Introduction to Endocrinology

Coordination of Body Functions by Chemical Messengers

The multiple activities of the cells, tissues, and organs of the body are coordinated by the interplay of several types of chemical messenger systems:

1. *Neurotransmitters* are released by axon terminals of neurons into the synaptic junctions and act locally to control nerve cell functions.
2. *Endocrine hormones* are released by glands or specialized cells into the circulating blood and influence the function of cells at another location in the body.
3. *Neuroendocrine hormones* are secreted by neurons into the circulating blood and influence the function of cells at another location in the body.
4. *Paracrines* are secreted by cells into the extracellular fluid and affect neighboring cells of a different type.
5. *Autocrines* are secreted by cells into the extracellular fluid and affect the function of the same cells that produced them by binding to cell surface receptors.
6. *Cytokines* are peptides secreted by cells into the extracellular fluid and can function as autocrines, paracrines, or endocrine hormones. Examples of cytokines include the *interleukins* and other *lymphokines* that are secreted by helper cells and act on other cells of the immune system (see Chapter 34). Cytokine hormones (e.g., *leptin*) produced by adipocytes are sometimes called *adipokines*.

In the next few chapters, we discuss mainly the endocrine and neuroendocrine hormone systems, keeping in mind that many of the body's chemical messenger systems interact with one another to maintain homeostasis. For example, the adrenal medullae and the pituitary gland secrete their hormones primarily in response to neural stimuli. The neuroendocrine cells, located in the hypothalamus, have axons that terminate in the posterior pituitary gland and median eminence and secrete several neurohormones, including *antidiuretic hormone (ADH), oxytocin,* and *hypophysiotropic hormones,* which control the secretion of anterior pituitary hormones.

The *endocrine hormones* are carried by the circulatory system to cells throughout the body, including the nervous system in some cases, where they bind with receptors and initiate many reactions. Some endocrine hormones affect many different types of cells of the body; for example, *growth hormone* (from the anterior pituitary gland) causes growth in most parts of the body, and *thyroxine* (from the thyroid gland) increases the rate of many chemical reactions in almost all the body's cells.

Other hormones affect only specific *target tissues,* because only these tissues have receptors for the hormone. For example, *adrenocorticotropic hormone (ACTH)* from the anterior pituitary gland specifically stimulates the adrenal cortex, causing it to secrete adrenocortical hormones, and the *ovarian hormones* have specific effects on the female sex organs as well as on the secondary sexual characteristics of the female body.

Figure 74–1 shows the anatomical loci of the major endocrine glands and endocrine tissues of the body, except for the placenta, which is an additional source of the sex hormones. Table 74–1 provides an overview of the different hormone systems and their most important actions.

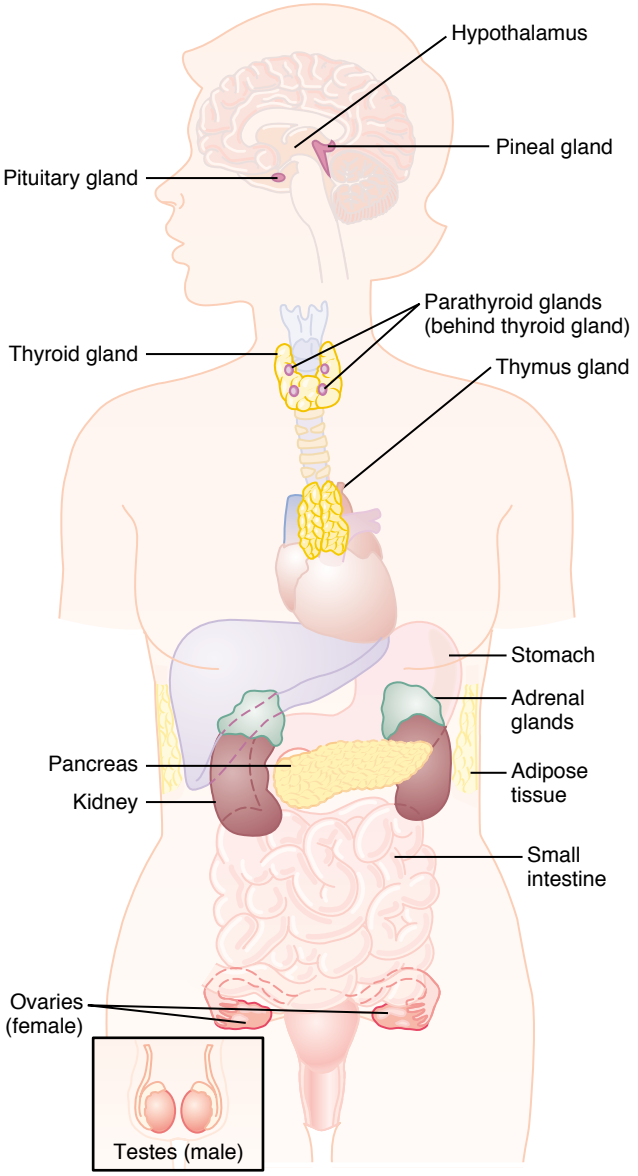

Figure 74–1

Anatomical loci of the principal endocrine glands and tissues of the body.

The multiple hormone systems play a key role in regulating almost all body functions, including metabolism, growth and development, water and electrolyte balance, reproduction, and behavior. For instance, without growth hormone, a person would be a dwarf. Without thyroxine and triiodothyronine from the thyroid gland, almost all the chemical reactions of the body would become sluggish, and the person would become sluggish as well. Without insulin from the pancreas, the body's cells could use little of the food carbohydrates for energy. And without the sex hormones, sexual development and sexual functions would be absent.

Chemical Structure and Synthesis of Hormones

There are three general classes of hormones:

1. *Proteins and polypeptides,* including hormones secreted by the anterior and posterior pituitary gland, the pancreas (insulin and glucagon), the parathyroid gland (parathyroid hormone), and many others (see Table 74–1).
2. *Steroids* secreted by the adrenal cortex (cortisol and aldosterone), the ovaries (estrogen and progesterone), the testes (testosterone), and the placenta (estrogen and progesterone).
3. *Derivatives of the amino acid tyrosine,* secreted by the thyroid (thyroxine and triiodothyronine) and the adrenal medullae (epinephrine and norepinephrine). There are no known polysaccharides or nucleic acid hormones.

Polypeptide and Protein Hormones Are Stored in Secretory Vesicles Until Needed. Most of the hormones in the body are polypeptides and proteins. These hormones range in size from small peptides with as few as 3 amino acids (thyrotropin-releasing hormone) to proteins with almost 200 amino acids (growth hormone and prolactin). In general, polypeptides with 100 or more amino acids are called proteins, and those with fewer than 100 amino acids are referred to as peptides.

Protein and peptide hormones are synthesized on the rough end of the endoplasmic reticulum of the different endocrine cells, in the same fashion as most other proteins (Figure 74–2). They are usually synthesized first as larger proteins that are not biologically active (*preprohormones*) and are cleaved to form smaller *prohormones* in the endoplasmic reticulum. These are then transferred to the Golgi apparatus for packaging into secretory vesicles. In this process, enzymes in the vesicles cleave the prohormones to produce smaller, biologically active hormones and inactive fragments. The vesicles are stored within the cytoplasm, and many are bound to the cell membrane until their secretion is needed. Secretion of the hormones (as well as the inactive fragments) occurs when the secretory vesicles fuse with the cell membrane and the granular contents are extruded into the interstitial fluid or directly into the blood stream by *exocytosis.*

In many cases, the stimulus for exocytosis is an increase in cytosolic calcium concentration caused by depolarization of the plasma membrane. In other instances, stimulation of an endocrine cell surface receptor causes increased cyclic adenosine monophosphate (cAMP) and subsequently activation of protein kinases that initiate secretion of the hormone. The peptide hormones are water soluble, allowing them to enter the circulatory system easily, where they are carried to their target tissues.

Steroid Hormones Are Usually Synthesized from Cholesterol and Are Not Stored. The chemical structure of steroid hormones is similar to that of cholesterol, and in most instances they are synthesized from cholesterol itself.

Table 74–1

Endocrine Glands, Hormones, and Their Functions and Structure

Gland/Tissue	Hormones	Major Functions	Chemical Structure
Hypothalamus (Chapter 75)	Thyrotropin-releasing hormone (TRH)	Stimulates secretion of TSH and prolactin	Peptide
	Corticotropin-releasing hormone (CRH)	Causes release of ACTH	Peptide
	Growth hormone–releasing hormone (GHRH)	Causes release of growth hormone	Peptide
	Growth hormone inhibitory hormone (GHIH) (somatostatin)	Inhibits release of growth hormone	Peptide
	Gonadotropin-releasing hormone (GnRH)	Causes release of LH and FSH	
	Dopamine or prolactin-inhibiting factor (PIF)	Inhibits release of prolactin	Amine
Anterior pituitary (Chapter 75)	Growth hormone	Stimulates protein synthesis and overall growth of most cells and tissues	Peptide
	Thyroid-stimulating hormone (TSH)	Stimulates synthesis and secretion of thyroid hormones (thyroxine and triiodothyronine)	Peptide
	Adrenocorticotropic hormone (ACTH)	Stimulates synthesis and secretion of adrenocortical hormones (cortisol, androgens, and aldosterone)	Peptide
	Prolactin	Promotes development of the female breasts and secretion of milk	Peptide
	Follicle-stimulating hormone (FSH)	Causes growth of follicles in the ovaries and sperm maturation in Sertoli cells of testes	Peptide
	Luteinizing hormone (LH)	Stimulates testosterone synthesis in Leydig cells of testes; stimulates ovulation, formation of corpus luteum, and estrogen and progesterone synthesis in ovaries	Peptide
Posterior pituitary (Chapter 75)	Antidiuretic hormone (ADH) (also called vasopressin)	Increases water reabsorption by the kidneys and causes vasoconstriction and increased blood pressure	Peptide
	Oxytocin	Stimulates milk ejection from breasts and uterine contractions	Peptide
Thyroid (Chapter 76)	Thyroxine (T_4) and triiodothyronine (T_3)	Increases the rates of chemical reactions in most cells, thus increasing body metabolic rate	Amine
	Calcitonin	Promotes deposition of calcium in the bones and decreases extracellular fluid calcium ion concentration	Peptide
Adrenal cortex (Chapter 77)	Cortisol	Has multiple metabolic functions for controlling metabolism of proteins, carbohydrates, and fats; also has anti-inflammatory effects	Steroid
	Aldosterone	Increases renal sodium reabsorption, potassium secretion, and hydrogen ion secretion	Steroid
Adrenal medulla (Chapter 60)	Norepinephrine, epinephrine	Same effects as sympathetic stimulation	Amine
Pancreas (Chapter 78)	Insulin (β cells)	Promotes glucose entry in many cells, and in this way controls carbohydrate metabolism	Peptide
	Glucagon (α cells)	Increases synthesis and release of glucose from the liver into the body fluids	Peptide
Parathyroid (Chapter 79)	Parathyroid hormone (PTH)	Controls serum calcium ion concentration by increasing calcium absorption by the gut and kidneys and releasing calcium from bones	Peptide
Testes (Chapter 80)	Testosterone	Promotes development of male reproductive system and male secondary sexual characteristics	Steroid
Ovaries (Chapter 81)	Estrogens	Promotes growth and development of female reproductive system, female breasts, and female secondary sexual characteristics	Steroid
	Progesterone	Stimulates secretion of "uterine milk" by the uterine endometrial glands and promotes development of secretory apparatus of breasts	Steroid
Placenta (Chapter 82)	Human chorionic gonadotropin (HCG)	Promotes growth of corpus luteum and secretion of estrogens and progesterone by corpus luteum	Peptide
	Human somatomammotropin	Probably helps promote development of some fetal tissues as well as the mother's breasts	Peptide
	Estrogens	See actions of estrogens from ovaries	Steroid
	Progesterone	See actions of progesterone from ovaries	Steroid
Kidney (Chapter 26)	Renin	Catalyzes conversion of angiotensinogen to angiotensin I (acts as an enzyme)	Peptide
	1,25-Dihydroxycholecalciferol	Increases intestinal absorption of calcium and bone mineralization	Steroid
	Erythropoietin	Increases erythrocyte production	Peptide
Heart (Chapter 22)	Atrial natriuretic peptide (ANP)	Increases sodium excretion by kidneys, reduces blood pressure	Peptide
Stomach (Chapter 64)	Gastrin	Stimulates HCl secretion by parietal cells	Peptide
Small intestine (Chapter 64)	Secretin	Stimulates pancreatic acinar cells to release bicarbonate and water	Peptide
	Cholecystokinin (CCK)	Stimulates gallbladder contraction and release of pancreatic enzymes	Peptide
Adipocytes (Chapter 71)	Leptin	Inhibits appetite, stimulates thermogenesis	Peptide

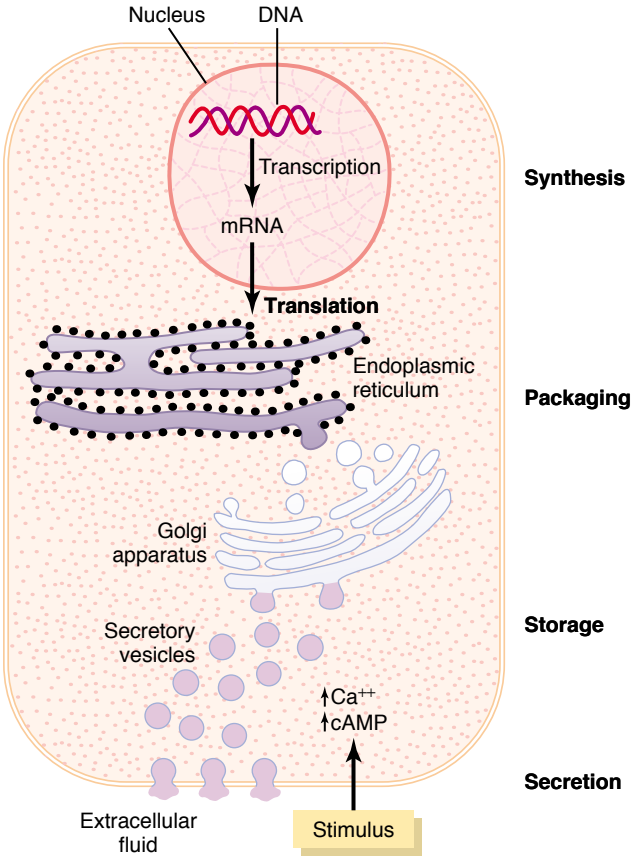

Figure 74–2

Synthesis and secretion of peptide hormones. The stimulus for hormone secretion often involves changes in intracellular calcium or changes in cyclic adenosine monophosphate (cAMP) in the cell.

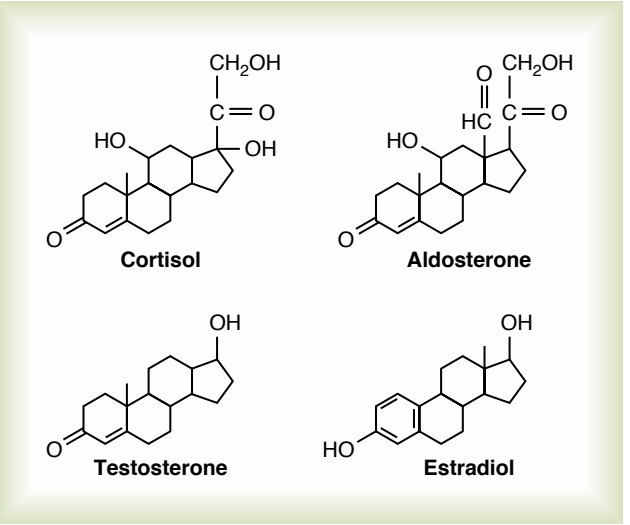

Figure 74–3

Chemical structures of several steroid hormones.

They are lipid soluble and consist of three cyclohexyl rings and one cyclopentyl ring combined into a single structure (Figure 74–3).

Although there is usually very little hormone storage in steroid-producing endocrine cells, large stores of cholesterol esters in cytoplasm vacuoles can be rapidly mobilized for steroid synthesis after a stimulus. Much of the cholesterol in steroid-producing cells comes from the plasma, but there is also de novo synthesis of cholesterol in steroid-producing cells. Because the steroids are highly lipid soluble, once they are synthesized, they simply diffuse across the cell membrane and enter the interstitial fluid and then the blood.

Amine Hormones Are Derived from Tyrosine. The two groups of hormones derived from tyrosine, the thyroid and the adrenal medullary hormones, are formed by the actions of enzymes in the cytoplasmic compartments of the glandular cells. The thyroid hormones are synthesized and stored in the thyroid gland and incorporated into macromolecules of the protein *thyroglobulin,* which is stored in large follicles within the thyroid gland. Hormone secretion occurs when the amines are split from thyroglobulin, and the free hormones are then released into the blood stream. After entering the blood, most of the thyroid hormones combine with plasma proteins, especially *thyroxine-binding globulin,* which slowly releases the hormones to the target tissues.

Epinephrine and norepinephrine are formed in the adrenal medulla, which normally secretes about four times more epinephrine than norepinephrine. Catecholamines are taken up into preformed vesicles and stored until secreted. Similar to the protein hormones stored in secretory granules, catecholamines are also released from adrenal medullary cells by exocytosis. Once the catecholamines enter the circulation, they can exist in the plasma in free form or in conjugation with other substances.

Hormone Secretion, Transport, and Clearance from the Blood

Onset of Hormone Secretion After a Stimulus, and Duration of Action of Different Hormones. Some hormones, such as norepinephrine and epinephrine, are secreted within seconds after the gland is stimulated, and they may develop full action within another few seconds to minutes; the actions of other hormones, such as thyroxine or growth hormone, may require months for full effect. Thus, each of the different hormones has its own characteristic onset and duration of action—each tailored to perform its specific control function.

Concentrations of Hormones in the Circulating Blood, and Hormonal Secretion Rates. The concentrations of hormones required to control most metabolic and endocrine functions are incredibly small. Their concentrations in the blood range from as little as 1 picogram (which is one millionth of one millionth of a gram) in each milliliter of blood up to at most a few micrograms (a few

millionths of a gram) per milliliter of blood. Similarly, the rates of secretion of the various hormones are extremely small, usually measured in micrograms or milligrams per day. We shall see later in this chapter that highly specialized mechanisms are available in the target tissues that allow even these minute quantities of hormones to exert powerful control over the physiological systems.

Feedback Control of Hormone Secretion

Negative Feedback Prevents Overactivity of Hormone Systems. Although the plasma concentrations of many hormones fluctuate in response to various stimuli that occur throughout the day, all hormones studied thus far appear to be closely controlled. In most instances, this control is exerted through *negative feedback mechanisms* that ensure a proper level of hormone activity at the target tissue. After a stimulus causes release of the hormone, conditions or products resulting from the action of the hormone tend to suppress its further release. In other words, the hormone (or one of its products) has a negative feedback effect to prevent oversecretion of the hormone or overactivity at the target tissue.

The controlled variable is often not the secretory rate of the hormone itself but the degree of activity of the target tissue. Therefore, only when the target tissue activity rises to an appropriate level will feedback signals to the endocrine gland become powerful enough to slow further secretion of the hormone. Feedback regulation of hormones can occur at all levels, including gene transcription and translation steps involved in the synthesis of hormones and steps involved in processing hormones or releasing stored hormones.

Surges of Hormones Can Occur with Positive Feedback. In a few instances, *positive feedback* occurs when the biological action of the hormone causes additional secretion of the hormone. One example of this is the surge of *luteinizing hormone (LH)* that occurs as a result of the stimulatory effect of estrogen on the anterior pituitary before ovulation. The secreted LH then acts on the ovaries to stimulate additional secretion of estrogen, which in turn causes more secretion of LH. Eventually, LH reaches an appropriate concentration, and typical negative feedback control of hormone secretion is then exerted.

Cyclical Variations Occur in Hormone Release. Superimposed on the negative and positive feedback control of hormone secretion are periodic variations in hormone release that are influenced by seasonal changes, various stages of development and aging, the diurnal (daily) cycle, and sleep. For example, the secretion of growth hormone is markedly increased during the early period of sleep but is reduced during the later stages of sleep. In many cases, these cyclical variations in hormone secretion are due to changes in activity of neural pathways involved in controlling hormone release.

Transport of Hormones in the Blood

Water-soluble hormones (peptides and catecholamines) are dissolved in the plasma and transported from their sites of synthesis to target tissues, where they diffuse out of the capillaries, into the interstitial fluid, and ultimately to target cells.

Steroid and thyroid hormones, in contrast, circulate in the blood mainly bound to plasma proteins. Usually less than 10 per cent of steroid or thyroid hormones in the plasma exist free in solution. For example, more than 99 per cent of the thyroxine in the blood is bound to plasma proteins. However, protein-bound hormones cannot easily diffuse across the capillaries and gain access to their target cells and are therefore biologically inactive until they dissociate from plasma proteins.

The relatively large amounts of hormones bound to proteins serve as reservoirs, replenishing the concentration of free hormones when they are bound to target receptors or lost from the circulation. Binding of hormones to plasma proteins greatly slows their clearance from the plasma.

"Clearance" of Hormones from the Blood

Two factors can increase or decrease the concentration of a hormone in the blood. One of these is the rate of hormone secretion into the blood. The second is the rate of removal of the hormone from the blood, which is called the *metabolic clearance rate.* This is usually expressed in terms of the number of milliliters of plasma cleared of the hormone per minute. To calculate this clearance rate, one measures (1) the rate of disappearance of the hormone from the plasma per minute and (2) the concentration of the hormone in each milliliter of plasma. Then, the metabolic clearance rate is calculated by the following formula:

$$\text{Metabolic clearance rate} = \frac{\text{Rate of disappearance of hormone from the plasma}}{\text{Concentration of hormone in each milliliter of plasma}}$$

The usual procedure for making this measurement is the following: A purified solution of the hormone to be measured is tagged with a radioactive substance. Then the radioactive hormone is infused at a constant rate into the blood stream until the radioactive concentration in the plasma becomes steady. At this time, the rate of disappearance of the radioactive hormone from the plasma equals the rate at which it is infused, which gives one the rate of disappearance. At the same time, the plasma concentration of the radioactive hormone is measured using a standard radioactive counting procedure. Then, using the formula just cited, the metabolic clearance rate is calculated.

Hormones are "cleared" from the plasma in several ways, including (1) metabolic destruction by the tissues, (2) binding with the tissues, (3) excretion by the liver into the bile, and (4) excretion by the kidneys into the urine. For certain hormones, a decreased metabolic clearance rate may cause an excessively high concentration of the hormone in the circulating body fluids. For instance, this occurs for several of the steroid hormones when the liver is diseased, because these hormones are conjugated mainly in the liver and then "cleared" into the bile.

Hormones are sometimes degraded at their target cells by enzymatic processes that cause endocytosis of the cell membrane hormone-receptor complex; the hormone is then metabolized in the cell, and the receptors are usually recycled back to the cell membrane.

Most of the peptide hormones and catecholamines are water soluble and circulate freely in the blood. They are usually degraded by enzymes in the blood and tissues and rapidly excreted by the kidneys and liver, thus remaining in the blood for only a short time. For example, the half-life of angiotensin II circulating in the blood is less than a minute.

Hormones that are bound to plasma proteins are cleared from the blood at much slower rates and may remain in the circulation for several hours or even days. The half-life of adrenal steroids in the circulation, for example, ranges between 20 and 100 minutes, whereas the half-life of the protein-bound thyroid hormones may be as long as 1 to 6 days.

Mechanisms of Action of Hormones

Hormone Receptors and Their Activation

The first step of a hormone's action is to bind to specific *receptors* at the target cell. Cells that lack receptors for the hormones do not respond. Receptors for some hormones are located on the target cell membrane, whereas other hormone receptors are located in the cytoplasm or the nucleus. When the hormone combines with its receptor, this usually initiates a cascade of reactions in the cell, with each stage becoming more powerfully activated so that even small concentrations of the hormone can have a large effect.

Hormonal receptors are large proteins, and each cell that is to be stimulated usually has some 2000 to 100,000 receptors. Also, each receptor is usually highly specific for a single hormone; this determines the type of hormone that will act on a particular tissue. The target tissues that are affected by a hormone are those that contain its specific receptors.

The locations for the different types of hormone receptors are generally the following:

1. *In or on the surface of the cell membrane.* The membrane receptors are specific mostly for the protein, peptide, and catecholamine hormones.
2. *In the cell cytoplasm.* The primary receptors for the different steroid hormones are found mainly in the cytoplasm.
3. *In the cell nucleus.* The receptors for the thyroid hormones are found in the nucleus and are believed to be located in direct association with one or more of the chromosomes.

The Number and Sensitivity of Hormone Receptors Are Regulated. The number of receptors in a target cell usually does not remain constant from day to day, or even from minute to minute. The receptor proteins themselves are often inactivated or destroyed during the course of their function, and at other times they are reactivated or new ones are manufactured by the protein-manufacturing mechanism of the cell. For instance, increased hormone concentration and increased binding with its target cell receptors sometimes cause the number of active receptors to decrease. This *down-regulation* of the receptors can occur as a result of (1) inactivation of some of the receptor molecules, (2) inactivation of some of the intracellular protein signaling molecules, (3) temporary sequestration of the receptor to the inside of the cell, away from the site of action of hormones that interact with cell membrane receptors, (4) destruction of the receptors by lysosomes after they are internalized, or (5) decreased production of the receptors. In each case, receptor down-regulation decreases the target tissue's responsiveness to the hormone.

Some hormones cause *up-regulation* of receptors and intracellular signaling proteins; that is, the stimulating hormone induces greater than normal formation of receptor or intracellular signaling molecules by the protein-manufacturing machinery of the target cell, or greater availability of the receptor for interaction with the hormone. When this occurs, the target tissue becomes progressively more sensitive to the stimulating effects of the hormone.

Intracellular Signaling After Hormone Receptor Activation

Almost without exception, a hormone affects its target tissues by first forming a hormone-receptor complex. This alters the function of the receptor itself, and the activated receptor initiates the hormonal effects. To explain this, let us give a few examples of the different types of interactions.

Ion Channel–Linked Receptors. Virtually all the neurotransmitter substances, such as acetylcholine and norepinephrine, combine with receptors in the postsynaptic membrane. This almost always causes a change in the structure of the receptor, usually opening or closing a channel for one or more ions. Some of these *ion channel–linked receptors* open (or close) channels for sodium ions, others for potassium ions, others for calcium ions, and so forth. The altered movement of these ions through the channels causes the subsequent effects on the postsynaptic cells. Although a few

hormones may exert some of their actions through activation of ion channel receptors, most hormones that open or close ions channels do this indirectly by coupling with G protein–linked or enzyme-linked receptors, as discussed next.

G Protein–Linked Hormone Receptors. Many hormones activate receptors that indirectly regulate the activity of target proteins (e.g., enzymes or ion channels) by coupling with groups of cell membrane proteins called *heterotrimeric GTP-binding proteins (G proteins)* (Figure 74–4). There are more than 1000 known G protein–coupled receptors, all of which have seven transmembrane segments that loop in and out of the cell membrane. Some parts of the receptor that protrude into the cell cytoplasm (especially the cytoplasmic tail of the receptor) are coupled to G proteins that include three (i.e., trimeric) parts—the α, β, and γ subunits. When the ligand (hormone) binds to the extracellular part of the receptor, a conformational change occurs in the receptor that activates the G proteins and induces intracellular signals that either (1) open or close cell membrane ion channels or (2) change the activity of an enzyme in the cytoplasm of the cell.

The trimeric G proteins are named for their ability to bind *guanosine nucleotides*. In their inactive state, the α, β, and γ subunits of G proteins form a complex that binds *guanosine diphosphate (GDP)* on the α subunit. When the receptor is activated, it undergoes a conformational change that causes the GDP-bound trimeric G protein to associate with the cytoplasmic part of the receptor and to exchange GDP for *guanosine triphosphate (GTP)*. Displacement of GDP by GTP causes the α subunit to dissociate from the trimeric complex and to associate with other intracellular signaling proteins; these proteins, in turn, alter the activity of ion channels or intracellular enzymes such as *adenylyl cyclase* or *phospholipase C,* which alters cell function.

The signaling event is rapidly terminated when the hormone is removed and the α subunit inactivates itself by converting its bound GTP to GDP; then the α subunit once again combines with the β and γ subunits to form an inactive, membrane-bound trimeric G protein.

Some hormones are coupled to *inhibitory G proteins* (denoted G_i proteins), whereas others are coupled to *stimulatory G proteins* (denoted G_s proteins). Thus, depending on the coupling of a hormone receptor to an inhibitory or stimulatory G protein, a hormone can either increase or decrease the activity of intracellular enzymes. This complex system of cell membrane G proteins provides a vast array of potential cell responses to different hormones in the various target tissues of the body.

Enzyme-Linked Hormone Receptors. Some receptors, when activated, function directly as enzymes or are closely associated with enzymes that they activate. These *enzyme-linked receptors* are proteins that pass through the membrane only once, in contrast to the seven-transmembrane G protein–coupled receptors. Enzyme-linked receptors have their hormone-binding site on the outside of the cell membrane and their catalytic or enzyme-binding site on the inside. When the hormone binds to the extracellular part of the receptor, an enzyme immediately inside the cell membrane is activated (or occasionally inactivated). Although many enzyme-linked receptors have intrinsic enzyme activity, others rely on enzymes that are closely associated with the receptor to produce changes in cell function.

One example of an enzyme-linked receptor is the *leptin receptor* (Figure 74–5). Leptin is a hormone

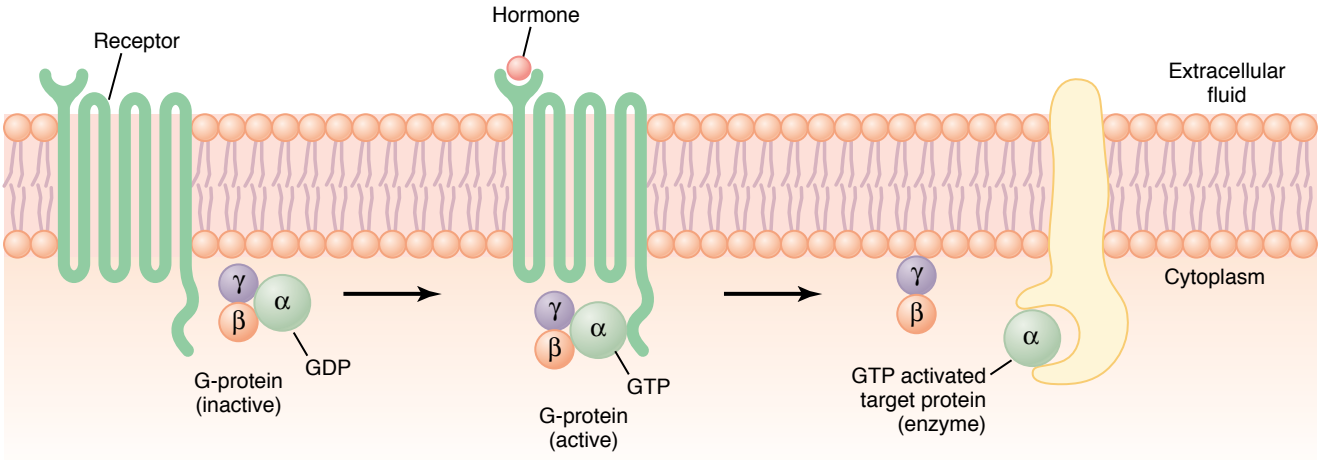

Figure 74–4

Mechanism of activation of a G protein–coupled receptor. When the hormone activates the receptor, the inactive α, β, and γ G protein complex associates with the receptor and is activated, with an exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP). This causes the α subunit (to which the GTP is bound) to dissociate from the β and γ subunits of the G protein and to interact with membrane-bound target proteins (enzymes) that initiate intracellular signals.

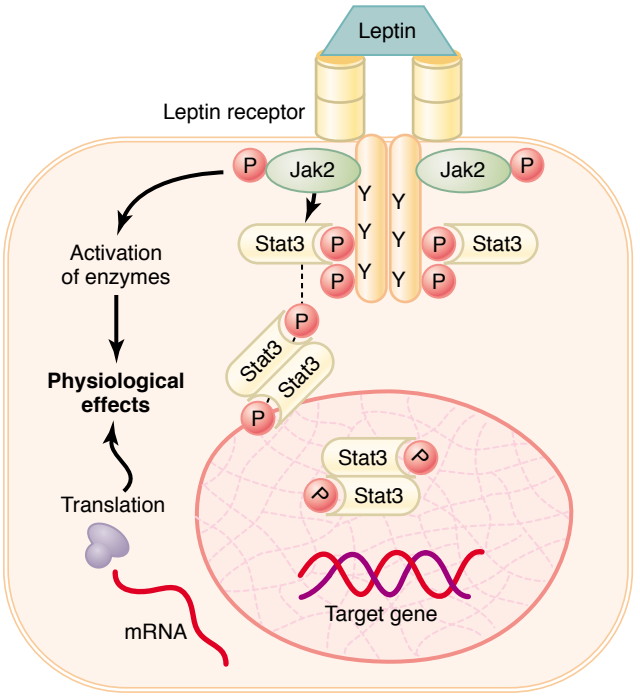

Figure 74–5

An enzyme-linked receptor—the leptin receptor. The receptor exists as a homodimer (two identical parts), and leptin binds to the extracellular part of the receptor, causing phosphorylation and activation of the intracellular associated janus kinase 2 (JAK2). This causes phosphorylation of signal transducer and activator of transcription (STAT) proteins, which then activates the transcription of target genes and the synthesis of proteins. JAK2 phosphorylation also activates several other enzyme systems that mediate some of the more rapid effects of leptin.

secreted by fat cells and has many physiological effects, but it is especially important in regulating appetite and energy balance, as discussed in Chapter 71. The leptin receptor is a member of a large family of *cytokine receptors* that do not themselves contain enzymatic activity but signal through associated enzymes. In the case of the leptin receptor, one of the signaling pathways occurs through a *tyrosine kinase* of the *janus kinase (JAK)* family, *JAK2*. The leptin receptor exists as a dimer (i.e., in two parts), and binding of leptin to the extracellular part of the receptor alters its conformation, enabling phosphorylation and activation of the intracellular associated JAK2 molecules. The activated JAK2 molecules then phosphorylate other tyrosine residues within the leptin receptor–JAK2 complex to mediate intracellular signaling. The intracellular signals include phosphorylation of *signal transducer and activator of transcription (STAT)* proteins, which activates transcription by leptin target genes to initiate protein synthesis. Phosphorylation of JAK2 also leads to activation of other intracellular enzyme pathways such as *mitogen-activated protein kinases (MAPK)* and *phosphatidylinositol 3-kinase (PI3K)*. Some of the effects of leptin occur rapidly as a result of activation of these intracellular enzymes, whereas other actions occur more slowly and require synthesis of new proteins.

Another example, one widely used in hormonal control of cell function, is for the hormone to bind with a special transmembrane receptor, which then becomes the activated enzyme *adenylyl cyclase* at the end that protrudes to the interior of the cell. This cyclase catalyzes the formation of cAMP, which has a multitude of effects inside the cell to control cell activity, as discussed in greater detail later. cAMP is called a *second messenger* because it is not the hormone itself that directly institutes the intracellular changes; instead, the cAMP serves as a second messenger to cause these effects.

For a few peptide hormones, such as atrial natriuretic peptide (ANP), *cyclic guanosine monophosphate (cGMP),* which is only slightly different from cAMP, serves in a similar manner as a second messenger.

Intracellular Hormone Receptors and Activation of Genes. Several hormones, including adrenal and gonadal steroid hormones, thyroid hormones, retinoid hormones, and vitamin D, bind with protein receptors inside the cell rather than in the cell membrane. Because these hormones are lipid soluble, they readily cross the cell membrane and interact with receptors in the cytoplasm or nucleus. The activated hormone-receptor complex then binds with a specific regulatory (promoter) sequence of the DNA called the *hormone response element,* and in this manner either activates or represses transcription of specific genes and formation of messenger RNA (mRNA) (Figure 74–6). Therefore, minutes, hours, or even days after the hormone has entered the cell, newly formed proteins appear in the cell and become the controllers of new or altered cellular functions.

Many different tissues have identical intracellular hormone receptors, but the genes that the receptors regulate are different in the various tissues. An intracellular receptor can activate a gene response only if the appropriate combination of gene regulatory proteins is present, and many of these regulatory proteins are tissue specific. Thus, the responses of different tissues to a hormone are determined not only by the specificity of the receptors but also by the genes that the receptor regulates.

Second Messenger Mechanisms for Mediating Intracellular Hormonal Functions

We noted earlier that one of the means by which hormones exert intracellular actions is to stimulate formation of the second messenger cAMP inside the cell membrane. The cAMP then causes subsequent intracellular effects of the hormone. Thus, the only direct effect that the hormone has on the cell is to activate a single type of membrane receptor. The second messenger does the rest.

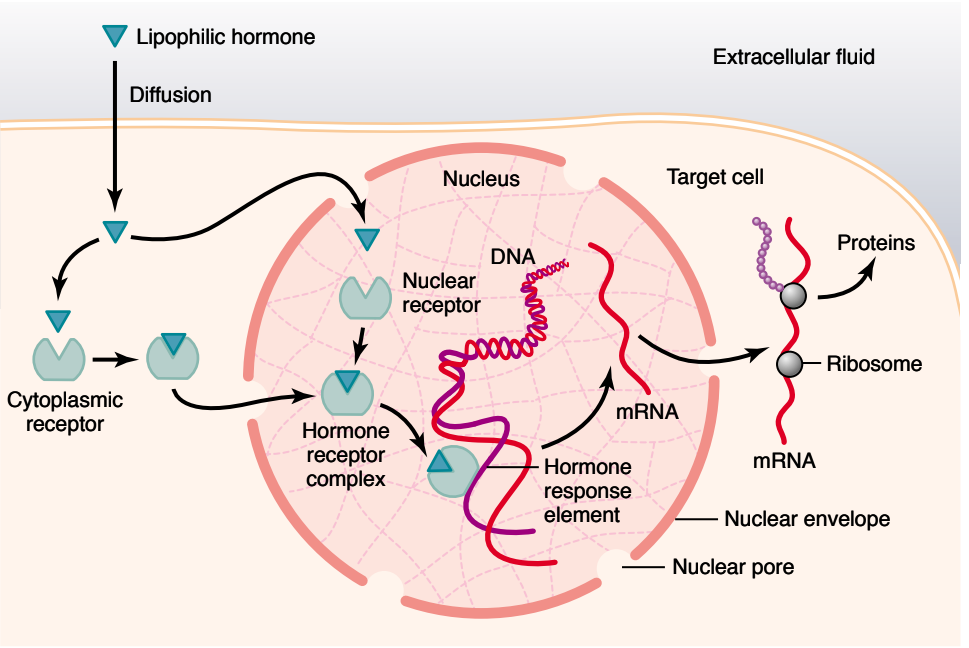

Figure 74–6

Mechanisms of interaction of lipophilic hormones, such as steroids, with intracellular receptors in target cells. After the hormone binds to the receptor in the cytoplasm or in the nucleus, the hormone-receptor complex binds to the hormone response element (promoter) on the DNA. This either activates or inhibits gene transcription, formation of messenger RNA (mRNA), and protein synthesis.

Table 74–2

Some Hormones That Use the Adenylyl Cyclase–cAMP Second Messenger System

Adrenocorticotropic hormone (ACTH)
Angiotensin II (epithelial cells)
Calcitonin
Catecholamines (β receptors)
Corticotropin-releasing hormone (CRH)
Follicle-stimulating hormone (FSH)
Glucagon
Human chorionic gonadotropin (HCG)
Luteinizing hormone (LH)
Parathyroid hormone (PTH)
Secretin
Somatostatin
Thyroid-stimulating hormone (TSH)
Vasopressin (V_2 receptor, epithelial cells)

cAMP is not the only second messenger used by the different hormones. Two other especially important ones are (1) calcium ions and associated *calmodulin* and (2) products of membrane phospholipid breakdown.

Adenylyl Cyclase–cAMP Second Messenger System

Table 74–2 shows a few of the many hormones that use the adenylyl cyclase–cAMP mechanism to stimulate their target tissues, and Figure 74–7 shows the adenylyl cyclase–cAMP second messenger system itself. Binding of the hormones with the receptor allows coupling of the receptor to a *G protein.* If the G protein stimulates the adenylyl cyclase–cAMP system, it is called a *G_s protein,* denoting a stimulatory G protein. Stimulation of adenylyl cyclase, a membrane-bound enzyme, by the G_s protein then catalyzes

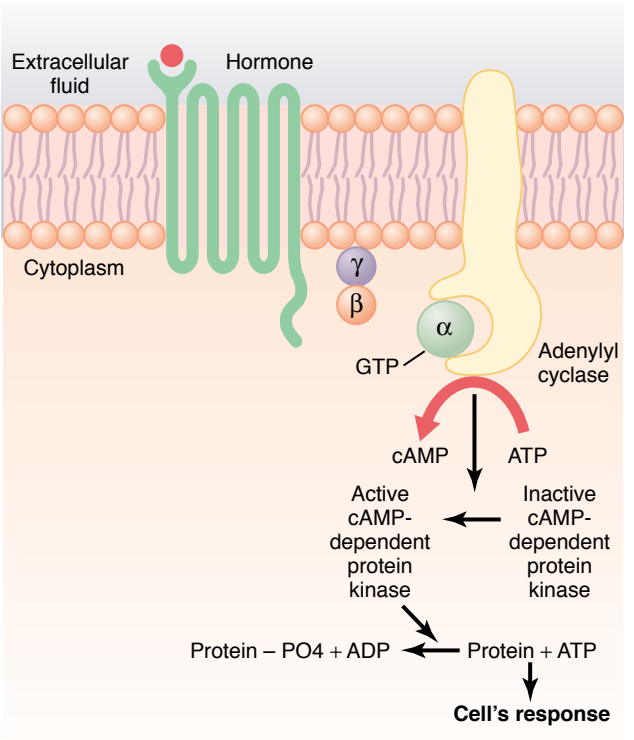

Figure 74–7

Cyclic adenosine monophosphate (cAMP) mechanism by which many hormones exert their control of cell function. ADP, adenosine diphosphate; ATP, adenosine triphosphate.

the conversion of a small amount of cytoplasmic *adenosine triphosphate* (ATP) into cAMP inside the cell. This then activates *cAMP-dependent protein kinase,* which phosphorylates specific proteins in the cell, triggering biochemical reactions that ultimately lead to the cell's response to the hormone.

Once cAMP is formed inside the cell, it usually activates a *cascade of enzymes.* That is, first one enzyme is activated, which activates a second enzyme, which activates a third, and so forth. The importance of this mechanism is that only a few molecules of activated adenylyl cyclase immediately inside the cell membrane can cause many more molecules of the next enzyme to be activated, which can cause still more molecules of the third enzyme to be activated, and so forth. In this way, even the slightest amount of hormone acting on the cell surface can initiate a powerful cascading activating force for the entire cell.

If binding of the hormone to its receptors is coupled to an inhibitory G protein (denoted G_i protein), adenylyl cyclase will be inhibited, reducing the formation of cAMP and ultimately leading to an inhibitory action in the cell. Thus, depending on the coupling of the hormone receptor to an inhibitory or a stimulatory G protein, a hormone can either increase or decrease the concentration of cAMP and phosphorylation of key proteins inside the cell.

The specific action that occurs in response to increases or decreases of cAMP in each type of target cell depends on the nature of the intracellular machinery—some cells have one set of enzymes, and other cells have other enzymes. Therefore, different functions are elicited in different target cells, such as initiating synthesis of specific intracellular chemicals, causing muscle contraction or relaxation, initiating secretion by the cells, and altering cell permeability.

Thus, a thyroid cell stimulated by cAMP forms the metabolic hormones thyroxine and triiodothyronine, whereas the same cAMP in an adrenocortical cell causes secretion of the adrenocortical steroid hormones. In epithelial cells of the renal tubules, cAMP increases their permeability to water.

The Cell Membrane Phospholipid Second Messenger System

Some hormones activate transmembrane receptors that activate the enzyme *phospholipase C* attached to the inside projections of the receptors (Table 74–3). This enzyme catalyzes the breakdown of some phospholipids in the cell membrane, especially *phosphatidylinositol biphosphate (PIP_2),* into two different second messenger products: *inositol triphosphate (IP_3)* and *diacylglycerol (DAG).* The IP_3 mobilizes calcium ions from mitochondria and the endoplasmic reticulum, and the calcium ions then have their own second messenger effects, such as smooth muscle contraction and changes in cell secretion.

DAG, the other lipid second messenger, activates the enzyme *protein kinase C (PKC),* which then phosphorylates a large number of proteins, leading to the cell's response (Figure 74–8). In addition to these effects, the lipid portion of DAG is *arachidonic acid,* which is the precursor for the *prostaglandins* and other local hormones that cause multiple effects in tissues throughout the body.

Table 74–3

Some Hormones That Use the Phospholipase C Second Messenger System

Angiotensin II (vascular smooth muscle)
Catecholamines (α receptors)
Gonadotropin-releasing hormone (GnRH)
Growth hormone–releasing hormone (GHRH)
Oxytocin
Thyroid-releasing hormone (TRH)
Vasopressin (V_1 receptor, vascular smooth muscle)

Calcium-Calmodulin Second Messenger System

Another second messenger system operates in response to the entry of calcium into the cells. Calcium entry may be initiated by (1) changes in membrane potential that open calcium channels or (2) a hormone interacting with membrane receptors that open calcium channels.

On entering a cell, calcium ions bind with the protein *calmodulin.* This protein has four calcium sites, and when three or four of these sites have bound with calcium, the calmodulin changes its shape and initiates multiple effects inside the cell, including activation or inhibition of protein kinases. Activation of

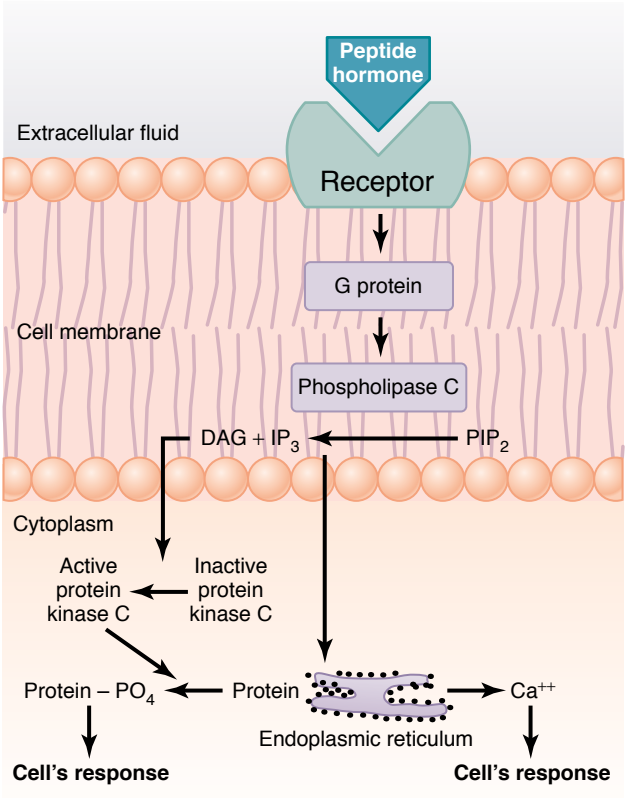

Figure 74–8

The cell membrane phospholipid second messenger system by which some hormones exert their control of cell function. DAG, diacylglycerol; IP_3, inositol triphosphate; PIP_2, phosphatidylinositol biphosphate.

calmodulin-dependent protein kinases causes, via phosphorylation, activation or inhibition of proteins involved in the cell's response to the hormone. For example, one specific function of calmodulin is to activate *myosin kinase,* which acts directly on the myosin of smooth muscle to cause smooth muscle contraction.

The normal calcium ion concentration in most cells of the body is 10^{-8} to 10^{-7} mol/L, which is not enough to activate the calmodulin system. But when the calcium ion concentration rises to 10^{-6} to 10^{-5} mol/L, enough binding occurs to cause all the intracellular actions of calmodulin. This is almost exactly the same amount of calcium ion change that is required in skeletal muscle to activate troponin C, which causes skeletal muscle contraction, as explained in Chapter 7. It is interesting that troponin C is similar to calmodulin in both function and protein structure.

Hormones That Act Mainly on the Genetic Machinery of the Cell

Steroid Hormones Increase Protein Synthesis

Another means by which hormones act—specifically, the steroid hormones secreted by the adrenal cortex, ovaries, and testes—is to cause synthesis of proteins in the target cells. These proteins then function as enzymes, transport proteins, or structural proteins, which in turn provide other functions of the cells.

The sequence of events in steroid function is essentially the following:

1. The steroid hormone diffuses across the cell membrane and enters the cytoplasm of the cell, where it binds with a specific *receptor protein.*
2. The combined receptor protein–hormone then diffuses into or is transported into the nucleus.
3. The combination binds at specific points on the DNA strands in the chromosomes, which activates the transcription process of specific genes to form mRNA.
4. The mRNA diffuses into the cytoplasm, where it promotes the translation process at the ribosomes to form new proteins.

To give an example, *aldosterone,* one of the hormones secreted by the adrenal cortex, enters the cytoplasm of renal tubular cells, which contain a specific aldosterone receptor protein. Therefore, in these cells, the sequence of events cited earlier ensues. After about 45 minutes, proteins begin to appear in the renal tubular cells and promote sodium reabsorption from the tubules and potassium secretion into the tubules. Thus, the full action of the steroid hormone is characteristically delayed for at least 45 minutes—up to several hours or even days. This is in marked contrast to the almost instantaneous action of some of the peptide and amino acid–derived hormones, such as vasopressin and norepinephrine.

Thyroid Hormones Increase Gene Transcription in the Cell Nucleus

The thyroid hormones *thyroxine* and *triiodothyronine* cause increased transcription by specific genes in the nucleus. To accomplish this, these hormones first bind directly with receptor proteins in the nucleus itself; these receptors are probably protein molecules located within the chromosomal complex, and they likely control the function of the genetic promoters or operators, as explained in Chapter 3.

Two important features of thyroid hormone function in the nucleus are the following:

1. They activate the genetic mechanisms for the formation of many types of intracellular proteins—probably 100 or more. Many of these are enzymes that promote enhanced intracellular metabolic activity in virtually all cells of the body.
2. Once bound to the intranuclear receptors, the thyroid hormones can continue to express their control functions for days or even weeks.

Measurement of Hormone Concentrations in the Blood

Most hormones are present in the blood in extremely minute quantities; some concentrations are as low as one billionth of a milligram (1 picogram) per milliliter. Therefore, it was very difficult to measure these concentrations by the usual chemical means. An extremely sensitive method, however, was developed about 40 years ago that revolutionized the measurement of hormones, their precursors, and their metabolic end products. This method is called *radioimmunoassay.*

Radioimmunoassay

The method of performing radioimmunoassay is as follows. First, an antibody that is highly specific for the hormone to be measured is produced.

Second, a small quantity of this antibody is (1) mixed with a quantity of fluid from the animal containing the hormone to be measured and (2) mixed simultaneously with an appropriate amount of purified standard hormone that has been tagged with a radioactive isotope. However, one specific condition must be met: There must be too little antibody to bind completely both the radioactively tagged hormone and the hormone in the fluid to be assayed. Therefore, the natural hormone in the assay fluid and the radioactive standard hormone *compete for the binding sites* of the antibody. In the process of competing, the quantity of each of the two hormones, the natural and the radioactive, that binds is proportional to its concentration in the assay fluid.

Third, after binding has reached equilibrium, the antibody-hormone complex is separated from the remainder of the solution, and the quantity of radioactive hormone bound in this complex is measured by radioactive counting techniques. If a large amount of radioactive hormone has bound with the antibody, it is clear that there was only a small amount of natural hormone to compete with the radioactive hormone, and therefore the concentration of the natural hormone in the assayed fluid was small. Conversely, if

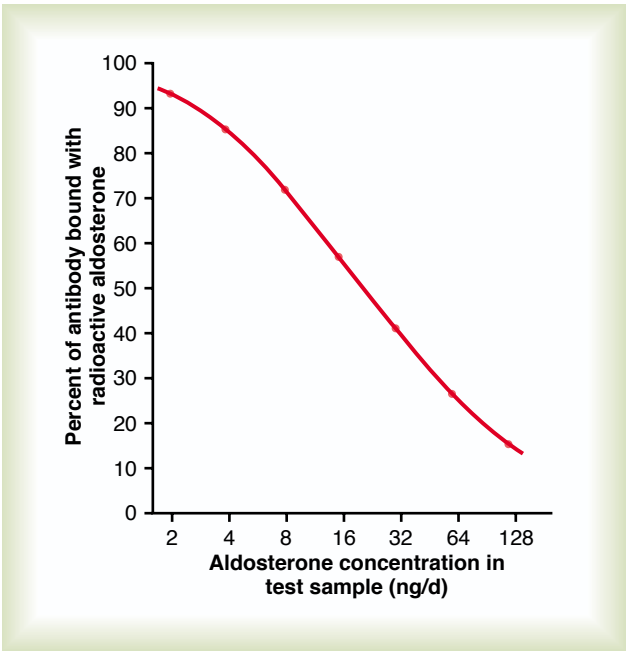

Figure 74–9

"Standard curve" for radioimmunoassay of aldosterone. (Courtesy Dr. Manis Smith.)

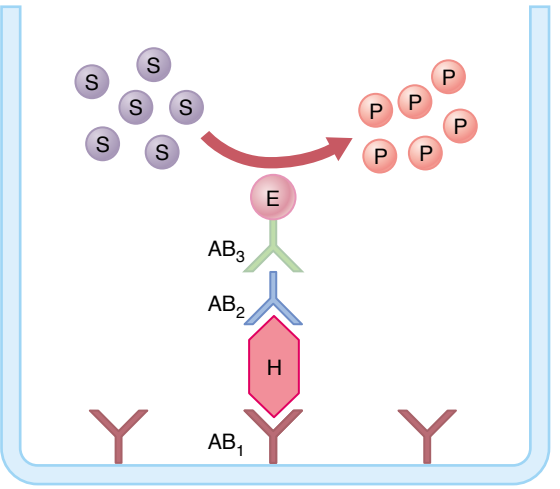

Figure 74–10

Basic principles of the enzyme-linked immunosorbent assay (ELISA) for measuring the concentration of a hormone (H). AB_1 and AB_2 are antibodies that recognize the hormone at different binding sites, and AB_3 is an antibody that recognizes AB_2. E is an enzyme linked to AB_3 that catalyzes the formation of a colored fluorescent product (P) from a substrate (S). The amount of the product is measured using optical methods and is proportional to the amount of hormone in the well if there are excess antibodies in the well.

only a small amount of radioactive hormone has bound, it is clear that there was a large amount of natural hormone to compete for the binding sites.

Fourth, to make the assay highly quantitative, the radioimmunoassay procedure is also performed for "standard" solutions of untagged hormone at several concentration levels. Then a "standard curve" is plotted, as shown in Figure 74–9. By comparing the radioactive counts recorded from the "unknown" assay procedures with the standard curve, one can determine within an error of 10 to 15 per cent the concentration of the hormone in the "unknown" assayed fluid. As little as billionths or even trillionths of a gram of hormone can often be assayed in this way.

Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assays (ELISAs) can be used to measure almost any protein, including hormones. This test combines the specificity of antibodies with the sensitivity of simple enzyme assays. Figure 74–10 shows the basic elements of this method, which is often performed on plastic plates that each have 96 small wells. Each well is coated with an antibody (AB_1) that is specific for the hormone being assayed. Samples or standards are added to each of the wells, followed by a second antibody (AB_2) that is also specific for the hormone but binds to a different site of the hormone molecule. A third antibody (AB_3) is added that recognizes AB_2 and is coupled to an enzyme that converts a suitable substrate to a product that can be easily detected by colorimetric or fluorescent optical methods.

Because each molecule of enzyme catalyzes the formation of many thousands of product molecules, even very small amounts of hormone molecules can be detected. In contrast to competitive radioimmunoassay methods, ELISA methods use excess antibodies so that all hormone molecules are captured in antibody-hormone complexes. Therefore, the amount of hormone present in the sample or in the standard is proportional to the amount of product formed.

The ELISA method has become widely used in clinical laboratories because (1) it does not employ radioactive isotopes, (2) much of the assay can be automated using 96-well plates, and (3) it has proved to be a cost-effective and accurate method for assessing hormone levels.

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