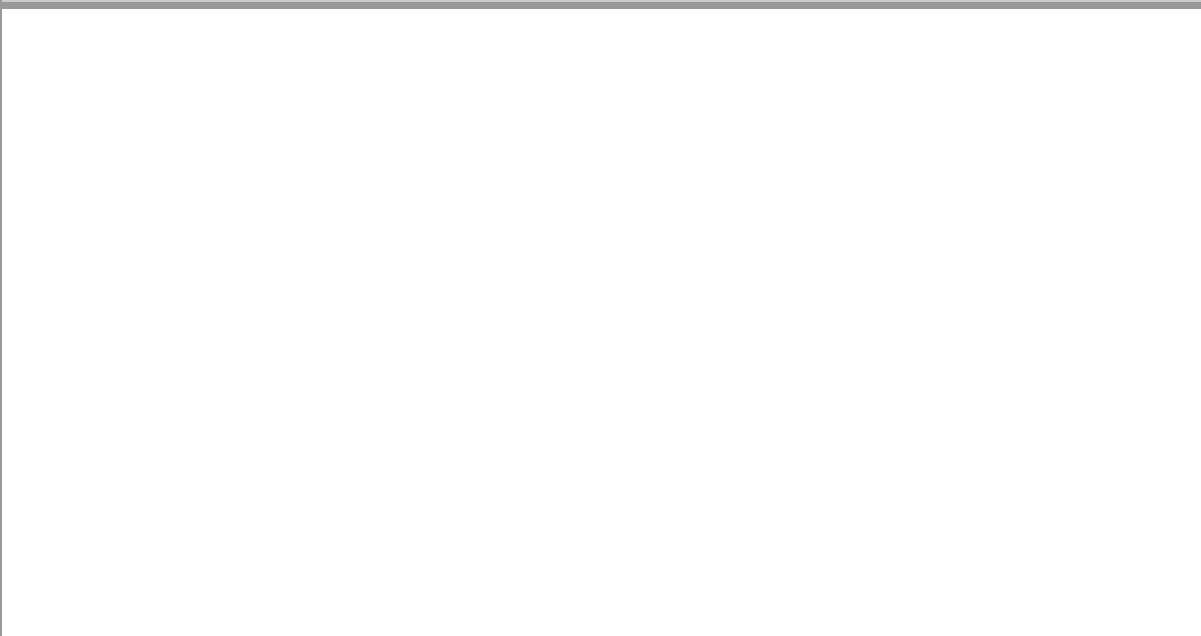
Hypothalamic-pituitary-ovarian axis. Gonadotropin synthesis and release and differential expression are under both positive and negative feedback control by ovarian steroid and peptide hormones. Ovarian

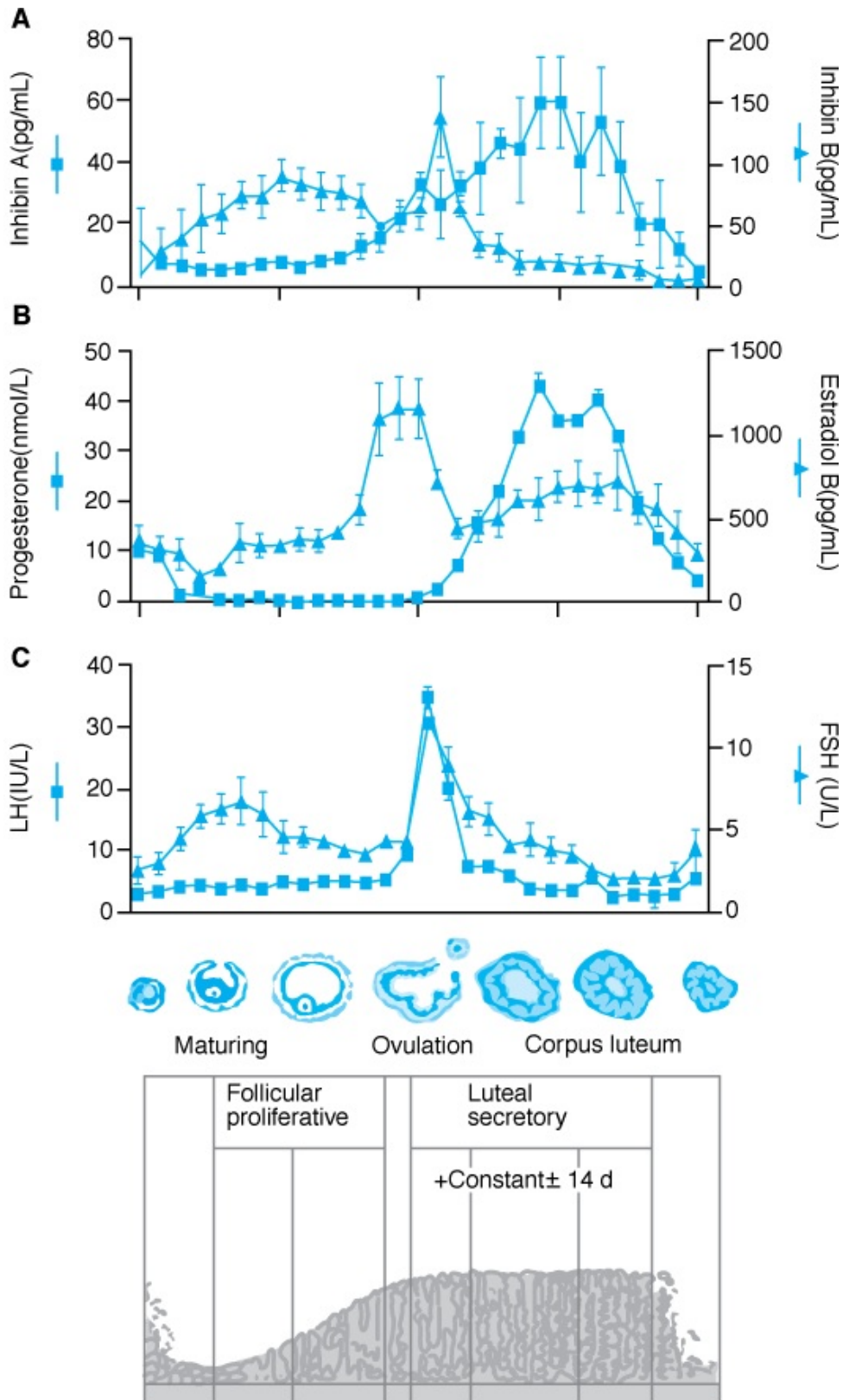
hormones can decrease gonadotropin release both by modulating gonadotropin-releasing hormone (GnRH) pulse frequency from the hypothalamus and by affecting the ability of GnRH to stimulate gonadotropin secretion from the pituitary itself. Estradiol enhances luteinizing hormone (LH) and inhibits follicle-stimulating hormone (FSH) release, whereas inhibins A and B (gonadal glycoprotein hormones) reduce FSH secretion. After ovulation, ovarian progesterone production predominates. Progesterone increases hypothalamic opioid activity and slows GnRH pulse secretion, favoring FSH production and decreasing LH release. Inhibin B peaks early in the follicular phase, whereas inhibin A peaks in the mid-luteal phase. The rising inhibin B levels in the mid-follicular phase act at the pituitary gonadotroph to offset activin signaling and suppress FSH biosynthesis from early follicular phase levels. The fall in inhibin A at the end of the luteal phase creates an environment in which FSH levels can again increase. DA, dopamine; IL-1, interleukin-1; GABA, γ -aminobutyric acid; NE, norepinephrine; NPY, neuropeptide Y.

FOLLICULAR PHASE

During this phase, the dominant follicle produces high concentrations of 17β -estradiol and inhibin B. As concentrations of estradiol rise, they act on the hypothalamus and pituitary to stimulate low-amplitude, high-frequency pulses (every 90 minutes) of LH, resulting in elevated circulating concentrations of LH that stimulate follicular development, induce ovulation, and decrease FSH secretion at the pituitary level (Figure 9–4). The mid-cycle LH surge is the result of positive feedback of estradiol resulting from an increased responsiveness of gonadotropic cells to GnRH (following exposure to rising estradiol), an increase in GnRH receptor number, and a GnRH surge, triggered by the effect on the hypothalamus of rising estradiol. Progesterone also exerts positive feedback by enhancing pituitary sensitivity to GnRH and increasing pulse frequency of neuropeptide Y release, which increases GnRH release from the hypothalamus. Inhibin B levels rise during the follicular phase and decrease immediately before the LH peak, with a brief surge occurring 2 days after ovulation. Inhibin A levels increase in the late follicular phase to reach a peak concentration on the day of the LH and FSH surge. The concentration then falls briefly before rising to reach a maximum concentration during the mid-luteal phase.

Figure 9–4.





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Hormonal events during the ovarian and endometrial cycles. Plasma concentrations of **A.** inhibins A and B, **B.** estradiol and progesterone, and **C.** luteinizing hormone (LH) and follicle-stimulating hormone (FSH) during the human menstrual cycle correspond to proliferative and secretory changes in the endometrium and to follicular development and ovulation.

LUTEAL PHASE

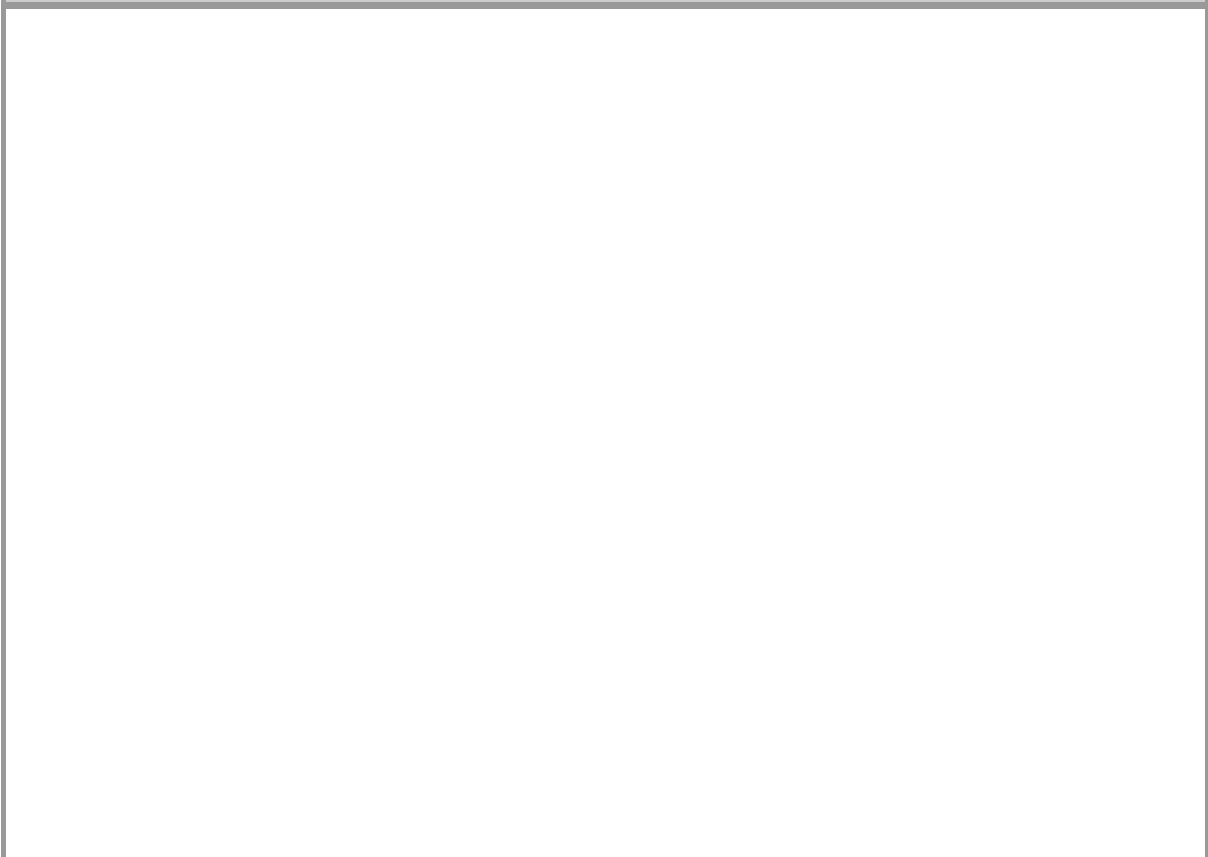
The surge in LH levels induces ovulation and promotes the survival of the corpus luteum during the luteal phase. Following ovulation, as the corpus luteum develops; high circulating concentrations of progesterone suppress the frequency and the amplitude of LH release, resulting in an overall decrease in LH. This negative feedback regulation of LH release by progesterone is mediated at both the hypothalamus and the pituitary. In the hypothalamus, progesterone blocks the surges of GnRH, whereas in the pituitary, it downregulates the number of GnRH receptors and decreases gene expression of the α - and β -subunits of both LH and FSH. Under the effect of progesterone, GnRH and LH pulse frequency is reduced to 1 pulse every 3–4 hours. This allows enrichment of FSH in the gonadotrope cells, which is secreted following lysis of the corpus luteum. In addition, negative feedback regulation by progesterone prevents a second LH surge delaying luteolysis. Thus, unless the corpus luteum is stimulated by human chorionic gonadotropin (hCG), a placental hormone (described later), it regresses. Inhibin B levels remain low during the luteal phase. During the luteal-follicular transition, following the mid-cycle rise in FSH secretion, the inhibin B concentration rises to a peak 4 days after the peak FSH concentration is reached. Inhibin A is secreted by the granulosa cells during the luteal phase, and its concentration falls during luteal regression synchronously with estradiol and progesterone, remaining low during the early follicular phase. In association with estradiol, inhibin A may then have a role in the control of FSH levels in the luteal-follicular transition and in the early follicular phase. The LH surge induces resumption of meiosis, luteinization of the follicular wall, and ovulation.

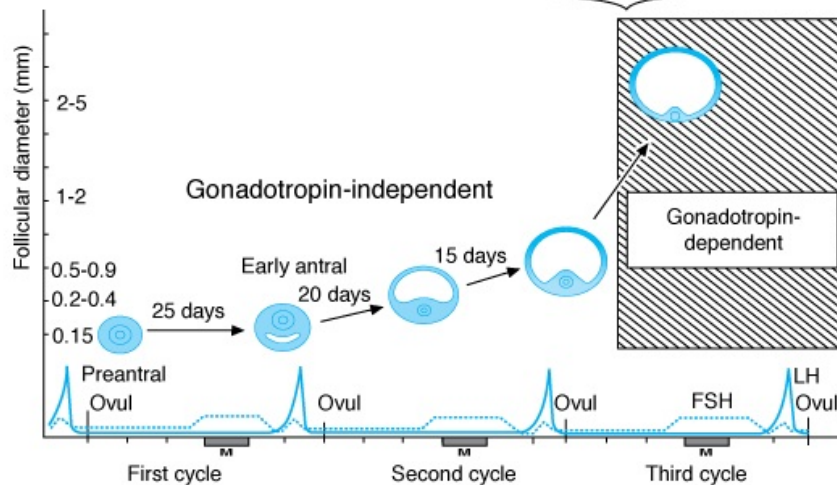
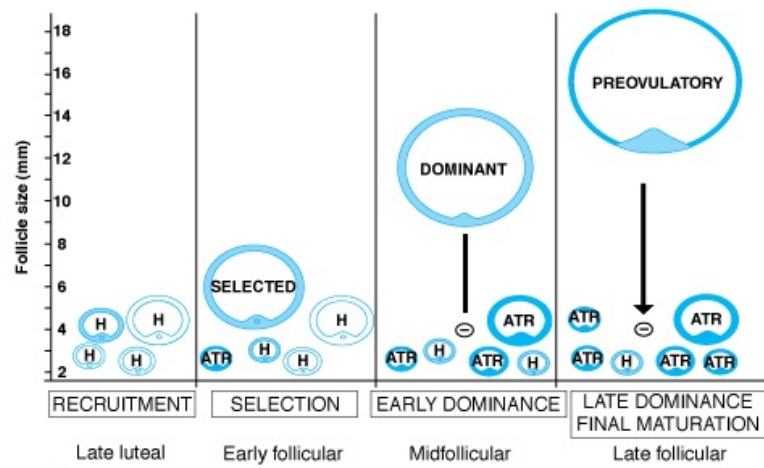
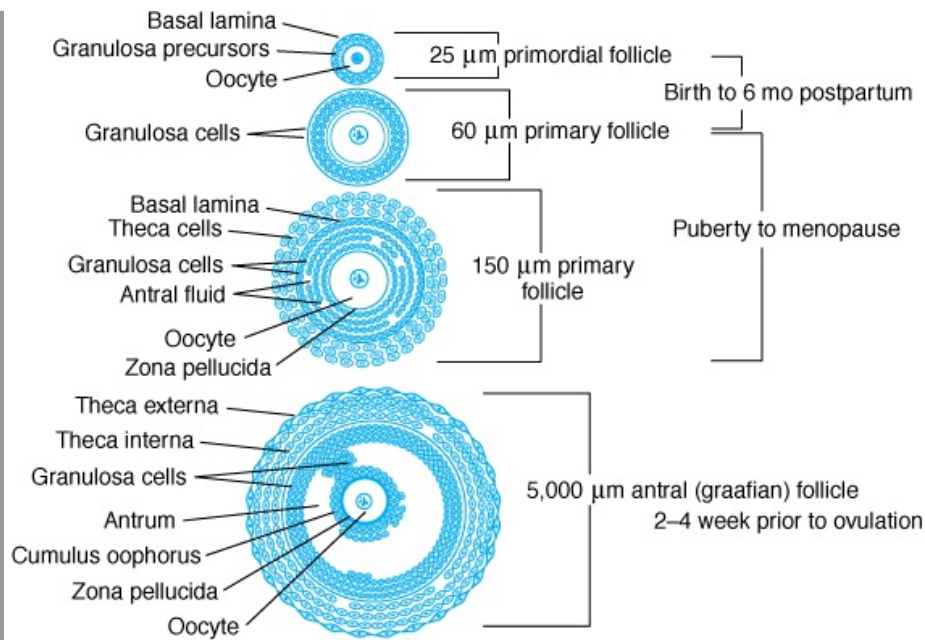
Oogenesis & Formation of the Dominant Follicle

Unlike the fetal testis, the fetal ovary begins germ cell development (oogenesis) early in fetal life. During early intrauterine development (15 weeks), primordial germ cells (oogonia) proliferate and migrate to the genital ridge. Upon their arrival in the fetal ovary, some of the oogonia continue mitotic proliferation and some begin to undergo apoptosis

(Figure 9–5). Some of these oogonia begin (but do not complete) meiosis and become oocytes. These cells have 2 X chromosomes. By 6 months postpartum, all oogonia have been converted to oocytes. At or near birth, the meiotic process is arrested at prophase of the first meiotic division. The oocytes remain arrested in the diplotene stage of the first meiotic prophase until they are recruited to grow and mature to produce an ovum or they undergo apoptosis. During the first days of postnatal life, the oocytes recruit somatic follicular cells, which are organized into a finite number of "resting" primordial follicles. Primordial follicles are composed of an outer layer of granulosa cells and a small oocyte, both enveloped in a basal lamina. The pool of primordial follicles in the female ovary reaches its maximum number at about 20 weeks of gestational age and then decreases in a logarithmic fashion throughout life until complete depletion occurs during menopause. When reproductive life is initiated, less than 10% of the primordial follicles are left.

Figure 9–5.





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Follicle growth and development. Folliculogenesis, or formation of the dominant follicle, consists of 2 stages: the gonadotropin-independent (preantral) period and the gonadotropin-dependent (antral or Graafian) period. Primordial follicular growth up to the antral stage occurs during fetal life and infancy and is gonadotropin independent. The final developmental phase of follicular growth, in which antral follicles are protected from apoptosis, begins approximately 85 days before ovulation. One dominant follicle is recruited in the luteal phase of the cycle preceding ovulation. The larger, more mature follicles are filled with a transparent albuminous fluid, consisting of an external fibrovascular coat, connected with the surrounding stroma of the ovary by a network of blood vessels; and an internal coat, consisting of several layers of nucleated cells (granulosa cells) anchored in the zona pellucida, a glycoprotein-rich eosinophilic material surrounding the oocyte. The zona pellucida forms the corona radiata, which close to the time of ovulation is separated from the granulosa cells and expelled with the oocyte during ovulation. Ovulation entails rupture of the wall of the follicle at the surface of the ovary, releasing the oocyte and corona radiata into the peritoneal cavity. Atr, atretic; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Folliculogenesis, or formation of the dominant follicle, consists of 2 stages: the gonadotropin-independent (preantral) period and the gonadotropin-dependent (antral or Graafian) period. Primordial follicular growth up to the antral stage (up to 0.2 mm) occurs during fetal life and infancy and is gonadotropin independent (Figure 9–5). Primary follicles are formed when the flattened epithelial cells become cuboidal and undergo mitosis. During the antral follicle growth phase, there is further proliferation of the granulosa cells, expression of FSH and steroid hormone receptor, and association of the theca cells with the growing follicle and granulosa cells, leading to formation of the secondary follicles. Tertiary follicles are formed following further theca cell hypertrophy and development. Their antrum is filled with estrogen-rich fluid, and the theca interstitial cells start to express FSH and LH receptors. The mechanisms that trigger initiation of follicular growth are still not completely understood, but are thought to involve bidirectional communication between germ and somatic cells through gap junctions

and paracrine factors, including cytokines and growth factors (insulin-like growth factor-I [IGF-I], epidermal and fibroblast growth factors, and interleukin-1 β). When follicles reach a size of 2–5 mm, approximately 50% are rescued from apoptosis and enter the selection growth phase. This final developmental phase of follicular growth, in which antral follicles are protected from apoptosis, begins approximately 85 days before ovulation in the luteal phase of the cycle preceding ovulation (Figure 9–5).



During this gonadotropin-regulated growth phase, follicles grow exponentially, and FSH stimulates estrogen production from granulosa cells through the induction of the aromatase system that catalyzes androgen conversion into estrogens, with stimulation of follicular fluid formation, cell proliferation, and LH receptor expression in the dominant follicle. The average time between primary follicle development and ovulation is 10–14 days (Figure 9–5).

Once a follicle is recruited for growth, the activation of gene transcription and the translation of key structural, metabolic, and signaling factors are sustained until the cell reaches full size. During follicular recruitment, the oocyte enters a growth phase that leads to the completion of the first meiotic division. The resumption of meiosis is mediated by the surge in LH. LH acts on mature follicles to terminate the program of gene expression associated with folliculogenesis. The transcription of genes that control granulosa cell proliferation (ie, IGF-I, FSH and estrogen receptors, cyclin D2) and those that encode steroidogenic enzymes is rapidly turned off by LH-mediated increases in intracellular cAMP. In addition, LH induces genes involved in ovulation (ie, progesterone receptor, cyclooxygenase-2) and luteinization (ie, cell cycle inhibitors, the enzymes steroidogenic acute regulatory protein and P450_{scc}, transcription factors, and protein kinases). At this stage, mRNA synthesis virtually ceases and does not resume until 1–3 days after the egg has been fertilized, when the final phases of meiosis are completed. One preovulatory follicle is selected every cycle, approximately 350 times during the female reproductive life span.

OVULATION

Ovulation involves rupture of the wall of the follicle at the surface of the ovary, releasing the oocyte and corona radiata into the peritoneal cavity, close to the opening of the fallopian tubes. After attaining a certain stage of development, the follicles gradually approach the surface of the ovary and rupture, releasing the ovum and fluid contents into the exterior of the ovary. Follicle rupture is an inflammatory process that involves regulated lysis of the extracellular matrix within the theca layers and tunica albuginea at the ovarian surface and release of the ovum. Several factors are involved in the rupture of the follicle, including cyclooxygenase-2, plasminogen activator, and metalloproteinases. The ovum is enclosed by an outer layer of cumulus cells and an inner, thick extracellular glycoprotein coat, the zona pellucida. Ciliary movement on the mucous membrane of the fimbria aids movement of the ovum into the fallopian tubes.

Throughout the preovulatory stage, the oocyte, granulosa cells, and theca cells acquire specific functional characteristics: The oocyte becomes competent to undergo meiosis; granulosa cells acquire the ability to produce estrogens and respond to LH via the LH receptor; and theca cells begin to synthesize increasing amounts of androgens that serve as substrates for the aromatase enzyme in the granulosa cells.

The sequence of temporal events that occurs during ovulation is initiated in a responsive preovulatory follicle by a surge of LH, which starts 34–36 hours before and peaks 12–24 hours before ovulation. This process causes both theca cells and granulosa cells to stimulate cAMP and activate selective protein kinase signaling cascades. These signaling pathways rapidly induce transcription of specific genes that are expressed transiently before follicle rupture. Their gene products initiate or alter additional cell signaling cascades, such as protease-driven cascades, which lead to follicular rupture and promote follicular remodeling to form a corpus luteum. In addition, the surge in LH stimulates resumption of meiosis up to the second meiotic metaphase following extrusion of the first polar body (remnants of 1 X chromosome) before ovulation. Meiosis is again arrested and then completed with the release of the second polar body following

fertilization.

FORMATION OF THE CORPUS LUTEUM



The surge in LH release produces reorganization of the follicle and formation of the corpus luteum, composed of small (theca-lutein) and large (granulosa-lutein) cells, fibroblasts, endothelial cells, and immune cells. Upon rupture of the follicle (following ovulation), small amounts of bleeding into the antral cavity lead to the formation of the corpus hemorrhagicum and the invasion by macrophages and mesenchymal cells, leading to revascularization of the corpus luteum. Capillaries and fibroblasts from the surrounding stroma proliferate in and penetrate the basal lamina. The granulosa-lutein cells transform into the corpus luteum a temporary endocrine gland. The corpus luteum continues to produce and secrete progesterone and estradiol, playing a key role in regulating the length of the ovarian cycle, maintaining gestation in its early stages, and suppressing LH and FSH release through the inhibition of GnRH release.

During the initial gestational period, placental production of hCG prevents the regression of the corpus luteum, transforming the corpus luteum of the ovarian cycle into the corpus luteum of pregnancy. hCG stimulates the granulosa-lutein cells to produce progesterone, 17-hydroxyprogesterone, estrogen, inhibin A, and relaxin, a polypeptide hormone from the insulin/IGF hormone family. Relaxin regulates the synthesis and release of metalloproteinases, mediators of tissue (uterus, mammary gland, fetal membranes, birth canal) growth and remodeling, in preparation for birth and lactation.

LUTEOLYSIS

Luteolysis is the process of lysis or regression of the corpus luteum and marks the end of the female reproductive cycle. The process involves an initial decline in progesterone secretion (functional luteolysis), followed by changes in the cellular structure leading to gradual corpus luteum involution (structural or morphologic luteolysis) to form a small scar of connective tissue known as the **corpus albicans**. This sequence of events takes place if fertilization does not occur within 1–2 days of ovulation. If fertilization occurs, the corpus luteum continues to grow and function for the first 2–3 months of pregnancy. Thereafter, it slowly regresses as the placenta assumes the role of hormone biosynthesis for the maintenance of pregnancy.

ENDOMETRIAL CYCLE



The ovarian cycle is accompanied by cyclic growth and shedding of the endometrium controlled by estrogen and progesterone (Figure 9–4). Three distinct phases can be identified in the endometrium throughout the menstrual cycle.

Proliferative Phase

The proliferative phase corresponds to the follicular phase of the ovary (Figure 9–5). It is characterized by estrogen-induced endometrial epithelial cell proliferation and upregulation of estradiol and progesterone receptor expression to reach a peak by the time of ovulation. This is the initial phase of endometrial maturation in preparation for implantation of the embryo. The preovulatory endometrial proliferation leads to relative hypertrophy of the uterine mucosa.

Secretory Phase

This phase corresponds to the ovarian luteal phase and is characterized by progesterone-induced differentiation of the endometrial epithelial cells into secretory cells. During the secretory phase, there is a short, well-defined period of uterine receptivity for embryo implantation (referred to as the "implantation window"). Toward the end of the secretory phase, glandular expression of estrogen receptors is markedly decreased, reflecting the suppressive effect of rising progesterone levels.

Menstrual Phase

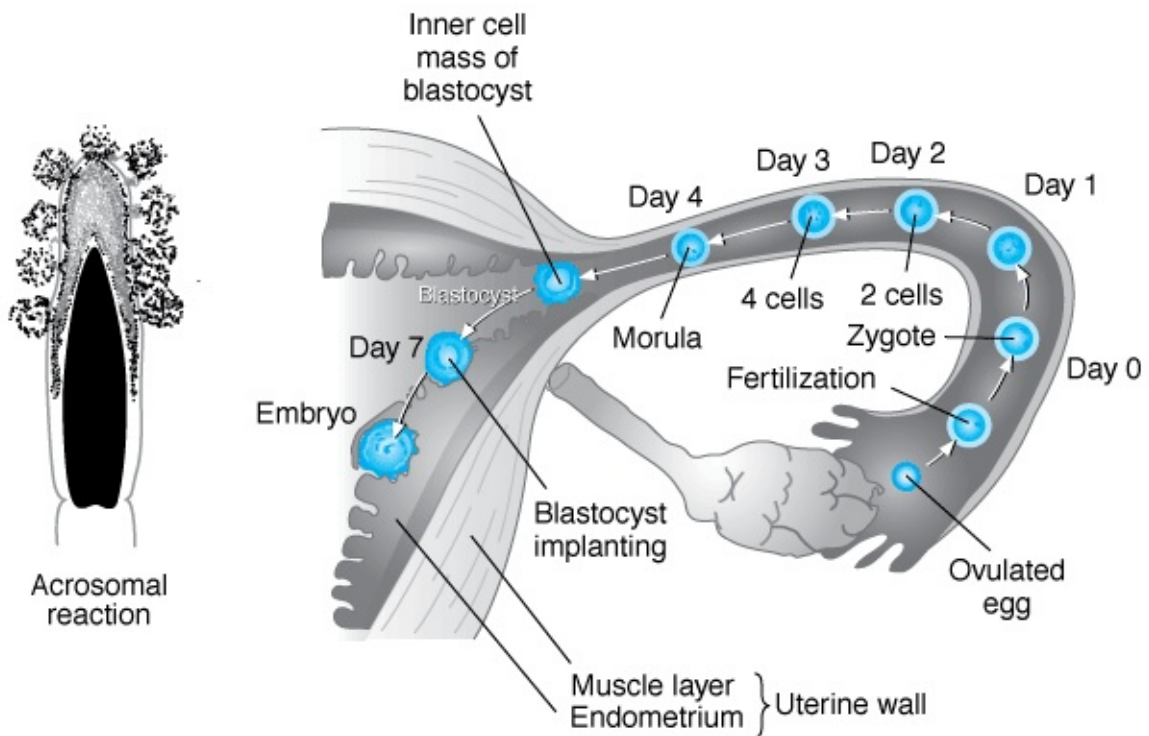
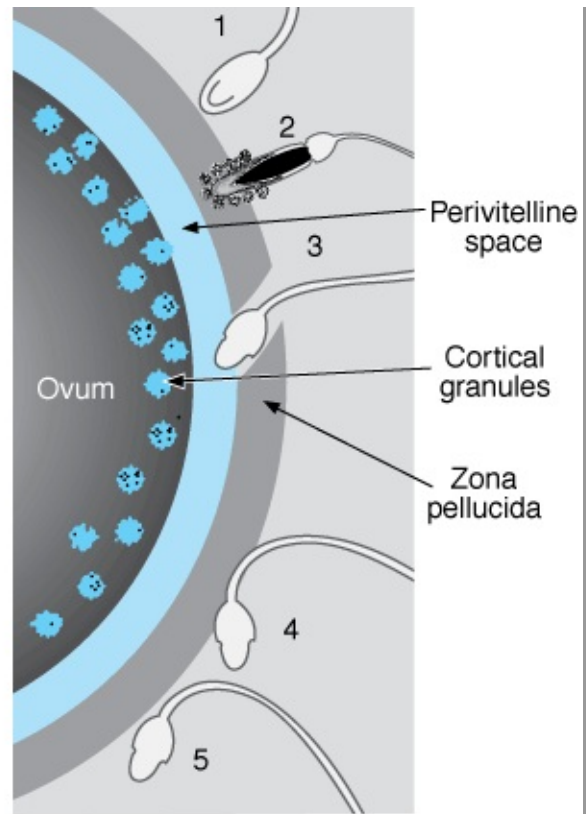
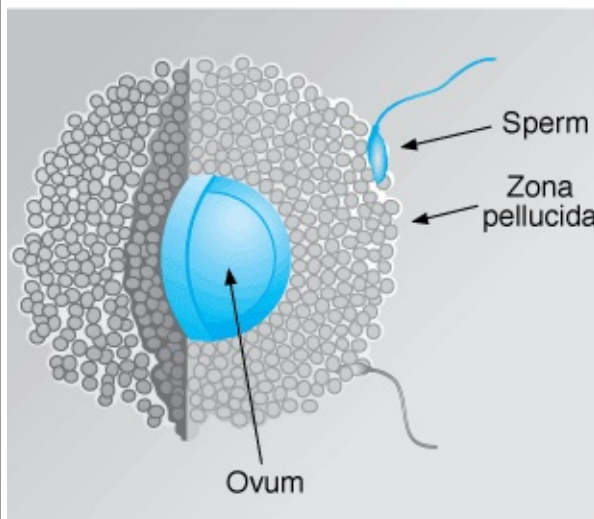
The menstrual period is characterized by shedding of the endometrium, resulting from proteolysis and ischemia in its superficial layer. Proteolytic enzymes accumulate in membrane-bound lysosomes during the first half of the postovulatory period. The integrity of the lysosomal membrane is lost with the decline in estrogen and progesterone on day 25, resulting in lysis of the glandular and stromal cells and the vascular endothelium. Ischemia due to vasoconstriction of endometrial vessels during the early part of the menstrual period results in rupture of the capillaries, leading to bleeding. In addition, a significant increase in prostaglandin $F_{2\alpha}$ in the late secretory endometrium contributes by releasing acid hydrolases from lysosomes and enhancing myometrial contractions, aiding in the expulsion of degenerated endometrium.

Fertilization

Fertilization is the union of the 2 germ cells, the ovum and the sperm, restoring chromosome number and initiating the development of a new individual. The final steps of mammalian oogenesis (and of spermatogenesis) prepare eggs (and sperm) for fertilization. In preparation for ovulation, fully grown oocytes undergo "meiotic maturation," preparing them to interact with sperm. A very low proportion (approximately 0.002%) of the sperm deposited into the vagina (approximately 10^7) migrate up the female reproductive tract to the site of fertilization in the ampullary-isthmic junction of the fallopian tubes (Figure 9–6). During this trajectory, sperm undergo activation or "capacitation," a series of changes in the sperm plasma membrane that increase its affinity for the zona pellucida, enabling the sperm to bind to the ovum and to undergo the acrosome reaction. In the fallopian tubes, sperm bind to the zona pellucida, leading to fusion of the ovum and sperm plasma membranes to form a single "activated" cell, the zygote (Figure 9–6). This simple process requires several events.

Figure 9–6.





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Fertilization and embryo migration. Sperm binds to the zona pellucida and undergoes the acrosome reaction, releasing its enzymatic contents, which are necessary for penetration of the zona pellucida. In addition, cortical granules in the ovum release their contents, preventing multiple sperm from fertilizing 1 ovum. Once the sperm penetrates the zona pellucida and begins entry into the perivitelline space, the sperm repositions itself from its original orientation with binding at the tip of the head to binding in an equatorial or sideways position, leading to fusion with the egg plasma membrane and formation of the zygote. This leads to completion of the meiotic division and initiation of mitotic divisions while the zygote is being propelled through the fallopian tubes through both ciliary movements by the epithelium and rhythmic contractions of the smooth muscle walls. The embryo enters the uterine cavity (where implantation occurs) as a blastocyst on day 4 following fertilization.

Sperm acrosome reaction and penetration of the ovum's zona pellucida—Sperm binding to the zona pellucida primes the sperm cell to initiate the acrosome reaction. The acrosome reaction involves calcium-induced fusion of the acrosome with the sperm plasma membrane and exocytosis of its enzymatic contents (proteases and glycosidases) and is required for sperm penetration. During or after the acrosome reaction, the fertilizing sperm detaches from the zona pellucida. It penetrates through the zona pellucida and fuses with the ovum plasma membrane. In this process, the sperm initially binds with the tip of its head to the ovum, which is followed by release in binding of the tip of the sperm head and an equatorial (parallel) binding, allowing incorporation of the acrosomal membrane into the ovum cytoplasm and fusion of the 2 cells.

Cortical and zona reaction—Fusion of the sperm and the ovum triggers the second meiotic division of the ovum, leading to the formation of the mature oocyte and the second polar body. In addition, this fusion triggers mechanisms that prevent fertilization of the ovum by multiple sperm, such as exocytosis of cortical granules (cortical reaction) from the oocyte, resulting in proteolysis of zona pellucida glycoproteins, as well as cross-linking of proteins forming the perivitelline barrier. The fusion of the sperm and ovum pro-nuclei

reconstitutes a diploid cell, called the zygote.

During migration of the zygote through the fallopian tubes toward the site of implantation in the uterine cavity, mitosis yields a morula and then a blastocyst. The outer cells of the blastocyst are the **trophoblast** cells, which participate in the implantation process and form the fetal components of the **placenta**. If fertilization does not take place, both the ovum and sperm degenerate relatively rapidly in the female reproductive tract.

Implantation

The human embryo (blastocyst) enters the uterus 3 days before implantation. As mentioned earlier, the "window of implantation" corresponds to the short period of endometrial receptivity for the embryo, between days 20 and 24 of the menstrual cycle. Outside of this period, implantation fails. Many of the physiologic events crucial to successful implantation are due to cyclic changes in ovarian hormone levels, leading to morphologic and functional maturation of the endometrium. Morphologically, the optimal period for implantation is characterized by the presence of columnar epithelium with microvilli and an increase in stromal cell proliferation. Implantation has been shown to occur when progesterone receptors in epithelial cells of the endometrium disappear. Implantation has many characteristics of an inflammatory process, including cytokine induction of adhesion molecule expression in endometrial cells, followed by invasion. Invasion requires enzymatic digestion of the extracellular matrix with simultaneous control of hemostasis and angiogenesis within decidual tissues. These enzymatic processes are mediated by proteinases, metalloproteinases, collagenases, gelatinases, stromelysins, metalloelastases, and urokinase. Both the growth of the trophoblast and invasion are required for successful embryo implantation and placental development.

PHYSIOLOGIC EFFECTS OF OVARIAN HORMONES

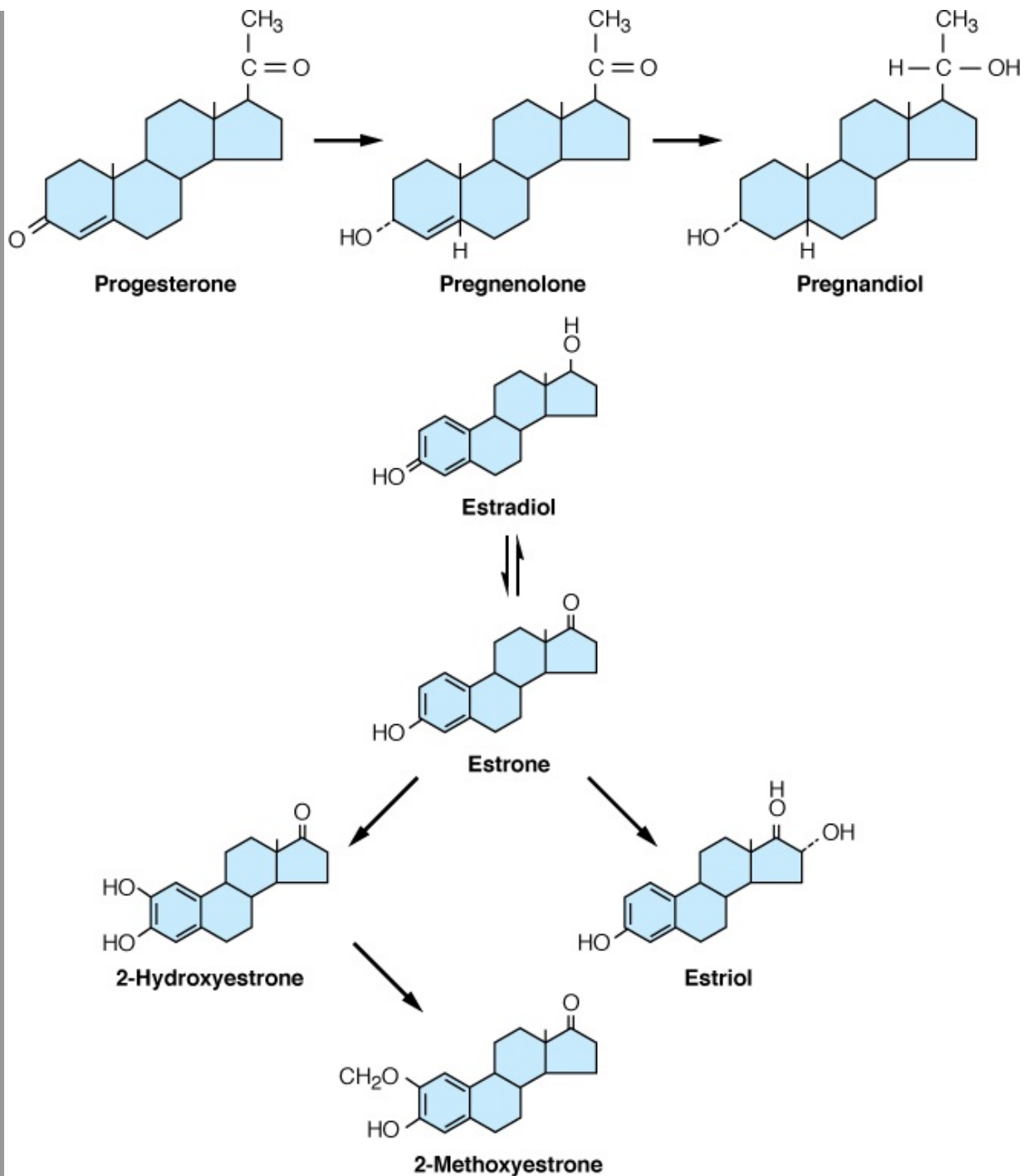
Estrogen

ESTROGEN SYNTHESIS, TRANSPORT, & METABOLISM

The primary source of estradiol in women is the granulosa cell of the ovaries. However, both granulosa and theca cells and both gonadotropins (FSH and LH) are required for the production of estrogen. The theca cells secrete androgens that diffuse to the granulosa cells to be aromatized to estrogens (Figure 9–2). In premenopausal women, 17 β -estradiol produced by the ovaries is the chief circulating estrogen. Serum estradiol concentrations are low in preadolescent girls and increase at menarche. In the adult woman, they range from about 100 pg/mL (367 pmol/L) in the follicular phase to about 600 pg/mL (2200 pmol/L) at the time of ovulation. Estradiol production varies cyclically throughout the menstrual cycle. The highest rates of production and serum concentrations are seen in the preovulatory phase and the lowest during the premenstrual phase (Figure 9–4). Estradiol levels rise to nearly 20,000 pg/mL (70,000 pmol/L) during pregnancy. After menopause, serum estradiol concentrations fall to values similar to or lower than those in men of a similar age (5–20 pg/mL [18–74 pmol/L]).

Most of the estradiol released into the blood circulates bound to sex hormone-binding globulin and to albumin, with only 2–3% circulating in the free form. Estradiol (as well as androstenedione) is converted to estrone (a weak estrogen) in peripheral tissues (Figure 9–7). Estrone is converted to estriol, primarily in the liver. Estrogens are metabolized by sulfation or glucuronidation, and the conjugates are excreted into the urine. Estrogen can also be metabolized through hydroxylation and subsequent methylation to form catechol- and methoxy-estrogens.

Figure 9–7.



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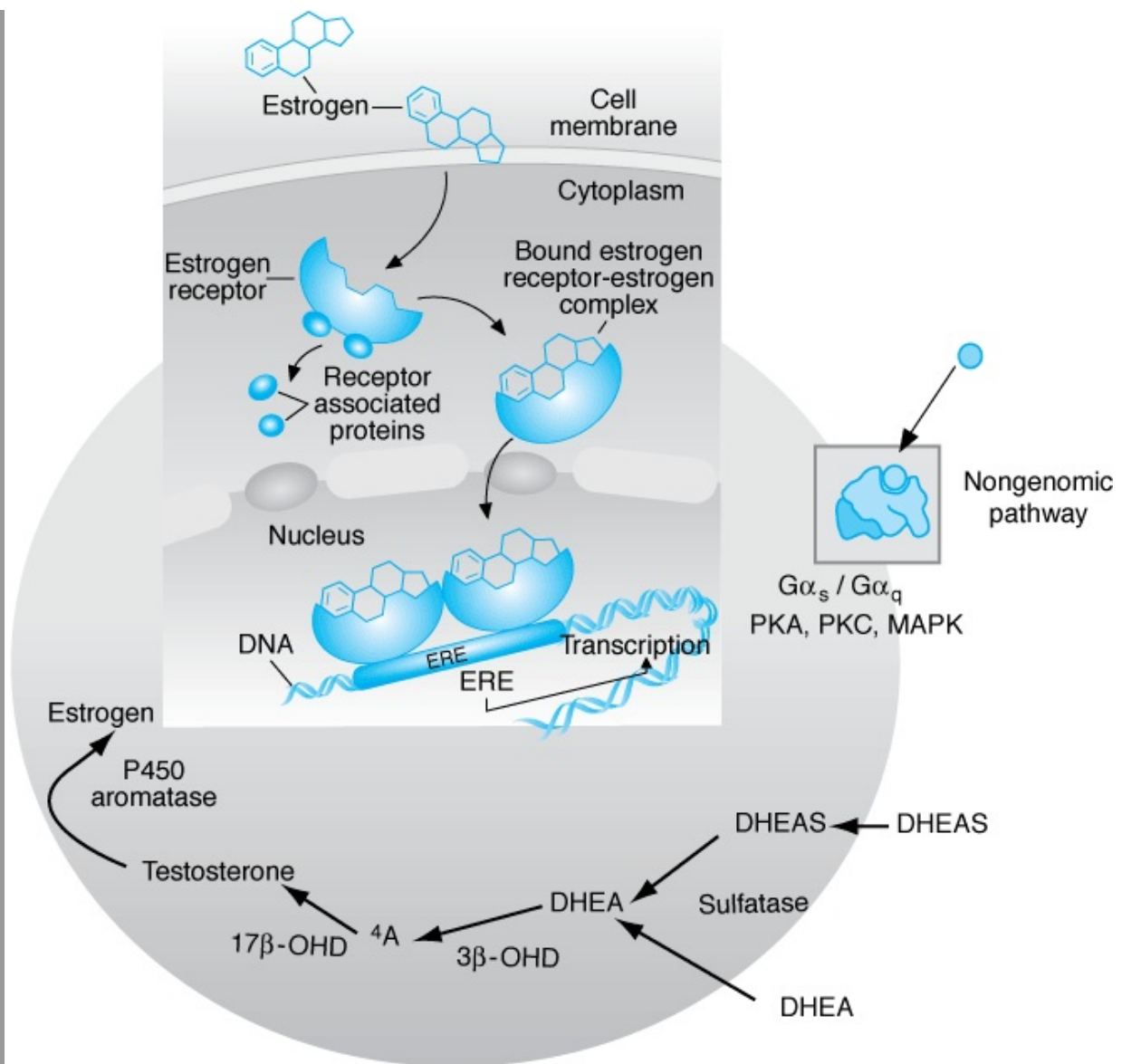
Metabolic fate of progesterone and estrogen. Progesterone and estrogen are degraded primarily in the liver. Estradiol and androstenedione are converted to estrone (a weak estrogen) in peripheral tissues. Estrone is converted to estriol, primarily in the liver.

Estrogens are metabolized by sulfation or glucuronidation, and the conjugates are excreted into the urine. Estrogen can also be metabolized through hydroxylation and subsequent methylation to form catechol-and methoxy-estrogens (not shown).

ESTROGEN RECEPTOR–MEDIATED (GENOMIC) EFFECTS

The estrogen receptors are members of the superfamily of steroid hormone receptors that function as transcription factors, altering gene expression upon activation. Like the androgen receptors, estrogen receptors have the N-terminal DNA-binding domain and the C-terminal ligand-binding domain (Figure 9–8). Two subtypes of estrogen receptors have been identified. They differ in structure, are encoded by different genes, and differ in tissue distribution. Estrogen receptor alpha (α) is considered the classic estrogen receptor. It is found predominantly in endometrium, breast cancer cells, and ovarian stroma. Estrogen receptor beta (β) is found predominantly in granulosa cells and developing spermatids, as well as in several nonreproductive target tissues, including the kidney, intestinal mucosa, lung parenchyma, bone marrow, bone, brain, endothelial cells, and prostate gland.

Figure 9–8.



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Genomic and nongenomic effects of estrogen. 17β -estradiol (E_2) diffuses into the cell and binds to either estrogen receptor (ER) $_{\alpha}$ or ER $_{\beta}$, forming homo- or heterodimers. Binding involves dissociation of the receptor from chaperone proteins and translocation into the nucleus, where the hormone-receptor complex binds to estrogen responsive elements and modulates gene transcription (genomic effects). Recently, it has been shown that estrogen can produce rapid effects that do not require gene transcription. These nongenomic mechanisms involve activation of an estrogen receptor localized in the plasma membrane

and linked to second-messenger systems, producing immediate responses as well as converging with the genomic pathway. The figure also illustrates intracrine extragonadal estrogen synthesis, prevalent during menopause. Estradiol is derived from testosterone, from androstenedione (Δ^4 A), and from dehydroepiandrosterone (DHEA) or dehydroepiandrosterone sulfate (DHEAS). The desulfated DHEA is converted to Δ^4 A by 3 β -hydroxysteroid dehydrogenase (3 β -OHD), and the Δ^4 A is transformed into testosterone by 17 β -hydroxysteroid dehydrogenase (17 β -OHD). Testosterone can then be converted into either estradiol or androstenedione, which in turn can be converted to estrone and then to estradiol by the type I isoenzyme. Some of the family of 17 β -hydroxysteroid dehydrogenase isoenzymes (eg, type II isoenzyme) can convert estradiol to estrone, providing an additional mechanism for the regulation of estrogen synthesis and metabolism. ERE, estrogen response element; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C. (Modified, with permission, from Gruber CJ *et al.* Mechanisms of disease: Production and actions of estrogens. *NEJM*. 2002;346:340. Copyright © Massachusetts Medical Society. All rights reserved.)

Estrogen receptors are mostly nuclear, but are also found in the cytoplasm. Similar to other steroid hormones, free estrogen diffuses into the cell and binds to the ligand-binding domain of the receptor, which dissociates from its cytoplasmic chaperone proteins. The estrogen-receptor complex then translocates into the cell nucleus, where it binds as homodimers or heterodimers to specific sequences of DNA called estrogen response elements, regulating gene transcription. The physiologic effects of estrogens that are mediated through transcriptional activation take minutes or hours to occur.

NONGENOMIC EFFECTS OF ESTROGEN

Some rapid effects of estrogen cannot be explained by a transcriptional mechanism (nongenomic) and are the result of direct estrogenic action on cell membranes mediated by cell membrane forms of estrogen receptor (Figure 9–8). Although these receptors remain largely uncharacterized, they are thought to resemble their intracellular counterparts. Alternatively, nongenomic effects may be exerted by the classic estrogen receptors in direct interaction with intracellular enzymes. Examples of some of the rapid nongenomic effects of estrogen include direct effects on the vasculature and activation of growth factor–related signaling pathways.

Estrogen can cause short-term vasodilatation by both endothelium-dependent and endothelium-independent pathways. Estrogen produces short-term vasodilatation and reduces vascular smooth muscle tone through the increased formation and release of endothelial nitric oxide and prostacyclin and the opening of calcium channels mediated by cyclic guanosine monophosphate (cGMP). Estrogen inhibits apoptosis of endothelial cells and promotes their angiogenic activity in vitro and may protect against atherosclerosis. At pharmacologic concentrations, estrogen inhibits the influx of extracellular calcium into vascular smooth muscle cells by an effect on L-type calcium channels.

Estrogen promotes rapid activation of growth factor–related signaling pathways through mitogen-activated protein kinase signaling cascades in several tissues, including osteoblasts, endothelial cells, neurons, and human breast cancer cells.

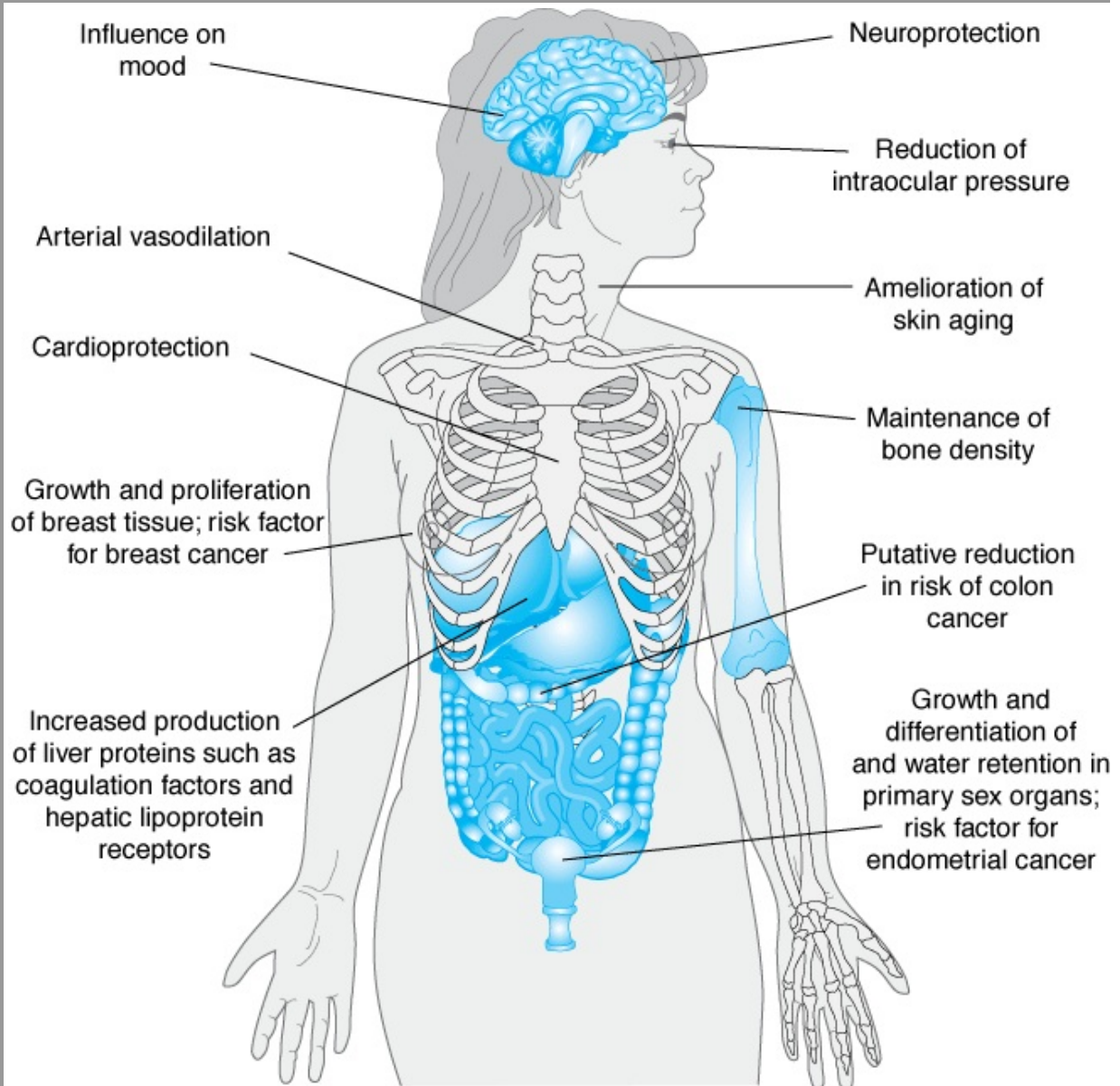
PHYSIOLOGIC ACTIONS OF ESTROGEN AT TARGET ORGANS

Reproductive System



Estrogen exerts multiple effects in reproductive organs (Figure 9–9).

Figure 9–9.



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Systemic effects of estrogen. In addition to its reproductive organ

effects, estrogen has neuroprotective effects and reduces perimenopausal mood fluctuations in women. Estrogen is cardioprotective and may protect against colon cancer, and it has vasodilatory effects. In the liver, estrogen stimulates the uptake of serum lipoproteins and the production of coagulation factors. Estrogen protects against bone loss. In the skin, it increases skin turgor and collagen production and reduces the depth of wrinkles. (Modified, with permission, from Gruber CJ *et al.* Mechanisms of disease: production and actions of estrogens. *NEJM*. 2002;346:340. Copyright © Massachusetts Medical Society. All rights reserved.)

Uterus—Estrogen promotes proliferation of the endometrium by stimulating mitosis of the stratum basale and angiogenesis of the stratum functionale. Estrogen sensitizes uterine smooth muscle to the effects of oxytocin by increasing the expression of oxytocin receptors and contractile proteins. Estrogen increases watery cervical mucus production.

Ovary—Estrogen exerts potent mitotic effects on granulosa cells.

Breast—Estrogen stimulates growth and differentiation of the ductal epithelium, induces mitotic activity of ductal cylindric cells, and stimulates the growth of connective tissue. The density of estrogen receptors in breast tissue is highest in the follicular phase of the menstrual cycle and falls after ovulation. Estrogen can also indirectly affect mammary gland development by elevating prolactin and progesterone levels and inducing progesterone receptors in mammary epithelium. The growth-promoting effects of estrogen have been implicated in breast and endometrial cancer.

Other body systems—Liver—Estrogen affects the expression of apoprotein genes and increases lipoprotein receptor expression, resulting in a decrease in serum concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol, increases in serum high-density lipoprotein cholesterol and triglyceride concentrations, and decreases in serum lipoprotein A concentrations. Estrogen regulates the hepatic expression of genes involved in coagulation and fibrinolysis. Estrogen decreases plasma concentrations of fibrinogen, antithrombin III and protein S, and plasminogen activator inhibitor type 1. Elevated

plasma estrogen levels are associated with an overall increase in the potential for fibrinolysis. Estrogen stimulates the synthesis of transport proteins (thyroxine-binding globulin and transcortin).

Central nervous system—Estrogen has neuroprotective actions, and its age-associated decline is associated with a decline in cognitive function.

Bone—Overall, the effects of estrogen are antiresorptive. Estrogen promotes bone maturation and closure of epiphysial plates in long bones. It conserves bone mass by suppressing bone turnover and maintaining balanced rates of bone formation and bone resorption. Estrogen affects the generation, life span, and functional activity of both osteoclasts and osteoblasts. It decreases osteoclast formation and activity and increases osteoclast apoptosis.

Progesterone

Progesterone is produced by both theca and granulosa cells and is the predominant ovarian hormone produced in the luteal phase, a period during which plasma concentrations rise 10-fold. Production of progesterone by the corpus luteum is mainly under LH stimulation. The majority (80%) of progesterone secreted circulates bound to albumin. The principal targets of progesterone are the reproductive tract and the hypothalamic-pituitary axis. The degradation of progesterone is similar to that of androgens and estrogens and occurs primarily in the liver.

PROGESTERONE RECEPTOR-MEDIATED EFFECTS

Progesterone appears to exert most of its effects by directly regulating transcription of genes through 2 specific progesterone receptor proteins, termed A and B. These progesterone receptor proteins arise from a single gene and act as ligand-inducible transcription factors, regulating the expression of genes by binding specific progesterone responsive elements on the DNA. The expression of progesterone receptors is upregulated by estrogen and downregulated by progesterone in most target tissues. Previous exposure to estrogen induces the production of receptors for progesterone and is required for progesterone to act on the reproductive tract.



Progesterone receptor expression in the uterus is increased during the first half of the menstrual cycle. During the second half, as serum progesterone levels increase, total progesterone receptor levels in the uterus decrease. During the follicular phase of the cycle, high levels of progesterone receptor are present in the nuclei of epithelial and stromal cells of the human endometrium and in myometrial smooth muscle cells. In the mid-and late-luteal phase, detection of progesterone receptor in the luminal and glandular epithelium declines markedly to undetectable levels; on the other hand, stromal and myometrial cells continue to express high levels of progesterone receptor despite high circulating progesterone and absent estrogen receptor.

In general, progesterone acts on the reproductive tract to prepare it for initiation and maintenance of pregnancy. The major physiologic roles of

progesterone are mediated in the uterus and ovary, where it stimulates the release of mature oocytes, facilitates implantation, and maintains pregnancy through stimulation of uterine growth and differentiation and suppression of myometrial contractility. In the brain, progesterone modulates sexual behavior. Increased progesterone levels during the luteal phase increase both core and skin temperatures. This results in a biphasic pattern of body core temperature throughout the menstrual cycle, with a higher temperature in the luteal phase of the cycle.

PHYSIOLOGIC ACTIONS OF PROGESTERONE AT TARGET ORGANS

Effects on the uterus during early pregnancy—Progesterone induces stromal differentiation; stimulates glandular secretions, changing the pattern of proteins secreted by endometrial cells; and modulates cyclic proliferation during the menstrual cycle.

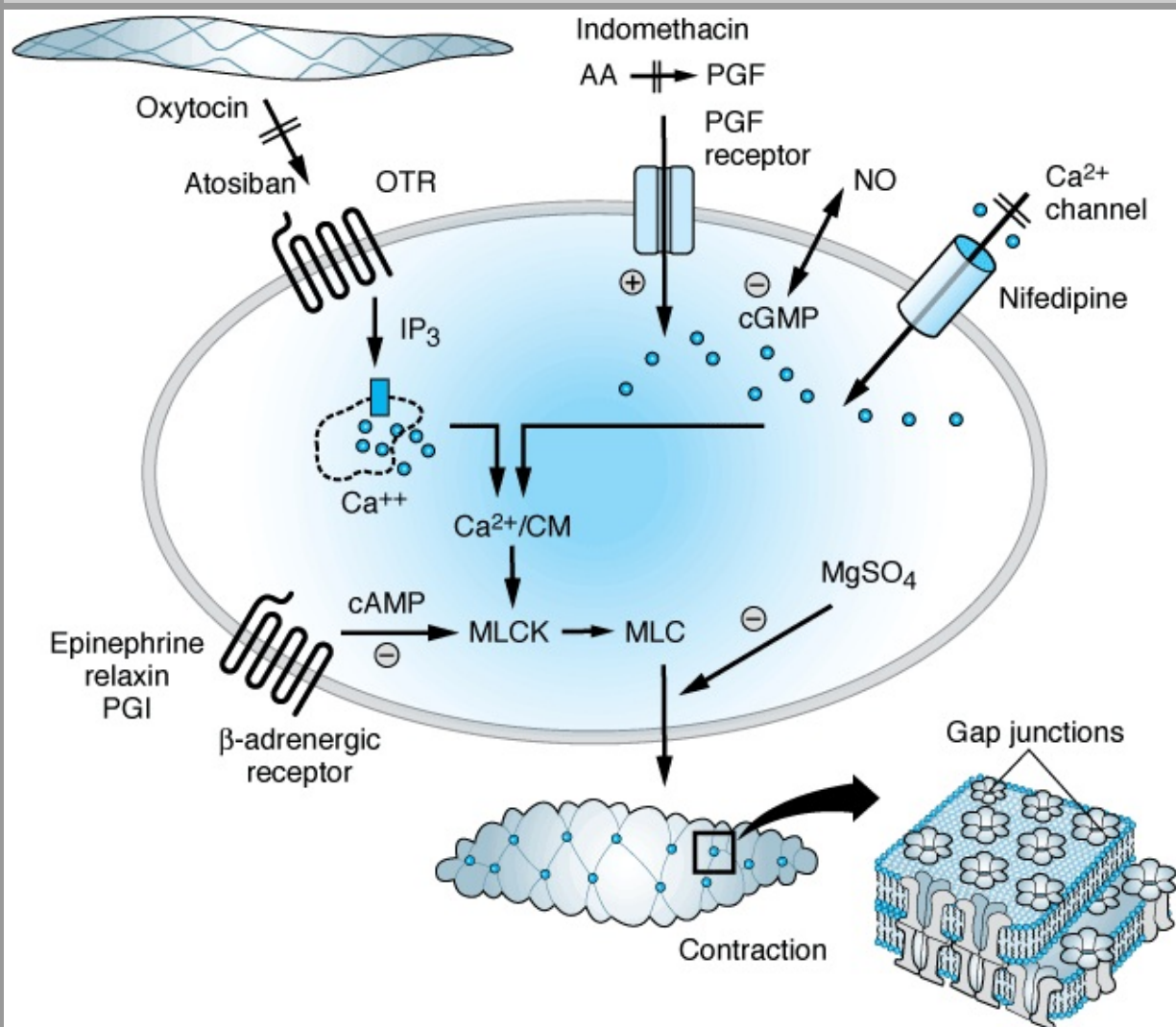
Progesterone induces uterine cell proliferation and differentiation in early pregnancy through regulation of localized growth factor synthesis and cell type–specific expression of their receptors, thereby regulating cellular sensitivity to the autocrine or paracrine effects of growth factors and producing an environment that supports early embryonic development.

Promotion and maintenance of implantation—Progesterone plays a major role in preparing the endometrium for implantation of a fertilized ovum. Progesterone facilitates implantation by stimulating the synthesis of enzymes responsible for lysis of the zona pellucida. Finally, it promotes and maintains implantation through effects on both the maternal uterus and the developing blastocyst.

Effects on uterine contractility—Progesterone induces quiescence of the myometrium by increasing resting membrane potential and preventing electrical coupling between myometrial cells (Figure 9–10). In addition, progesterone decreases uptake of extracellular calcium required for contraction of myometrial cells by downregulating the expression of genes that encode subunits of voltage-dependent calcium channels. Progesterone also prevents uterine contractions by blocking the ability of estradiol to induce membrane expression of α -adrenergic receptors (α -adrenergic activation causes contractions). Progesterone

decreases prostaglandin synthesis and increases the rate of prostaglandin inactivation through the stimulation of prostaglandin 15-dehydrogenase and opposes the stimulatory effects of estrogen on endometrial prostaglandin F2 α expression in the luteal phase of the menstrual cycle. Progesterone maintains the levels of relaxin, inhibiting spontaneous or prostaglandin-induced myometrial contraction, and contributes to the maintenance of implantation and early pregnancy by increasing the collagen framework and distensibility of the uterus.

Figure 9–10.



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Factors that affect uterine contractility. Smooth muscle contraction is

mediated through Ca^{2+} -calmodulin activation (CAM) of myosin light chain kinase (MLCK), the enzyme that phosphorylates myosin light chain (MLC). Uterine contraction is stimulated by prostaglandin F (PGF) and oxytocin (OT). Uterine contraction is decreased by β -adrenergic agonists, nitric oxide (NO), relaxin, and PGI_2 . The mechanism involves an increase in either cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP). Indomethacin inhibits prostaglandin synthesis from arachidonic acid (AA); Atosiban inhibits the oxytocin receptor (OTR antagonist). cAMP activates protein kinase A, which then phosphorylates MLCK to reduce its ability to bind Ca^{2+} -calmodulin, or phosphorylates a membrane-binding site for Ca^{2+} that increases Ca^{2+} binding and reduces free intracellular Ca^{2+} concentrations. DAG, diacylglycerol; IP_3 , inositol 1,4,5-trisphosphate; PIP_2 , phosphatidylinositol 4,5-bisphosphate.

At the end of pregnancy, the fall in progesterone levels is associated with increased prostaglandin synthase activity and prostaglandin $\text{F}_{2\alpha}$ production, enhancing uterine contractility. The antiprogestin mifepristone antagonizes all of the actions of progesterone on prostaglandin synthesis and catabolism and stimulates prostaglandin production, thereby producing its abortive effects.

Effects on lactation—In the mammary gland, progesterone stimulates lobular-alveolar development in preparation for milk secretion but suppresses milk protein synthesis before parturition. Progesterone antagonizes prolactin's effects in mid-to late-pregnancy, preventing the synthesis of milk proteins. The sudden fall in circulating progesterone that occurs with parturition is associated with a concurrent increase in prolactin secretion and the onset of lactation.

Antiestrogen actions—Progesterone antagonizes estrogen induction of many of the known hormone-responsive genes. This effect is mediated by downregulation of cytoplasmic and nuclear estrogen receptor protein concentrations, decreasing the active estrogen concentration (and antagonizing the action of estrogen receptor at the molecular level), particularly in the uterus.

The Placenta

STRUCTURE AND PHYSIOLOGIC FUNCTION

The placenta is derived from 2 major cell types, which are the source of the principal placental hormones. The outer cell mass of the blastocyst, the precursor to the trophoblast, is in contact with the endometrium and undergoes proliferation and tissue penetration during implantation. The trophoblast has 2 cell populations: an inner cytotrophoblast and an outer invasive syncytiotrophoblast. The maternal side of the placenta contains fetal chorionic villi that provide an extensive surface area for nutrient and gas exchange between the fetal and maternal circulation. The villi are covered with multinucleated syncytiotrophoblast and trophoblast stem cells, stromal cells, and blood vessels. The villous cytotrophoblast cells are entirely secluded from maternal elements, with the exception of any molecules that might be transported across the placenta by the syncytiotrophoblast. By contrast, the extravillous trophoblast cells are continuously exposed to maternal tissues. The middle layer of the placenta consists of densely packed cytotrophoblast cell columns and serves as structural support for the underlying villi.

The physiologic functions of the placenta can be classified as follows:

- Supportive, allowing embryo implantation into the uterus and transporting nutrients and oxygen necessary for fetal growth
- Immune, suppressing the local immune system to prevent immunologic rejection of the fetus by the mother
- Endocrine, including hormone synthesis, transport, and metabolism to promote fetal growth and survival

Inability of the placental unit to perform these functions leads to multiple complications of human pregnancy, including abortion, miscarriage, impaired fetal growth, and preeclampsia.

ENDOCRINE FUNCTION OF THE PLACENTA

The placenta produces cytokines, hormones, and growth factors that are essential for regulation of the fetomaternal unit. In addition, the placenta expresses enzymes involved in hormone metabolism, playing an important role in protection of the fetus from maternal adrenal-derived androgens through aromatase activity and from glucocorticoids through the activity of type II 11β -hydroxysteroid dehydrogenase (Chapter 6). The principal placental hormones are as follows.

Human chorionic gonadotropin—hCG is a heterodimeric glycoprotein from the same hormone family as LH, FSH, and TSH. It is produced by the syncytiotrophoblast and released into the fetal and maternal circulation. It is known as the hormone of pregnancy and is the basis for the pregnancy test. hCG is detected in serum at day 6–8 after implantation, and its levels peak at 60–90 days of gestation, declining thereafter. hCG has structural and functional similarity to LH, has a much longer half-life, and exerts its physiologic effects through binding to the LH receptors. The main function of hCG is to maintain the corpus luteum to ensure the production of progesterone until placental production takes over. hCG plays an important role in fetal development through the regulation of testosterone production by the fetal Leydig cells (Chapter 8). Regulation of hCG release from the placenta is not completely understood, but evidence indicates that its paracrine regulation involves placenta-derived GnRH, activin, and inhibin. Maternal hCG levels are a useful index of functional status of the trophoblast (placental health).

Human placental lactogen and growth hormone—The human growth hormone (hGH) and human placental lactogen (hPL) gene family is important in the regulation of maternal and fetal metabolism and the growth and development of the fetus. hPL is produced by the syncytiotrophoblast and is secreted into both the maternal and fetal circulations after the sixth week of pregnancy. In the fetus, hPL acts via lactogenic receptors and possibly a unique hPL receptor to modulate embryonic development; regulate intermediary metabolism; and stimulate the production of IGFs, insulin, adrenocortical hormones, and pulmonary surfactant. During pregnancy, hGH-V, a GH variant expressed by the placenta, becomes the predominant GH in the mother. This hormone has structural and functional similarity to pituitary GH (it

differs by 13 amino acids) and is not released into the fetus. Starting from the 15th to the 20th week of gestation up to term of pregnancy, placental GH gradually replaces maternal pituitary GH, which becomes undetectable. hGH-V stimulates IGF-I production and modulates maternal intermediary metabolism, increasing the availability of glucose and amino acids to the fetus. Placental GH is not detectable in the fetal circulation, and thus it does not appear to have a direct effect on fetal growth. However, its physiologic role is thought to involve modulation of placental development through an autocrine or paracrine mechanism because of GH receptor expression by the placenta.

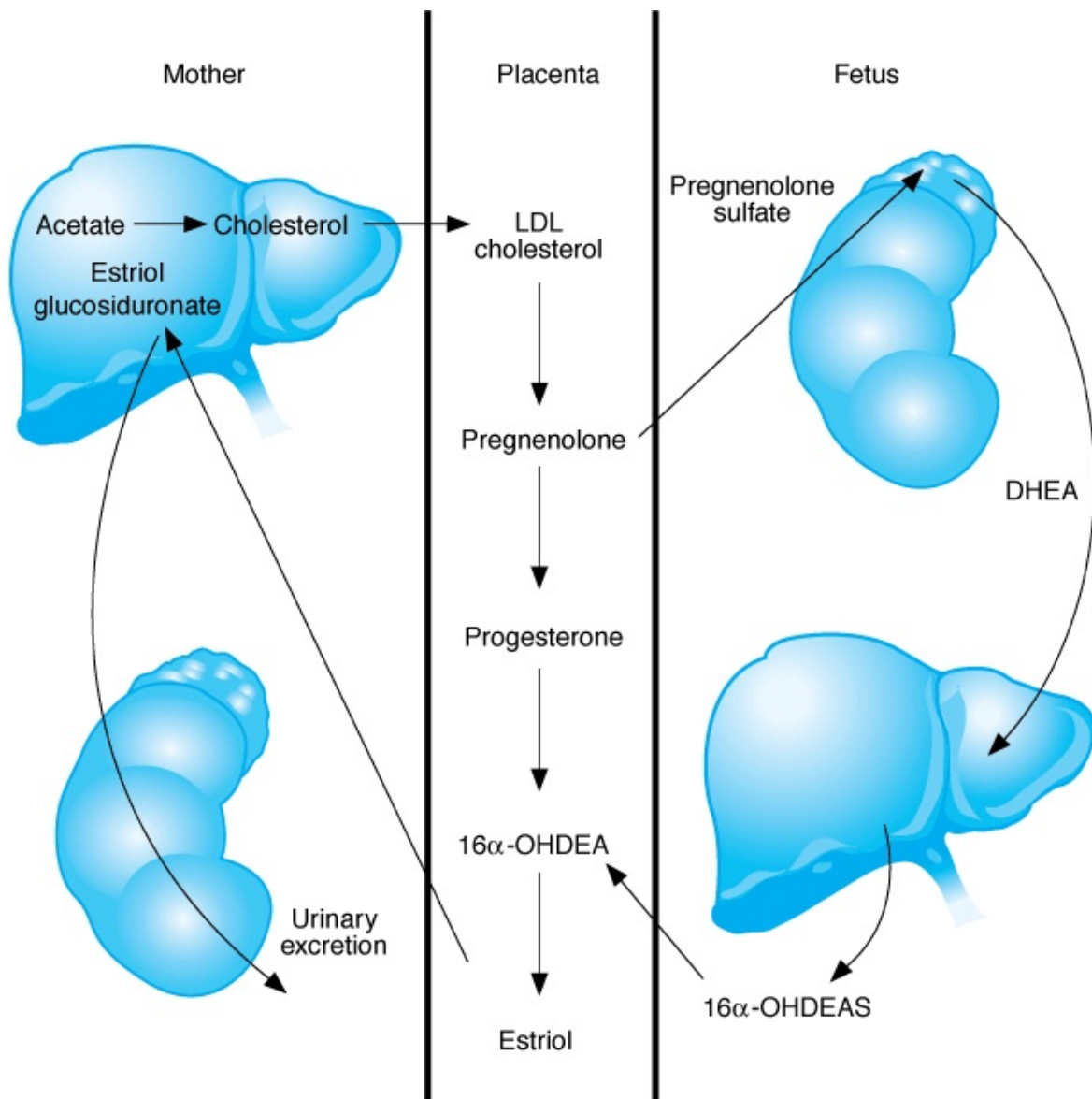
Progesterone—The major source of progesterone during the initial phase of pregnancy is the corpus luteum under hCG regulation. Starting from about week 8 of gestation, the placenta (syncytiotrophoblast) becomes the principal source of progesterone, leading to rising levels of maternal progesterone from 25 ng/mL during the luteal phase to 150 ng/mL during the last trimester of pregnancy. Because the placenta is unable to produce cholesterol from acetate, cholesterol for placental progesterone synthesis is derived from circulating LDL. LDL binds to the LDL receptor in trophoblast cells and undergoes endocytosis; cholesterol is released and processed through the steroidogenic hormone pathway (Chapter 6). As discussed earlier, progesterone plays an important role in maintaining uterine quiescence during pregnancy, inhibiting prostaglandin synthesis, and modulating the immune response to preserve pregnancy.

Estrogen—The main source of estrogen during the initial phase of pregnancy is the corpus luteum, being replaced later by placental production. The principal estrogen produced by the syncytiotrophoblast cells of the placenta is estriol. Plasma and urinary levels of estriol increase significantly throughout pregnancy to about 25 ng/mL during the last trimester. The production of estrogen by the placenta requires coordinated interaction between fetal and maternal adrenal gland steroid hormone production (fetoplacental unit of steroid biosynthesis). The placenta lacks 17 α -hydroxylase and 17, 20-desmolase and is thus unable to convert progesterone to estrogen or to produce androgens. This lack of placental androgen production protects the female fetus from masculinization; protection is also aided by the strong aromatase

activity that inactivates maternal and fetal adrenal-derived androgens. Therefore, maternal and fetal adrenal-derived androgens (dehydroepiandrosterone sulfate, DHEAS) are required for 17β -estradiol and estriol production. Estriol is synthesized through the aromatization of 16α -hydroxyandrostenedione derived from 16α -hydroxyepiandrosterone sulfate produced by the fetal liver and desulfated in the placenta (Figure 9–11); 16α -hydroxyepiandrosterone sulfate is derived from DHEAS produced in the fetal adrenal gland. The enzymes involved are placental sulfatase (DHEAS deconjugation), 3β -hydroxysteroid dehydrogenase (pregnenolone to progesterone conversion), and aromatase. The principal physiologic effects of estrogen during pregnancy include stimulation of uterine growth, prostaglandin synthesis, thickening of the vaginal epithelium, sensitization to oxytocin effects, growth and development of the mammary epithelium, and inhibition of milk production.

Figure 9–11.





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Fetoplacental unit hormone synthesis. The production of estrogen by the placenta requires the coordinated interaction between fetal and maternal adrenal gland steroid hormone production. The placenta lacks 17α -hydroxylase and is thus unable to convert progesterone to estrogen or to produce androgens. Maternal and fetal adrenal-derived androgens (dehydroepiandrosterone sulfate, DHEAS) are required for 17β -estradiol and estrone production. Estriol is synthesized through the aromatization of 16α -hydroxyandrostenedione derived from 16α -hydroxyepiandrosterone sulfate (16α -OHDEAS) produced by the fetal liver and desulfated in the placenta; 16α -hydroxyepiandrosterone

sulfate in turn is derived from DHEAS produced in the fetal adrenal gland. LDL, low-density lipoprotein; 16 α -OHDEA, 16 α -hydroxyepiandrosterone.

Corticotropin-releasing hormone—Corticotropin-releasing hormone (CRH) is produced by the syncytiotrophoblast and trophoblast cells of the placenta. Its structure and function are similar to those of hypothalamus-derived CRH. The CRH concentration increases exponentially throughout pregnancy and peaks during labor. Placental production of CRH has been linked to the length of gestation in humans. CRH is secreted into the maternal circulation in large amounts during the third trimester of pregnancy and may play an important role in the onset of labor. CRH exerts a number of functions within the intrauterine environment, such as induction of prostaglandin production and maintenance of placental blood flow.

Pregnancy & Lactation

HORMONAL CONTROL OF PARTURITION

Uterine contractility during pregnancy and parturition can be divided into at least 4 distinct phases.

Phase 0—During pregnancy, the uterus is maintained in a relatively quiescent state, mainly through the effects of progesterone. Additional factors involved in modulation of uterine activity during this period are prostacyclin, relaxin, parathyroid hormone-related peptide, and CRH. In general, their effects are the result of increased intracellular concentrations of cAMP or cGMP and inhibition of intracellular calcium release, resulting in decreased myosin light chain kinase activity. The initiation of parturition results from the transition from the quiescent phase (phase 0) to a phase of activation (phase 1).

Phase 1—This phase of parturition is associated with activation of uterine function and is characterized by release from the inhibitory mechanisms maintaining uterine quiescence throughout pregnancy and activation of factors promoting uterine activity. These factors include uterine stretch and tension caused by the fully grown fetus, activation of the fetal hypothalamic-pituitary-adrenal axis, and increased prostaglandin synthesis. Mechanical stretch or hormonal priming leads to upregulation of gene expression of proteins that facilitate smooth muscle contraction, including connexins (key components of gap junctions), prostaglandin and oxytocin receptors, and ion channel proteins.

Phase 2—This phase of parturition is a period of active uterine contraction and is stimulated by prostaglandins, oxytocin, and CRH. Prostaglandins, particularly those produced in the intrauterine tissues, play a central role in the initiation and progression of labor. They induce myometrial contractility and help produce the changes associated with cervical softening at the onset of labor.

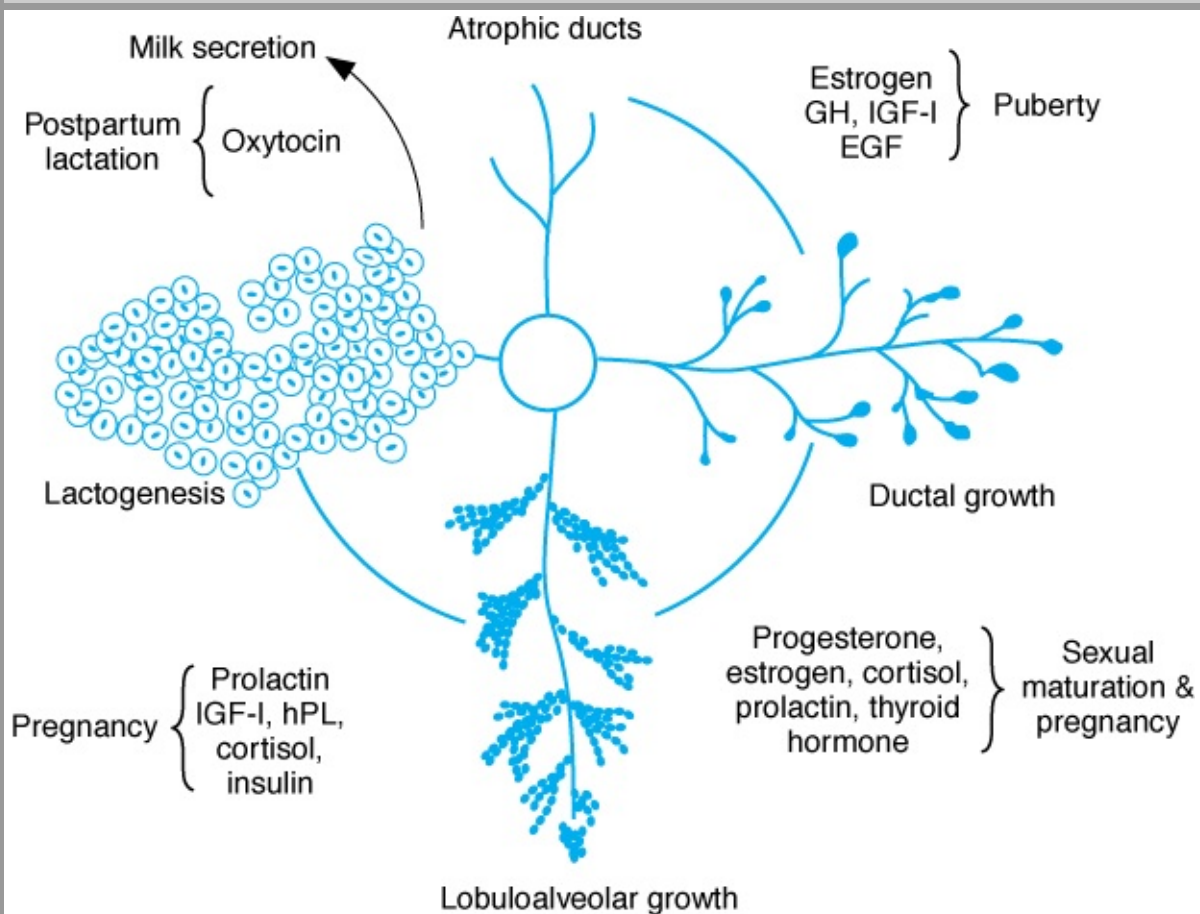
Phase 3—This postpartum phase involves uterine involution after delivery of the fetus and placenta and is mainly due to the effects of oxytocin.

MAMMARY GLAND DEVELOPMENT



Mammary gland development represents a complex program of cell proliferation, cell differentiation, and morphogenesis. Most mammary gland development occurs postnatally and involves branching and extension of ductal growth points and secretory lobules into a fatty stroma. Ductal development in the mammary gland supports the establishment of alveolar structures during pregnancy before the onset of lactogenesis and is regulated by the associated alterations in key hormones and growth factors during the various reproductive states (Figure 9–12).

Figure 9–12.



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Hormonal regulation of breast development and lactogenesis. Mammary gland development is initiated at puberty through the actions of estradiol and growth factors and is further regulated during pregnancy through the effects of prolactin and human placental lactogen (hPL). Throughout pregnancy, progesterone inhibits lactogenesis. This inhibitory effect is removed following parturition, when prolactin levels act unopposed to stimulate lactogenesis. Through neuroendocrine reflexes, suckling stimulates the release of oxytocin from the posterior pituitary, producing the milk "let-down" reflex. EGF, epidermal growth factor; GH, growth hormone; IGF-I, insulin-like growth factor-I.

Ductal elongation is mediated by estrogen, GH, IGF-I, and epidermal growth factor. At the onset of puberty, estrogen production stimulates the complex developmental process from the immature breast consisting of a nipple, a few small ductal elements, and an underlying fat pad. The terminal end bud directs ductal growth, elongation, and branching into the fat pad.

Ductal branching and alveolar budding are influenced by progesterone, prolactin, and thyroid hormone. Progesterone stimulates ductal side branching and alveolar development. Prolactin acts directly upon mammary epithelium to induce alveolar development. Both progesterone and prolactin synergize to stimulate proliferation of ductal epithelium. In the postpubertal female, during the luteal phase, progesterone stimulates the sprouting of alveolar structures from the sides of the ducts. During pregnancy, prolactin, progesterone, and hPL levels rise and the terminal duct lobular units undergo expansion and differentiation of the secretory function.

The stage of mammary differentiation into a secretory function in preparation for lactogenesis is called stage I lactogenesis. During pregnancy, progesterone, estrogen, prolactin, hPL, and GH act synergistically to prepare the breast for lactation by promoting lobuloalveolar development. The elevated levels of progesterone prevent milk production during this period. With the progression of pregnancy, the circulating levels of prolactin continue to increase. During the third trimester, prolactin levels increase from less than 25 ng/mL to a range of 200–450 ng/mL, and hPL rises to 6000 ng/mL. The

rise in prolactin levels is the result of estrogen-induced increase in lactotrophs in the anterior pituitary during gestation.

The second stage (stage II lactogenesis) is initiated after termination of pregnancy. The sudden fall in circulating progesterone that accompanies parturition in association with the concurrent increase in prolactin secretion mark the onset of lactation. The removal of the inhibition of synthesis of α -lactalbumin, and β -casein by the decrease in progesterone action allow prolactin, insulin, and glucocorticoids to stimulate the synthesis of the milk proteins. Continuous milk production is maintained by prolactin secretion from the anterior pituitary (Chapter 3) throughout the period of lactation. Prolactin is the main regulator of milk protein synthesis. This effect is mediated through the prolactin receptor located on mammary epithelial cells and requires the presence of glucocorticoids and insulin. Pharmacologic analogs of dopamine such as bromocriptine inhibit lactogenesis through the inhibition of prolactin release. Weaning, or cessation of the lactation period, is followed by involution of the terminal duct lobular units mediated by alveolar cell apoptosis and gland remodeling, returning the breast to its mature quiescent state.

HORMONAL CONTROL OF MILK SECRETION AND EJECTION

The onset of adequate milk production during the postpartum period requires developed mammary epithelium, persistent elevation in plasma prolactin (approximately 200 ng/mL), and a decrease in circulating levels of progesterone. Milk secretion from the mammary glands is triggered by stimulation of tactile receptors in the nipples by suckling. Sensory impulses are transmitted to the secretory oxytocinergic neurons in the hypothalamus, which in response release oxytocin into the systemic circulation (Chapter 2). Oxytocin produces contraction of the myoepithelial cells of the lactiferous ducts, sinuses, and breast tissue alveoli. The immediate process (30 seconds to 1 minute) of milk flow in response to suckling is called the milk let-down reflex, and persists throughout the lactation period. Milk production increases during the first 36 hours postpartum from a volume of less than 100 mL/d to an average of 500 mL/d at approximately day 4 postpartum. The composition of breast milk changes considerably

during this period as well.

AGE-RELATED CHANGES IN THE FEMALE REPRODUCTIVE SYSTEM

Puberty

Female puberty is initiated by low-amplitude nocturnal pulses of gonadotropin release, which increase serum estradiol concentrations to 15–35 pg/mL (55–128 pmol/L). The increased synthesis and secretion of estrogen by the ovary cause the progressive skeletal maturation that eventually leads to epiphysial fusion and the termination of linear growth. The onset of puberty causes a rapid increase in bone mass that correlates with bone age. The initial stage of puberty (at age 8–13) in girls involves breast development, accompanied by ovarian and follicular growth. This is followed by androgen-plus estrogen-induced pubic and axillary hair growth and the onset of menses (approximately at age 13), indicating sufficient estrogen production to stimulate endometrial proliferation. The first cycles are usually anovulatory, becoming fully ovulatory after 2–3 years. In girls, leptin serum concentrations increase dramatically as pubertal development progresses, and this rise in leptin levels parallels the increase in body fat mass. As mentioned in Chapter 8, leptin is considered to play a permissive role in the initiation of puberty.

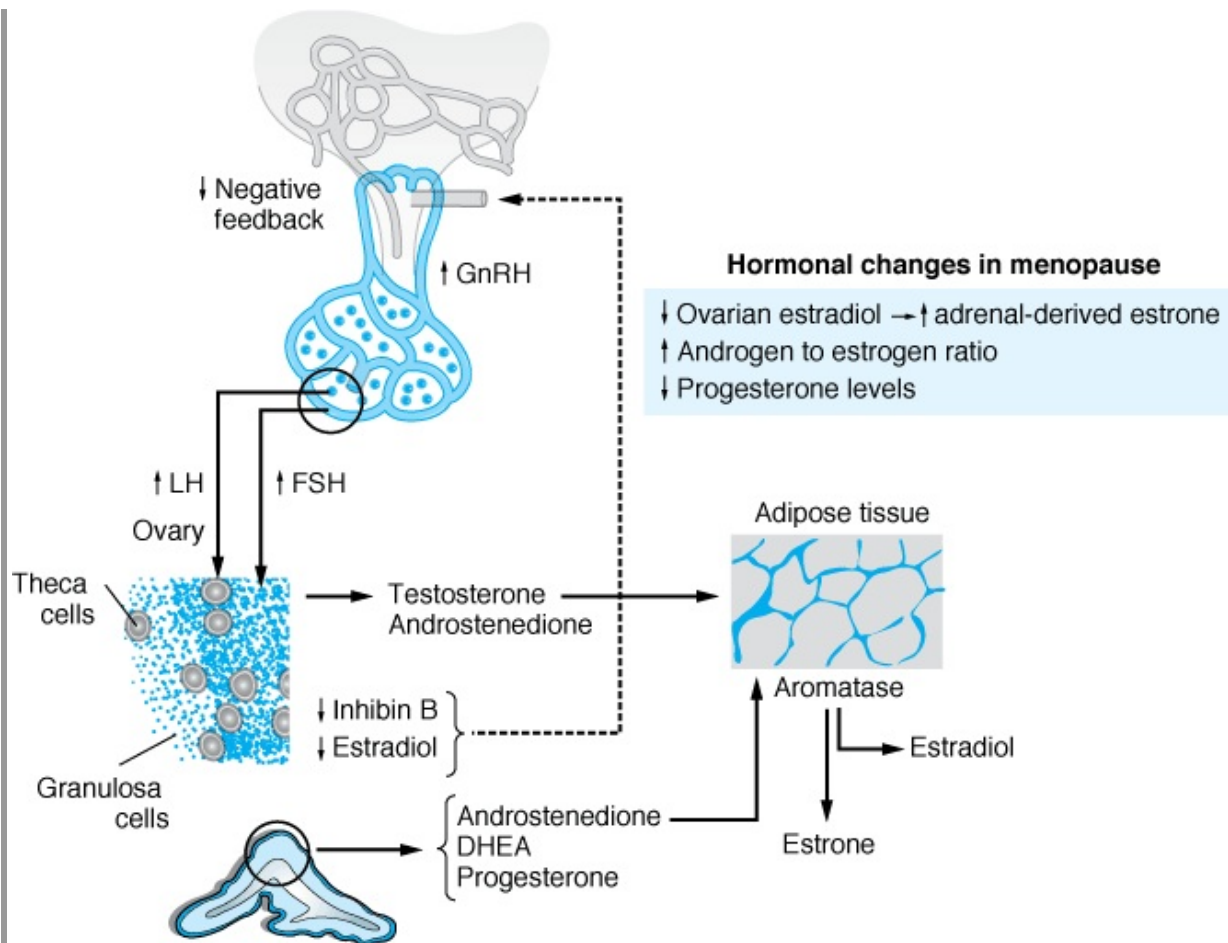
Menopause

Menopause is the permanent cessation of menstruation resulting from loss of ovarian follicular activity. Menopause is preceded by a perimenopausal period, starting when the first features of impending menopause begin (ie, irregular menstrual bleeding and cycle frequency) and lasting until at least 1 year after the final menstrual period. During the menopausal transition, gonadotropins, estradiol, and inhibin show a marked degree of variability in their circulating levels. Within 1–2 years after the final menstrual period or the onset of menopause, FSH levels are markedly elevated, LH levels are moderately high, and estradiol and inhibin levels are low or undetectable. Postmenopausally, adrenal androstenedione is the major source of estrogen, and serum testosterone levels fall moderately.

Starting from age 36, ovarian follicular apoptosis accelerates, leading to a steady decline in ovarian estradiol production (Figure 9–13). This loss of ovarian function results in a 90% loss of circulating estradiol; serum estradiol concentrations are often lower than 20 pg/mL. However, extragonadal estrogen synthesis increases as a function of age and body weight, and most of the estradiol is formed by extragonadal conversion of testosterone. The predominant estrogen in menopausal women is the weak estrogen estrone, produced through aromatase conversion of androstenedione.

Figure 9–13.





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Changes in gonadotropin and ovarian hormone production associated with aging. Lower levels of inhibin B and estradiol result in impaired negative feedback regulation of gonadotropin release, increasing FSH and LH. Production of androstenedione and testosterone during early menopause continues, with some conversion to estradiol by aromatase activity in adipose tissue. Adrenal-derived androstenedione is converted to estrone, principally in adipose tissue.

The decline in ovarian function associated with the perimenopausal period is also responsible for an early decline in the release of inhibin B (inhibitor of FSH secretion), leading to an increase in follicular phase FSH. The decrease in serum inhibin B is believed to reflect the age-related decrease in ovarian follicle reserve, which is the primary source of serum inhibin B. The later rise in serum LH during the menopausal transition is due to the cessation of ovarian follicle development. Despite

a 30% decrease in GnRH pulse frequency with aging, there is an increase in the overall amount of GnRH secreted. FSH levels gradually increase with age in women who continue to cycle regularly. The consequences of loss of ovarian function during reproductive life may be severe. Symptoms include hot flushes, night sweats, vaginal dryness and dyspareunia (painful intercourse), loss of libido, loss of bone mass with subsequent osteoporosis, and abnormalities of cardiovascular function, including a substantial increase in the risk of ischemic heart disease. As already mentioned, estrogens (like androgens) have general metabolic roles that are not directly involved in reproductive processes. These include actions on vascular function, lipid and carbohydrate metabolism, and bone mineralization and epiphyseal closure at sites such as breast, bone, vasculature, and brain. Within these sites, aromatase action can generate high levels of estradiol locally without significantly affecting circulating levels. Circulating C₁₉ steroid precursors are essential substrates for extragonadal estrogen synthesis. The levels of these androgenic precursors decline markedly with advancing age in women, possibly from the mid-to late-reproductive years. This is thought to contribute to the greater risk of bone mineral loss and fracture, and possibly the decline in cognitive function, in women as compared with men.

CONTRACEPTION & THE FEMALE REPRODUCTIVE TRACT

The multiple steps involved in the regulation of ovarian hormone production, the consequent modifications of the endometrium, and the regulation of uterine motility are all under tight control, ensuring ovulation, fertilization, implantation, and maintenance of pregnancy. Multiple approaches have been implemented for contraception. Some of the principal interventions are summarized in Table 9–2.

Table 9–2. Principal Contraceptive Methods

Method	Mechanism involved
Steroid contraceptives	Combination estrogen-progestin: constant concentrations over a 21-day period followed by 7-

	day rest Phasic estrogen-progestin: varying concentrations throughout the 21-day period and 7 days of placebo Progestin only: constant progesterone dose daily
Intrauterine devices	Prevent blastocyst implantation by altering the endometrial lining. Some release progesterone, modifying the endometrial lining
Barrier methods: condoms, foam, and diaphragms	Prevent fertilization by either interfering with the access of sperm to the uterine cavity or destroying sperm in the vaginal cavity
Sterilization	Surgically disrupts the continuity of the fallopian tubes, impairing access of the fertilized ovum to the uterine cavity and implantation
Abortive	Antiprogesterone mifepristone produces a rise in prostaglandin $F_{2\alpha}$ synthesis, leading to expulsion of the embryo
Rhythm	Relies on changes in mucus thickness and body temperature throughout the menstrual cycle, indicating a "safe" period for intercourse

Combined oral contraceptive pills contain synthetic estrogen (ethinyl estradiol or mestranol) together with one of several synthetic progestogens and produce a range of effects on the female reproductive tract. Their main mode of action is the inhibition of ovulation; both estrogen and progestogen inhibit the ability of estrogen to produce a preovulatory surge of LH. Estrogen also suppresses FSH and follicular development and ovulation. The degree of follicular activity occurring during oral contraceptive use depends on the type and dose of steroid. The dose of estrogen used in modern contraceptive preparations is likely to be the minimal amount (20 μ g ethinyl estradiol) that will reliably suppress FSH enough to prevent the growth of an ovulatory follicle.

Natural methods, such as the rhythm method, are the least effective for contraception. This method is based on the identification of a "safe"

period for intercourse that relies on knowledge of the life span of an ovum (24 hours) and of sperm (3 days) and of the interval between ovulation and menstruation, which is generally constant (14 days). The time of ovulation is determined by charting basal core temperature or using sensors for urinary LH levels throughout the cycle. The likelihood of conception is greatest during the follicular or preovulatory phase, during ovulation, and during the immediate postovulatory phase. Thus, for a typical 28-to 30-day cycle, the abstinence period would fall at about days 10–19 of the menstrual cycle.

DISEASES OF OVERPRODUCTION & UNDERSECRETION OF OVARIAN HORMONES

Alterations in female reproductive endocrine function are of multiple etiologies and produce manifestations that range from precocious puberty to infertility, depending on the age at presentation. The most frequent are abnormalities in the menstrual cycle, consisting of either absent menstruation (amenorrhea) or excess bleeding (metrorrhagia), and infertility. Abnormalities of ovarian development and function are usually caused by gonadal dysgenesis and rarely by defects in the synthesis of ovarian steroids. In general, increased ovarian hormone production can be due to increased gonadotropin release (hypergonadotropic hypergonadism) related to tumors, brain inflammatory diseases, and head injury, among other causes, or it can result from excess hormone production by ovarian tumors.

Hypogonadotropic hypergonadism may be caused by congenital adrenal hyperplasia, activating mutations of the α -subunit of the Gs protein, and excess aromatase (the last enzymatic step in estrogen synthesis) activity. Decreased ovarian hormone production can be genetic (eg, FSH and LH receptor gene mutations, mutation of the β -subunit of FSH, enzymatic deficiencies), or acquired (eg, radiation) despite adequate gonadotropin release (hypergonadotropic hypogonadism). Decreased ovarian hormone production due to impaired gonadotropin release (hypogonadotropic hypogonadism) is rare and may result from GnRH receptor gene mutations, lesions in the hypothalamic area, and other causes.

KEY CONCEPTS



Gonadotropin release is under negative and positive feedback regulation by ovarian steroid and peptide hormones.



Estrogen synthesis requires LH and FSH regulation of coordinated metabolism by granulosa and theca cells of the ovarian follicle.



LH and FSH rescue selected ovarian follicles from apoptosis and stimulate their growth and maturation.



The corpus luteum is a temporary endocrine organ that plays a central role during the initial stages of pregnancy.



The ovarian cycle produces cyclic changes in steroid hormone production, which in parallel produce marked morphologic and functional changes in the endometrium, preparing it for embryo implantation.



Estrogen has important systemic effects affecting the risk of cardiovascular disease, osteoporosis, and endometrial and breast cancer.



Progesterone ensures uterine quiescence and prevents lactogenesis during pregnancy.



Mammary gland morphologic development occurs during puberty and is functionally modified during pregnancy by prolactin and hPL, ensuring lactogenesis.

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OBJECTIVES

- ▶ *Identify the normal range of plasma glucose concentrations and the hormonal regulation of its metabolism, storage, and mobilization.*
- ▶ *Identify the specific roles of insulin, glucagon, glucocorticoids, catecholamines, growth hormone, and thyroid hormone in the regulation of energy substrate utilization, storage, and mobilization.*
- ▶ *Describe the hormonal regulation of energy substrate metabolism during the fed and fasted states and understand the consequences of its dysregulation.*
- ▶ *Identify the mechanisms involved in the maintenance of long-term energy balance.*
- ▶ *Identify the normal range of dietary sodium intake, its body distribution, and routes of excretion. Explain the roles of antidiuretic hormone, aldosterone, angiotensin, and atrial natriuretic hormone in the regulation of sodium balance.*
- ▶ *Identify the normal range of dietary potassium intake, its body distribution, and routes of excretion. Explain the hormonal regulation of plasma potassium concentration, distribution, and balance in the acute and chronic settings.*
- ▶ *Identify the normal range of dietary calcium intake, its body distribution, and routes of excretion. Explain the hormonal regulation of plasma calcium concentration through bone resorption, renal excretion, and intestinal absorption.*
- ▶ *Identify the normal range of dietary phosphate intake, its body distribution, and routes of excretion. Explain the hormonal regulation of plasma phosphate concentration through exchange with bone, renal excretion, and dietary intake and absorption.*

ENDOCRINE INTEGRATION OF ENERGY & ELECTROLYTE BALANCE: INTRODUCTION

In the first chapter, several of the key functions of the endocrine system that maintain homeostasis were outlined. Subsequent chapters described the specific physiologic effects of individual hormones, the

mechanisms that regulate their production and release, and the consequences of isolated excess or deficiency. The presentation of this material would not be complete without an attempt to integrate some of these actions into the overall regulation of specific functions. Although a complete description of the integrative control of physiologic function is beyond the scope of this book, this chapter integrates many of the concepts already presented. It describes how the different arms of the neuroendocrine system interact to regulate and maintain basic functions, which include energy substrate balance, blood volume and blood pressure, and preservation of bone mineral density (BMD). Finally, it presents an integrated discussion of the neuroendocrine mechanisms involved in mediating the stress response.

NEUROENDOCRINE REGULATION OF ENERGY STORAGE, MOBILIZATION, & UTILIZATION

Two distinct phases directly related to the ingestion of a meal alternate throughout the day in the regulation of energy metabolism. The **fed state** reflects overall **anabolic metabolism**, during which energy is stored in the form of energy-rich compounds (adenosine triphosphate [ATP], phosphocreatinine), glycogen, fat, and proteins. The **fasted or catabolic phase** is the period during which endogenous energy sources are utilized.

The anabolic and catabolic phases alternate to preserve adequate glucose supply to the brain as well as sufficient energy to maintain body functions, such as thermoregulation (maintaining a constant core temperature), food digestion, and physical activity. The 2 hormones at the core of maintaining this balance are insulin and glucagon (Chapter 7); in particular, their ratio plays a critical role in the dynamic regulation of substrate metabolism (summarized in Table 10–1). However, several other established and newly discovered hormones participate in the regulation of energy metabolism to different extents, according to age, sex, nutritional state, and metabolic demands of the individual.

Table 10–1. Regulation of Metabolic Processes by

Insulin/Glucagon Ratios

Anabolic ↑ I:G	Metabolic process	Catabolic ↓ I:G
↑	Glycogen synthesis (liver and muscle)	↓
↓	Glycogen breakdown	↑
↓	Gluconeogenesis	↑
↑	Fatty acid synthesis and triglycerides (hepatocytes and adipose tissue)	↓
↑	Muscle protein synthesis	↓
↑	Lipogenesis and triglyceride formation	↓
↓	Lipolysis	↑
↓	Free fatty acid oxidation	↑
↓	Ketone body formation	↑
↓	Muscle proteolysis	↑

G, glucagon; I, insulin.



Because the autonomic nervous system also interacts with the endocrine system in the modulation of glucose and fat metabolism, the system is in fact under neuroendocrine regulation. The autonomic nervous system exerts its effects both directly and indirectly. For example, activation of the sympathetic nervous system through norepinephrine release directly stimulates skeletal muscle glycogenolysis and hepatic glucose output. The indirect effects of the autonomic nervous system are exemplified by sympathetic activation of the adrenal medulla (Chapter 6), stimulating the release of epinephrine. Epinephrine stimulates the release of glucagon and suppresses the release of insulin, resulting in an increase in the glucagon to insulin ratio and an overall increase in hepatic glucose production.

To simplify the discussion of the neuroendocrine regulation of substrate metabolism, a brief summary of overall substrate regulation and the principal hormones involved will be presented as they pertain to the fed

(anabolic) and fasted (catabolic) states.

Neuroendocrine Regulation of Energy Metabolism during the Fed State

GLUCOSE

Blood glucose regulation occurs through interactions among hormonal, neural, and hepatic autoregulatory mechanisms. As described in detail in Chapter 7, the pancreatic hormones insulin and glucagon play central roles in the tight control of blood glucose concentrations.

Following a meal (postprandial state), in response to the rise in pancreatic insulin release, glucose uptake is increased in muscle, fat, and the hepatosplanchnic bed; hepatic glucose output is suppressed; and glycogen synthesis is increased.

Glucose disposal by insulin-sensitive tissues is regulated initially by an increase in glucose transport and enzyme phosphorylation leading to the activation of glycogen synthase, phosphofructokinase, and pyruvate dehydrogenase (Figure 7–5). The majority of insulin-stimulated glucose taken up is stored as glycogen. Hormonally induced changes in intracellular fructose 2,6-bisphosphate concentrations play a key role in muscle glycolytic flux and both glycolytic and gluconeogenic flux in the liver.

FAT



Most of the body's energy reserve is stored in adipose tissue in the form of triglycerides. During periods of caloric excess or abundance, fat is stored in the form of triacylglycerol in the adipocytes. The principal hormone involved in lipogenesis is insulin, through activation of lipogenic and glycolytic enzymes. Opposing the effects of insulin are growth hormone (GH, discussed in Chapter 3) and leptin (described later), which inhibit lipogenesis. The balance between lipogenesis and lipolysis followed by fatty acid oxidation determines the overall accumulation of body fat. Both processes are under hormonal and cytokine regulation.

PROTEIN

The whole-body protein pool, as well as that of individual tissues, is determined by the balance between protein synthesis and degradation. These in turn are principally regulated by interactions among hormonal, nutritional, neural, and inflammatory mediators. Hormonally, regulation of protein metabolism is predominantly under the influence of insulin, GH, and insulin-like growth factor-I (IGF-I). During the fed state, insulin acts primarily to inhibit proteolysis, and GH stimulates protein synthesis. IGF-I has antiproteolytic effects during the postabsorptive state that progress to stimulation of protein synthesis in the fed state or when amino acids are provided. GH and testosterone are of particular importance during growth and development, as well as during adulthood and senescence. Thyroid hormones are also required for normal growth and development. Thyroid hormones stimulate bone growth indirectly by increasing secretion of GH and IGF-I and directly by activating gene transcription.

Neuroendocrine Regulation of Energy Metabolism during the Fasted State

During the fasted postabsorptive state, catabolism of stored energy sources provides the energy required for bodily functions. The total amount of energy produced per unit of time by a given individual is referred to as the **metabolic rate**. The amount of energy expended by an awake, resting individual, measured 12–14 hours following the last meal, at normal (or thermoneutral) body temperature is called the **basal metabolic rate** (BMR). The BMR is the amount of energy required to maintain breathing, brain activity, enzymatic activity, and other functions without any physical movement of the individual. Any deviation from the basal condition, such as changes in body temperature (fever or hypothermia), the level of activity of the individual (exercise or sleeping), or time from the last meal (fed or fasted), will affect the metabolic rate. In addition, BMR can be directly affected by hormone action, particularly thyroid hormones, which increase body temperature and increase Na^+/K^+ -ATPase activity, resulting in an increase in the BMR. In a healthy individual, BMR averages 2000 kcal/d. Hence, the recommended dietary allowance of calories is

derived from the BMR, age, sex, and level of activity of any given individual. The BMR can be estimated clinically by measuring the amount of oxygen consumed with the use of indirect calorimetry.

GLUCOSE

In the resting postabsorptive state, release of glucose from the liver through glycogenolysis and gluconeogenesis is the key regulated process. During fasting, hepatic glucose production is increased and peripheral glucose utilization is inhibited. Initially, hepatic glucose output is derived from breakdown of hepatic glycogen stores (a maximum of 70–80 g in humans) through glycogenolysis. Following an overnight fast, glycogenolysis provides approximately 50% of the overall hepatic glucose output. As hepatic glycogen stores are depleted during a period of prolonged fasting (approximately 60 hours), the contribution of glycogenolysis to hepatic glucose output becomes negligible, with hepatic gluconeogenesis predominating.

Glycogenolysis depends on the relative activities of glycogen synthase and phosphorylase, the latter being the more important (Figure 7–5).

Gluconeogenesis is regulated by the activities of fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase, pyruvate kinase, and pyruvate dehydrogenase, and by the availability of the principal gluconeogenic precursors, lactate, glycerol, glutamine, and alanine.

A smaller, yet significant amount (approximately 25%) of systemic glucose production in the postabsorptive state is derived from renal gluconeogenesis. Overall, the kidney is not a net producer of glucose. The proximal tubule cells produce glucose at a rate similar to that of glucose utilization by the renal medulla. Thus, in the postabsorptive state, blood glucose homeostasis is the result of hormonal regulation of glycogenolysis, gluconeogenesis, and glucose uptake.

FAT

After an overnight fast, most of the resting energy requirement is provided by oxidation of fatty acids derived from adipose tissue. Lipolysis in adipose tissue is mostly dependent on the concentrations of hormones (epinephrine stimulates lipolysis, and insulin inhibits lipolysis). During a period of acute energy deprivation or prolonged starvation, lipolysis mobilizes triglycerides, providing nonesterified fatty acids as energy substrates for tissues such as muscle, heart, and liver; and substrates for glucose (glycerol) and lipoprotein (free fatty acids) synthesis to the liver. Unlike most other tissues, the brain cannot utilize fatty acids for energy when blood glucose levels become compromised. In this case, ketone bodies (discussed in Chapter 7) provide the brain with an alternative source of energy, amounting to nearly two thirds of the brain's energy needs during periods of prolonged fasting and starvation.

The release of glycerol and free fatty acids from adipose tissue is under negative regulation by insulin and is stimulated primarily by catecholamines (Chapter 7). During fasting, plasma insulin levels decrease and plasma GH and glucagon both increase. As the fasting period progresses, or more frequently during periods of acute glucose deficiency (insulin-induced hypoglycemia) or increased energy demand (as with strenuous exercise), catecholamines play an important role in the stimulation of lipolysis.

The amount of energy stored as triglycerides in adipose tissue is substantial. For example, an adult with 15 kg of body fat has enough energy to support the whole-body energy requirements (8.37 MJ; 2000 kcal) for about 2 months.

PROTEIN

Unlike excess fat and glucose, which are stored as fat and glycogen in adipose tissue, liver, and muscle, there is no storage pool for body protein. Therefore, under catabolic conditions, essential proteins are catabolized. Glucagon, cortisol, and epinephrine together favor muscle protein breakdown and hepatic amino acid uptake, some of which can be utilized for gluconeogenesis. The effects of glucagon are predominantly mediated through increased hepatic uptake of amino acids. Epinephrine increases production of the gluconeogenic amino acid alanine by muscle and its uptake by the splanchnic bed.

Prolonged changes in either the protein synthetic or degradative processes (or both) leading to loss of lean body mass have been shown to increase morbidity and mortality from several diseases, including cancer and AIDS.

Counterregulatory Hormone Effects

GLUCAGON

Glucagon plays a primary role in energy metabolism. This hormone primarily stimulates hepatic glycogenolysis, as well as gluconeogenesis resulting in an overall increase in hepatic glucose output.

GROWTH HORMONE AND CORTISOL

GH and cortisol facilitate glucose production and limit glucose utilization, but neither of them plays a critical role. Their effects are not immediate (delayed for about 6 hours); thus, they are mostly involved in defense against prolonged hypoglycemia. Cortisol contributes to the regulation of gluconeogenic substrate supply through permissive effects on the lipolytic action of catecholamines and GH in adipose tissue and on the glycogenolytic action of catecholamines in skeletal muscle. In addition, it induces hepatic enzymatic gene expression required for enhanced gluconeogenic rates and exerts permissive effects on the stimulation of gluconeogenesis in the liver by glucagon and epinephrine.

EPINEPHRINE

Epinephrine stimulates hepatic glycogenolysis and hepatic and renal gluconeogenesis, largely by mobilizing gluconeogenic precursors including lactate, alanine, glutamine, and glycerol. Epinephrine also limits glucose utilization by insulin-sensitive tissues. Although epinephrine helps increase hepatic glucose output, its role is critical only when glucagon release is deficient. Together, glucagon and epinephrine act within minutes to raise plasma glucose concentrations.

The increased activation of the sympathetic nervous system and the associated release of epinephrine and norepinephrine suppress pancreatic insulin release and stimulate glucagon release. Thus, with insulin release suppressed, the stimulation of glycogenolysis and inhibition of glycogen synthesis by glucagon and epinephrine as well as the glucagon-stimulated hepatic gluconeogenesis proceed unopposed.

The contribution of the activation of the autonomic nervous system is easier understood when described in the context of acute and severe hypoglycemia. Because of the clear advantages of glycemic control in preventing organ injury in diabetic patients, tight regulation of plasma glucose levels is desired and recommended in this patient population. However, the most prevalent problem associated with tight glycemic control is the development of insulin-induced hypoglycemia. The decrease in plasma glucose concentrations (hypoglycemia) within and below the physiologic postabsorptive concentration range of about 70–110 mg/dL (3.9–6.1 mmol/L) triggers the activation of a counterregulatory neuroendocrine response. Acute insulin-induced hypoglycemia increases neuronal activity in the nucleus tractus solitarius and lateral hypothalamic glucosensors, resulting in increased sympathetic activity.



Enhanced sympathetic activity at the liver increases hepatic glucose output. The release of counterregulatory hormones glucagon, epinephrine, GH, and cortisol also contributes to the increase in hepatic glucose output and the suppression of tissue glucose uptake, partly through an increase in tissue fatty acid oxidation. As plasma

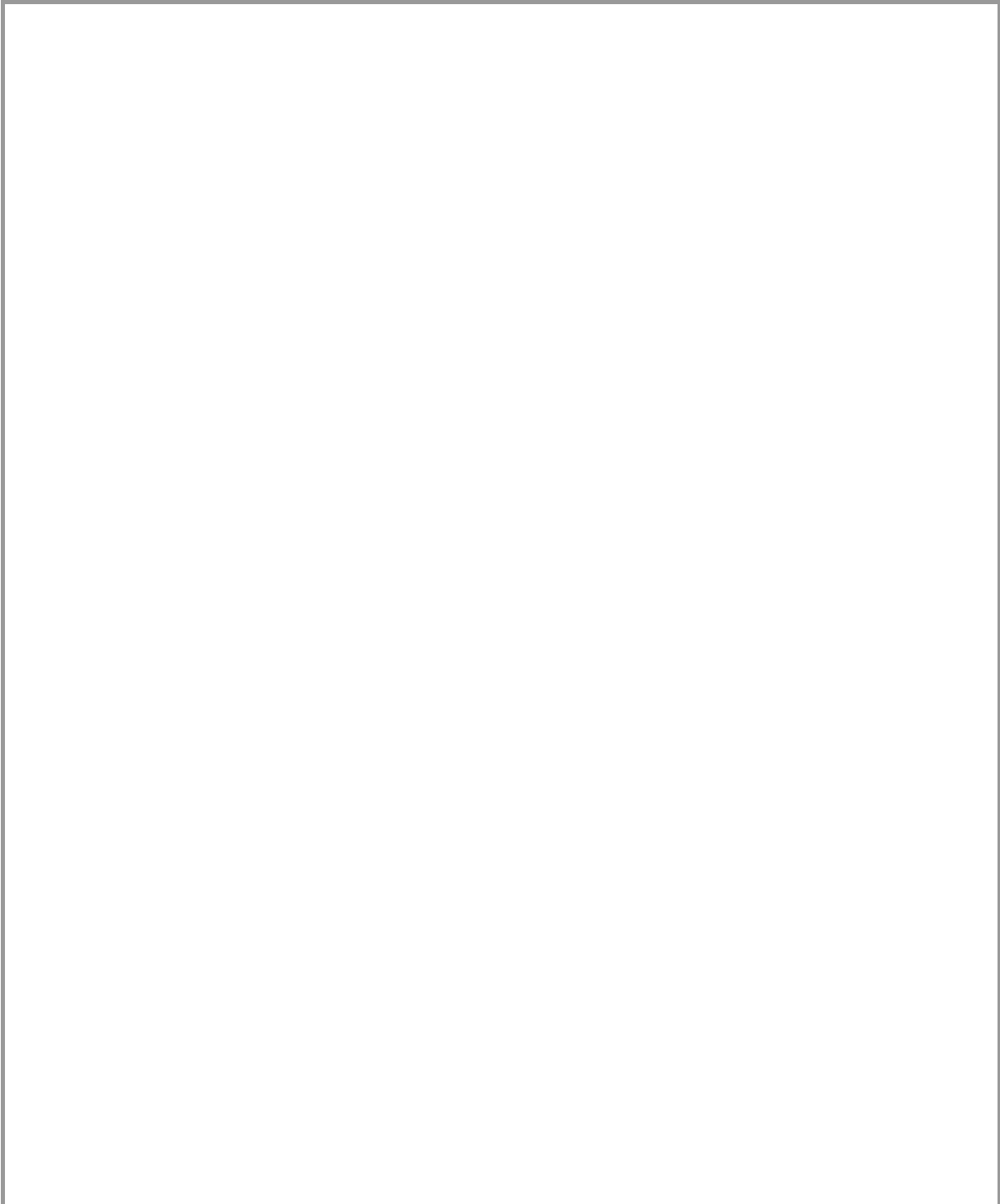
glucose levels are restored, peripheral glucose sensors in the portal vein, small intestine, and liver decrease firing. This afferent signal is transmitted to the hypothalamus and to the nucleus solitarius in the medulla through the vagus nerve, conveying information on the prevailing peripheral glucose levels. The central nervous system integrates these signals and initiates an appropriate response through the inhibition of hepatic and adrenal nerve activity, with consequently decreased release of adrenomedullary catecholamines, removing the inhibition on pancreatic insulin release and thus allowing hyperglycemia to induce pancreatic insulin secretion. Thus, in this system, glucose acts as a feedback signal contributing to integration of the neuroendocrine mechanisms that regulate its homeostasis.

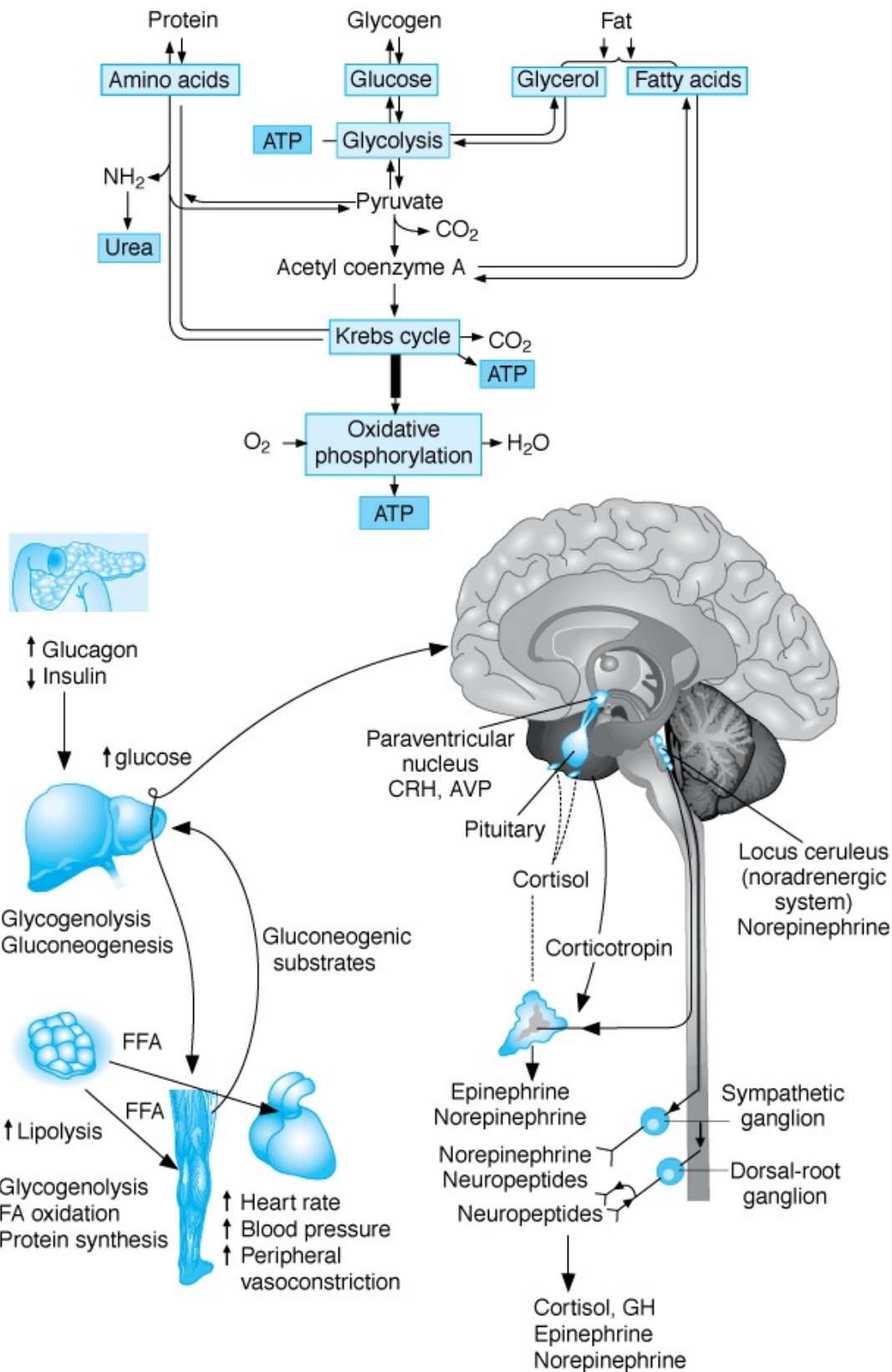
Regulation of Energy Metabolism during Exercise

During exercise, cardiac contractility and output are increased, respiration is enhanced, and blood flow is redirected to provide adequate perfusion to the brain and skeletal muscle (Figure 10–1). The neuroendocrine response to exercise is targeted to provide for the increased energy demands of the exercising muscle and includes activation of the sympathetic nervous system, release of GH, activation of the hypothalamic-pituitary-adrenal axis with consequent release of catecholamines and cortisol, suppression of insulin release, and stimulation of glucagon release. This neuroendocrine response stimulates lipolysis, hepatic and muscle glycogenolysis leading to an increase in free fatty acids, gluconeogenic substrate mobilization, and hepatic glucose output (due to an increase in both gluconeogenesis and glycogenolysis). Both hepatic and skeletal muscle glycogenolysis are stimulated by the increase in catecholamine release. However, because muscle lacks glucose-6-phosphatase, the glucose-6-phosphate produced by muscle glycogenolysis is either oxidized in muscle cells or released into the circulation as lactate. The increased lactate delivered to the liver is then used for hepatic gluconeogenesis. Hepatic glycogenolysis predominates during intense exercise, but gluconeogenesis contributes substantially with prolonged exercise as the liver glycogen stores decline and the supply of gluconeogenic precursors increases. As exercise intensity increases from mild to

moderate and intense, energy substrate selection switches from lipid to carbohydrate dependence. GH and cortisol contribute only minimally to the exercise-induced rise in liver glucose output.

Figure 10–1.





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Neuroendocrine response to exercise. The principal pathways activated by stress are the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system, resulting in the release of corticotropin-releasing hormone (CRH), arginine vasopressin (AVP), catecholamines, endorphins, and growth hormone (GH). In the periphery, increased production and release of cortisol, glucagon, and catecholamines and suppressed release of insulin favor an overall catabolic response. Stimulation of hepatic glycogenolysis and gluconeogenesis, muscle glycogenolysis, and adipose tissue lipolysis ensures the production and mobilization of energy stores to meet the enhanced metabolic demands of the individual. Reproductive and growth functions are inhibited, conserving energy to sustain fundamental processes that ensure survival. ATP, adenosine triphosphate; FA, fatty acid; FFA, free fatty acid.

Skeletal muscle is the main site of oxidation of fatty acids, and endogenous triacylglycerols represent an important source of energy both at rest and during low-and moderate-intensity exercise. During moderate-intensity exercise, lipolysis increases approximately 3-fold, mainly because of an increase in β -adrenergic stimulation, leading to an increased release of fatty acids. The increase in adipose tissue and muscle blood flow decreases fatty acid reesterification and facilitates the delivery of fatty acids to skeletal muscle. In combination with the decrease in insulin and increase in lipolysis and in sympathetic outflow, this leads to a progressive increase in triacylglycerol oxidation, which serves as the main energy substrate for muscle. As the intensity of exercise increases, fat oxidation increases further until exercise intensities reach about 65% VO_2 max. After this point, the rate of fat oxidation declines, most likely because of reduced fatty acid delivery from adipose tissue to muscle.

The contribution of amino acid oxidation to total energy expenditure is negligible during short-term intense exercise, and accounts for 3–6% of the total ATP supplied during prolonged exercise in humans. Although it is not quantitatively important in terms of energy supply, the intermediary metabolism of several amino acids, notably glutamate, alanine, and the branched-chain amino acids, influences the availability of tricarboxylic acid cycle intermediates. Sustained dynamic

exercise stimulates amino acid oxidation, chiefly of the branched-chain amino acids, and ammonia production in proportion to exercise intensity. If the exercise is intense enough, it causes a net loss of muscle protein (as a result of decreased protein synthesis, increased breakdown, or both); some of the amino acids are oxidized as energy, whereas the rest provide substrates for gluconeogenesis and possibly for acid-base regulation.

When the fasted state is prolonged into a state of starvation, or under conditions of increased energy substrate demands, the contributions of the counterregulatory hormones cortisol, epinephrine, norepinephrine, and glucagon become evident, favoring overall catabolic effects. During such conditions, insulin effects are practically abolished. Because of the important anabolic effects of insulin, it follows that the metabolic responses that predominate are those that lead to catabolism of stored energy (glycogenolysis, lipolysis) and of lean tissues (muscle proteolysis), leading to loss of lean body mass, or wasting. It is important to note that energy metabolism can be pushed to a starvation-like state not only by discontinuation of food intake, in the classic sense of starvation, but also during conditions of stress, such as from a surgical intervention, cancer, severe infection, sepsis, burns, and traumatic injury. Under these conditions, additional factors such as inflammatory cytokines (eg, tumor necrosis factor [TNF], interleukin-1) interact with mediators of the neuroendocrine response. In addition, the production of GH, IGF-I, and the respective binding proteins becomes dysregulated, also contributing to the lack of anabolic processes and dominance of catabolic responses. Overall, the increased release of stress hormones and proinflammatory cytokines and the impairment in the release and action of insulin and growth factors, in association with altered release of androgens, lead to an overall catabolic response that affects the liver, adipose tissue, muscle, and, in extreme circumstances, visceral tissues. The deleterious impact of the loss of lean body mass on survival from disease underscores the relevance of understanding the hormonal mechanisms involved in the regulation of energy metabolism and their importance in the management of the critically ill patient.

Maintenance of Long-Term Energy Balance &

Fat Storage

The neuroendocrine mechanisms involved in maintaining energy balance have been described earlier. Furthermore, the hormonal regulation of the 2 principal energy stores in the body—hepatic glycogen and adipose tissue triglycerides—has been outlined. The balanced transition from fed to fasted and the consumption of adequate energy commensurate with the level of physical activity of a given individual ensure that adequate energy reserves are available for short-term increases in metabolic demands, such as those described for exercise. An imbalance in either energy intake or expenditure leads to 1 of 2 extremes: loss of lean body mass or wasting syndrome, or obesity. In the absence of chronic physical or psychiatric illness, development of a wasting syndrome is infrequent. Obesity is an important health problem that increases the risk of several diseases. Because of the current trend toward a rising incidence of obesity in our society, a brief discussion follows on the endocrine physiologic responses implicated in the development of this condition.

OBESITY

Obesity is defined as a significant increase above the ideal weight (the weight that maximizes life expectancy). The increase in body mass index (BMI), an indicator of the adiposity or fatness that accompanies obesity, has become an important health problem for western civilization. Life expectancy is reduced when body mass index is significantly increased above the ideal level. Obesity is associated with an increased risk of diabetes, dyslipidemia, hypertension, heart disease, diabetes, and cancer. Approximately 20% of the US population is considered obese, according to the definition of the World Health Organization (Table 10–2).

Table 10–2. Classification of Overweight According to the World Health Organization

BMI (kg/m ²) ^a	Definition	WHO classification
<18.5	Thin	
18.5–24.9	Healthy or normal	
25.0–29.9	Overweight	Grade 1 overweight
30.0–39.9	Obese	Grade 2 overweight
≥40.0	Morbidly obese	Grade 3 overweight

BMI, body mass index; WHO, World Health Organization.

^aBMI is defined as mass in kilograms divided by the square of the height in meters.

Body weight and the excess weight gain leading to obesity are determined by interactions among genetic, environmental, and psychosocial factors that affect the physiologic mediators of energy intake and expenditure, several of which pertain to the endocrine system. The altered balance between energy intake and energy expenditure leads to excess weight gain and obesity. Energy expended by the individual can be in the form of work (physical activity) or heat production (thermogenesis), which can be affected by environmental temperature, diet, and the neuroendocrine system (catecholamines

and thyroid hormone). The uncoupling of ATP production from mitochondrial respiration dissipates heat and affects the efficiency with which the body utilizes energy substrates. The expression of proteins involved in this process (uncoupling protein-1 expressed in brown adipose tissue and uncoupling protein-3 in skeletal muscle) is modulated by catecholamines, thyroid hormones, and leptin. Studies are currently under way to establish their roles in the regulation of BMR and in the development of obesity.

The role of genetics in the predisposition to obesity has been demonstrated convincingly. Susceptibility genes have been identified that increase the risk of developing obesity, and their relevance has been shown in studies in which pairs of twins were exposed to periods of positive and negative energy balance. The differences in the rate of weight gain, the proportion of weight gained, and the sites of fat deposition showed greater similarity within pairs than between pairs, indicating a close genetic relationship. Although a clear correlation between energy expenditure and weight gain has not been demonstrated, increasing physical activity; which represents 20–50% of total energy expenditure, has been actively promoted as an approach to prevent obesity and improve insulin responsiveness. Environmental factors are also thought to unmask genetic tendencies toward obesity.

From the standpoint of endocrine physiology, it is important to note that the responsiveness to hormones that regulate lipolysis varies according to the distribution of fat depots. The lipolytic response to noradrenaline is greater in abdominal than in gluteal or femoral adipose tissue in both men and women. The exaggerated release of free fatty acids from abdominal adipocytes directly into the portal system, an increased hepatic gluconeogenesis, and hepatic glucose release, and hyperinsulinemia are hallmarks of patients with upper-body obesity. The endocrine properties of the different fat pads may be more important than the anatomic location of the fat pad. The severity of medical complications is more closely related to body fat distribution; being greater in individuals with upper-body obesity than those with an excess total-body fat. Differential fat deposition leading to upper-body or abdominal obesity is reflected in a high waist-hip ratio, an index

used for predicting risks associated with fat accumulation. The presence of visceral obesity, insulin resistance, dyslipidemia, and hypertension is collectively termed the **metabolic syndrome** or **syndrome X**.

Excess energy intake in relation to the energy expended by the organism leads to the accumulation of fat. The fat mass itself is determined by the balance between breakdown (lipolysis) and synthesis (lipogenesis). The sympathetic nervous system is the principal stimulator of lipolysis, leading to a decrease in fat stores, particularly when the energy demands of the individual are increased. When intake exceeds energy utilization, lipogenesis occurs in liver and adipose tissue. Lipogenesis is influenced by diet (increased by carbohydrate-rich diets) and hormones (principally GH, insulin, and leptin) through modification of transcription factors (eg, peroxisome proliferator–activated receptor- γ [PPAR- γ]). The main hormones involved in fat storage are insulin (which stimulates lipogenesis), and GH and leptin (which reduce lipogenesis). The transcription factor PPAR, the target for the insulin sensitizer thiazolidinedione drugs (Chapter 7), affects gene transcription of several enzymes involved in glucose and fat metabolism and is involved in preadipocyte differentiation into mature fat cells. Other hormones involved in the regulation of body fat stores include testosterone, dehydroepiandrosterone, and thyroid hormone.

REGULATION OF ENERGY INTAKE

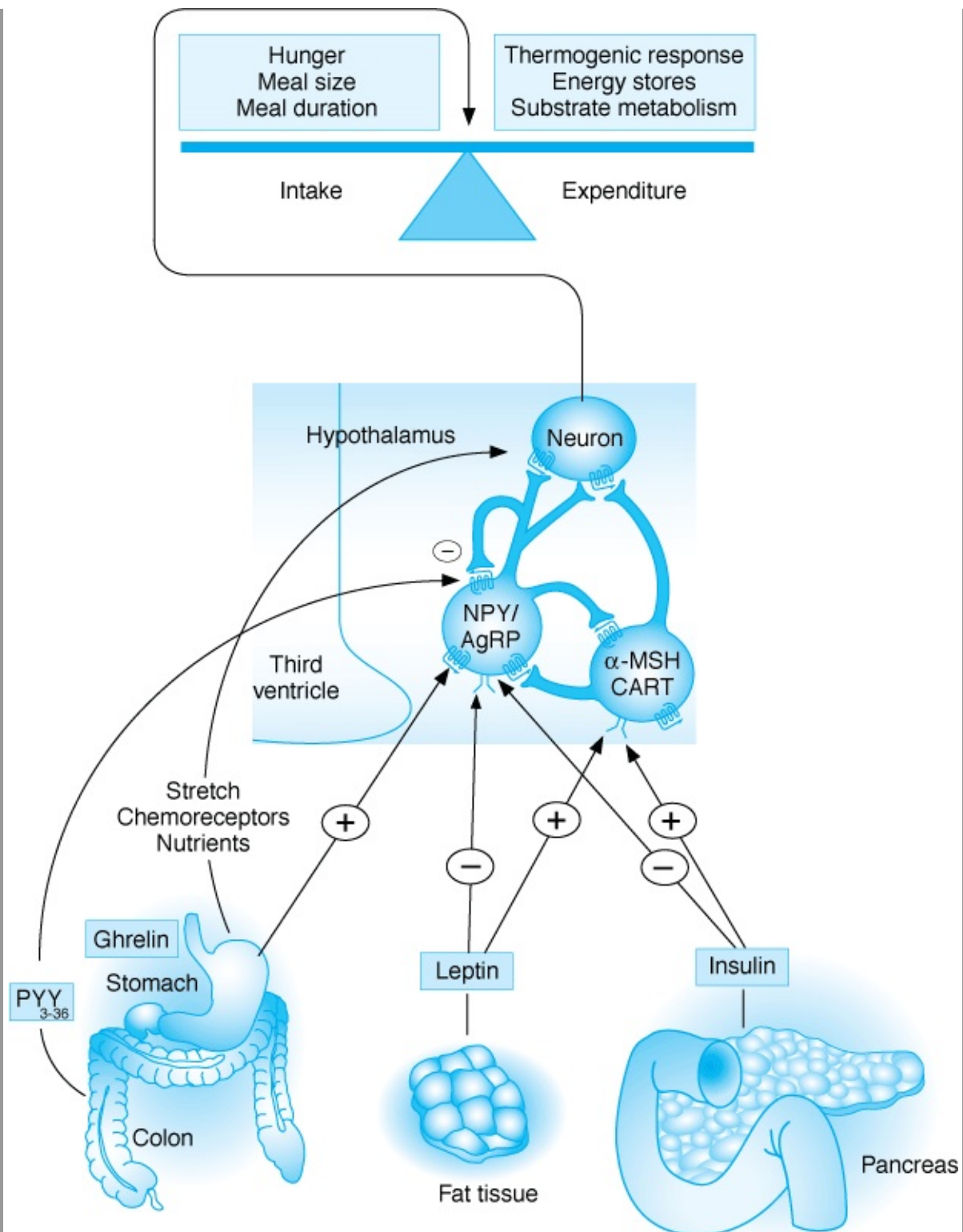
Regulation of energy intake is mediated by several factors. Central integration of peripheral signals, including those mediated by mechanoreceptors and chemoreceptors, signals the presence and energy density of food in the gastrointestinal tract. Hypothalamic glucose sensors monitor fluctuations in circulating glucose concentrations. Hormones signal the central release of peptides that regulate appetite and satiety. Two hormones that have been identified as crucial in the long-term regulation of energy balance are insulin (Chapter 7) and **leptin**, the product of the *ob* gene (discussed later). Both hormones are released in proportion to body fat. They are transported into the brain, where they modulate the expression of hypothalamic neuropeptides known to regulate feeding behavior and body weight, resulting in inhibition of food intake and increase in energy expenditure. While insulin release is directly correlated to meals; that of leptin does not correlate with food intake but reflects body fat mass.

HYPOTHALAMIC INTEGRATION

The hypothalamus receives innervation from several areas, notably the nucleus tractus solitarius and area postrema in the brainstem. These areas relay many neural and hormonal signals from the gastrointestinal tract. Mechanical stretch receptors sense stretch of the stomach and other areas of the intestine. The principal hormone associated with control of satiety is cholecystokinin (CCK). CCK, a peptide released from the duodenum is released in response to the presence of lipids or protein in the intestinal lumen (Figure 10–2). This hormone acts via local sensory receptors in the duodenum sending signals to the brain regarding the intestinal nutritional content. The nucleus tractus solitarius also relays taste information to the hypothalamus and other centers. Other signals regarding smell, sight, memory of food, and the social context under which it is ingested are also integrated and may also influence energy intake by modulating output from the hypothalamus. Integration of these signals results in the activation of gene expression of mediators implicated in the regulation of satiety and development of obesity. These genes control thermogenesis (uncoupling proteins), hormone synthesis (**ghrelin**, leptin, and cholecystokinin and adiponectin), and neurotransmitter (**neuropeptide Y**) availability, as summarized in Table 10–3.

Figure 10–2.





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Hypothalamic integration of energy intake. The hypothalamus

receives innervation from several areas, notably the nucleus tractus solitarius and area postrema in the brainstem, that relay neural and hormonal signals from the gastrointestinal tract, such as mechanical signals indicating stretch of the stomach and other areas of the intestine, and hormonal signals, such as cholecystokinin, indicating the presence of food in the gut. Additional signals regarding smell, sight, memory of food, and the social context under which it is ingested are also integrated and may influence energy intake by modulating output from the hypothalamus. Hormones also alter hypothalamic gene expression, resulting in modulation of energy intake. Leptin and insulin decrease appetite by inhibiting the production of neuropeptide Y (NPY) and agouti-related protein (AgRP), while stimulating melanocortin-producing neurons in the arcuate nucleus of the hypothalamus. Neuropeptide Y and agouti-related protein stimulate eating, whereas melanocortins inhibit eating. Ghrelin stimulates appetite by activating the NPY/AgRP-expressing neurons. PYY_{3–36}, released from the colon, inhibits these neurons and transiently decreases appetite. Integration of these signals results in the activation of gene expression of mediators implicated in the regulation of satiety, control of thermogenesis, and energy expenditure. α -MSH, α -melanocyte-stimulating hormone; CART, cocaine and amphetamine-regulated transcript; PYY, polypeptide YY.

Table 10–3. Mediators Implicated in Regulation of Energy Intake

Mediator	Regulation and target effect
Gastrointestinal tract	
Cholecystokinin	Released in the duodenum during a meal. Stimulates the vagus nerve projecting to the NTS and signals within the hypothalamus to induce satiety.
Ghrelin	Released from the GI tract before meals. Plasma levels are low in obese patients. Stimulates growth hormone release, decreases fat oxidation, increases food intake and adiposity. Overall has anti-leptin action.

PYY ₃₋₃₆	Member of the neuropeptide Y family, released in the distal small intestine and colon in response to food. Blood levels remain elevated between meals. Decreases food intake at the arcuate nucleus in the hypothalamus.

Adipose tissue

Adiponectin (adipocyte complement-related protein of 30 kDa, AdipoQ)	Produced by adipose tissue (decreased in obese patients; plasma levels correlate negatively with triglycerides). Increases insulin sensitivity and tissue fat oxidation, resulting in reduced circulating fatty acid levels and reduced intramyocellular and liver triglyceride content.
Acylation-stimulating protein	Produced by adipose tissue. Paracrine signal increases efficiency of triacylglycerol synthesis in adipocytes, resulting in more rapid postprandial lipid clearance.
Leptin	Produced in adipose tissue. Acts on neuropeptide Y, AgRP-containing neurons, and α -MSH neurons in the arcuate nucleus of the hypothalamus to decrease food intake.
Resistin	Peptide hormone induced during adipogenesis. It antagonizes insulin action.

Hypothalamus

Neuropeptide Y (NPY)	Produced by hypothalamic neurons that express AgRP. Release is under leptin, insulin, and cortisol regulation. Stimulates food intake via the NPY5 receptor.
α -MSH	Product of POMC in hypothalamic neuronal subset under leptin regulation. Decreases food intake through melanocortin-4 receptors in the hypothalamus.
Cocaine-and amphetamine-regulated transcript (CART)	Leptin and amphetamines stimulate production of this peptide by hypothalamic POMC-expressing neurons. Reduces food intake.

Agouti-related peptide (AgRP)	Released from hypothalamic NPY-expressing neurons. Inhibits neuronal melanocortin-4 receptors and increases food intake.
Orexins (A and B)	Produced by neurons in the lateral hypothalamus perifornical area. Regulated by glucose, leptin, neuropeptide Y, and POMC neurons. They stimulate food intake.

AgRP, agouti-related peptide; GI, gastrointestinal; NTS, nucleus tractus solitarius; MSH, melanocyte-stimulating hormone; POMC, proopiomelanocortin; PYY, polypeptide YY.

The relative contributions of these mediators to the regulation of caloric intake, energy expenditure, body weight, and fat mass are not completely understood. However, important new discoveries, such as the secretory function of adipose tissue, have provided new insight into potential factors contributing to obesity. Adipose tissue is now classified not just as an energy storage tissue but as an endocrine tissue participating in a complex network regulating energy homeostasis, glucose and lipid metabolism, vascular homeostasis, immune response, and even reproduction. Among the hormones identified that are produced by adipose tissue are leptin, cytokines (TNF- α , interleukin-6), adiponectin, and acylation-stimulating protein, angiotensinogen, plasminogen activator inhibitor-1, resistin, and steroid hormones (Table 10–3). Secretion of almost all of these hormones and cytokines is dysregulated as a consequence of both excess and deficiency in the mass of adipose tissue, suggesting that they are involved in the pathophysiology of both obesity and cachexia. Of particular interest are the contributions of the proinflammatory cytokines to the development of insulin resistance in obese individuals and the potential role of leptin as a regulator of fat mass.

TNF produced by adipose tissue has been implicated in producing insulin insensitivity, both indirectly and directly. Indirectly, TNF stimulates stress hormone production. Directly, TNF decreases insulin-induced receptor substrate 1 tyrosine phosphorylation and its association with the downstream signaling mediators

(phosphatidylinositol trisphosphate kinase), and it also inhibits PPAR. These pathways are currently under intense investigation and will likely lead to a new understanding of the role of adipose tissue–produced factors in the regulation of intermediary metabolism and regulation of fat mass.

LEPTIN

Leptin is a peptide hormone (146 amino acids) thought to serve as an indicator (lipostat) of energy stores, as well as a modulator of energy balance. The specific effects of leptin on fat metabolism are as follows:

- Decrease in fat storage
- Increase in sympathetic-mediated energy expenditure
- Increase in expression of uncoupling proteins
- Decrease in triglyceride content by increasing fatty acid oxidation
- Decrease in activity and expression of esterification and lipogenic enzymes
- Decrease in lipogenic activity of insulin, favoring lipolysis.

The overall effect of leptin is to deplete fat stores and promote leanness in a feedback regulatory system. In this feedback loop, leptin produced by white adipose tissue functions as a sensor that monitors the level of energy stores (adipose tissue mass). The signal is received and integrated by hypothalamic neurons, and an effector response, most likely involving modulation of appetite centers and sympathetic nervous system activity, regulates the 2 main determinants of energy balance: intake and expenditure. Leptin secretion exhibits a circadian rhythm, with a nocturnal rise over daytime secretion. Leptin plasma concentrations are low at about noon and begin to rise toward 3:00 pm; they reach maximal values during the night. These changes in leptin plasma concentrations are not influenced by meal ingestion and meal-induced increases in the circulating insulin concentration.

The effects of leptin are mediated through the leptin receptor, a member of the gp130 family of cytokine receptors, which activates a gene transcription factor on 2 populations of hypothalamic neurons. This process results in reduced expression of 2 orexigenic (feeding-inducing) neuropeptides, neuropeptide Y and agouti-related peptide; and enhanced expression of 2 anorexigenic peptides, α -melanocyte-stimulating hormone and cocaine-and amphetamine-regulated transcript. Thus, leptin-induced inhibition of food intake results from both the suppression of orexigenic and the induction of anorexigenic

neuropeptides.

The feedback regulatory loop for leptin's effects has been well established in rodents; however, many unsolved questions remain about its applicability to body weight regulation in humans. The role of leptin in humans appears to be mostly one of adaptation to low energy intake rather than a brake on overconsumption and obesity. Leptin concentrations decrease during fasting and energy-restricted diets, independent of body fat changes, stimulating an increase in food intake before body energy stores become depleted. Because leptin levels do not rise in response to individual meals, it is not thought to serve as a meal-related satiety signal. Finally, it is notable that obese individuals have high plasma leptin concentrations that do not result in the expected reduction in food intake and increase in energy expenditure, suggesting that obesity may be related to leptin resistance and not leptin deficiency.

GHRELIN

Ghrelin is a hormone produced by the enteroendocrine cells of the stomach, and to lesser extent by the placenta, pituitary, and hypothalamus. Circulating levels of ghrelin decrease during meals and are highest in the fasted state. Ghrelin levels are decreased in obese individuals and increased in individuals consuming low calorie diets, chronic strenuous exercise, cancer anorexia, anorexia nervosa and in patients with Prader-Willi syndrome. In humans, ghrelin has been shown to be a potent growth hormone secretagogue and appetite stimulant.

ELECTROLYTE BALANCE

Regulation of Sodium Balance



Sodium is the primary electrolyte that regulates extracellular fluid (ECF) levels and osmolarity in the body. Sodium is an essential mineral required for the integrity of multiple organ functions, particularly through the regulation of fluid balance and ultimately the regulation of blood pressure. It is essential for maintaining hydration, water balance, osmotic equilibrium, plasma volume, and acid-base balance and for preserving nerve impulses and muscle contractions. Sodium concentration determines the ECF tonicity, which reflects the balance between sodium and water in the ECF. The osmolarity (the amount of solute per unit volume) of bodily fluids is tightly regulated by balancing the intake and excretion of sodium with those of water. Extreme variation in osmolarity causes cells to shrink or swell, damaging or destroying cellular structure and disrupting normal cellular function.

Regulation of extracellular Na^+ concentration controls the distribution of water between the ECF and intracellular fluid (ICF) and maintains cell volume, ensuring normal physiologic function. Sodium is maintained in the ECF by the action of Na^+/K^+ -ATPase, whereas water crosses cell membranes through ubiquitously expressed aquaporins (maintaining ICF and ECF isotonicity). Water balance is maintained by matching the amount of water consumed in food and drink (and generated by metabolism) to the volume of water excreted. Consumption is regulated by central nervous system stimulation of thirst and salt craving, whereas excretion is principally regulated hormonally at the kidney. Additional loss of water (1 L/d) occurs through the skin, lungs, and feces.

The overall mass of Na^+ is under aldosterone regulation, whereas the Na^+ concentration in plasma is under antidiuretic hormone (ADH) regulation. Thus, low Na^+ concentrations do not necessarily mean that total Na^+ mass is low. In chronic heart failure, osmolarity can be low yet Na^+ mass can be high because of excess water and Na^+ in the ECF, with greater increases in total-body water than in Na^+ mass.

INTAKE, DISTRIBUTION, AND EXCRETION OF SODIUM

Sodium concentrations average 140 mmol/L in plasma and 10 mmol/L intracellularly. The concentration of sodium in gut secretions and sweat is similar to that in the ICF (10–50 mmol/L). Sodium concentrations and intravascular volume are physiologically controlled in parallel. For a given amount of total-body water, intravascular volume is determined by sodium concentrations, which control the distribution between ICF and ECF.

The minimum recommended intake of sodium is 500 mg/d; which is markedly exceeded by the average diet in the United States (average 4–5 g/d). One teaspoon of table salt contains approximately 6 g of sodium chloride (2.3 mg of sodium). Sodium intake is usually considered to be unregulated; however, specific hypothalamic areas are involved in salt appetite, although their physiology is not completely understood.

Sodium balance is maintained primarily through hormonal regulation of renal sodium excretion. Fecal loss is small (0.8–8 mmol/d) even when sodium intake is high. Skin loss is small except under conditions of excessive sweating. Small changes in percentage renal reabsorption of sodium cause a large change in the amount of sodium excreted. The total amount of filtered sodium load (about 25,200 mmol/d) is equal to the glomerular filtration rate (180 L/d) x plasma sodium concentrations (140 mmol/L). Therefore, to maintain sodium balance on a dietary intake of 150 mmol/d, a total of 25,050 mmol (ie, 99.4% of the filtered load) must be reabsorbed. About 60–70% of the filtered sodium is reabsorbed in the proximal tubule. An additional 20–30% of filtered sodium is reabsorbed in the ascending limb of the loop of Henle. Sodium reabsorption in the thick ascending limb is passive, down an electrical gradient set up by the active transport of chloride ions, and is the site of inhibition by loop diuretics. Thus, a significant (80–95%) obligatory reabsorption of filtered sodium occurs in the renal tubule before reaching the distal tubule, preventing large renal sodium losses. The majority of the remaining sodium (5–10%) is reabsorbed in the distal tubule and collecting duct. It is here that the finer regulation of sodium excretion through aldosterone occurs, and this is the site of inhibition by the diuretic hydrochlorothiazide (Chapter 6).

HORMONAL REGULATION OF SODIUM AND WATER

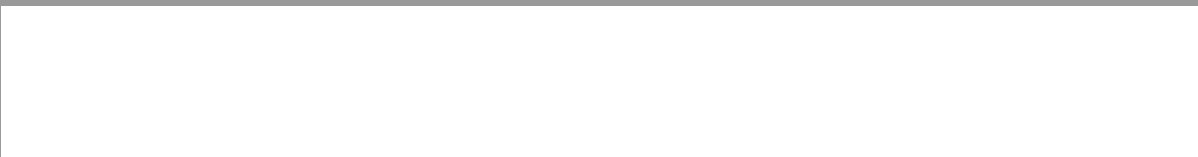
BALANCE

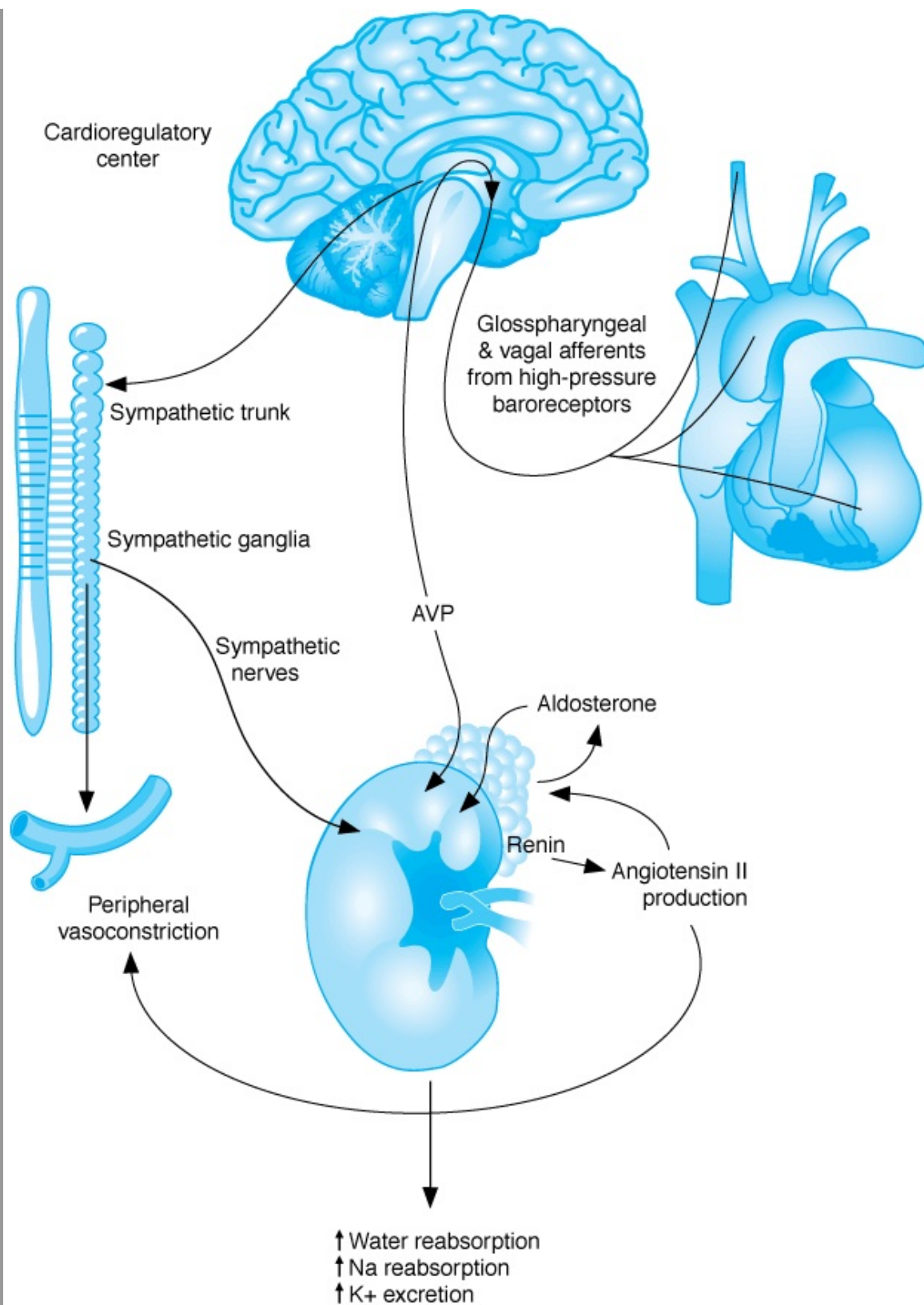
The system that controls total-body water is a negative feedback homeostatic mechanism, of which thirst and ADH are the major effectors. Two stimuli regulate the system: tonicity of the ECF through osmoreceptors, and intravascular volume through stretch or baroreceptors.

As discussed in Chapter 3, the system works primarily to maintain intravascular volume and to a lesser extent to maintain tonicity. Thirst is stimulated by an increase in tonicity (1–2% changes are sufficient to elicit thirst) and by reductions in the ECF volume. Water intake is inhibited by hypotonicity and ECF volume expansion.

Sudden decreases in blood volume are sensed by mechanoreceptors in the left ventricle, carotid sinus, aortic arch, and renal afferent arterioles (Figure 10–3; Figure 2–7). These mechanoreceptors respond to decreased stretch resulting from decreases in systemic arterial pressure, stroke volume, renal perfusion, or peripheral vascular resistance by triggering an increase in sympathetic outflow from the central nervous system, activation of the renin-angiotensin-aldosterone system, and nonosmotic release of arginine vasopressin, as well as stimulation of thirst. Low blood pressure results in decreased renal perfusion pressure and lower glomerular filtration rates, which stimulate the release of renin from juxtaglomerular cells in the afferent and efferent arterioles. Renin is an enzyme synthesized in the juxtaglomerular cells of the kidney that cleaves angiotensinogen (a peptide produced by the liver) to angiotensin I, which is later converted by angiotensin-converting enzyme to angiotensin II. This renin-angiotensin system is part of an extremely powerful feedback system for long-term control of blood pressure and volume homeostasis. Together, angiotensin II, aldosterone, and ADH (arginine vasopressin) produce vasoconstriction, and renal retention of Na^+ and water.

Figure 10–3.





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Neuroendocrine control of blood volume. Sudden decreases in blood volume are sensed by mechanoreceptors in the left ventricle, carotid sinus, aortic arch, and renal afferent arterioles, triggering an increase in sympathetic outflow from the central nervous system, activation of the renin-angiotensin-aldosterone system, nonosmotic release of arginine vasopressin (AVP), and stimulation of thirst. The decreases in renal perfusion pressure and glomerular filtration rates stimulate the release of renin, the enzyme responsible for the conversion of angiotensinogen to angiotensin I (later converted by angiotensin-converting enzyme to angiotensin II). Angiotensin II, aldosterone, and ADH produce vasoconstriction, venoconstriction, and renal retention of Na^+ and water.

Antidiuretic hormone—ADH directly controls water excretion by the kidneys (Chapter 2). ADH secretion and compensatory thirst are stimulated by hypothalamic osmoreceptors and by decreased stimulation of aortic and carotid stretch receptors. Release of ADH is inhibited by increased stretch of mechanoreceptors (stretch receptors) in the atria of the heart. ADH stimulates insertion of aquaporins into the cell membrane, increases water reabsorption in the collecting ducts, and concentrates excreted urine.

Angiotensin II—Angiotensin II elevates blood pressure by several mechanisms, including direct vasoconstriction, potentiation of the activity of the sympathetic nervous system at both the central and peripheral levels, stimulation of aldosterone synthesis and release with consequent sodium reabsorption by the kidney (Chapter 6), stimulation of ADH release and increased water retention, intrarenal efferent arteriolar constriction (which maintains glomerular filtration rate when renal perfusion is impaired, also known as "glomerular tubular feedback"). Because of the effectiveness of the renin-angiotensin system in regulating blood pressure, blockade of the system with angiotensin-converting enzyme inhibitors offers a powerful therapeutic tool in diseases such as hypertension and congestive heart failure.

Aldosterone—Aldosterone increases sodium reabsorption and potassium excretion in the distal tubule and the collecting duct of the nephron, playing a central role in determining total-body Na^+ mass,

and thus long-term blood pressure regulation. Aldosterone release from the adrenals is under positive regulation by Angiotensin II.

Integrity of the arterial circulation, as determined by cardiac output and peripheral vascular resistance, is the primary determinant of renal sodium and water excretion in health and disease. Specifically, either a primary decrease in cardiac output or arterial vasodilatation causes arterial underfilling, which results in the activation of neurohumoral reflexes that stimulate sodium and water retention. The renal excretion of sodium and water normally parallels sodium and water intake, so that an increase in plasma and blood volume is associated with increased renal sodium and water excretion. The increase in blood volume results in pressure diuresis and a pressure natriuresis (loss of water and sodium), restoring blood volume to normal. Atrial natriuretic factor is a peptide hormone produced in atrial myocardial cells and released in response to increased stretch, usually resulting from increased intravascular volume. Atrial natriuretic factor increases renal Na^+ excretion and water loss through an increase in glomerular filtration rate and a decrease in Na^+ reabsorption in the medullary collecting duct.

ABNORMALITIES IN SODIUM AND WATER BALANCE

Abnormalities in sodium and water balance can be classified into 4 categories. Excess Na^+ is characterized by expansion of the ECF volume and frequently by low effective circulating blood volume (ie, heart failure, hypoalbuminemia, renal insufficiency). Deficit in Na^+ is characterized by reduced ECF volume. Excess water is due to either excess intake or enhanced ADH release and is manifested by hyponatremia and hypoosmolarity. Water deficit is due to lack of intake or excess loss (renal and nonrenal) and is manifested by hypernatremia and hyperosmolarity.

Regulation of Potassium Balance

Potassium is the most abundant cation in the body and the main intracellular electrolyte. Most (98%) of the potassium in the body (about 40–50 mmol/kg body weight) is sequestered within cells. The ratio of extracellular to intracellular potassium (1:10) is maintained by a sodium-potassium pump and is the major determinant of resting membrane potential. Serum potassium levels range between 3.6 and 5.0 mmol/L. Small losses (1%, or 35 mmol) of total-body potassium content can seriously disturb the delicate balance between intracellular and extracellular potassium and can result in profound physiologic changes. Because only a small percentage of the total-body stores is present in the ECF, however, hypokalemia (serum levels less than 3.6 mmol/L) is not necessarily synonymous with whole-body potassium deficiency. The tissues most severely affected by potassium imbalance are muscle and renal tubular cells. Manifestations of hypokalemia include generalized muscle weakness, paralytic ileus, and cardiac arrhythmias.

INTAKE, DISTRIBUTION, AND EXCRETION OF POTASSIUM



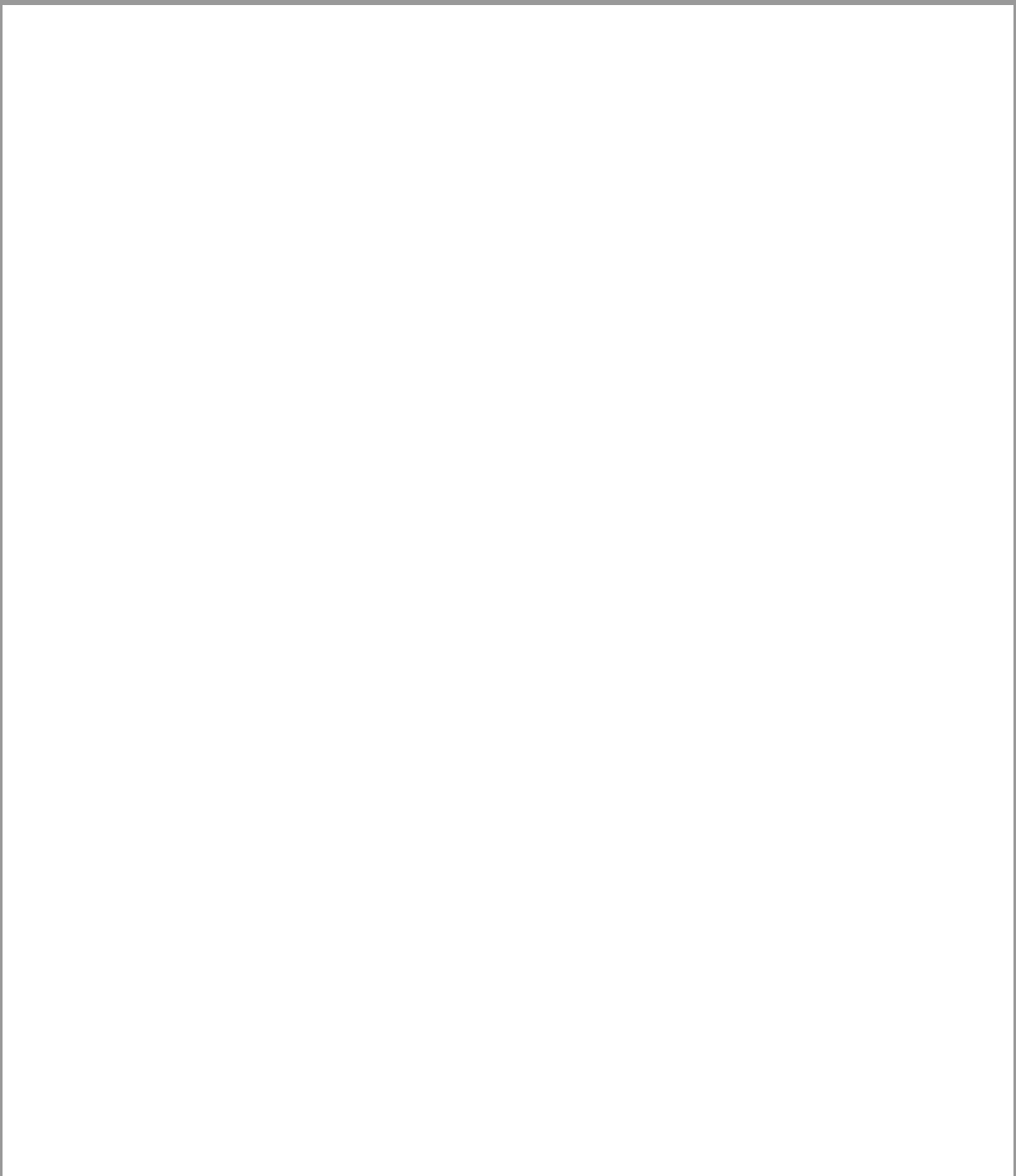
The minimum daily requirement of potassium is approximately 1600–2000 mg (40–50 mmol or mEq). The daily intake of potassium in the western diet is about 80–120 mmol. Only a small fraction (10%) of potassium is excreted through the gastrointestinal tract. The majority is excreted by the kidney, accounting for 90% of daily potassium losses. Thus, the kidney is responsible for long-term potassium homeostasis, as well as for regulating the serum potassium concentration. On a short-term basis, serum potassium is also regulated by the shift of potassium between the ICF and ECF. This short-term regulation of serum potassium is principally controlled by insulin and catecholamines through regulation of the transcellular distribution of potassium. Dietary potassium, which is rapidly absorbed by the gut, increases serum potassium transiently. The release of insulin and catecholamines during a meal quickly shifts the potassium into the cells.

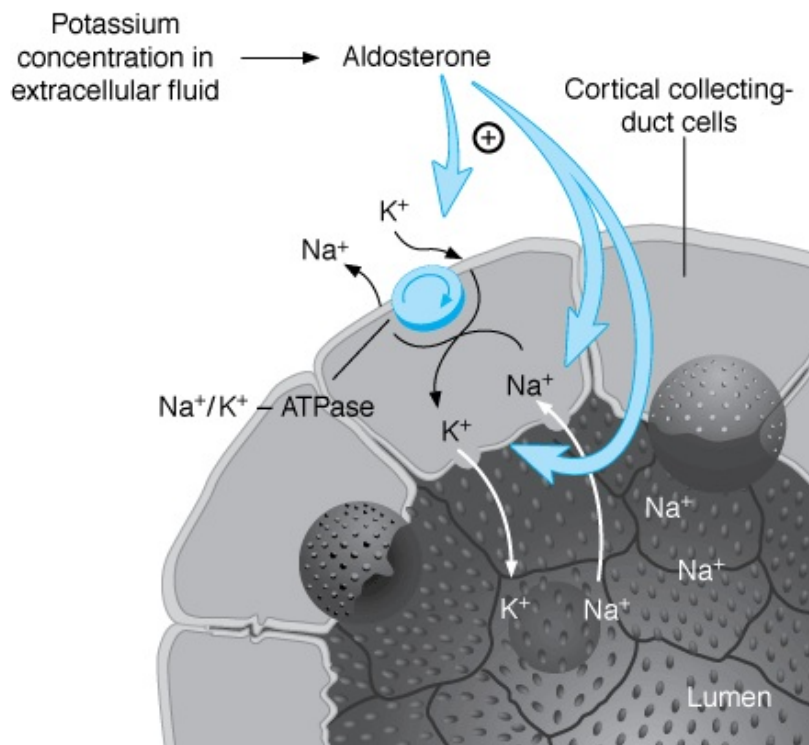
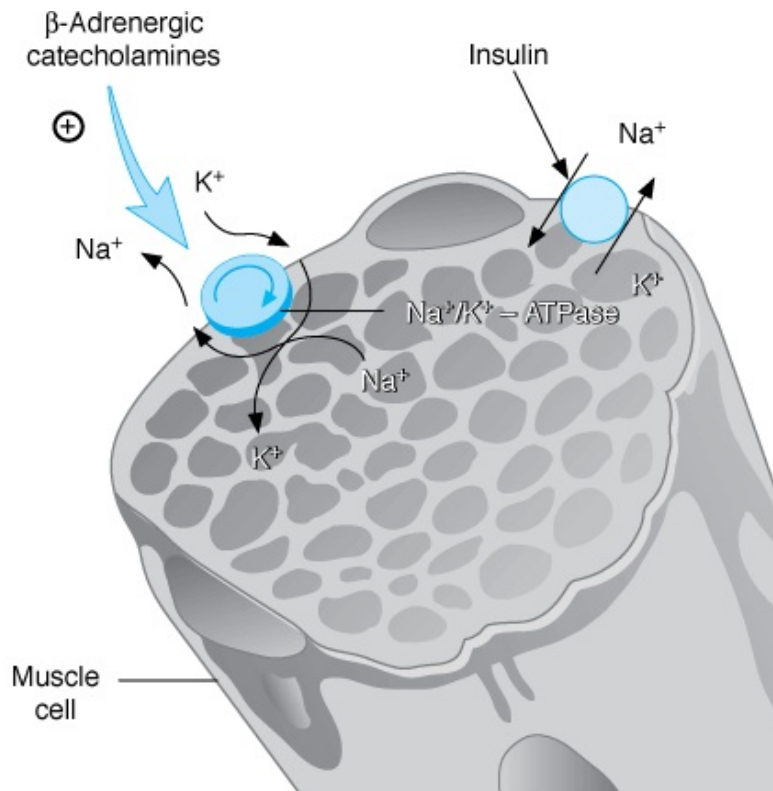
Potassium excretion by the kidney is tightly regulated and is determined primarily by events beyond the early distal tubule, where either reabsorption or secretion of K^+ can occur. The filtered potassium (approximately 700–800 mmol/d) is largely reabsorbed by proximal nephron segments, including the proximal convoluted tubules and thick loop of Henle. Only about 10% of filtered K^+ reaches the distal convoluted tubule. Excretion of potassium occurs mainly through secretion by distal segments, predominantly the distal convoluted tubule and the collecting duct, and is mediated primarily by apical membrane K^+ channels in the principal cells. Potassium excretion almost always exceeds the amount delivered to the early distal tubule (except under conditions of sustained K^+ depletion), indicating that the rate of secretion is the key determinant of K^+ excretion. The principal site for the regulation of K^+ excretion is the distal tubule, where secretion is indirectly but tightly coupled to sodium reabsorption via the amiloride-sensitive sodium channel; and under regulation by aldosterone. Increased sodium reabsorption increases potassium secretion, whereas decreased sodium reabsorption decreases potassium secretion. Any condition that decreases the activity of renal potassium channels results in hyperkalemia (eg, amiloride intake or aldosterone deficiency) whereas increased activity results in hypokalemia (eg, primary aldosteronism or Liddle syndrome; Chapter 6). Because the kidney is the major regulator of potassium homeostasis, renal dysfunction results in abnormal levels of serum potassium.

Potassium contributes to regulation of its balance by stimulating aldosterone secretion by the glomerulosa cells of the adrenal cortex (Figure 10–4). This effect is facilitated by angiotensin II released in response to activation of the renin-angiotensin system when renal perfusion pressure is decreased. Aldosterone enhances renal and colonic K^+ secretion, promoting the loss of K^+ in the urine and stool. Sustained hyperkalemia does not occur in individuals with normal renal function despite marked increases in potassium intake because of an adaptive change in distal tubular K^+ secretion, such that intake is matched by rapid and equivalent increases in K^+ excretion. The mechanisms involved in the chronic adaptation to increased levels of

K^+ include changes in apical K^+ and Na^+ conductance and in basolateral Na^+/K^+ -ATPase pump activity, an increase in apical Na^+ delivery and reabsorption, and an increase in K^+ excretion per nephron to match K^+ intake.

Figure 10–4.





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Key hormones involved in normal potassium homeostasis. An increase in the ECF potassium concentration stimulates the secretion of aldosterone, and a decrease in potassium inhibits its secretion. Aldosterone promotes potassium excretion through its effects on the Na^+/K^+ -adenosine triphosphatase (ATPase) and epithelial sodium and potassium channels in collecting-duct cells. Angiotensin II has a synergistic effect on the stimulation of aldosterone production induced by hyperkalemia. Insulin stimulates entry of K^+ into the cell through the activation of the electroneutral Na^+/K^+ antiporter. The rise in intracellular Na^+ produced by insulin triggers the activation of the electrogenic Na^+/K^+ -ATPase, which extrudes Na^+ from the cell in exchange for K^+ . Catecholamines (β -adrenergic receptor stimulation) increase cellular potassium uptake by stimulating cell membrane Na^+/K^+ -ATPase. Stimulation of the β -adrenergic receptor shifts K^+ out of the cell. (Modified, with permission, from Gennari F. Current concepts: Hypokalemia. *NEJM*. 1998;339:451. Copyright © Massachusetts Medical Society. All rights reserved.)

HORMONAL REGULATION OF POTASSIUM BALANCE

Total-body stores of potassium and its cellular distribution in the body are closely regulated by key hormones.

Aldosterone—Aldosterone is the major regulator of body stores of potassium through its effect on the excretion of potassium by the kidney. Aldosterone increases the synthesis and activity of Na^+/K^+ -ATPase in the basolateral membrane of the distal tubule, promoting the exchange of cytosolic Na^+ for K^+ . The overall result is an increase in Na^+ reabsorption and an increase in K^+ excretion.



Insulin—Insulin stimulates entry of K^+ into the cell through activation of the electroneutral Na^+/H^+ antiporter, leading to Na^+ influx. The rise in intracellular Na^+ produced by insulin triggers the activation of the electrogenic Na^+/K^+ -ATPase, which extrudes Na^+ from the cell in exchange for K^+ . The treatment of patients with diabetic ketoacidosis with high insulin doses produces a significant influx of K^+ into the cells that may result in hypokalemia, manifested by changes in the electrocardiogram.

Catecholamines—Catecholamines (β -adrenergic receptor stimulation) increase cellular potassium uptake by stimulating cell membrane Na^+/K^+ -ATPase. Indirectly, catecholamines stimulate glycogenolysis, resulting in a rise in plasma glucose concentrations, release of insulin from the pancreas, and insulin-mediated effects on K^+ redistribution. Stimulation of the β -adrenergic receptor shifts K^+ out of the cell and can also affect K^+ distribution through inhibition of pancreatic insulin release.

Insulin and catecholamines are both stimulated by the ingestion of glucose- and potassium-rich foods, thereby maintaining K^+ homeostasis despite large dietary intake. These hormones are essential in moving potassium primarily into the intracellular compartment of the liver and striated muscle cells.

ACID-BASE AND OSMOLAR REGULATION OF POTASSIUM DISTRIBUTION

Intracellular potassium homeostasis is also affected by changes in acid-base balance and osmolarity. Sudden changes in plasma osmolarity redistribute water between the ICF and ECF. This movement of water out of a cell creates a solvent drag phenomenon, pulling K^+ out of the cell and therefore increasing serum potassium. Similarly, metabolic acidosis caused by a loss of bicarbonate or a gain in hydrogen ion concentration $[H^+]$ leads to a shift of K^+ across cell membranes and hyperkalemia. However, integrity of renal function and stimulation of aldosterone release rapidly correct this imbalance. These examples do not entail net changes in body K^+ . In contrast, in diabetic ketoacidosis, there is a net loss of K^+ from the body because of osmotic diuresis, despite elevations in ECF K^+ concentrations (hyperkalemia), because of insulin deficiency. Following aggressive insulin treatment, hypokalemia becomes apparent. Opposite effects are observed during alkalosis. In metabolic alkalosis, the excess bicarbonate causes H^+ in the ECF to fall, leading to entry of Na^+ into the cell in exchange for H^+ . Na^+ is pumped out of the cell by the Na^+/K^+ -ATPase in exchange for K^+ movement into the cell creating a shift of K^+ into the cells.

Low serum potassium concentration (hypokalemia; less than 3.6 mmol/L) is perhaps the most common electrolyte abnormality encountered in clinical practice. Hypokalemia is almost always the result of potassium depletion induced by abnormal fluid losses (ie, vomiting, colonic diarrhea, profuse sweating, diuretic use, or nasogastric suction). Patients present with muscle weakness and changes in the electrocardiogram. More rarely, hypokalemia occurs because of an abrupt shift of potassium from the ECF into cells, frequently as an effect of prescription drugs.

Regulation of Calcium Balance

Serum calcium concentrations are tightly regulated and concentrations are held constant at 1 mmol of ionized calcium or 10 mg/dL of total calcium. Calcium accounts for 1–2% of adult human body weight, with the majority (99%) found in bones and teeth. Calcium helps maintain the cell membrane electrical potential and is involved in signaling mechanisms, enzymatic activity, coagulation cascade, neurotransmitter release, and intercellular communication. Because of its role in these and other critical functions, its tight regulation is important in preventing diseases such as osteoporosis, renal and heart disease, and hypertension.

The recommended calcium intake varies with age, sex, and reproductive stage. Higher intakes are recommended for children, adolescents, pregnant and lactating mothers, postmenopausal women, and the elderly (1200–1500 mg/d) than for healthy adults up to age 65 years (1000 mg/d). A positive correlation between protein intake and urinary calcium has been established. This relationship may explain the apparently higher calcium requirement of the western diet as compared with that of underdeveloped countries. The catabolism of dietary protein generates ammonium ion and sulfates from sulfur-containing amino acids, leading to the acidification of plasma. This decrease in pH triggers bone resorption to supply buffers such as citrate and carbonate, with the consequent release of calcium into the circulation, resulting in calciuria.

HORMONAL REGULATION OF CALCIUM BALANCE



Calcium homeostasis is maintained by the complex interaction of several hormones, particularly vitamin D, parathyroid hormone (PTH), and calcitonin (Chapter 4). Because the major reservoir for calcium is the bone, and this is the pool of calcium that is involved in actively maintaining serum plasma concentrations within a normal range, the factors that influence bone metabolism such as estrogen, growth factors, glucocorticoids, thyroid hormone, and cytokines also contribute significantly to the overall metabolic control of Ca^{2+} stores. However, it is important to note that it is the "free" calcium in serum that is under tight hormonal regulation, principally by PTH.

Parathyroid hormone—PTH activates 25-hydroxyvitamin D-1 α -hydroxylase, the enzyme that converts 25-hydroxyvitamin D₃ to the active form of vitamin D (1,25(OH)₂D₃), resulting in a marked elevation of plasma levels of vitamin D (calcitriol). PTH also stimulates renal reabsorption of calcium and inhibits the reabsorption of phosphate, causing phosphate diuresis.

Vitamin D—Vitamin D (1,25(OH)₂D₃) increases dietary Ca^{2+} absorption in the small intestine and increases osteoblast activity, resulting in stimulation of osteoclast-mediated resorption. Vitamin D plays an important role in differentiation of the promyelocyte to the osteoclast precursor to mature osteoclast through osteoblast-generated osteoclast differentiation factor (Chapter 5). These effects of vitamin D provide bone calcium for the plasma and also cause bone resorption coupled with formation as part of the bone-remodeling process. Thus, vitamin D strengthens bone and repairs microfractures that may have occurred during bone usage. The result is calcium mobilization by the skeleton into the plasma compartment by the action of the vitamin D hormone and PTH. Both PTH and vitamin D are required for this system to operate. In the distal renal tubule, PTH and vitamin D act in concert to produce virtually complete reabsorption of the filtered load of calcium. These sources of calcium cause a rise in serum calcium, which, through feedback inhibition at the parathyroid gland, decreases PTH secretion (Chapter 5).

Calcitonin—Calcitonin counteracts the effects of PTH and vitamin D. It prevents hypercalcemia by directly inhibiting osteoclast activity, thereby reducing calcium mobilization and release from the skeleton.

Regulation of Phosphate Balance

Phosphorus (Pi), in the form of phosphate (HPO_4^{2-}), accounts for more than 50% of bone mineral mass. Osteoblasts are unique among all other cell types in that they create a mineral trap (calcium phosphate) in bone matrix after it has been deposited. This trap depletes the ECF around the osteoblast of both calcium and phosphorus, and if the local concentration of phosphorus drops too low, osteoblasts become phosphorus starved. Throughout the body, phosphorus is found as a component of nucleic acids, phospholipids, signaling molecules (inositol 1,4,5-trisphosphate, phosphatidylinositol 4,5-bisphosphate), and cofactors involved in cellular energy metabolism (ATP, GTP), playing numerous vital roles in cell function. Most food products, whether plant or animal, contain relatively abundant quantities of phosphorus. Normal balanced diets provide 800–1500 mg of phosphorus per day. The skeleton contains 85% of the body's phosphorus; the rest is distributed in the ECF and ICF (0.5–0.8 g). Total extracellular phosphorus (about 12 mg/dL) is found in an ionized form and a nonionized form (8.5 mg/dL is in the organic form and 3.5 mg/dL is in the inorganic form). The inorganic form may be found ionized or free in the form of HPO_4^{2-} (50%); complexed with Ca^{2+} , Mg^{2+} , and Na^+ (35%); or bound to protein (15%).

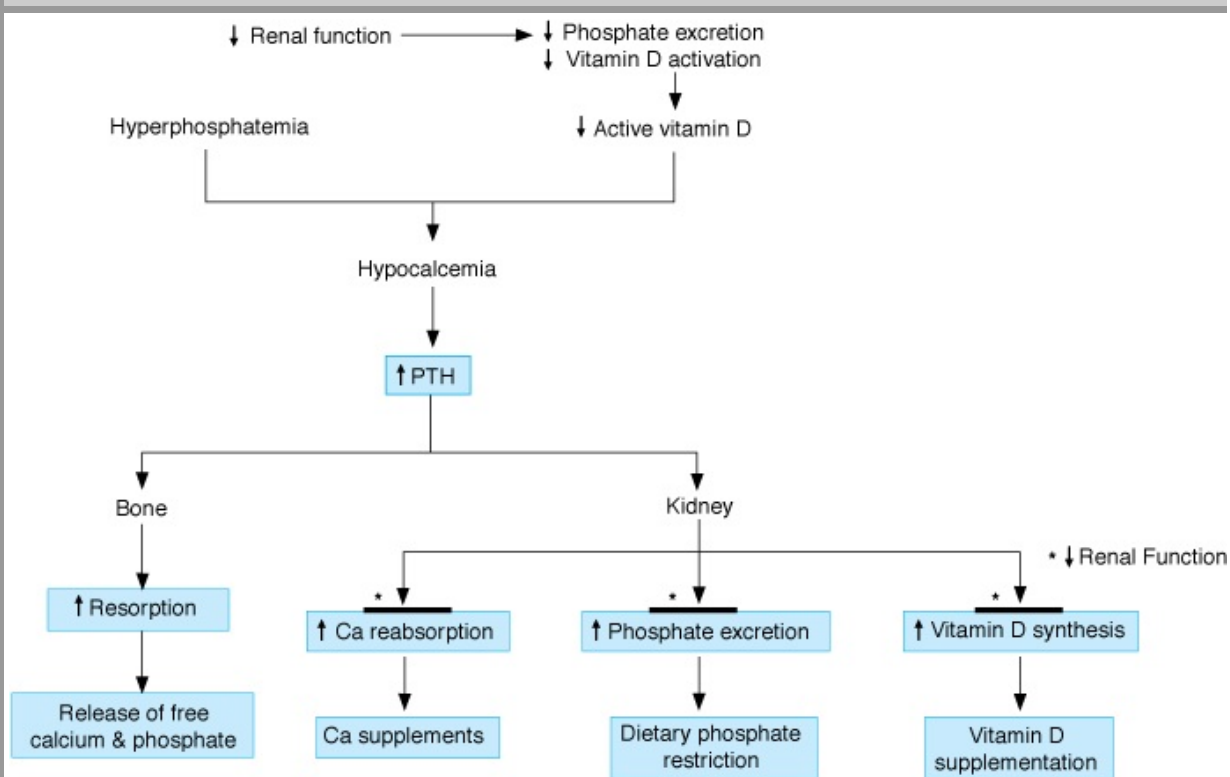
Pi homeostasis is maintained by intestinal absorption, renal excretion, balance of Pi exchange in and out of the cells, and hormonal regulation. Following intestinal absorption from the diet, most Pi undergoes urinary excretion. Because of its critical role in energy-requiring physiologic functions, the extracellular Pi concentration is maintained in a narrow range, principally through regulation of urinary Pi excretion. Under normal physiologic conditions, urinary Pi excretion corresponds roughly to phosphate intake and absorption from the upper small intestine.

Alterations in extracellular Pi concentrations lead to rapid adjustments in renal Pi excretion and slower and less regulated adjustments in intestinal absorption. Under normal physiologic conditions, approximately 80–90% of the filtered load of Pi is reabsorbed primarily in the proximal tubules, with higher rates of reabsorption at early segments. Low dietary Pi intake can lead to almost 100% reabsorption of filtered Pi, whereas high dietary Pi intake decreases proximal tubular Pi reabsorption.



Pi excretion by the kidney is stimulated by PTH through the inhibition of brush-border membrane Na-Pi cotransport activity (Figure 10–5). Pi reabsorption by the kidney is increased by vitamin D and insulin through stimulation of brush-border membrane Na-Pi cotransport and inhibition of the phosphaturic action of PTH. Vitamin D also regulates intestinal Pi absorption by stimulating the brush-border membrane Na-Pi cotransport in the upper small intestine. Thus, PTH promotes Pi excretion, whereas vitamin D and insulin promote Pi renal reabsorption and intestinal absorption. Vitamin D deficiency leads to increased Pi renal excretion and decreased intestinal Pi and Ca^{2+} absorption, resulting in a severe loss of both Ca^{2+} and Pi from bone (the major site of both of these mineral stores) because of enhanced PTH activity, resulting in loss of bone mineral and osteomalacia. This is in contrast to osteoporosis induced by Ca^{2+} deficiency.

Figure 10–5.



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Integration of phosphate balance. Alterations in extracellular Pi concentrations lead to rapid adjustments in renal Pi excretion. When renal function is compromised, impaired Pi excretion by the kidney leads to hyperphosphatemia and the stimulation of parathyroid hormone (PTH) release from the parathyroid gland. PTH stimulates the resorption and release of calcium phosphate that can lead to osteoporosis. Because of the impairment in renal function, PTH is unable to stimulate calcium reabsorption, phosphate excretion, and vitamin D activation shown by the black line and *. Management of these patients requires control of dietary phosphate intake and supplementation with calcium and vitamin D. Normal renal function allows rapid adaptation of the body to sudden changes in serum phosphate levels.

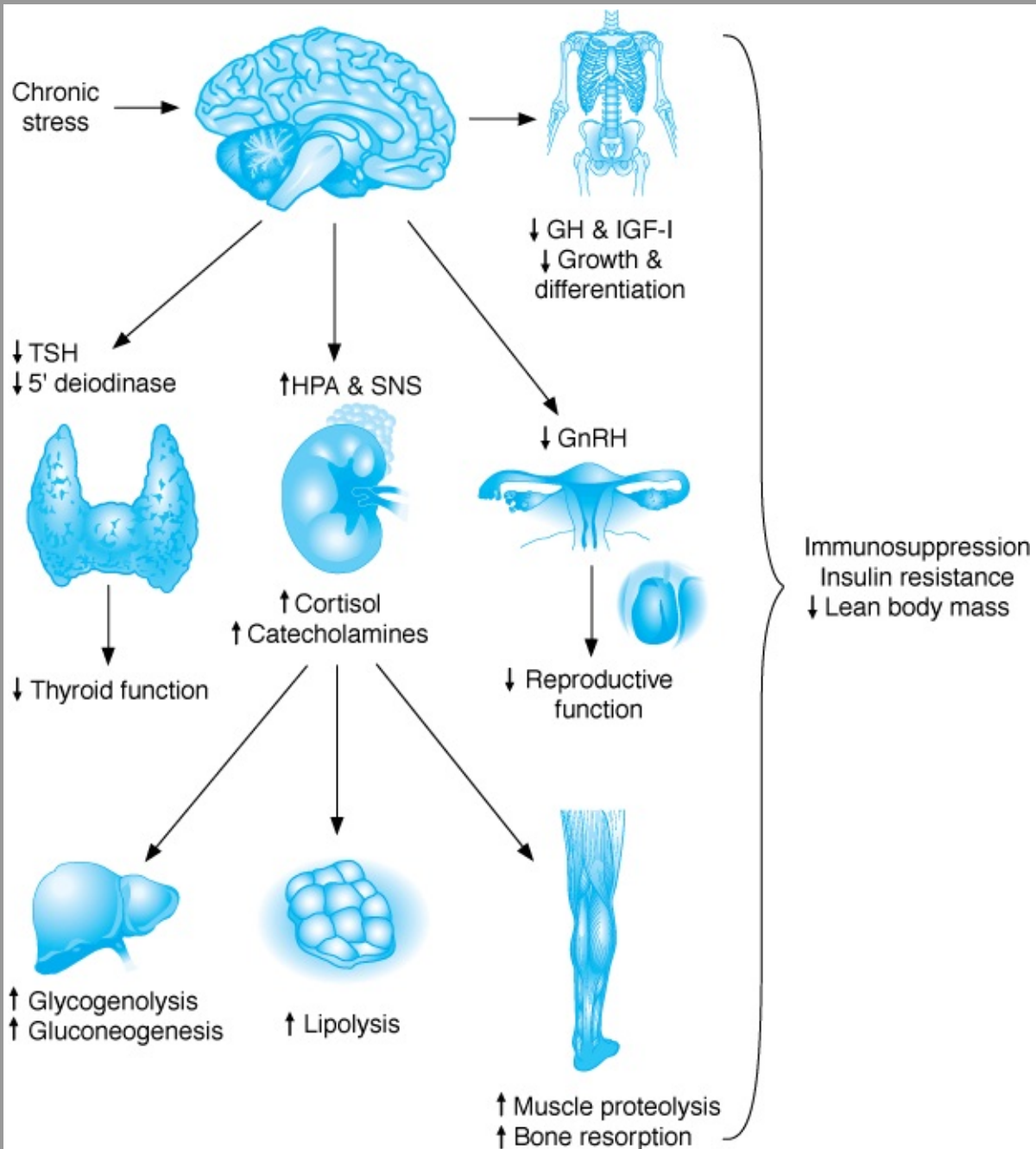
NEUROENDOCRINE REGULATION OF THE STRESS RESPONSE

The contributions of neuroendocrine mechanisms in maintaining homeostasis have been discussed individually throughout the previous chapters and in a more integrated approach in the present chapter. Alterations in the environment or in the host that require adaptation involve the synchronized interaction of virtually all aspects of neuroendocrine function that have been described. The adaptation to a biologic, psychosocial, or environmental insult to the host is referred to as the stress response; in the acute setting, it is also termed the "fight or flight" response. It is now clear that in modern life, this stress response can be chronic, with a significant cost to the health of the individual. This wear and tear of chronic adaptation to daily stressors constitutes the allostatic load of the individual; it is the "pathologic" chronic homeostasis through which we achieve stability at the expense of psychosocial and physical well being.

Chronic activation of the mechanisms that restore homeostasis results in excessive and, in some cases, inadequate responses that ultimately alter the function of virtually all organ systems (eg, hypertension, autoimmune disorders, metabolic syndrome) (Figure 10–6). Many of the effects of this dysregulated state are mediated by chronic activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic

nervous system, producing marked alterations in endocrine function, such as the following.

Figure 10–6.



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Neuroendocrine responses to chronic or severe stress. Chronic

activation of the neuroendocrine response to restore homeostasis influences virtually all organ systems. The short-term activation of these stress-response mechanisms ensures that energy substrates are available to meet the increased metabolic demands of the individual. However, prolonged duration and increased magnitude of these activities lead to erosion of lean body mass and tissue injury. GH, growth hormone; GnRH, gonadotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; IGF-I, insulin-like growth factor-I; SNS, sympathetic nervous system; TSH, thyroid-stimulating hormone.

Inhibition of reproduction function—Enhanced release of corticotropin-releasing hormone (CRH) and β -endorphin suppresses GnRH release directly and indirectly through the release of glucocorticoids. Glucocorticoids decrease the release of luteinizing hormone and produce gonadotropin resistance at the gonads. This suppression in gonadal function is evident in patients with anorexia nervosa, athletes, and ballet dancers.

Inhibition of the GH-IGF-I axis—Chronic activation of the HPA axis suppresses GH release and inhibits the effects of IGF-I at target tissues.

Suppression of thyroid function—CRH and cortisol suppress the production of thyroid-stimulating hormone and inhibit the activity of peripheral 5'-deiodinase, leading to the euthyroid sick syndrome.

Dysregulation of energy substrate metabolism—An increase in catecholamines stimulates lipolysis and decreases triglyceride synthesis in white adipose tissue. In the liver, increased epinephrine levels stimulate hepatic glycogenolysis and, together with high cortisol levels, increase hepatic glucose output. High cortisol levels resulting from activation of the HPA increase gluconeogenesis, produce insulin resistance in peripheral tissues, inhibit the lipolytic action of GH, and inhibit bone osteoblastic activation (remodeling) by sex steroids. This leads to increases in visceral adiposity and loss of BMD and lean body mass. This aspect of the stress response may be of particular importance in the treatment of diabetic patients during stressful periods such as surgery or infection.

Alterations in the Immune Response—The significant rise in circulating cortisol levels affects virtually all aspects of the immune

response, including cytokine production, leukocyte trafficking and recruitment, and production of chemokines. Overall, glucocorticoids exert an anti-inflammatory response. CRH may have direct proinflammatory effects on cells of the immune system. Activation of the autonomic nervous system also affects the immune response through effects on neutrophil demargination and cytokine production.

Short-term activation of these stress-response mechanisms ensures that energy substrates are available to meet the increased metabolic demands of the individual. However, prolonged duration and increased magnitude of these activities lead to erosion of lean body mass and tissue injury. Nevertheless, impaired activation or lack of responsiveness of the HPA and autonomic nervous system can also be deleterious, as in the case of the critically ill patient. Thus, the overall regulation of the neuroendocrine responses that mediate the physiologic functions involved in maintaining and restoring homeostasis is critically important in situations such as illness, trauma, surgery, or fasting.

KEY CONCEPTS



Energy substrate mobilization, utilization, and storage are under neuroendocrine regulation.



Hepatic glycogen and adipose tissue triglycerides are the principal sites of energy storage.



The central nervous system integrates the counterregulatory response to acute decreases in energy substrate availability.



Regulation of sodium balance determines blood volume and blood pressure control.



The kidney is responsible for long-term potassium homeostasis and serum potassium concentration.



Insulin and catecholamines regulate the cellular distribution of potassium.



Serum calcium levels are tightly regulated through hormone-mediated effects on bone.



Bone mineral density and mass are under nutritional and hormonal control.



Phosphate is regulated principally through effects on renal excretion.

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NORMAL VALUES OF METABOLIC PARAMETERS AND TESTS OF ENDOCRINE FUNCTION: PLASMA AND SERUM VALUES

Table 1. Normal Values of Metabolic Parameters and Tests of Endocrine Function: Plasma and Serum Values.

Hormone	Time/condition of sample	Normal value or range: SI (traditional units)*
ACTH	8:00 AM	1.3–16.7 pmol/L (6–76 pg/mL)
Aldosterone	8:00 AM	55–250 pmol/L (2–9 ng/dL)
Potassium		3.5–5.0 mmol/L (3.5–5.0 mEq/L)
Angiotensin II	8:00 AM	10–30 nmol/L (10–30 pg/mL)
Arginine vasopressin	Random fluid intake	1.5–5.6 pmol/L (1.5–6 ng/L)
Plasma osmolality		285–295 mOsm/kg H ₂ O
Sodium		136–145 mmol/L (136–145 mEq/L)
Cortisol	8:00 AM	138–690 nmol/L (5–25 µg/dL)
	noon–8:00 PM	138–414 nmol (5–15 µg/dL)
DHEA		6–43 nmol/L (180–

		1250 ng/dL)
DHEAS		1.3–6.7 μ mol/L (10–619 μ g/dL)
17-Hydroxyprogesterone	Follicular	0.6–3 nmol/L (0.20–1 μ g/L)
	Luteal	3–15 nmol/L (100–500 ng/dL)
	Men	0.2–9 nmol/L (0.06–3 μ g/L)
Calcium	Plasma	2.2–2.6 mmol/L (9–10.5 mg/dL)
Phosphorus	Inorganic	1.0–1.4 mmol/L (3–4.5 mg/dL)
PTH		10–60 pg/mL (10–60 ng/L)
PTH-related protein		<1.3 pmol/L
Somatostatin		<25 pg/ml (<25 ng/L)
Vitamin D		40–160 pmol/L (16–65 pg/mL)
Calcitonin		3–26 ng/L (3–26 pg/mL)
Glucagon		20–100 ng/L (20–100 pg/mL)
Insulin	Overnight fast	14–144 pmol/L (2–20 μ U/mL)
Glucose	Overnight fast	4.2–6.4 mmol/L (75–115 mg/dL)
β -OH butyrate		<300 μ mol/L (<3 mg/dL)
Lactate	Venous plasma	0.6–2.2 mmol/L (5–15 mg/dL)
Fatty acids	Free	0.17–0.95 mmol/L (<18 mg/dL)
Androstenedione		1.75–8.73 nmol/L (5–

		25 ng/mL)
Estradiol	Women	70–220 pmol/L (20–60 pg/mL)
	Men	<180 pmol/L (50 pg/mL)
Progesterone	Women: luteal peak	>16 nmol/L (75 ng/mL)
	Men, prepubescent girls, postmenopausal women	<6 nmol/L (<2 ng/mL)
Testosterone	Women	<3.5 nmol/L (0.6–8.6 ng/mL)
	Men	10–35 nmol/L (3–10 ng/mL)
	Prepubertal boys & girls	0.17–0.7 nmol/L (0.05–0.2 ng/mL)
FSH	Women	
	Reproductive age	5–20 IU/L (5–20 mIU/mL)
	Ovulatory surge	9–26 IU/L (9–26 mIU/mL)
	Postmenopausal	18–153 IU/L (18–153 mIU/mL)
	Mature men	1–12 IU/L (1–12 mIU/mL)
LH	Women	
	Reproductive age	5–25 IU/L (5–25 mIU/mL)
	Ovulatory surge	25–100 IU/L (25–100 mIU/mL)
	Postmenopausal	>50 IU/L (>50 mIU/mL)
	Mature men	5–20 IU/L (5–20 mIU/mL)

β-hCG	Men and nonpregnant women	<3 IU/L
Oxytocin	Random	1–4 pmol/L (1.25–5 pg/mL)
	Ovulatory peak	4–8 pmol/L (5–10 ng/mL)
Prolactin		0–20 µg/L (0–20 ng/mL)
Growth hormone	Fasting	<5 µg/L (<5 ng/mL)
	Provocative stimuli	>7 µg/L (>7 µg/mL)
TSH		0.4–5 mU/L (0.4–5 µU/mL)
Total thyroxine (T ₄)		64–154 nmol/L (5–12 µg/dL)
Triiodothyronine (T ₃)		1.1–2.9 nmol/L (70–190 ng/dL)
Reverse T ₃		0.15–0.61 nmol/L (10–40 ng/dL)
Resin T ₃ uptake		25–35%

SI, International System of Units; ACTH, adrenocorticotrophic hormone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; PTH, parathyroid hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; hCG, human chorionic gonadotropin; TSH, thyroid-stimulating hormone.

*Conversions: mmol/L = (mg/dL × 10)/atomic weight; mg/dL = (mmol/L × atomic weight)/10

NORMAL VALUES OF METABOLIC PARAMETERS AND TESTS OF ENDOCRINE FUNCTION: URINARY LEVELS

Table 2. Normal Values of Metabolic Parameters and Tests of Endocrine Function: Urinary Levels.

Hormone or metabolite	Amount excreted
Aldosterone	14–53 nmol/d (5–19 µg/d)
Free cortisol	55–275 nmol/d (20–100 µg/d)
17-Hydroxycorticosteroids	5.5–28 µmol/d (2–10 mg/d)
17-Ketosteroids	Men: 24–88 µmol/d (7–25 mg/d) Women: 14–52 µmol/d (4–15 mg/d)
Free catecholamines	<590 nmol/d (<100 µg/d)
Epinephrine	<275 nmol/d (<50 µg/d)
Metanephrines	<7 µmol/d (<1.3 mg/d)
Vanillylmandelic acid	<40 µmol/d (<8 mg/d)