PART IX Endocrine Physiology

CHAPTER 31

Endocrine Control Mechanisms

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CHAPTER OUTLINE

- GENERAL CONCEPTS OF ENDOCRINE CONTROL
- THE NATURE OF HORMONES
- MECHANISMS OF HORMONE ACTION

KEY CONCEPTS

1. Hormones are chemical substances, involved in cell-to-cell communication, that promote the maintenance of homeostasis.
2. There are six classes of steroid hormones, based on their primary actions.
3. Most polypeptide hormones are initially synthesized as preprohormones.
4. Steroid hormones and thyroid hormones are generally transported in the bloodstream bound to carrier proteins, whereas most peptide and protein hormones are soluble in the plasma and are carried free in solution.
5. RIA and ELISA have provided major advancements in the field of endocrinology, but each type of assay has limitations.
6. Altered hormone-receptor interactions may lead to endocrine abnormalities.

Endocrinology is the branch of physiology concerned with the description and characterization of processes involved in the regulation and integration of cells and organ systems by a group of specialized chemical substances called hormones. The diagnosis and treatment of a large number of endocrine disorders is an important aspect of any general medical practice. Certain endocrine disease states, such as diabetes mellitus, thyroid disorders, and reproductive disorders, are fairly common in the general population; therefore, it is likely that they will be encountered repeatedly in the practice of medicine.

In addition, because hormones either directly or indirectly affect virtually every cell or tissue in the body, a number of other prominent diseases not primarily classified as endocrine diseases may have an important endocrine component. Atherosclerosis, certain forms of cancer, and even certain psychiatric disorders are examples of conditions in which an endocrine disturbance may contribute to the progression or severity of disease.

GENERAL CONCEPTS OF ENDOCRINE CONTROL

Hormones are bloodborne substances involved in regulating a variety of processes. The word "hormone" is derived from the Greek *hormaein,* which means to "excite" or to "stir up." The endocrine system forms an important communication system that serves to regulate, integrate, and coordinate a variety of different physiological processes. The processes that hormones regulate fall into four areas: (1) the digestion, utilization, and storage of nutrients; (2) growth and development; (3) ion and water balance; and (4) reproductive function.

Hormones Regulate and Coordinate Many Functions

It is difficult to describe hormones in absolute terms. As a working definition, however, it can be said that hormones serve as regulators and coordinators of various biological

functions in the animals in which they are produced. They are highly potent, specialized, organic molecules produced by endocrine cells in response to specific stimuli and exert their actions on specific target cells. These target cells are equipped with receptors that bind hormones with high affinity and specificity; when bound, they initiate characteristic biological responses by the target cells.

In the past, definitions or descriptions of hormones usually included a phrase indicating that these substances were secreted into the bloodstream and carried by the blood to a distant target tissue. Although many hormones travel by this mechanism, we now realize that there are many hormones or hormone-like substances that play important roles in cell-to-cell communication that are not secreted directly into the bloodstream. Instead, these substances reach their target cells by diffusion through the interstitial fluid. Recall the discussion of autocrine and paracrine mechanisms in Chapter 1.

Hormone Receptors Determine Whether a Cell Will Respond to a Hormone

In the endocrine system, a hormone molecule secreted into the blood is free to circulate and contact almost any cell in the body. However, only **target cells**, those cells that possess specific receptors for the hormone, will respond to that hormone. A **hormone receptor** is the molecular entity (usually a protein or glycoprotein) either outside or within a cell that recognizes and binds a particular hormone. When a hormone binds to its receptor, biological effects characteristic of that hormone are initiated. Therefore, in the endocrine system, the basis for specificity in cell-to-cell communication rests at the level of the receptor. Similar concepts apply to autocrine and paracrine mechanisms of communication.

A certain degree of specificity is ensured by the restricted distribution of some hormones. For example, several hormones produced by the hypothalamus regulate hormone secretion by the anterior pituitary. These hormones are carried via small blood vessels directly from the hypothalamus to the anterior pituitary, prior to entering the general systemic circulation. The anterior pituitary is, therefore, exposed to considerably higher concentrations of these hypothalamic hormones than the rest of the body; as a result, the actions of these hormones focus on cells of the anterior pituitary. Another mechanism that restricts the distribution of active hormone is the local transformation of a hormone within its target tissue from a less active to a more active form. An example is the formation of dihydrotestosterone from testosterone, occurring in such androgen target tissues as the prostate gland. Dihydrotestosterone is a much more potent androgen than testosterone. Because the enzyme that catalyzes this conversion is found only in certain locations, its cell or tissue distribution partly localizes the actions of the androgens to these sites. Therefore, while receptor distribution is the primary factor in determining the target tissues for a specific hormone, other factors may also focus the actions of a hormone on a particular tissue.

Feedback Regulation Is an Important Part of Endocrine Function

The endocrine system, like many other physiological systems, is regulated by feedback mechanisms. The mechanism is usually negative feedback, although a few positive feedback mechanisms are known. Both types of feedback control occur because the endocrine cell, in addition to synthesizing and secreting its own hormone product, has the ability to sense the biological consequences of secretion of that hormone. This enables the endocrine cell to adjust its rate of hormone secretion to produce the desired level of effect, ensuring the maintenance of homeostasis.

Hormone secretion may be regulated via simple first-order feedback loops or more complex multilevel second- or third-order feedback loops. Since negative feedback is most prevalent in the endocrine system, only examples of this type are illustrated here.

Simple Feedback Loops.

First-order feedback regulation is the simplest type and forms the basis for more complex modes of regulation. Figure 31.1A illustrates a simple first-order feedback loop. In this example, an endocrine cell secretes a hormone that produces a specific biological effect in its target tissue. It also senses the magnitude of the effect produced by the hormone. As the biological response increases, the amount of hormone secreted by the endocrine cell is appropriately decreased.

Complex Feedback Loops.

More commonly, feedback regulation in the endocrine system is complex, involving second- or third-order feedback loops. For example, multiple levels of feedback regulation may be involved in regulating hormone production by various endocrine glands under the control of the anterior pituitary (Fig. 31.1B). The regulation of target gland hormone secretion, such as adrenal steroids or thyroid hormones, begins with production of a releasing hormone by the hypothalamus. The releasing hormone stimulates production of a trophic hormone by the anterior pituitary, which, in turn, stimulates the production of the target gland hormone by the target gland. As indicated by the dashed lines in Figure 31.1B, the target gland hormone may have negative-feedback effects to inhibit secretion of both the trophic hormone from the anterior pituitary and the releasing hormone from the hypothalamus. In addition, the trophic hormone may inhibit releasing hormone secretion from the hypothalamus, and in some cases, the releasing hormone may inhibit its own secretion by the hypothalamus.

The more complex multilevel form of regulation appears to provide certain advantages compared with the simpler system. Theoretically, it permits a greater degree of fine-tuning of hormone secretion, and the multiplicity of regulatory steps minimizes changes in hormone secretion in the event that one component of the system is not functioning normally.

It is important to bear in mind the normal feedback relationships that control the secretion of each individual hormone are discussed in the chapters that follow. Clinical diagnoses are often made based on the evaluation of

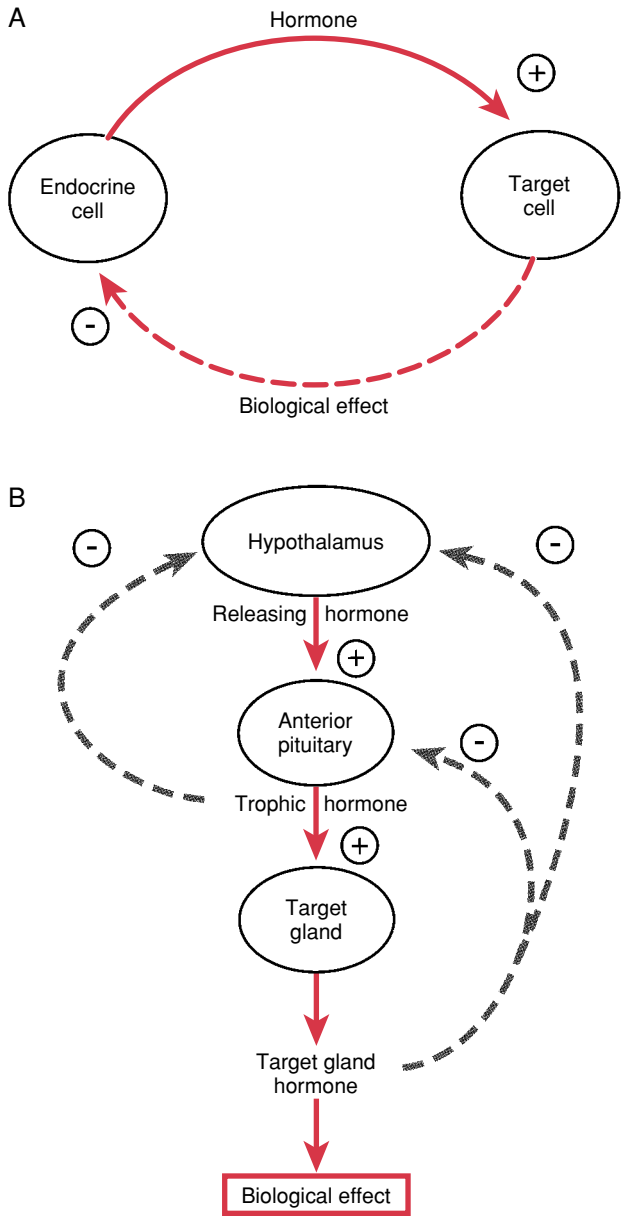

FIGURE 31.1 **Simple and complex feedback loops in the endocrine system.** **A**, A simple first-order feedback loop. **B**, A complex, multilevel feedback loop: the hypothalamic-pituitary-target gland axis. Solid lines indicate stimulatory effects; dashed lines indicate inhibitory, negative-feedback effects.

hormone-effector pairs relative to normal feedback relationships. For example, in the case of anterior pituitary hormones, measuring both the trophic hormone and the target gland hormone concentration provides important information to help determine whether a defect in hormone production exists at the level of the pituitary or at the level of the target gland. Furthermore, most dynamic tests of endocrine function performed clinically are based on our knowledge of these feedback relationships. Dynamic tests involve a prescribed perturbation of the feedback relationship(s); the range of response in a normal individual is well established, while a response outside the normal range is indicative of abnormal function at some level and greatly enhances information gained from static measurements of hormone concentrations (see Clinical Focus Box 31.1).

Signal Amplification Is an Important Characteristic of the Endocrine System

Another important feature of the endocrine system is **signal amplification**. Blood concentrations of hormones are exceedingly low, generally, 10^{-9} to 10^{-12} mol/L. Even at the higher concentration of 10^{-9} mol/L, only one hormone molecule would be present for roughly every 50 billion water molecules. Therefore, for hormones to be effective regulators of biological processes, amplification must be part of the overall mechanism of hormone action.

Amplification generally results from the activation of a series of enzymatic steps involved in hormone action. At each step, many times more signal molecules are generated than were present at the prior step, leading to a cascade of ever-increasing numbers of signal molecules. The self-multiplying nature of the hormone action pathways provides the molecular basis for amplification in the endocrine system.

Pleiotropic Hormone Effects and Multiplicity of Regulation Also Characterize the Endocrine System

Most hormones have multiple actions in their target tissues and are, therefore, said to have **pleiotropic effects**. For example, insulin exhibits pleiotropic effects in skeletal muscle, where it stimulates glucose uptake, stimulates glycolysis, stimulates glycogenesis, inhibits glycogenolysis, stimulates amino acid uptake, stimulates protein synthesis, and inhibits protein degradation.

In addition, some hormones are known to have different effects in several different target tissues. For example, testosterone, the male sex steroid, promotes normal sperm formation in the testes, stimulates growth of the accessory sex glands, such as the prostate and seminal vesicles, and promotes the development of several secondary sex characteristics, such as beard growth and deepening of the voice.

Multiplicity of regulation is also common in the endocrine system. The input of information from several sources allows a highly integrated response to a variety of stimuli, which is of ultimate benefit to the whole animal. For example, liver glycogen metabolism may be regulated or influenced by several different hormones, including insulin, glucagon, epinephrine, thyroid hormones, and adrenal glucocorticoids.

Hormones Are Often Secreted in Definable Patterns

The secretion of any particular hormone is either stimulated or inhibited by a defined set of chemical substances in

CLINICAL FOCUS BOX 31.1

Growth Hormone and Pulsatile Hormone Secretion

Growth hormone is a 191-amino acid protein hormone that is synthesized and secreted by somatotrophs of the anterior lobe of the pituitary gland. As described in Chapter 32, the hormone plays a role in regulating bone growth and energy metabolism in skeletal muscle and adipose tissue. A deficiency in growth hormone production during adolescence results in dwarfism and overproduction results in gigantism. Measurements of circulating growth hormone levels are, therefore, desirable in children whose growth rate is not appropriate for their age.

Like many other peptide hormones, growth hormone secretion occurs in a pulsatile fashion. The most consistent pulse occurs just after the onset of deep sleep and lasts for about 1 hour. There are usually 4 to 6 irregularly timed pulses throughout the remainder of the day. In order to obtain reliable information about growth hormone secretion, endocrinologists employ a dynamic test of growth hormone secretory capacity. There are several variations of this test that are used at different hospitals. In one test, a bolus of arginine, which is known to stimulate growth hormone secretion, is given and a blood sample is taken a short time later for the measurement of growth hormone concentrations. Another test makes use of the fact that hypoglycemia is a known stimulus for growth hormone secretion. Mild hypoglycemia is induced by an injection of insulin, and a blood sample is drawn a short time later. Regardless of which test is used, by perturbing the system in a well-prescribed fashion, the endocrinologist is able to gain important information about growth hormone secretion that would not be possible if a random blood sample were used.

the blood or environmental factors. In addition to these specific **secretagogues**, many hormones are secreted in a defined, rhythmic pattern. These rhythms can take several forms. For example, they may be pulsatile, episodic spikes in secretion lasting just a few minutes, or they may follow a daily, monthly, or seasonal change in overall pattern. Pulsatile secretion may occur in addition to other longer secretory patterns.

For these reasons, a single randomly drawn blood sample for determining a certain hormone concentration may be of little or no diagnostic value. A dynamic test of endocrine function in which hormone secretion is specifically stimulated by a known agent often provides much more meaningful information.

THE NATURE OF HORMONES

Hormones can be categorized by a number of criteria. Grouping them by chemical structure is convenient, since in many cases, hormones with similar structures also use similar mechanisms to produce their biological effects. In addition, hormones with similar chemical structures are usually produced by tissues with similar embryonic origins. Hormones can generally be classed as one of three chemical types.

The Simplest Hormones, in Terms of Structure, Consist of One or Two Modified Amino Acids

Hormones derived from one or two amino acids are small in size and often hydrophilic. These hormones are formed by conversion from a commonly occurring amino acid; epinephrine and thyroxine, for example, are derived from tyrosine. Each of these hormones is synthesized by a particular sequence of enzymes that are primarily localized in the endocrine gland involved in its production. The synthesis of amino acid-derived hormones can, therefore, be influenced in a relatively specific fashion by a variety of environmental or pharmacological agents. The steps involved in the synthesis of these hormones are discussed in detail in later chapters.

Many Hormones Are Polypeptides

Hormones in the polypeptide group are quite diverse in size and complexity. They may be as small as the tripeptide thyrotropin-releasing hormone (TRH) or as large as human chorionic gonadotropin (hCG), which is composed of separate alpha and beta subunits, has a molecular weight of approximately 34 kDa, and is a glycoprotein comprised of 16% carbohydrate by weight.

Within the polypeptide class of hormones are a number of families of hormones, some of which are listed in Table 31.1. Hormones can be grouped into these families as a result of considerable homology with regard to amino acid sequence and structure. Presumably, the similarity of struc-

TABLE 31.1 Examples of Peptide Hormone Families

Insulin Family
Insulin
Insulin-like growth factor I
Insulin-like growth factor II
Relaxin
Glycoprotein Family
Luteinizing hormone (LH)
Follicle-stimulating hormone (FSH)
Thyroid-stimulating hormone (TSH)
Human chorionic gonadotropin (hCG)
Growth Hormone Family
Growth hormone (GH)
Prolactin (PRL)
Human placental lactogen (hPL)
Secretin Family
Secretin
Vasoactive intestinal peptide (VIP)
Glucagon
Gastric inhibitory peptide (GIP)

ture in these families resulted from the evolution of a single ancestral hormone into each of the separate and distinct hormones. In many cases, there is also considerable homology among receptors for the hormones within a family.

Steroid Hormones Are Derived From Cholesterol

Steroids are lipid-soluble, hydrophobic molecules synthesized from cholesterol. They can be classified into six categories, based on their primary biological activity. An example of each category is shown in Figure 31.2.

Glucocorticoids, such as cortisol, are primarily produced in cells of the adrenal cortex and regulate processes involved in glucose, protein, and lipid homeostasis. Glucocorticoids generally produce effects that are catabolic in nature. Aldosterone, a primary example of a **mineralocorticoid**, is produced in cells of the outermost portion of the adrenal cortex. Aldosterone is primarily involved in regulating sodium and potassium balance by the kidneys and is the principal mineralocorticoid in the body.

Androgens, such as testosterone, are primarily produced in the testes, but physiologically significant amounts can be synthesized by the adrenal cortex as well. The primary female sex hormone is estradiol, a member of the **estrogen** family, produced by the ovaries and placenta. **Progestins**, such as progesterone, are involved in maintenance of pregnancy and are produced by the ovaries and placenta.

The **calciferols**, such as 1,25-dihydroxycholecalciferol, are involved in the regulation of calcium homeostasis. 1,25-dihydroxycholecalciferol is the hormonally active form of vitamin D and is formed by a sequence of reactions occurring in skin, liver, and kidneys.

Polypeptide and Protein Hormones Are Synthesized in Advance of Need and Stored in Secretory Vesicles

Steroid hormones are synthesized and secreted on demand, but polypeptide hormones are typically stored prior to secretion. Steroid hormone synthesis and secretion are dis-

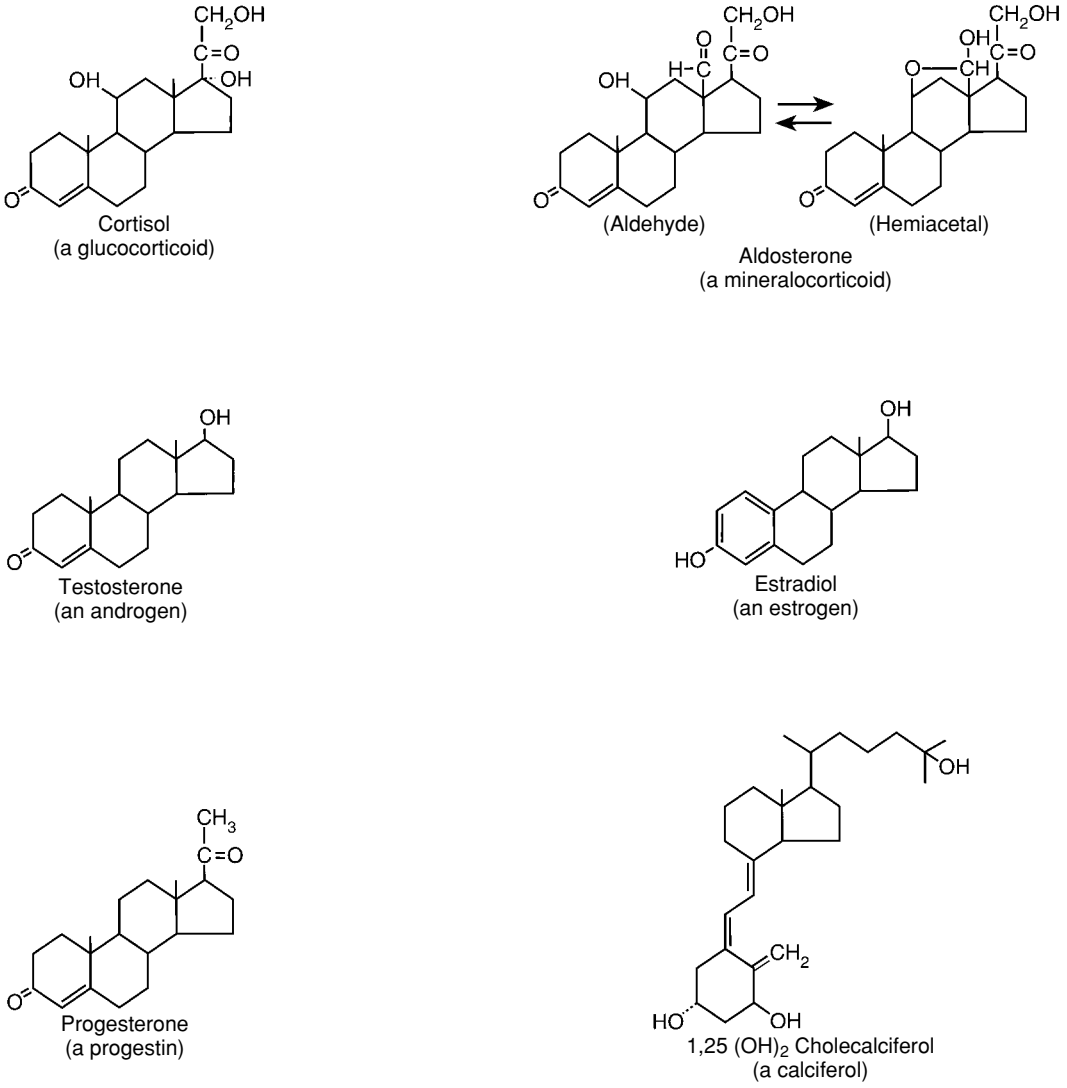

FIGURE 31.2 Examples of the six types of naturally occurring steroids.

cussed in Chapter 34; the discussion here is confined to the synthesis and secretion of polypeptide hormones.

Preprohormones and Prohormones. Like other proteins destined for secretion, polypeptide hormones are synthesized with a *pre-* or *signal* peptide at their amino terminal end that directs the growing peptide chain into the cisternae of the rough ER. Most, if not all, polypeptide hormones are synthesized as part of an even larger precursor or **preprohormone**. The prepeptide is cleaved off upon entry of the preprohormone into the rough ER, to form the **prohormone**. As the prohormone is processed through the Golgi apparatus and packaged into secretory vesicles, it is proteolytically cleaved at one or more sites to yield active hormone. In many cases, preprohormones may contain the sequences for several different biologically active molecules. These active elements may, in some cases, be separated by inactive spacer segments of peptide.

Examples of prohormones that are the precursors for polypeptide hormones, which illustrate the multipotent nature of these precursors, are shown schematically in Figure 31.3. Note, for example, that proopiomelanocortin (POMC) actually contains the sequences for several biologically active signal molecules. Propressophysin serves as the precursor for the nonapeptide hormone arginine vasopressin (AVP). The precursor for TRH contains five repeats of the TRH tripeptide in one single precursor molecule.

In general, two basic amino acid residues, either lys-arg or arg-arg, demarcate the point(s) at which the prohormone will be cleaved into its biologically active components. Presumably, these two basic amino acids serve as specific recognition sites for the trypsin-like endopeptidases thought to be responsible for cleavage of the prohormones. Although somewhat rare, there are documented cases of inherited diseases in which a point mutation involving an amino acid residue at the cleavage site results in an inability to convert the prohormone into active hormone, resulting in a state of hormone deficiency. Partially cleaved precursor molecules having limited biological activity may be found circulating in the blood in some of these cases.

In some disease states, large amounts of intact precursor molecules are found in the circulation. This situation may be the result of endocrine cell hyperactivity or even uncontrolled production of hormone precursor by nonendocrine tumor cells. Although precursors usually have relatively low biological activity, if they are secreted in sufficiently high amounts, they may still produce biological effects. In some cases, these effects may be the first recognized sign of neoplasia.

Tissue-specific differences in the processing of prohormones are well known. Although the same prohormone gene may be expressed in different tissues, tissue-specific differences in the way the molecule is cleaved give rise to different final secretory products. For example, within alpha cells of the pancreas, proglucagon is cleaved at two positions to yield three peptides, illustrated in Figure 31.4 (left). Glucagon, an important hormone in the regulation of carbohydrate metabolism, is the best characterized of the three peptides. In contrast, in other cells of the gastrointestinal (GI) tract in which proglucagon is also produced, the molecule is cleaved at three different positions such that glicentin, glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2) are produced (Fig. 31.4, right).

Intracellular Movement of Secretory Vesicles and Exocytosis. Upon insertion of the preprohormone into the cisternae of the ER, the prepeptide or signal peptide is rapidly cleaved from the amino terminal end of the molecule. The resulting prohormone is translocated to the Golgi appara-

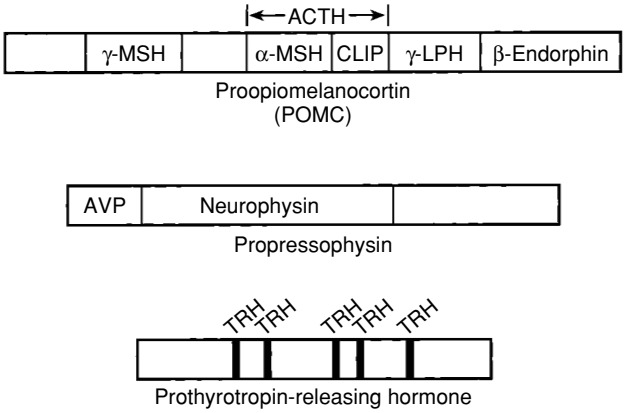

FIGURE 31.3 **The structure of three prohormones.** Relative sizes of individual peptides are only approximations. MSH = melanocyte-stimulating hormone; CLIP = corticotropin-like intermediate lobe peptide; LPH = lipotropin; AVP = arginine vasopressin; TRH = thyrotropin-releasing hormone.

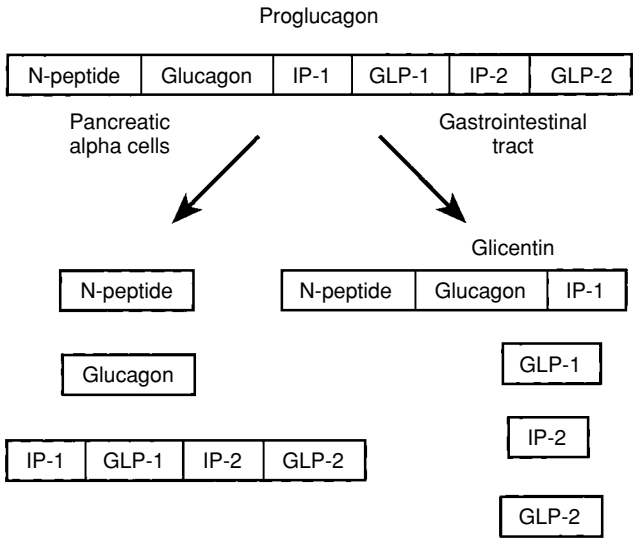

FIGURE 31.4 **The differential processing of prohormones.** In alpha cells of the pancreas (left), the major bioactive product formed from proglucagon is glucagon itself. It is not currently known whether the other peptides are processed to produce biologically active molecules. In intestinal cells (right), proglucagon is cleaved to produce the four peptides shown. Glicentin is the major glucagon-containing peptide in the intestine. IP-1, intervening peptide 1; IP-2, intervening peptide 2; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide 2.

CLINICAL FOCUS BOX 31.2

Pancreatic Beta Cell Function and C-Peptide

Beta cells of the human pancreas produce and secrete **insulin**. The product of the insulin gene is a peptide known as preproinsulin. As with other secretory peptides, the prepeptide or signal peptide is cleaved off early in the biosynthetic process, yielding proinsulin. Proinsulin is an 86-amino acid protein that is subsequently cleaved at two sites to yield insulin and a 31-amino acid peptide known as **C-peptide**. Insulin and C-peptide are, therefore, localized within the same secretory vesicle and are co-secreted into the bloodstream.

For these reasons, measurements of circulating C-peptide levels can provide a valuable indirect assessment of beta cell insulin secretory capacity. In diabetic patients who are receiving exogenous insulin injections, the measurement of circulating insulin levels would not provide any useful information about their own pancreatic function because it would primarily be the injected insulin that would be measured. However, an evaluation of C-peptide levels in such patients would provide an indirect measure of how well the beta cells were functioning with regard to insulin production and secretion.

tus, where it is processed and packaged for export. After processing in the Golgi apparatus, peptide hormones are stored in membrane-enclosed secretory vesicles. Secretion of the peptide hormone occurs by exocytosis; the secretory vesicle is translocated to the cell surface, its membrane fuses with the plasma membrane, and its contents are released into the extracellular fluid. Movement of the secretory vesicle and membrane fusion are triggered by an increase in cytosolic calcium stemming from an influx of calcium into the cytoplasm from internal organelles or the extracellular fluid. In some cells, an increase in cAMP and the subsequent activation of protein kinases is also involved in the stimulus-secretion coupling process. Elements of the microtubule-microfilament system play a role in the movement of secretory vesicles from their intracellular storage sites toward the cell membrane.

The cleavage of prohormone into active hormone molecules typically takes place during transit through the Golgi apparatus or, perhaps, soon after entry into secretory vesicles. Secretory vesicles, therefore, contain not only active hormone but also the excised biologically inactive fragments. When active hormone is released into the blood, a quantitatively similar amount of inactive fragment is also released. In some instances, this forms the basis for an indirect assessment of hormone secretory activity (see Clinical Focus Box 31.2). Other types of processing of peptide hormones that may occur during transit through the Golgi apparatus include glycosylation and coupling of subunits.

Many Hormones Reach Their Target Cells by Transport in the Bloodstream

According to the classical definition, hormones are carried by the bloodstream from their site of synthesis to their target tissues. However, the manner in which different hormones are carried in the blood varies.

Transport of Amino Acid-Derived and Polypeptide Hormones. Most amino acid-derived and polypeptide hormones dissolve readily in the plasma, and thus no special mechanisms are required for their transport. Steroid and thyroid hormones are relatively insoluble in plasma. Mechanisms are present to promote their solubility in the aqueous phase of the blood and ultimate delivery to a target cell.

Transport of Steroid and Thyroid Hormones. In most cases, 90% or more of steroid and thyroid hormones in the blood are bound to plasma proteins. Some of the plasma proteins that bind hormones are specialized, in that they have a considerably higher affinity for one hormone over another, whereas others, such as serum albumin, bind a variety of hydrophobic hormones. The extent to which a hormone is protein-bound and the extent to which it binds to specific versus nonspecific transport proteins vary from one hormone to another. The principal binding proteins involved in specific and nonspecific transport of steroid and thyroid hormones are listed in Table 31.2. These proteins are synthesized and secreted by the liver, and their production is influenced by changes in various nutritional and endocrine factors.

Typically, for hormones that bind to carrier proteins, only 1 to 10% of the total hormone present in the plasma exists free in solution. However, only this free hormone is biologically active. Bound hormone cannot directly interact with its receptor and, thus, is part of a temporarily inactive pool. However, free hormone and carrier-bound hormone are in a dynamic equilibrium with each other (Fig. 31.5). The size of the free hormone pool and, therefore, the amount available to receptors are influenced not only by changes in the rate of secretion of the hormone but also by the amount of carrier protein available for hormone binding and the rate of degradation or removal of the hormone from the plasma.

TABLE 31.2 Circulating Transport Proteins

Transport Protein	Principal Hormone(s) Transported
Specific	
Corticosteroid-binding globulin (CBG, transcortin)	Cortisol, aldosterone
Thyroxine-binding globulin (TBG)	Thyroxine, triiodothyronine
Sex hormone-binding globulin (SHBG)	Testosterone, estrogen
Nonspecific	
Serum albumin	Most steroids, thyroxine, triiodothyronine
Transthyretin (prealbumin)	Thyroxine, some steroids

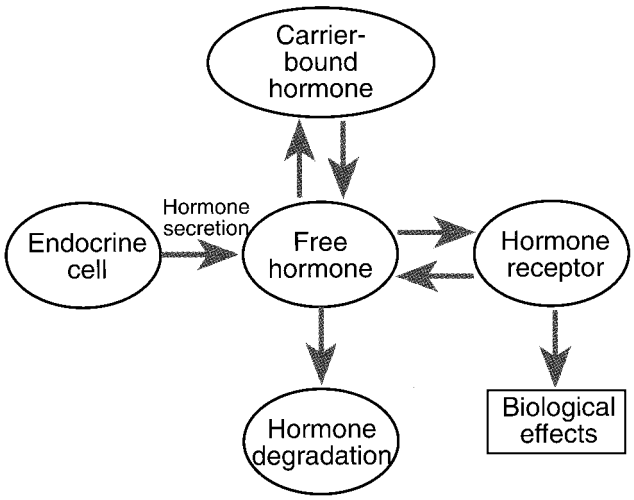

FIGURE 31.5 The relationship between hormone secretion, carrier protein binding, and hormone degradation. This relationship determines the amount of free hormone available for receptor binding and the production of biological effects.

In addition to increasing the total amount of hormone that can be carried in plasma, transport proteins also provide a relatively large reservoir of hormone that buffers rapid changes in free hormone concentrations. As unbound hormone leaves the circulation and enters cells, additional hormone dissociates from transport proteins and replaces free hormone that is lost from the free pool. Similarly, following a rapid increase in hormone secretion or the therapeutic administration of a large dose of hormone, the majority of newly appearing hormone is bound to transport proteins, since under most conditions these are present in considerable excess.

Protein binding greatly slows the rate of clearance of hormones from plasma. It not only slows the entry of hormones into cells, slowing the rate of hormone degradation, but also prevents loss by filtration in the kidneys.

From a diagnostic standpoint, it is important to recognize that most hormone assays are reported in terms of total concentration (i.e., the sum of free and bound hormone), not just free hormone concentration. The amount of transport protein and the total plasma hormone content are known to change under certain physiological or pathological conditions, while the free hormone concentration may remain relatively normal. For example, increased concentrations of binding proteins are seen during pregnancy and decreased concentrations are seen with certain forms of liver or kidney disease. Assays of total hormone content might be misleading, since free hormone concentrations may be in the normal range. In such cases, it is helpful to determine the extent of protein binding, so free hormone concentrations can be estimated.

The proportion of a hormone that is free, bound to a specific transport protein, and bound to albumin varies depending on its solubility, its relative affinity for the two classes of transport proteins, and the relative abundance of the transport proteins. For example, the affinity of cortisol for corticosteroid-binding globulin (CBG) is more than 1,000 times greater than its affinity for albumin, but albumin is present in much higher concentrations than CBG. Therefore, about 70% of plasma cortisol is bound to CBG, 20% is bound to albumin, and the remaining 10% is free in solution. Aldosterone also binds to CBG, but with a much lower affinity, such that only 17% is bound to CBG, 47% associates with albumin, and 36% is free in solution.

As this example indicates, more than one hormone may be capable of binding to a specific transport protein. When several such hormones are present simultaneously, they compete for a limited number of binding sites on these transport proteins. For example, cortisol and aldosterone compete for CBG binding sites. Increases in plasma cortisol result in displacement of aldosterone from CBG, raising the unbound (active) concentration of aldosterone in the plasma. Similarly, prednisone, a widely used synthetic corticosteroid, can displace about 35% of the cortisol normally bound to CBG. As a result, with prednisone treatment, the free cortisol concentration is higher than might be predicted from measured concentrations of total cortisol and CBG.

Peripheral Transformation, Degradation, and Excretion of Hormones, in Part, Determine Their Activity

As a general rule, hormones are produced by their gland or tissue of origin in an active form. However, for a few notable exceptions, the peripheral transformation of a hormone plays a very important role in its action.

Peripheral Transformation of Hormones. Specific hormone transformations may be impaired because of a congenital enzyme deficiency or drug-induced inhibition of enzyme activity, resulting in endocrine abnormalities. Well-known transformations are the conversion of testosterone to dihydrotestosterone (see Chapter 37) and the conversion of thyroxine to triiodothyronine (see Chapter 33). Other examples are the formation of the octapeptide angiotensin II from its precursor, angiotensinogen (see Chapter 34), and the formation of 1,25-dihydroxycholecalciferol from cholecalciferol (see Chapter 36).

Mechanisms of Hormone Degradation and Excretion. As in any regulatory control system, it is necessary for the hormonal signal to dissipate or disappear once appropriate information has been transferred and the need for further stimulus has ceased. As described earlier, steady-state plasma concentrations of hormone are determined not only by the rate of secretion but also by the rate of degradation. Thus, any factor that significantly alters the degradation of a hormone can potentially alter its circulating concentration. Commonly, however, secretory mechanisms can compensate for altered degradation such that plasma hormone concentrations remain within the normal range. Processes of hormone degradation show little, if any, regulation; alterations in the rates of hormone synthesis or secretion in most cases provide the primary mechanism for altering circulating hormone concentrations.

For most hormones, the liver is quantitatively the most important site of degradation; for a few others, the kidneys play a significant role as well. Diseases of the liver and kid-

neys may, therefore, indirectly influence endocrine status as a result of altering the rates at which hormones are removed from the circulation. Various drugs also alter normal rates of hormone degradation; thus, the possibility of indirect drug-induced endocrine abnormalities also exists. In addition to the liver and kidneys, target tissues may take up and degrade quantitatively smaller amounts of hormone. In the case of peptide and protein hormones, this occurs via receptor-mediated endocytosis.

The nature of specific structural modification(s) involved in hormone inactivation and degradation differs for each hormone class. As a general rule, however, specific enzyme-catalyzed reactions are involved. Inactivation and degradation may involve complete metabolism of the hormone to entirely different products, or it may be limited to a simpler process involving one or two steps, such as a covalent modification to inactivate the hormone. Urine is the primary route of excretion of hormone degradation products, but small amounts of intact hormone may also appear in the urine. In some cases, measuring the urinary content of a hormone or hormone metabolite provides a useful, indirect, noninvasive means of assessing endocrine function.

The degradation of peptide and protein hormones has been studied only in a limited number of cases. However, it appears that peptide and protein hormones are inactivated in a variety of tissues by proteolytic attack. The first step appears to involve attack by specific peptidases, resulting in the formation of several distinct hormone fragments. These fragments are then metabolized by a variety of nonspecific peptidases to yield the constituent amino acids, which can be reused.

The metabolism and degradation of steroid hormones has been studied in much more detail. The primary organ involved is the liver, although some metabolism also takes place in the kidneys. Complete steroid metabolism generally involves a combination of one or more of five general classes of reactions: reduction, hydroxylation, side chain cleavage, oxidation, and esterification. Reduction reactions are the principal reactions involved in the conversion of biologically active steroids to forms that possess little or no activity. Esterification (or conjugation) reactions are also particularly important. Groups added in esterification reactions are primarily glucuronate and sulfate. The addition of such charged moieties enhances the water solubility of the metabolites, facilitating their excretion. Steroid metabolites are eliminated from the body primarily via the urine, although smaller amounts also enter the bile and leave the body in the feces.

At times, quantitative information concerning the rate of hormone metabolism is clinically useful. One index of the rate at which a hormone is removed from the blood is the **metabolic clearance rate** (MCR). The metabolic clearance of a hormone is analogous to that of renal clearance (see Chapter 23). The MCR is the volume of plasma cleared of the hormone in question per unit time. It is calculated from the equation:

$$\text{MCR} = \frac{\text{Hormone removed per unit time (mg/min)}}{\text{Plasma concentration (mg/mL)}} \quad (1)$$

and is expressed in mL plasma/min.

One approach to measuring MCR involves injecting a small amount of radioactive hormone into the subject and then collecting a series of timed blood samples to determine the amount of radioactive hormone remaining. Based on the rate of disappearance of hormone from the blood, its half-life and MCR can be calculated. The MCR and half-life are inversely related—the shorter the half-life, the greater the MCR. The half-lives of different hormones vary considerably, from 5 minutes or less for some to several hours for others. The circulating concentration of hormones with short half-lives can vary dramatically over a short period of time. This is typical of hormones that regulate processes on an acute minute-to-minute basis, such as a number of those involved in regulating blood glucose. Hormones for which rapid changes in concentration are not required, such as those with seasonal variations and those that regulate the menstrual cycle, typically have longer half-lives.

The Measurement of Hormone Concentrations Is an Important Tool in Endocrinology

The concentration of hormone present in a biological fluid is often measured to make a clinical diagnosis of a suspected endocrine disease or to study basic endocrine physiology. Substantial advancements have been made in measuring hormone concentrations.

Bioassay.

Even before hormones were chemically characterized, they were quantitated in terms of biological responses they produced. Thus, early assays for measuring hormones were bioassays that depended on a hormone's ability to produce a characteristic biological response. As a result, hormones came to be quantitated in terms of units, defined as an amount sufficient to produce a response of specified magnitude under a defined set of conditions. A unit of hormone is, thus, arbitrarily determined. Although bioassays are rarely used today for diagnostic purposes, many hormones are still standardized in terms of **biological activity units**. For example, commercial insulin is still sold and dispensed based on the number of units in a particular preparation, rather than by the weight or the number of moles of insulin.

Bioassays in general suffer from a number of shortcomings, including a relative lack of specificity and a lack of sensitivity. In many cases, they are slow and cumbersome to perform, and often they are expensive, since biological variability often requires the inclusion of many animals in the assay.

Radioimmunoassay.

Development of the **radioimmunoassay** (RIA) in the late 1950s and early 1960s was a major step forward in clinical and research endocrinology. Much of our current knowledge of endocrinology is based on this method. A RIA or closely related assay is now available for virtually every known hormone. In addition, RIAs have been developed to measure circulating concentrations of a variety of other biologically relevant proteins, drugs, and vitamins.

The RIA is a prototype for a larger group of assays termed **competitive binding assays**. These are modifica-

tions and adaptations of the original RIA, relying to a large degree on the principle of competitive binding on which the RIA is based. It is beyond the scope of this text to describe in detail the competitive binding assays currently used to measure hormone concentrations, but the principles are the same as those for the RIA.

The two key components of a RIA are a specific antibody (Ab) that has been raised against the hormone in question and a radioactively labeled hormone (H*). If the hormone being measured is a peptide or protein, the molecule is commonly labeled with a radioactive iodine atom (^{125}I or ^{131}I) that can be readily attached to tyrosine residues of the peptide chain. For substances lacking tyrosine residues, such as steroids, labeling may be accomplished by incorporating radioactive carbon (^{14}C) or hydrogen (^{3}H). In either case, the use of the radioactive hormone permits detection and quantification of very small amounts of the substance.

The RIA is performed *in vitro* using a series of test tubes. Fixed amounts of Ab and of H* are added to all tubes (Fig. 31.6A). Samples (plasma, urine, cerebrospinal fluid, etc.) to be measured are added to individual tubes. Varying known concentrations of unlabeled hormone (the standards) are added to a series of identical tubes. The principle of the RIA, as indicated in Figure 31.6B, is that labeled and unlabeled hormone compete for a limited number of antibody binding sites. The amount of each hormone that is bound to antibody is a proportion of that present in solution. In a sample containing a high concentration of hormone, less radioactive hormone will be able to bind to the antibody, and less antibody will be able to bind to the radioactive hormone. In each case, the amount of radioactivity present as antibody-bound H* is determined. The response produced by the standards is used to generate a **standard curve** (Fig. 31.7). Responses produced by the unknown samples are then compared to the standard curve to determine the amount of hormone present in the unknowns (see dashed lines in Fig. 31.7).

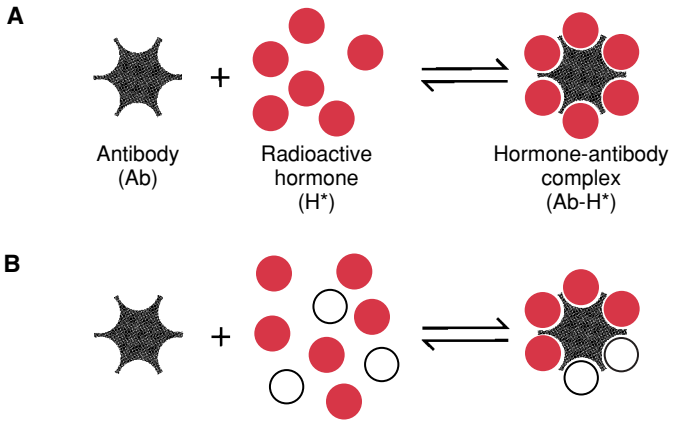

FIGURE 31.6 **The principles of radioimmunoassay (RIA).** **A,** Specific antibodies (Ab) bind with radioactive hormone (H*) to form hormone-antibody complexes (Ab-H*). **B,** When unlabeled hormone (open circles) is also introduced into the system, less radioactive hormone binds to the antibody. (Modified from Hedge GA, Colby HD, Goodman RL. Clinical Endocrine Physiology. Philadelphia: WB Saunders, 1987.)

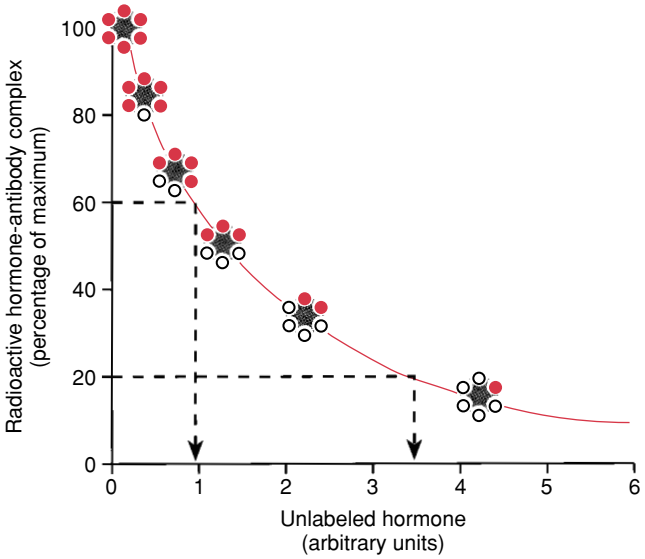

FIGURE 31.7 **A typical RIA standard curve.** As indicated by the dashed lines, the hormone content in unknown samples can be deduced from the standard curve. (Modified from Hedge GA, Colby HD, Goodman RL. Clinical Endocrine Physiology. Philadelphia: WB Saunders, 1987.)

One major limitation of RIAs is that they measure immunoreactivity, rather than biological activity. The presence of an immunologically related but different hormone or of heterogeneous forms of the same hormone can complicate the interpretation of the results. For example, POMC, the precursor of ACTH, is often present in high concentrations in the plasma of patients with bronchogenic carcinoma. Antibodies for ACTH may cross-react with POMC. The results of a RIA for ACTH in which such an antibody is used may suggest high concentrations of ACTH, when actually POMC is being detected. Because POMC has less than 5% of the biological potency of ACTH, there may be little clinical evidence of significantly elevated ACTH. If appropriate measures are taken, however, such possible pitfalls can be overcome in most cases, and reliable results from the RIA can be obtained.

One important modification of the RIA is the **radioreceptor assay**, which uses specific hormone receptors rather than antibodies as the hormone-binding reagent. In theory, this method measures biologically active hormone, since receptor binding rather than antibody recognition is assessed. However, the need to purify hormone receptors and the somewhat more complex nature of this assay limit its usefulness for routine clinical measurements. It is more likely to be used in a research setting.

ELISA. The **enzyme-linked immunosorbent assay** (ELISA) is a solid-phase, enzyme-based assay whose use and application have increased considerably over the past two decades. A typical ELISA is a colorimetric or fluorometric assay, and therefore, the ELISA, unlike the RIA, does not produce radioactive waste, which is an advantage, considering environmental concerns and the rapidly increasing cost of radioactive waste disposal. In addition, because it is

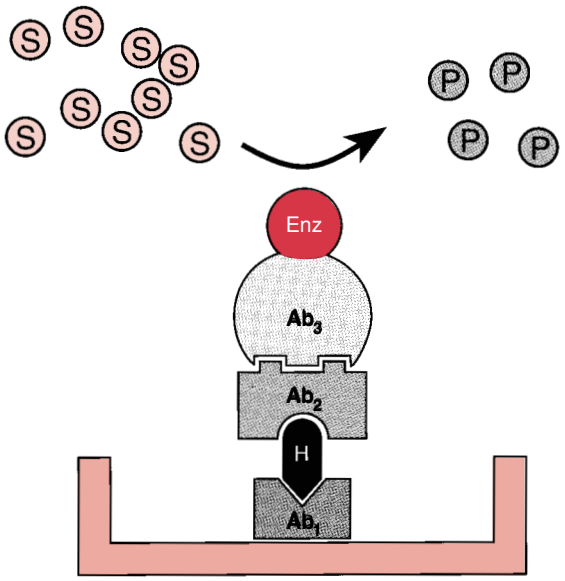

FIGURE 31.8 **The basic components of an ELISA.** A typical ELISA is performed in a 3 × 5-inch plastic plate containing 96 small wells. Each well is precoated with an antibody (Ab_1) that is specific for the hormone (H) being measured. Unknown samples or standards are introduced into the wells, followed by a second hormone-specific antibody (Ab_2). A third antibody (Ab_3), which recognizes Ab_2, is then added. Ab_3 is coupled to an enzyme that will convert an appropriate substrate (S) into a colored or fluorescent product (P). The amount of product formed can be determined using optical methods. After the addition of each antibody or sample to the wells, the plates are incubated for an appropriate period of time to allow antibodies and hormones to bind. Any unbound material is washed out of the well before the addition of the next reagent. The amount of colored product formed is directly proportional to the amount of hormone present in the standard or unknown sample. Concentrations are determined using a standard curve. For simplicity, only one Ab_1 molecule is shown in the bottom of the well, when, in fact, there is an excess of Ab_1 relative to the amount of hormone to be measured.

a solid-phase assay, the ELISA can be automated to a large degree, which reduces costs. Figure 31.8 shows a relatively simple version of an ELISA. More complex assays using similar principles have been developed to overcome a variety of technical problems, but the basic principle remains the same. In recent years the RIA has been the primary assay used clinically; its use has expanded considerably, and it will likely be the predominant assay in the future because of the advantages listed above.

MECHANISMS OF HORMONE ACTION

As indicated earlier, hormones are one mechanism by which cells communicate with one another. Fidelity of communication in the endocrine system depends on each hormone's ability to interact with a specific receptor in its target tissues. This interaction results in the activation (or inhibition) of a series of specific events in cells that results in precise biological responses characteristic of that hormone.

The binding of a hormone to its receptor with subsequent activation of the receptor is the first step in hormone action and also the point at which specificity is determined within the endocrine system. Abnormal interactions of hormones with their receptors are involved in the pathogenesis of a number of endocrine disease states, and therefore, considerable attention has been paid to this aspect of hormone action.

The Kinetics of Hormone-Receptor Binding Determines, in Part, the Biological Response

The probability that a hormone-receptor interaction will occur is related to both the abundance of cellular receptors and the receptor's affinity for the hormone relative to the ambient hormone concentration. The more receptors available to interact with a given amount of hormone, the greater the likelihood of a response. Similarly, the higher the affinity of a receptor for the hormone, the greater the likelihood that an interaction will occur. The circulating hormone concentration is, of course, a function of the rate of hormone secretion relative to hormone degradation.

The association of a hormone with its receptor generally behaves as if it were a simple, reversible chemical reaction that can be described by the following kinetic equation:

$$[H] + [R] \rightleftharpoons [HR] \quad (2)$$

where [H] is the free hormone concentration, [R] is the unoccupied receptor concentration, and [HR] is the **hormone-receptor complex** (also referred to as bound hormone or occupied receptor).

Assuming a simple chemical equilibrium, it follows that

$$K_a = [HR]/[H] \times [R] \quad (3)$$

where K_a is the association constant. If R_0 is defined as the total receptor number (i.e., [R] + [HR]), then after substituting and rearranging, we obtain the following relationship:

$$[HR]/[H] = -K_a[HR] + K_aR_0 \quad (4)$$

Literally translated, this equation states:

$$\text{Bound hormone} = -K_a \times \text{Bound hormone} + K_a \times \text{Total receptor number} \quad (5)$$
$$\text{Free hormone}$$

Notice that equations 4 and 5 have the general form of an equation for a straight line: $y = mx + b$.

To obtain information regarding a particular hormone-receptor system, a fixed number of cells (and, therefore, a fixed number of receptors) is incubated *in vitro* in a series of test tubes with increasing amounts of hormone. At each higher hormone concentration, the amount of receptor-bound hormone is increased until all receptors are occupied by hormone. Receptor number and affinity can be obtained by using the relationships given in equation 5 above and plotting the results as the ratio of receptor-bound hormone to free hormone ([HR]/[H]) as a function of the amount of bound hormone ([HR]). This type of analysis is known as a **Scatchard plot** (Fig. 31.9). In theory, a Scatchard plot of simple, reversible equilibrium binding is a straight line (Fig. 31.9A), with the slope of the line being equal to the negative of the association constant ($-K_a$) and the x-intercept

being equal to the total receptor number (R_0). Other equally valid mathematical and graphic methods can be used to analyze hormone-receptor interactions, but the Scatchard plot is probably the most widely used.

In practice, Scatchard plots are not always straight lines but instead can be curvilinear (Fig. 31.9B). Insulin is a classic example of a hormone that gives curved Scatchard plots. One interpretation of this result is that cells contain two separate and distinct classes of receptors, each with a different binding affinity. Typically, one receptor population has a higher affinity but is fewer in number compared to the second population. Therefore, as indicated in Figure 31.9B, $Ka_1 > Ka_2$, but $R0_2 > R0_1$. Computer analysis is often required to fit curvilinear Scatchard plots accurately to a two-site model.

Another explanation for curvilinear Scatchard plots is that occupied receptors influence the affinity of adjacent, unoccupied receptors by **negative cooperativity**. According to this theory, when one hormone molecule binds to its receptor, it causes a decrease in the affinity of nearby unoccupied receptors, making it more difficult for additional hormone molecules to bind. The greater the amount of hormone bound, the lower the affinity of unoccupied receptors. Therefore, as shown in Figure 31.9B, as bound hormone increases, the affinity (slope) steadily decreases. Whether curvilinear Scatchard plots in fact result from two-site receptor systems or from negative cooperativity between receptors is unknown.

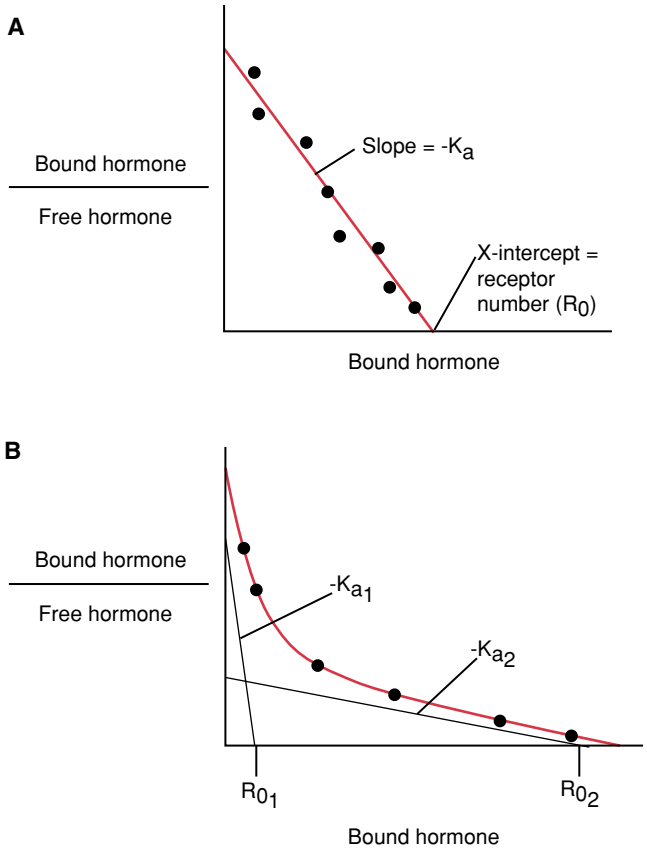

FIGURE 31.9 **Scatchard plots of hormone-receptor binding data.** **A**, A straight-line plot typical of hormone binding to a single class of receptors. **B**, A curvilinear Scatchard plot typical of some hormones. Several models have been proposed to account for nonlinearity of Scatchard plots. (See text for details).

Dose-Response Curves Are Useful in Determining Whether There Has Been a Change in Responsiveness or Sensitivity

Hormone effects are generally not all-or-none phenomena—that is, they generally do not switch from totally off to totally on, and then back again. Instead, target cells exhibit graded responses proportional to the concentration of free hormone present.

The dose-response relationship for a hormone generally exhibits a sigmoid shape when plotted as the biological response on the y-axis versus the log of the hormone concentration on the x-axis (Fig. 31.10). Regardless of the biological pathway or process being considered, cells typically exhibit an intrinsic **basal level** of activity in the absence of added hormone, even well after any previous exposure to hormone. As the hormone concentration surrounding the cells increases, a minimal **threshold concentration** must be present before any measurable increase in the cellular response can be produced. At higher hormone concentrations, a **maximal response** by the target cell is produced, and no greater response can be elicited by increasing the hormone concentration. The concentration of hormone required to produce a response half-way between the maximal and basal responses, the **median effective dose** or ED_{50}, is a useful index of the **sensitivity** of the target cell for that particular hormone (see Fig. 31.10).

For some peptide hormones, the maximal response may occur when only a small percentage (5 to 10%) of the total receptor population is occupied by hormone. The remaining 90 to 95% of the receptors are called **spare receptors**

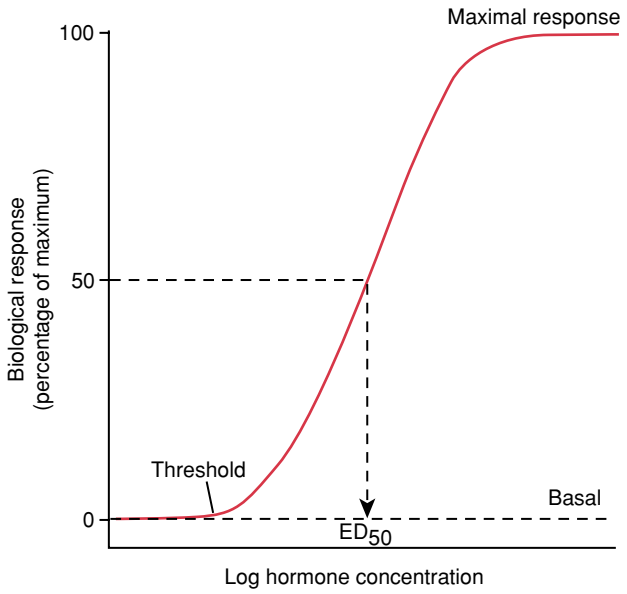

FIGURE 31.10 **A normal dose-response curve of hormone activity.**

because on initial inspection they do not appear necessary to produce a maximal response. This term is unfortunate, because the receptors are not "spare" in the sense of being unused. While at any one point in time only 5 to 10% of the receptors may be occupied, hormone-receptor interactions are an equilibrium process, and hormones continually dissociate and reassociate with their receptors. Therefore, from one point in time to the next, different subsets of the total population of receptors may be occupied, but presumably all receptors participate equally in producing the biological response.

Physiological or pathophysiological alterations in target tissue responses to hormones can take one of two general forms, as indicated by changes in their dose-response curves (Fig. 31.11). Although changes in dose-response curves are not routinely assessed in the clinical setting, they can serve to distinguish between a **receptor abnormality** and a **postreceptor abnormality** in hormone action, providing useful information regarding the underlying cause of a particular disease state. A change in responsiveness is indicated by an increase or decrease in the maximal response of the target tissue and may be the result of one or more factors (Fig. 31.11A). Altered responsiveness can be caused by a change in the number of functional target cells in a tissue, by a change in the number of receptors per cell for the hormone in question or, if receptor function itself is not rate-limiting for hormone action, by a change in the specific rate-limiting postreceptor step in the hormone action pathway.

A change in sensitivity is reflected as a right or left shift in the dose-response curve and, thus, a change in the ED_{50}; a right shift indicates decreased sensitivity and a left shift indicates increased sensitivity for that hormone (Fig. 31.11B). Changes in sensitivity reflect (1) an alteration in receptor affinity or, if submaximal concentrations of hormone are present, (2) a change in receptor number. Dose-response curves may also reflect combinations of changes in responsiveness and sensitivity in which there is both a right or left shift of the curve (a sensitivity change) and a change in maximal biological response to a lower or higher level (a change in responsiveness).

Cells can regulate their receptor number and/or function in several ways. Exposing cells to an excess of hormone for a sustained period of time typically results in a decreased number of receptors for that hormone per cell. This phenomenon is referred to as **down-regulation**. In the case of peptide hormones, which have receptors on cell surfaces, a redistribution of receptors from the cell surface to intracellular sites usually occurs as part of the process of down-regulation. Therefore, there may be fewer total receptors per cell, and a smaller percentage may be available for hormone binding on the cell surface. Although somewhat less prevalent than down-regulation, **up-regulation** may occur when certain conditions or treatments cause an increase in receptor number compared to normal. Changes in rates of receptor synthesis may also contribute to long-term down- or up-regulation.

In addition to changing receptor number, many target cells can regulate receptor function. Chronic exposure of cells to a hormone may cause the cells to become less responsive to subsequent exposure to the hormone by a process termed **desensitization**. If the exposure of cells to a hormone has a desensitizing effect on further action by that same hormone, the effect is termed **homologous desensitization**. If the exposure of cells to one hormone has a desensitizing effect with regard to the action of a different hormone, the effect is termed **heterologous desensitization**.

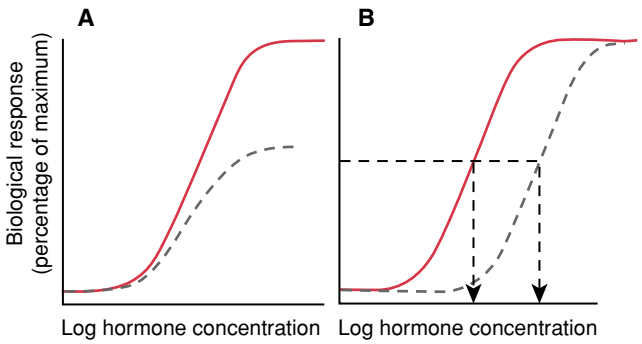

FIGURE 31.11 Altered target tissue responses reflected by dose-response curves. A, Decreased target tissue responsiveness. B, Decreased target tissue sensitivity.

REVIEW QUESTIONS

DIRECTIONS: Each of the numbered items or incomplete statements in this section is followed by answers or by completions of the statement. Select the ONE lettered answer or completion that is BEST in each case.

1. A shift to the right in the biological activity dose-response curve for a hormone with no accompanying change in the maximal response indicates
   (A) Decreased responsiveness *and* decreased sensitivity
   (B) Increased responsiveness
   (C) Decreased sensitivity
   (D) Increased sensitivity *and* decreased responsiveness
   (E) Increased sensitivity
2. Within the endocrine system, specificity of communication is determined by
   (A) The chemical nature of the hormone
   (B) The distance between the endocrine cell and its target cell(s)
   (C) The presence of specific receptors on target cells
   (D) Anatomical connections between the endocrine and target cells
   (E) The affinity of binding between the hormone and its receptor
3. The principal mineralocorticoid in the body is
   Aldosterone
   (A) Testosterone
   (B) Progesterone
   (C) Prostaglandin E_2
   (D) Cortisol
4. An index of the binding affinity of a hormone for its receptor can be obtained by examining the
   (A) Y-intercept of a Scatchard plot
   (B) Slope of a Scatchard plot
   (C) Maximum point on a biological dose-response curve
   (D) X-intercept of a Scatchard plot
   (E) The threshold point of a biological dose-response curve
5. Most peptide and protein hormones are synthesized as
   (A) A secretagogue
   (B) A pleiotropic hormone
   (C) Proopiomelanocortin (POMC)
   (D) A preprohormone
   (E) Propressophysin
6. The primary form of cortisol in the plasma is that which is
   (A) Bound to albumin
   (B) Bound to transthyretin
   (C) Free in solution
   (D) (D Bound to cortisol receptors
   (E) Bound to corticosteroid-binding globulin (CBG)
7. The ability of hormones to be effective regulators of biological function despite circulating at very low concentrations results from
   (A) The multiplicity of their effects Transport proteins
   (B) Pleiotropic effects
   (C) Signal amplification
   (D) Competitive binding

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