Alquds University

College of Health Professions

Department of Medical Laboratory Science

Endocrinology Manual

By

Dr. Akram Kharroubi

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THE ENDOCRINE SYSTEM

The endocrine system controls the flow of information between different cells and tissues (Figure 1–1). The term "endocrine" denotes internal secretion of biologically active substances—in contrast to "exocrine," which denotes secretion outside the body (e.g., through sweat glands or ducts that lead into the gastrointestinal tract). The endocrine system releases hormones into the circulation to convey information to target cells that contain cognate hormone receptors. This system is subject to complex regulatory mechanisms that govern hormone synthesis, release, transport, metabolism and delivery to the interior of the target cells, and expression and activity of the hormone receptor itself and its downstream signaling machinery. The endocrine system also has complex relationships with the other major system of communication between cells, the nervous system, as well as with the immune system. It exerts widespread effects on development, growth, and metabolism.

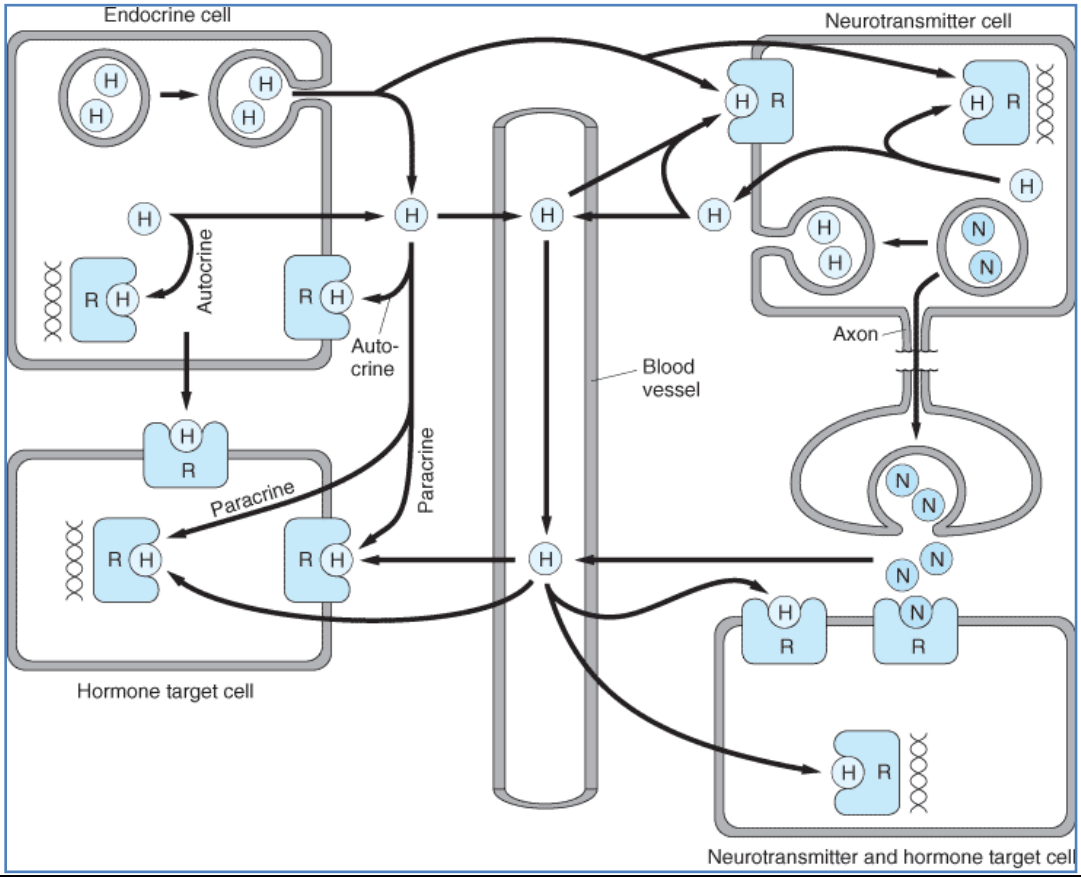


Figure 1-1. **Actions of hormones and neurotransmitters**. Endocrine and neurotransmitter cells synthesize hormones and release them by specialized secretory pathways or by diffusion. The hormones act on the producer cell (autocrine) or on neighboring target cells, including neurotransmitter cells, without entering the circulation (paracrine). They may go to the target cell through the circulation (hormonal). Neurotransmitter cells release neurotransmitters from nerve terminals. The same neurotransmitters can be released to act as hormones through the synaptic junctions or directly by the cell (H, hormone; R, receptor; N, neurotransmitter).

Hormones: Endocrine, Paracrine, Autocrine, & Intracrine Actions

Hormones can function in the traditional way to circulate through the bloodstream to target tissues or can act locally by binding to receptors expressed by cells close to the site of release. When hormones act on neighboring cells, the action is called "paracrine," as illustrated by actions of sex steroids in the ovary and angiotensin II in the kidney. When hormone is released and acts on receptors located on the same cell, the action is referred to as "autocrine." Autocrine actions may be important in promoting unregulated growth of cancer cells. Hormones can act inside the cell without being released, "an intracrine" effect. An example of autocrine actions are those of insulin and somatostatin that can inhibit their own release from pancreatic B and D cells, respectively.

Endocrine Organs

The field of endocrinology traditionally encompassed a small number of "classic" endocrine glands that produce and release hormones. In the brain, these include the anterior pituitary gland, which produces corticotropin (ACTH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), and prolactin (PRL); the posterior pituitary gland, which produces antidiuretic hormone (ADH) and oxytocin; the hypothalamus, which produces releasing and inhibitory factors that regulate secretion of the anterior pituitary hormones; and the pineal gland, which produces melatonin. In the periphery, classic endocrine glands are the thyroid gland (thyroid hormones), parathyroid gland (parathyroid hormone [PTH]), adrenal gland (corticosteroids, epinephrine), ovaries and testis (sex steroids), and pancreas (insulin, glucagon and somatostatin).

In addition, organs that are not traditionally considered to be part of the endocrine system also produce hormones. Thus, the kidney produces renin that converts angiotensinogen to angiotensin I and erythropoietin that stimulates blood cell production. Adipose tissue produces several hormones, including leptin, an important mediator of body weight, and resistin, which regulates sensitivity to insulin and may be involved in the pathology of type 2 diabetes. The heart produces natriuretic peptides. Some molecules traditionally thought to be metabolic intermediates, such as fatty acids and bile acids, are now known to function as hormones.

Target Organs

Target organs mount a response to a given hormone. They must express an appropriate cognate hormone receptor protein and molecules that mediate the receptors' downstream effects.

Hormone Receptors

Hormone receptors are proteins with bifunctional properties of recognition of the hormone (e.g., ability to distinguish the hormone from other molecules to which they are exposed) and transduction of the information from binding to downstream receptor effects. The hormone acts as an allosteric effector that alters receptor conformation, and this conformational alteration transmits (transduces) binding information into postreceptor events that influence cellular function. Some hormones (e.g., insulin, GH, PRL, leptin, catecholamines) bind cell surface

receptors, whereas others (e.g., steroids, thyroid hormones) bind intracellular receptors that act in the nucleus. Some hormones (e.g., estrogens and progestins) bind multiple receptors, which are present on both the cell surface and inside the cell.

Chemical Composition of Hormones

Hormones derive from the major classes of biological molecules (Figures 1–2 and 1–3). Thus, hormones can be proteins (including glycoproteins), peptides or peptide derivatives, amino acid analogs, or lipids. Polypeptide hormones are direct translation products of specific mRNAs, cleavage products of larger precursor proteins, or modified peptides. Catecholamines and thyroid hormones are amino acid derivatives. Steroid hormones, bile acids and vitamin D are derived from cholesterol. Retinoids are derived from carotenoids in the diet. Eicosanoids are derived from fatty acids.

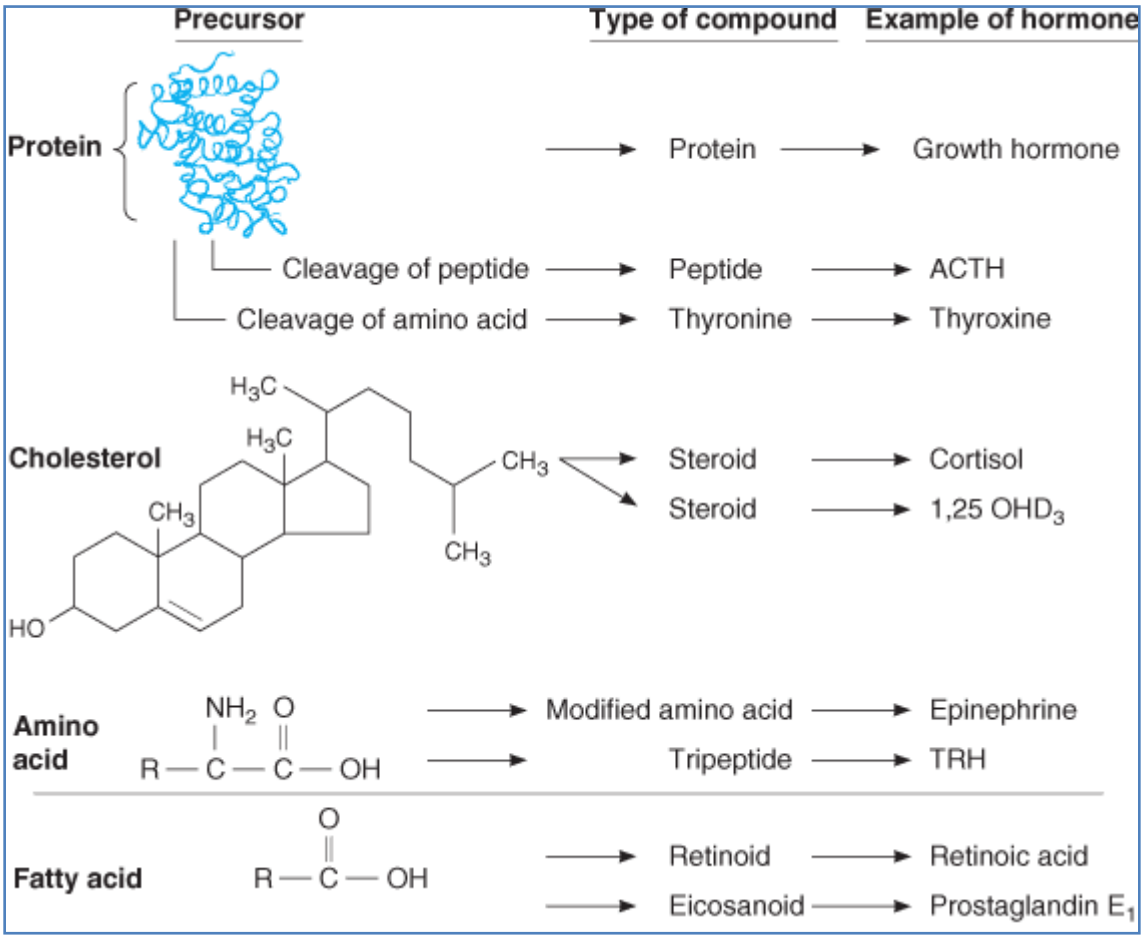


Figure 1-2. **Precursors of hormones**. Shown are representations of the sources of the major hormones, with examples of different hormones that reflect each chemical type.

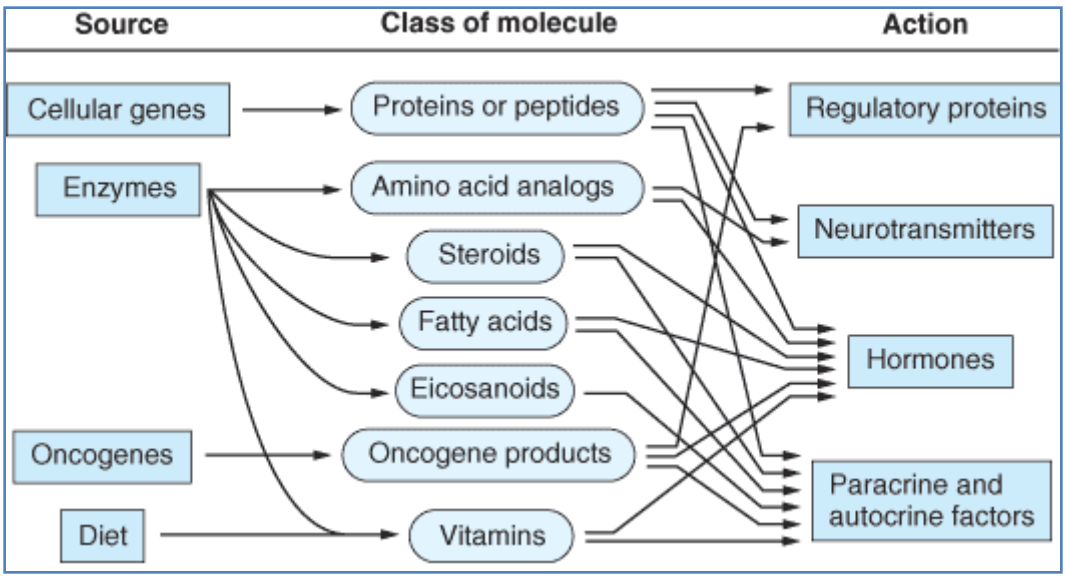


Figure 1-3. Relations between the source, class, and actions of various molecules involved in the endocrine system. These molecules are or become hormones, eicosanoids, oncogene products, and vitamins. Normal genes encode proteins that are regulatory proteins, neurotransmitters, hormones, and paracrine or autocrine factors (or polypeptides from which these are derived). Normal genes also encode enzymes which generate amino acid analogs that can be neurotransmitters, hormones, and autocrine or paracrine factors; steroids that can be hormones or autocrine or paracrine factors; or eicosanoids that can be autocrine or paracrine factors. Oncogenes encode proteins that can be regulatory proteins, hormones, or autocrine or paracrine factors. Vitamin D can be obtained from the diet or synthesized by the body. It can act as a hormone or as an autocrine or paracrine factor.

Relationships between Hormones & Other Signaling Molecules

Hormones are part of a large complement of small intercellular signaling molecules. The following sections review parallels and overlaps between the endocrine and other signaling systems.

Neurotransmitters & Hormones

The endocrine system is distinguished from the nervous system by the fact that endocrine signals are released systemically, whereas the nervous system is directly connected to target tissues through neurons (Figure 1–1). Hormones are widely distributed, and reliance is placed on the receptor to distinguish the hormone from other molecules and then to generate responses in specific cells (Figure 1–1). By contrast, neurotransmitters are synthesized in the cell body of the neuron and travel down the axon, where they are stored in synaptic vesicles, released on depolarization, and bind specific receptors on the postsynaptic neuron. Here, specificity of response is dependent largely on targeted local release of signaling molecules, although the presence, levels, and activities of the receptors are an important component of the response.

There are strong similarities between signaling mechanisms in the endocrine and nervous systems. Indeed, the same molecule can be both a hormone and a neurotransmitter (Figure 1–1).

Catecholamines are hormones when released by the adrenal medulla and neurotransmitters when released by nerve terminals. Furthermore, catecholamines utilize the same types of adrenergic receptors and the same postreceptor intracellular signaling pathways in peripheral tissues and the central nervous system. Other molecules behave as hormones and neurotransmitters. Thyrotropin-releasing hormone (TRH) is a hormone when it is produced by the hypothalamus, but it has diverse neurotransmitter actions in the central nervous system. Dopamine, corticotropin-releasing hormone (CRH), calcitonin gene-related peptide (CGRP), somatostatin, gonadotropin-releasing hormone (GnRH), vasoactive intestinal peptide (VIP), gastrin, secretin, cholecystokinin (CCK), and steroids (neurosteroids) and their receptors are also found in various parts of the brain.

There is also overlap between the function of endocrine glands and the nervous system. For example, the hypothalamus contains specialized neurons that secrete hormones into the circulation.

Vitamins & Hormones

Vitamins (Figure 1–3) are essential substances derived from the diet. They are required in small quantities and are utilized by the body as cofactors and regulators. Although this definition is acceptable, the body produces molecules that have been described as "vitamins," and vitamins have actions that resemble hormones. For example, vitamin D is produced in individuals who are exposed to sunlight, and supplementation is required only when there is inadequate exposure to sunlight. Further, the active product of vitamin D is a derivative of the ingested vitamin. Vitamins also act in ways that resemble hormones. For example, vitamin D and the retinoids (retinoic acid, 9-*cis*-retinoic acid, and others) act through nuclear hormone receptors.

Eicosanoids: Prostaglandins & Related Compounds

Eicosanoids (including prostaglandins, prostacyclins, leukotrienes, and thromboxanes; Figures 1–2 and 1–3) are derived from polyunsaturated fatty acids with 18-, 20-, or 22-carbon skeletons. Arachidonic acid (*cis*-5,8,11,14-eicosatetraenoic acid) is the most abundant eicosanoid precursor in humans. Eicosanoids are produced by most cells, released with little storage, cleared rapidly from the circulation, and thought to act in a paracrine or autocrine fashion. Their mechanisms of action resemble those of hormones and they act through both cell surface and nuclear receptors.

There is significant cross-talk between eicosanoids and endocrine systems. Eicosanoids regulate hormone release and actions. For example, prostaglandin E (PGE) inhibits GH and PRL release from the pituitary. Eicosanoid synthesis is also frequently stimulated by hormones and in these instances eicosanoids act as downstream mediators of hormone action.

Oncogenes & Hormones

Oncogenes are mutated versions of normal genes that promote cancer (Figure 1–3). Oncogenes were originally identified in oncogenic viruses that captured genes from their host's genome. In some cases, oncogenes are mutated analogs of genes encoding hormones, hormone receptors, or factors that are downstream of the hormone receptor.

The Immune System & Hormones

Interrelationships between the endocrine and immune systems are illustrated in Figure 1–4. Many immune signaling events resemble events of endocrine signaling. Thus, antigen recognition and the mechanisms that inhibit the recognition of self-antigens involve ligand-receptor interactions similar to those of peptide hormones. Moreover, immune cells release peptide signaling molecules that function largely through paracrine, autocrine, and intracrine mechanisms. These include cytokines (e.g., interleukins, interferons, tumor necrosis factor [TNF], and plasminogen activator) that bind to receptors on target cells and stimulate growth, mediate cytotoxicity, or suppress antibody production by B cells as well as lymphokines that attract macrophages and neutrophils to an area of infection. In some cases, immune cells produce peptides that have been traditionally considered as hormones (e.g., ACTH, prolactin, GnRH). The roles of these immune cell-produced classic hormones are not yet clear in all cases, although GnRH appears to play a role in lymphocyte homing.

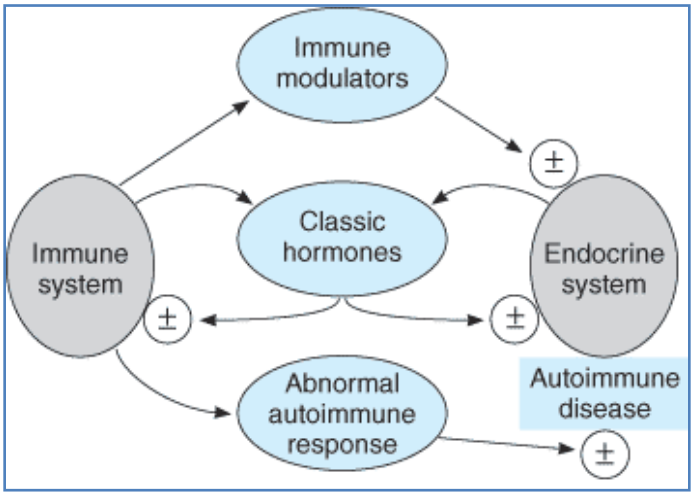


Figure 1-4. Interrelations between the endocrine and immune systems. The plus or minus signs indicate that the influences can be stimulatory or inhibitory.

There is also extensive cross-talk between the regulation of the endocrine and immune systems. Hormones regulate actions of the immune system and immune system signals can affect the function of the endocrine system. For example, TNF influences release and metabolism of thyroid hormones. Finally, autoimmune-induced disorders of the endocrine glands comprise a major component of endocrine practice such as autoimmune destruction of glands as seen in type 1 diabetes mellitus and Addison's disease, and autoimmune stimulation as in the most common form of hyperthyroidism.

Modulation of Hormone Levels

The response of a particular target organ to hormone is governed by local concentration of the hormone. Hormone concentration, in turn, is influenced by many factors; these include rate of hormone synthesis and release from the endocrine organ, transport in the circulation, efficiency of delivery into target cells, whether it is actively exported by target cells and metabolic modifications. In many cases, hormones and their actions have short half-lives. Thus, response can be rapidly initiated or terminated by modulating hormone concentration.

Regulation of Hormone Production

Hormones are produced by endocrine organs in response to diverse signals, including other hormones, innervation of the endocrine organ and environmental signals. Many hypothalamic and anterior pituitary hormones are regulated by classic feedback loops. Here, anterior pituitary hormones stimulate release of other hormones within peripheral endocrine organs. These hormones, in turn, inhibit production of their respective trophic hormones by the hypothalamus and anterior pituitary (Figure 1–5). Examples of this type of regulation include ACTH-dependent production of corticosteroids in the adrenals, FSH- and LH-stimulated production of sex steroids in the gonads and TSH-stimulated production of thyroid hormone in the thyroid gland. This type of regulatory network tends to control hormone levels within a narrow range, referred to as the set point.

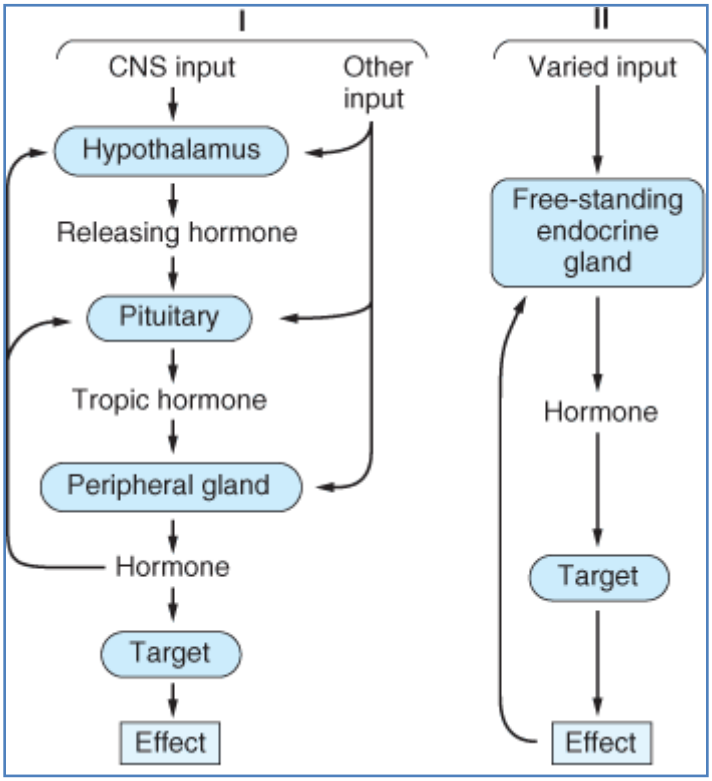


Figure 1-5. The two major types of control of endocrine gland function. ***I:*** The hypothalamic-pituitary-target gland systems involve central nervous system regulation of releasing hormones from the hypothalamus, which stimulate the pituitary to release trophic hormones that act on peripheral glands to release hormones. These hormones can be regulated by other factors. The hormones from the peripheral glands exert feedback control on the hypothalamus and pituitary. ***II:*** Free-standing endocrine glands (eg, parathyroid and islet cells) release hormones that stimulate a target tissue to produce an effect (eg, a rise in serum calcium or a fall in blood sugar) which in turn modifies the function of the gland.

Other systems are more freestanding (Figure 1–5). For example, PTH increases plasma Ca^{2+} concentration, and this exerts a dominant feedback inhibition on the release of PTH by binding Ca^{2+} sensing receptors in the membrane of PTH-producing cells (Chapter 9). Insulin production leads to decreased glucose levels, and this effect leads to cessation of the stimulus to release more insulin.

Innervation regulates hormone production in both the central nervous system and the periphery. Nervous inputs regulate release of hypothalamic hormones and, consequently, downstream target hormones in the anterior pituitary. The pineal gland at the base of the brain provides an interface free of the blood-brain barrier between the brain, the cerebral circulation, and the cerebrospinal fluid. It receives photosensory information through sympathetic innervation that influences

production of melatonin from serotonin. Melatonin regulates circadian rhythms, can have antireproductive actions through blockade of GnRH-induced LH release, and has other effects on hormone release.

Hormone production in peripheral endocrine organs can also be regulated through innervation. For example, stress-related catecholamine release by the adrenal medulla is regulated by autonomic innervation.

Finally, hormone release can be regulated directly by environmental signals such as levels of nutrients. Thus, increased glucose levels in the pancreatic B cell are a potent stimulator of insulin release.

In general, hormone production is regulated by multiple regulatory inputs. For example, insulin release is primarily regulated by glucose concentration, but other signals, including β-adrenergic stimulation through nervous inputs to the pancreas, can amplify the signal and promote higher levels of insulin release. Regulators of hormone production can have either positive or negative influences and include all types of regulators, such as trophic and counter-regulatory hormones, traditional growth factors, eicosanoids, and ions.

Hormone synthesis

Endoplasmic Reticulum (ER): structure and function

The endoplasmic reticulum (ER) is an extensive closed membrane system consisting of tubular and saccular structures. In the area of the nucleus, the ER turns into the external nuclear membrane. Morphologically, a distinction is made between the **rough ER** (rER) and the **smooth ER** (sER). Large numbers of ribosomes are found on the membranes of the rER, which are lacking on the sER. On the other hand, the sER is rich in membrane-bound **enzymes**, which catalyze partial reactions in the lipid metabolism as well as biotransformations.

A. Rough endoplasmic reticulum and the Golgi apparatus

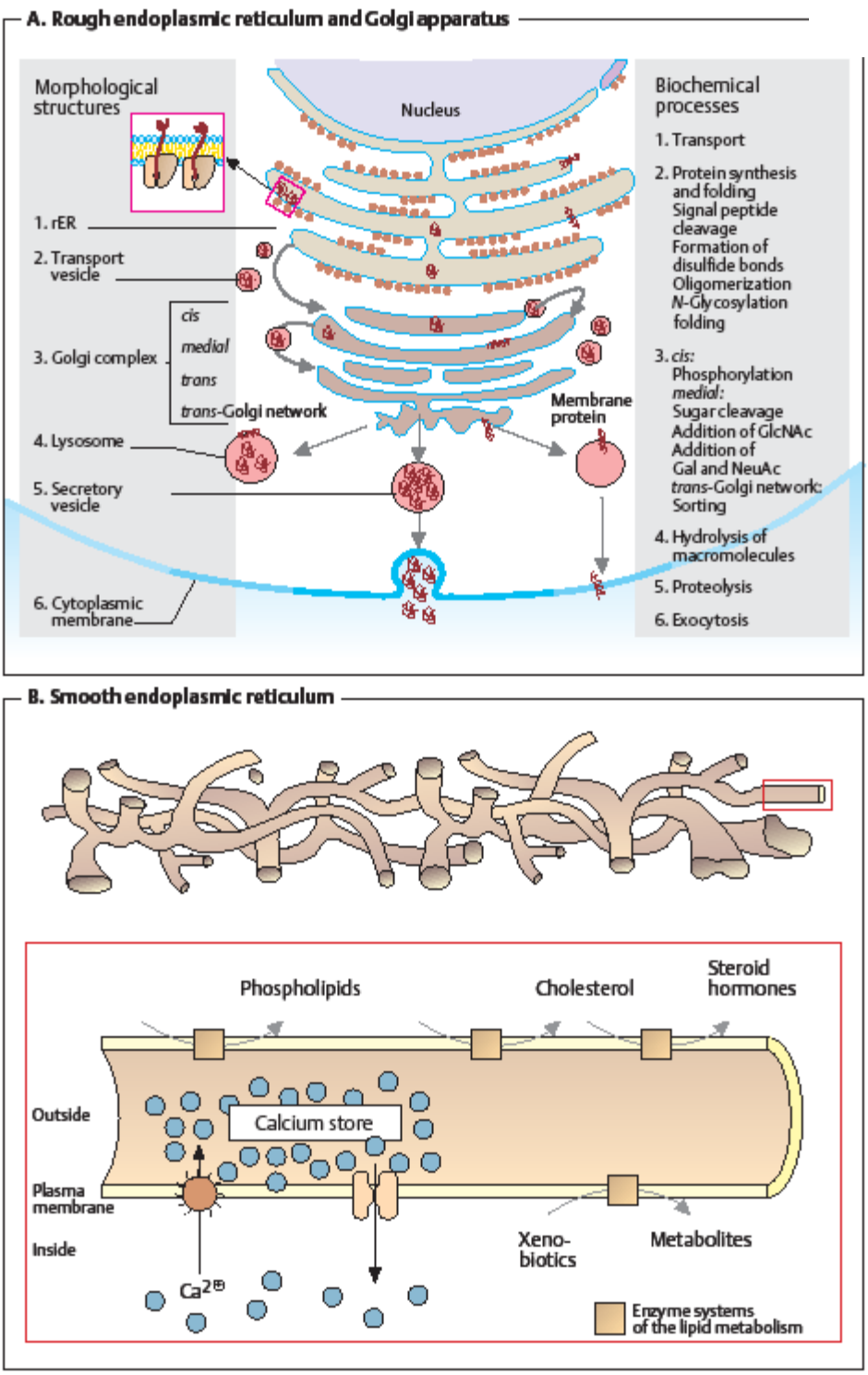
The **rER** (**1**) is a site of active *protein biosynthesis.* This is where proteins destined for membranes, lysosomes, and export from the cell are synthesized. The remaining proteins are produced in the cytoplasm on ribosomes that are not bound to membranes. Proteins synthesized at the rER (**1**) are folded and modified after translation. They remain either in the rER as membrane proteins, or pass with the help of transport vesicles (**2**) to the Golgi apparatus (**3**). Transport vesicles are formed by budding from existing membranes, and they disappear again by fusing with them.

The **Golgi apparatus** (**3**) is a complex network, also enclosed, consisting of flattened membrane saccules ("cisterns"), which are stacked on top of each other in layers. Proteins mature here and are sorted and packed. A distinction is made between the *cis*, *medial*, and *trans* Golgi regions, as well as a *trans* Golgi network (tGN). The *post–translational modification of proteins,* which starts in the ER, continues in these sections. From the Golgi apparatus, the proteins are transported by vesicles to various targets in the cells—e. g., to lysosomes (**4**), the plasma membrane (**6**), and secretory vesicles (**5**) that release their contents into the extracellular space

by fusion with the plasma membrane (**exocytosis**). Protein transport can either proceed continuously (*constitutive*), or it can be *regulated* by chemical signals. The decision regarding which pathway a protein will take and whether its transport will be constitutive or regulated depends on the signal sequences or signal structures that proteins carry with them like address labels. In addition to proteins, the Golgi apparatus also transports membrane lipids to their targets.

B. Smooth endoplasmic reticulum

Regions of the ER that have no bound ribosomes are known as the **smooth endoplasmic reticulum** (sER). In most cells, the proportion represented by the sER is small. A marked sER is seen in cells that have an active lipid metabolism, such as hepatocytes and Leydig cells. The sER is usually made up of branching, closed tubules. Membrane-located enzymes in the sER catalyze **lipid synthesis**. In endocrine cells that form *steroid hormones,* a large proportion of the reaction steps involved also take place in the sER. In the liver's hepatocytes, the proportion represented by the sER is particularly high. It contains enzymes that catalyze so-called **biotransformations**. These are reactions in which non-polar foreign substances, as well as endogenous substances—e. g., steroid hormones— are chemically altered in order to inactivate them and/or prepare them for conjugation with polar substances. Numerous *cytochrome P450 enzymes* are involved in these conversions and can therefore be regarded as the major molecules of the sER. The sER also functions as an intracellular **calcium store**, which normally keeps the Ca^{2+} level in the cytoplasm low. This function is particularly marked in the *sarcoplasmic reticulum*, a specialized form of the sER in muscle cells. For release and uptake of Ca^{2+}, the membranes of the sER contain signal-controlled Ca^{2+} channels and energy-dependent Ca^{2+} ATPases.



Hormone synthesis

Protein sorting

A. Protein sorting

The biosynthesis of all proteins starts on free ribosomes (top). However, the paths that the proteins follow soon diverge, depending on which target they are destined for. Proteins that carry a *signal peptide for the ER* (**1**) follow the *secretory pathway* (right). Proteins that do not have this signal follow the *cytoplasmic pathway* (left).

Secretory pathway. Ribosomes that synthesize a protein with a signal peptide for the ER settle on the ER. The peptide chain is transferred into the lumen of the rER. The presence or absence of other signal sequences and signal regions determines the subsequent transport pathway. Proteins that have *stop-transfer sequences* (**4**) remain as integral membrane proteins in the ER membrane. They then pass into other membranes via vesicular transport. From the rER, their pathway then leads to the Golgi apparatus and then on to the plasma membrane. Proteins destined to remain in the rER—e. g., enzymes—find their way back from the Golgi apparatus to the rER with the help of a *retention signal* (**2**). Other proteins move from the Golgi apparatus to the lysosomes (**3**), to the cell membrane (integral membrane proteins or constitutive exocytosis), or are transported out of the cell (**9;** signal-regulated exocytosis) by secretory vesicles (**8**).

Cytoplasmic pathway. Proteins that do not have a signal peptide for the ER are synthesized in the cytoplasm on free ribosomes, and remain in that compartment. Special signals mediate further transport into the mitochondria (**5**), the nucleus (**6**) or peroxisomes (**7**).

B. Translocation signals

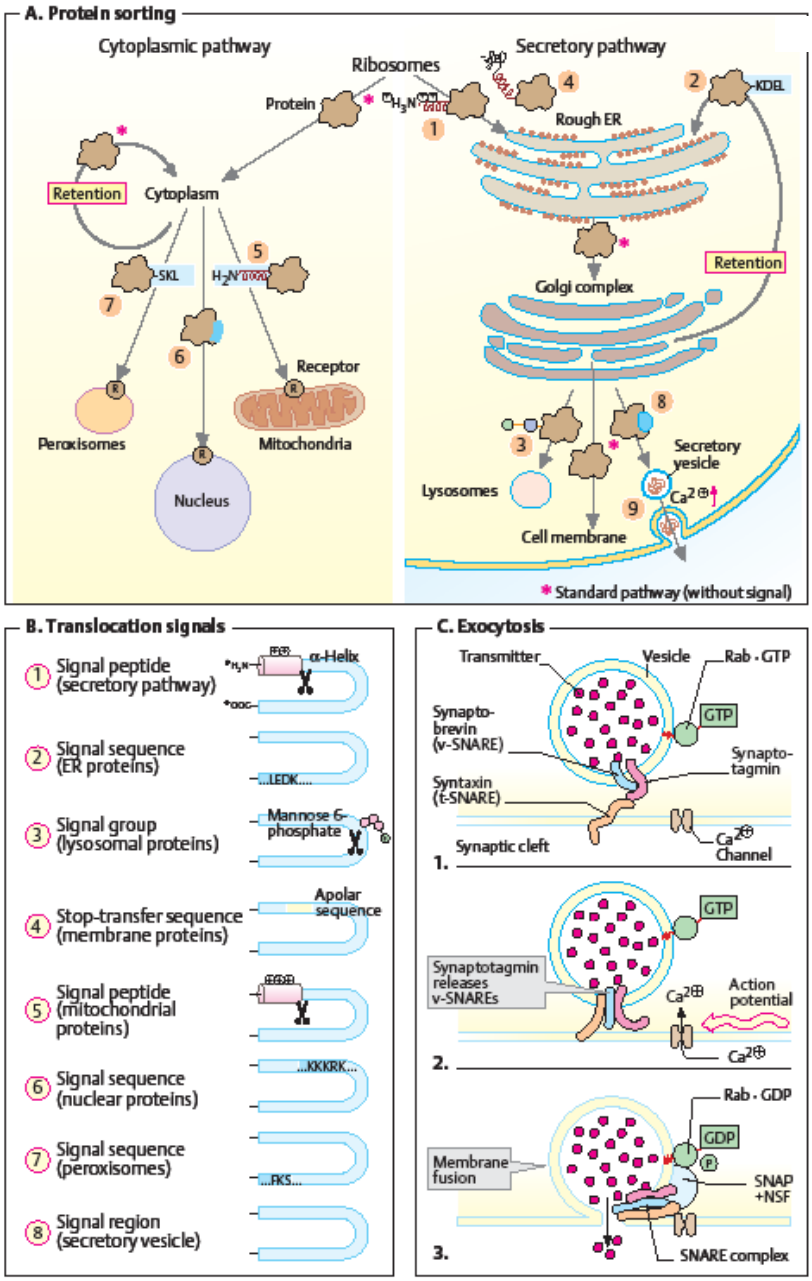
Signal peptides are short sections at the N or C terminus, or within the peptide chain. Areas on the protein surface that are formed by various sections of the chain or by various chains are known as **signal regions**. Signal peptides and signal regions are *structural signals* that are usually recognized by *receptors* on organelles (see **A**). They move the proteins, with the help of additional proteins, into the organelles (*selective protein transfer*). Structural signals can also activate *enzymes* that modify the proteins and thereby determine their subsequent fate. Examples include lysosomal proteins and membrane proteins with lipid anchors. After they have been used, signal peptides at the N terminus are cleaved off by specific hydrolases (symbol: scissors). In proteins that contain several successive signal sequences, this process can expose the subsequent signals. By contrast, signal peptides that have to be read several times are not cleaved.

C. Exocytosis

Exocytosis is a term referring to processes that allow cells to expel substances (e. g., hormones or neurotransmitters) quickly and in large quantities. Using a complex protein machinery, secretory vesicles fuse with the plasma membrane and release their contents. Exocytosis is usually *regulated* by chemical or electrical signals. As an example, the mechanism by which neurotransmitters are released from synapses is shown here, although only the most important proteins are indicated.

The decisive element in exocytosis is the interaction between proteins known as SNAREs that are located on the vesicular membrane (v-SNAREs) and on the plasma membrane (t-SNAREs).

In the resting state (**1**), the v-SNARE *synaptobrevin* is blocked by the vesicular protein *synaptotagmin.* When an action potential reaches the presynaptic membrane, voltage-gated Ca^{2+} channels open. Ca^{2+} flows in and triggers the machinery by conformational changes in proteins. Contact takes place between synaptobrevin and the t-SNARE *synaptotaxin* (**2**). Additional proteins known as SNAPs bind to the SNARE complex and allow fusion between the vesicle and the plasma membrane (**3**). The process is supported by the hydrolysis of GTP by the auxiliary protein *Rab.* The toxin of the bacterium *Clostridium botulinum*, one of the most poisonous substances, destroys components of the exocytosis machinery in synapses through enzymatic hydrolysis, and in this way blocks neurotransmission.



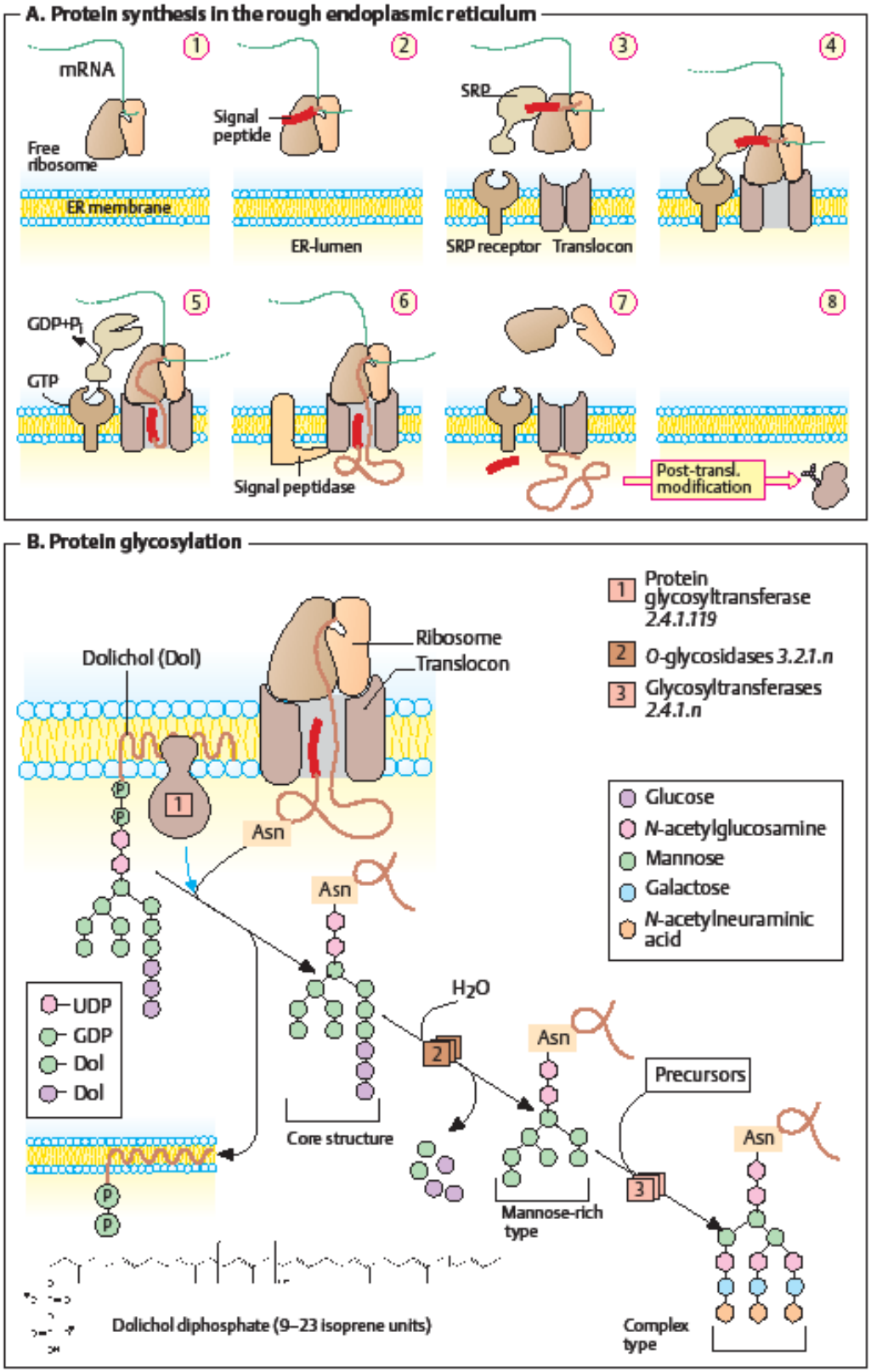
Protein synthesis and maturation

A. Protein synthesis in the rER

With all proteins, protein biosynthesis (**Translation**) starts on free ribosomes in the cytoplasm (**1**). Proteins that are exported out of the cell or into lysosomes, and membrane proteins of the ER and the plasma membrane, carry a **signal peptide for the ER** at their N-terminus. This is a section of 15–60 amino acids. As soon as the signal peptide (red) appears on the surface of the ribosome (**2**), an RNA-containing *signal recognition particle* (**SRP**, green) binds to the sequence and initially interrupts translation (**3**). The SRP then binds to an **SRP receptor** in the rER membrane, and in this way attaches the ribosome to the ER (**4**). After this, the SRP dissociates from the signal peptide and from the SRP receptor and is available again for step 3. This endergonic process is driven by GTP hydrolysis (**5**). Translation now resumes. The remainder of the protein, still unfolded, is gradually introduced into a channel (the **translocon**) in the lumen of the rER (**6**), where a *signal peptidase* located in the inner ER membrane cleaves the signal peptide while translation is still taking place (**7**). This converts the **preprotein** into a **proprotein**, from which the mature protein finally arises after additional post-translational modifications (**8**) in the ER and in the Golgi apparatus. If the growing polypeptide contains a **stop-transfer signal**, then this hydrophobic section of the chain remains stuck in the membrane outside the translocon, and an *integral membrane protein* arises. In the course of translation, an additional signal sequence can re-start the transfer of the chain through the translocon. Several repetitions of this process produce integral membrane proteins with several transmembrane helices.

B. Protein glycosylation

Most extracellular proteins contain covalently bound oligosaccharide residues. For example, all plasma proteins with the exception of albumin are glycosylated. Inside the ER, the carbohydrate parts of the glycoproteins are cotranslationally transferred to the growing chain, and are then converted into their final form while passing through the ER and Golgi apparatus. **N-bound oligosaccharides** are always bound to the amide group of asparagine residues. In **glycosylation,** the carrier molecule to the peptide **is dolichol diphosphate**. As the proprotein passes through the ER, *glycosidases* [2] remove the glucose residues completely and the mannoses partially ("trimming"), thereby producing the **mannose-rich type** of oligosaccharide residues. Subsequently, various *glycosyltransferases* [3] transfer additional monosaccharides (e.g., GlcNAc, galactose, fucose, and N-acetylneuraminic acid) to the mannose-rich intermediate and thereby produce the **complex type** of oligosaccharide. The structure of the final oligosaccharide depends on the type and activity of the glycosyltransferases present in the ER of the cell concerned, and is therefore genetically determined (although indirectly).


A. Protein synthesis in the rough endoplasmic reticulum
mRNA
Free ribosome
ER membrane
Signal peptide
ER-lumen
SRP
SRP receptor
Translocon
GDP+Pi
GTP
Signal peptidase
Post-transl. modification
B. Protein glycosylation
Protein glycosyltransferase 2.4.1.119
O-glycosidases 3.2.1.n
Glycosyltransferases 2.4.1.n
Glucose
N-acetylglucosamine
Mannose
Galactose
N-acetylneuraminic acid
Dolichol (Dol)
Ribosome
Translocon
Asn
UDP
GDP
Dol
Dol
H2O
Core structure
Precursors
Mannose-rich type
Complex type
Dolichol diphosphate (9–23 isoprene units)

Protein maturation

After translation, proteins destined for the secretory pathway first have to fold into their native conformation within the rER. During this process they are supported by various *auxiliary proteins.*

A. Protein folding in the rER

To prevent incorrect folding of the growing protein during protein biosynthesis, **chaperones** in the lumen of the rER bind to the peptide chain and stabilize it until translation has been completed. Binding protein (**BiP**) is an important chaperone in the ER. Many secretory proteins contain several disulfide bonds that are only formed oxidatively from SH groups after translation. Incorrect pairings can block further folding or lead to unstable or insoluble conformations. The enzyme *protein disulfide isomerase* accelerates the equilibration between paired and unpaired cysteine residues, so that incorrect pairs can be quickly split before the protein finds its final conformation.

B. Chaperones and chaperonins

Most proteins fold spontaneously into their native conformation, even in the test tube. In the cell, where there are very high concentrations of proteins, this is more difficult. In the unfolded state, the nonpolar regions of the peptide chain tend to aggregate—due to the hydrophobic effect—with other proteins or with each other to form insoluble products. In addition, unfolded proteins are susceptible to proteinases. To protect partly folded proteins, there are auxiliary proteins called **chaperones** because they guard immature proteins against damaging contacts. Chaperones are formed increasingly during temperature stress and are therefore also known as **heat-shock proteins** (hsp). Several classes of hsp are distinguished. Chaperones of the hsp70 type are common, as are type hsp60 **chaperonins**. Class hsp90 chaperones have special tasks. While small proteins can often reach their native conformation without any help, larger molecules require hsp70 proteins for protection against aggregation which bind as monomers and can dissociate again, dependent on ATP. By contrast, type hsp60 chaperonins form large, barrel-shaped complexes with 14 subunits in which proteins can fold independently while shielded from their environment (4). The function of hsp60 has been investigated in detail in the bacterial chaperonin **GroEL**. The barrel has two chambers, which are closed with a lid (**GroES**) during folding of the guest protein. Driven by ATP hydrolysis, the chambers open and close alternately—i. e., the release of the fully folded protein from one chamber is coupled to the uptake of an unfolded peptide in the second chamber.

Lysosomes

A. Structure and contents

Animal lysosomes are organelles with a diameter of 0.2–2.0 μm with various shapes that are surrounded by a single membrane. There are usually several hundred lysosomes per cell. ATP-driven V-type proton pumps are active in their membranes. As these accumulate H^+ in the lysosomes, the content of lysosomes with pH values of 4.5–5 is much more acidic than the cytoplasm (pH 7–7.3). The lysosomes are the cell's "stomach," serving to break down various cell components. For this purpose, they contain some 40 different types of **hydrolases**, which are

capable of breaking down every type of macromolecule. The marker enzyme of lysosomes is *acid phosphatase.* The pH optimum of lysosomal enzymes is adjusted to the acid pH value and is also in the range of pH 5. At neutral pH, as in the cytoplasm, lysosomal enzymes only have low levels of activity. This appears to be a mechanism for protecting the cells from digesting themselves in case lysosomal enzymes enter the cytoplasm at any time. In plants and fungi, the **cell vacuoles** have the function of lysosomes.

C. Synthesis and transport of lysosomal proteins

Primary lysosomes arise in the region of the Golgi apparatus. **Lysosomal proteins** are synthesized in the rER and are glycosylated there as usual. The next steps are specific for lysosomal proteins (right part of the illustration). In a two-step reaction, terminal mannose residues (Man) are phosphorylated at the C–6 position of the mannose. First, *N*-acetylglucosamine 1-phosphate is transferred to the OH group at C-6 in a terminal mannose residue, and *N*-acetylglucosamine is then cleaved again. Lysosomal proteins therefore carry a terminal **mannose 6–phosphate** (Man6–P; **2**). The membranes of the Golgi apparatus contain receptor molecules that bind Man 6–P. They recognize lysosomal proproteins by this residue and bind them (**3**). With the help of *clathrin,* the receptors are concentrated locally. This allows the appropriate membrane sections to be pinched off and transported to the endolysosomes with the help of transport vesicles (**4**), from which primary lysosomes arise through maturation (**5**). Finally, the phosphate groups are removed from Man 6–P (**6**). The *Man 6–P receptors* are reused. The fall in the pH value in the endolysosomes releases the receptors from the bound proteins (**7**) which are then transported back to the Golgi apparatus with the help of transport vesicles.

Molecular Mechanisms Regulating Hormone Release

Essentially, hormone production is regulated by one or more of three different steps.

1. Hormone synthesis. This can occur through alterations in the synthesis of the protein itself, for example through increased transcription of the gene encoding the hormone, or through conversion of hormone precursors to the hormone, usually through regulation of the synthesis or activity of enzymes that are rate-limiting for hormone synthesis.
2. Hormone release. Hormones can accumulate in the gland until an appropriate signal stimulates their release into the bloodstream. This mechanism permits rapid release of large quantities of hormone that are greater than can be achieved through modification of synthetic rate alone.
3. Functional synthesis and secretory capacity of the hormone-producing organ. Increases (or decreases) in the number and size of endocrine cells actively producing hormone can occur, sometimes as a result of effects of trophic hormones or growth factors on endocrine organs.

Because of the diverse chemical nature of hormones, hormone production involves a large variety of different synthetic pathways and specific regulatory mechanisms.

Peptide Hormones

Peptide hormones are products of specific genes. Thus, the messenger RNA (mRNA) encoding the hormone is translated and the nascent translated product is then targeted to secretory vesicles, via endoplasmic reticulum and Golgi apparatus. In many cases, including with GH, PRL, and PTH, the mature hormone is ready for secretion at this point. Other peptide hormones, including insulin, ACTH, CRH, and glucagon, are present as precursors at this stage. An additional step or steps is required, such as cleavage of the precursor through actions of proteases (in some cases specific proteases) to yield the mature peptide hormone.

Peptide hormone production can be regulated at several different levels. For example, thyroid hormone inhibits TSH gene transcription to ensure feedback inhibition of its own synthesis. Production of angiotensin II is regulated by renin, which cleaves its precursor, angiotensinogen to angiotensin I, which, in turn, is subsequently cleaved to release angiotensin II. Release of insulin stored in secretory vesicles is regulated by glucose that promotes fusion of the secretory vesicles with the plasma membrane, thereby delivering hormone into the circulation. Glucose also stimulates insulin synthesis. Calcium ion regulates both synthesis and release of PTH.

Catecholamines

Catecholamine release is regulated at the level of secretion. Catecholamines are produced by chemical modification of phenylalanine. Cytosolic catecholamines are loaded into secretory granules by actions of specific transporters. Vesicular fusion and catecholamine release is promoted by stimuli that enhance intracellular free calcium mobilization.

Thyroid Hormone

Thyroid hormone is derived from a large protein, thyroglobulin, which is produced by the thyroid gland. The gland selectively takes up iodine that is used to iodinate tyrosine residues within thyroglobulin. There is then "coupling" of iodinated tyrosine residues to link two aromatic rings through an oxygen moiety to form the two-ring thyronines of thyroid hormone. Iodinated thyroglobulin is secreted into the lumen of the thyroid follicle, where it is stored in a form called colloid. TSH promotes endocytosis of iodinated thyroglobulin across basolateral cell membranes, on which the iodinated thyroglobulin is degraded by proteases to release free thyroid hormone, which crosses the vesicular membrane and is released constitutively.

Thyroid hormone is mostly released in a form that contains four iodines, called 3,5,3',5'-tetraiodo-L-thyronine (thyroxine; T_4). However, a significant proportion of thyroid hormone that is released from the gland lacks one or more of the iodine groups, including the major active form of the hormone, which binds nuclear thyroid hormone receptors (TRs) with high affinity, 3,5,3'-triiodo-L-thyronine (triiodothyronine T_3).

Steroids

Steroid hormones are produced mainly in the adrenals, gonads, placenta, and nervous system, although other tissues, including adipocytes, produce steroids in smaller quantities. Steroids are

not stored to a significant degree in endocrine organs. Consequently, hormone release is regulated at the level of synthesis.

Steroid hormones are derived from cholesterol via actions of a series of steroidogenic enzymes of the cytochrome P450 class of oxidases. The first reaction is the primary rate-limiting and hormonally regulated step in biosynthesis of all steroid hormones. It involves cleavage of the cholesterol side chain residues to yield pregnenolone. The reaction is catalyzed by a steroid hydroxylating enzyme P450scc within the mitochondrion. The primary regulatory event is to provide increased substrate for the enzyme. This is achieved through tropic hormone-induced stimulation of the transcription of a gene encoding a protein called steroidogenic acute regulator (StAR). StAR acts at the outer surface of the mitochondrion and promotes increased transport of the cholesterol substrate across the mitochondrial membrane to the steroid synthetic enzyme.

Conversion of cholesterol to pregnenolone is followed by a variety of enzymatic modifications to yield individual steroid hormones. Expression of particular steroidogenic enzymes that catalyze these reactions is regulated at the level of transcription in a tissue-specific manner, thereby ensuring production of the appropriate steroid by the appropriate organ.

Vitamin D_3

Like steroids, vitamin D is derived from cholesterol. Cholesterol is converted to 7-dehydrocholesterol and then to cholecalciferol (vitamin D_3) in skin in response to light. Vitamin D_3 undergoes two additional hydroxylations by mitochondrial cytochrome P450 enzymes in the liver (25-hydroxylation) and kidney (1-hydroxylation) to yield the active form of vitamin D, 1,25-$(OH)_2$-cholecalciferol. The 1-hydroxylation step is rate-limiting and is regulated by PTH and phosphate ion concentrations. Vitamin D_3 can also be obtained from dietary animal sources. In addition, ergocalciferol (vitamin D_2), which differs only slightly from vitamin D_3, can be obtained from plant sources and is metabolized to a form that is approximately equal to 1,25-$(OH)_2$-cholecalciferol in activity.

Eicosanoids

Eicosanoids are mostly derived from arachidonic acid, which, in turn, is synthesized from precursors such as linoleic acid, an essential fatty acid that is stored in cell membranes. Arachidonic acid can be converted to a variety of signaling molecules, leukotrienes, hydroxyeicosatetraenoic acid (HETE) or prostaglandin H_2. The latter is the precursor for other signaling molecules including prostaglandins, prostacyclins, and thromboxanes. The relative abundance of each species depends on the lipid composition of each cell type and activities of individual converting enzymes, which, in turn, are regulated by a variety of stimuli.

Hormone Transport in the Circulation

Secreted hormones move through the bloodstream to endocrine target organs, which are often distant from the initial site of hormone release. The efficiency of delivery from the endocrine organ to the target cell depends on several factors, including the extent to which the hormone is

free in the circulation or bound to transport proteins, hormone metabolism, and rates of clearance of hormone from the circulation.

Most peptide hormones circulate at low concentrations and are not bound to transport proteins and, thus, are completely free for interactions with cell surface receptors. Exceptions include GH, which binds to a protein identical to the hormone-binding portion of the GH receptor; insulin-like growth factor (IGF-I) and IGF-II, which bind to a variety of IGF-binding proteins; and vasopressin and oxytocin, which are bound to neurophysins.

Circulating steroids, thyroid hormones, and vitamin D are bound to plasma proteins via noncovalent interactions (Figure 1–6). The major plasma binding proteins are corticosteroid-binding globulin (CBG), which binds cortisol and progesterone; sex hormone-binding globulin (SHBG), which binds testosterone and estradiol; thyroxine-binding globulin (TBG); and vitamin D-binding protein.

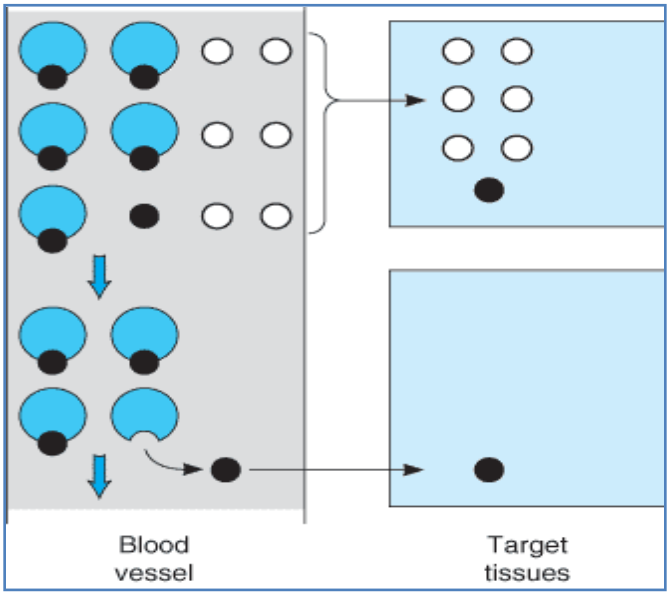


Figure 1-6. Role of plasma binding in delivery of hormones to peripheral tissues. Shown are examples with a hormone that is bound (solid circles) to a plasma protein (large circles) and a hormone that is not bound (open circles). With the bound hormone, only the free fraction is available for tissue uptake. As the free fraction is taken up, additional hormone dissociates from the plasma-binding protein as the blood moves to more distal portions of the tissue and becomes available for tissue uptake. In contrast, all of the hormone that does not bind to plasma proteins is available for uptake by the proximal part of the tissue.

Free hormone that is not bound by plasma proteins is available for receptor binding; dictates feedback inhibition of hormone release; is cleared from the circulation; and in most situations, correlates best with clinical states of hormone excess and deficiency. In most clinical situations, the best measurement of hormone excess or deficiency is that of plasma levels of free hormone.

Generally, transport proteins bind most soluble circulating hormone, so that free hormone is a small portion of the total. Nevertheless, the binding capacity of transport proteins barely exceeds the normal concentrations of the hormone in plasma. Thus, modest decreases in transport proteins or modest elevations of hormone concentrations lead to large increases in free active hormone. Thus, low or high levels of hormone transport proteins generally do not lead to clinical abnormalities but do influence interpretation of laboratory tests.

Transport proteins are thought to facilitate even delivery of hormones across target tissues (Figure 1–6). Thus, a free hormone might be completely sequestered in the proximal portions of

a tissue as the blood flows through it. By contrast, if the hormone is bound to transport proteins, only the free hormone would be sequestered in proximal portions of the tissue, and additional hormone is released from the binding protein as the blood moves distally, making the hormone available for all tissue regions. Differences in the uniformity of hormone delivery probably explain why deletion of the plasma vitamin D-binding protein affects vitamin D action.

Transport of Hormones Across the Membrane

Peptide hormones act on cell surface receptors and therefore do not need to traverse the membrane to signal. Frequently, the hormone-receptor complex is internalized by endocytosis and degraded. This event curtails the immediate hormone signaling event and serves to limit hormone responsiveness through degradation of receptors on the cell surface.

For most nuclear receptor actions, hormones must traverse the cell membrane. Most nuclear receptor ligands are hydrophobic and can diffuse across the lipid bilayer. However, as mentioned above, steroid hormones are sometimes taken up by cells as complexes with plasma binding proteins. Moreover, the influx and efflux of many hormones can vary in different cell types pointing toward active import and export mechanisms. Specific differences in transport in target cells may therefore regulate hormone concentration and action.

Thyroid hormones, in particular, are subject to active import and export mechanisms. These amino acid derivatives can be transported by both amino acid and organic anion transporters. A widely expressed X-linked monocarboxylate transporter 8 (MCT8) can enhance import of T_4, T_3. This protein is mutated in a rare disorder in which patients have high serum levels of thyroid hormone and severe mental retardation. It is believed that MCT8 is required for thyroid hormone uptake into brain neurons and that defects in this transporter cause tissue-specific hypothyroidism in the brain during development.

Metabolism & Elimination of Hormones

Once released, hormones are subject to metabolic modifications. These processes frequently degrade hormones and hormone precursors to inactive forms, limiting the exposure of the tissue to active hormone. However, metabolic modification can serve to convert inactive hormone precursors to active hormones or generate more active hormone products.

Mechanisms of Hormone Action

Hormone Receptors

Hormones bind specifically to hormone receptors, usually with high affinity. Hormone binding promotes allosteric changes within the receptor molecule that translate the signal into biologic activities. The receptors can be expressed on the cell surface or within the cell.

Cell Surface Receptors

Cell surface receptors have ligand recognition domains that are exposed on the outer surface of the cell membrane, one or more membrane-spanning domains, and a ligand-regulated

cytoplasmic effector domain. This organization allows the cell to sense extracellular events and to pass this information to the intracellular environment.

Cell surface receptors can be divided into four types.

(1) Seven-transmembrane domain receptors—also known as GPRs—mediate actions of catecholamines, prostaglandins, ACTH, glucagon, PTH, TSH, LH, and others. They contain a surface-exposed amino terminal domain followed by seven transmembrane domains that span the lipid bilayer and a cytoplasmic hydrophilic carboxyl terminal domain. These receptors are coupled to the guanyl nucleotide binding "G proteins." Binding of ligand to the receptor activates G proteins, which in turn act on effectors such as adenylyl cyclase and phospholipase C and in that way initiate production of second messengers with resultant influences on cell organization, enzymatic activities, and transcription. Ligand also promotes binding of β-arrestins, which limit the primary signaling event. β-arrestins also promote interactions with G protein kinases that trigger alternate second-messenger events with different downstream effects from initial G protein-dependent signaling actions.

(2) Receptors with intrinsic ligand-regulated enzymatic activities mediate actions of growth factors, atrial natriuretic peptide (ANP), and transforming growth factor (TGF)-β. Each contains an amino terminal surface exposed ligand-binding domain, a single membrane-spanning domain, and a carboxyl terminal catalytic domain. Growth factor receptors, including those for insulin and epidermal growth factor (EGF), possess tyrosine kinase activity. Ligand binding results in dimerization, activation of tyrosine kinase, and autophosphorylation. ANP receptors possess intrinsic ligand-regulated guanylyl cyclase activity, which generates the second-messenger guanosine 3',5'-cyclic monophosphate (cGMP).

(3) Cytokine receptors are part of a large family of proteins that bind signaling molecules such as TNF-α. It is now apparent that this class of receptors also mediates the actions of hormones, including GH and leptin. Like growth factor receptors, this class contains a surface-exposed amino terminal domain that binds ligand, a single membrane-spanning domain, and a carboxyl terminal effector domain. Also, like growth factor receptors, cytokine receptors function as dimers. However, unlike growth factor receptors, the cytokine receptors do not possess intrinsic enzymatic activity. Instead, liganded receptors associate with cytoplasmic tyrosine kinases (such as the Janus kinases; JAKs) that mediate downstream signaling events.

(4) Ligand-regulated transporters can bind ligands such as acetylcholine and respond by opening the channel for ion flow. In this case, the ion flux acts as the second-messenger.

Other cell surface molecules resemble "receptors" but transport hormones into cells for degradation. Examples include the type C (clearance) ANP receptor and those for low-density lipoprotein (LDL), mannose 6-phosphate, and transferrin. Each of these proteins shares a short cytoplasmic domain with no known signal transduction function. Because most of these receptors are engaged in internalization by endocytosis and degradation of ligands, they are often called "transporters" rather than receptors.

Nuclear Receptors

Nuclear receptors mediate actions of steroid hormones, vitamin D, thyroid hormones, retinoids, fatty acids, bile acids, eicosanoids, and other molecules. The human genome contains 48 distinct nuclear receptor genes.

In addition to regulation by ligand, nuclear receptors can be activated by second messenger signaling pathways. These ligand-independent activation pathways represent a means by which membrane receptors and nuclear receptors communicate, and a mechanism by which the cellular environment can modulate nuclear receptor function.

Activated nuclear receptors act by binding to DNA response elements in the promoters (sites where transcription is initiated) of target genes or to other transcription factors associated with target genes, or some combination of both. Nuclear receptors recruit large corepressor and coactivator complexes (depending on the state of activation and the cellular environment) that modulate gene expression by modifying chromatin or contacting the basal transcription machinery. The DNA binding elements are termed hormone response elements (HREs) and are specific sequences, usually a repeated hexanucleotide separated by a variable number of nucleotides and aligned either as a direct repeat, a palindrome, or a reverse palindrome. Nuclear receptor-protein interactions modulate activities of heterologous DNA-bound transcription factors leading primarily to ligand-dependent repression of the activities of these factors.

Nuclear receptors have similar overall structures and functions. Each is composed of three domains that can act somewhat independently. The amino terminal domain is not well conserved and mediates effects on transcription. The DNA-binding domain is well conserved throughout the receptor family and mediates HRE recognition and dimerization and contributes to modulation of heterologous transcription factor activity. The carboxyl terminal domain is also well conserved and mediates ligand binding, dimerization, and effects on transcription.

In spite of the overall similarities among nuclear receptors, there are subclasses that differ in the details of their actions. Unliganded steroid receptors form inactive cytoplasmic or nuclear complexes with heat shock proteins. Ligand binding promotes dissociation from the heat shock protein complex and formation of active receptor homodimers that translocate to the nucleus where they recruit coactivators. By contrast, unliganded thyroid hormone, retinoid, vitamin D, and peroxisomal proliferator-activated receptors bind tightly to chromatin, usually as heterodimers with the retinoid X receptor. Here, at positively regulated genes, ligand promotes dissociation of corepressors that have been repressing the gene and subsequent recruitment of coactivators thereby inducing a shift from repression to activation.

There are complex interrelations between nuclear receptors, membrane receptors and their ligands. Activation of nuclear receptors by second messenger signaling is discussed above. Conversely, nuclear receptor ligands can exert rapid effects on the cell membrane. These effects generally occur on time scales that are too fast to be accounted for by alterations in gene transcription. Sometimes these effects involve specialized membrane receptors. Thus, progesterone can antagonize the action of oxytocin by direct and highly selective binding to G protein-coupled membrane oxytocin receptors. Estrogens can bind to a G protein-coupled receptor.

Hormone Binding

Hormones usually bind their receptors with high affinity and trigger biologic effects. The affinity of the hormone for the receptor is usually expressed as the equilibrium dissociation constant, K_D, which is the same as the concentration of the hormone that saturates one half of the available receptors and can be determined experimentally.

Classes of Hormone Action

Hormones and hormone analogs can be classified according to the receptor through which ligand acts (Figure 1–8) or by the type of activity of the ligand (e.g., agonist, antagonist). Thus, a compound that produces estrogenic effects on breast through the estrogen receptor (ER) is an ER agonist. Conversely, a compound that binds to the ER and blocks binding of estrogens but does not allow the receptor to adopt a functionally active state is said to be an ER antagonist.

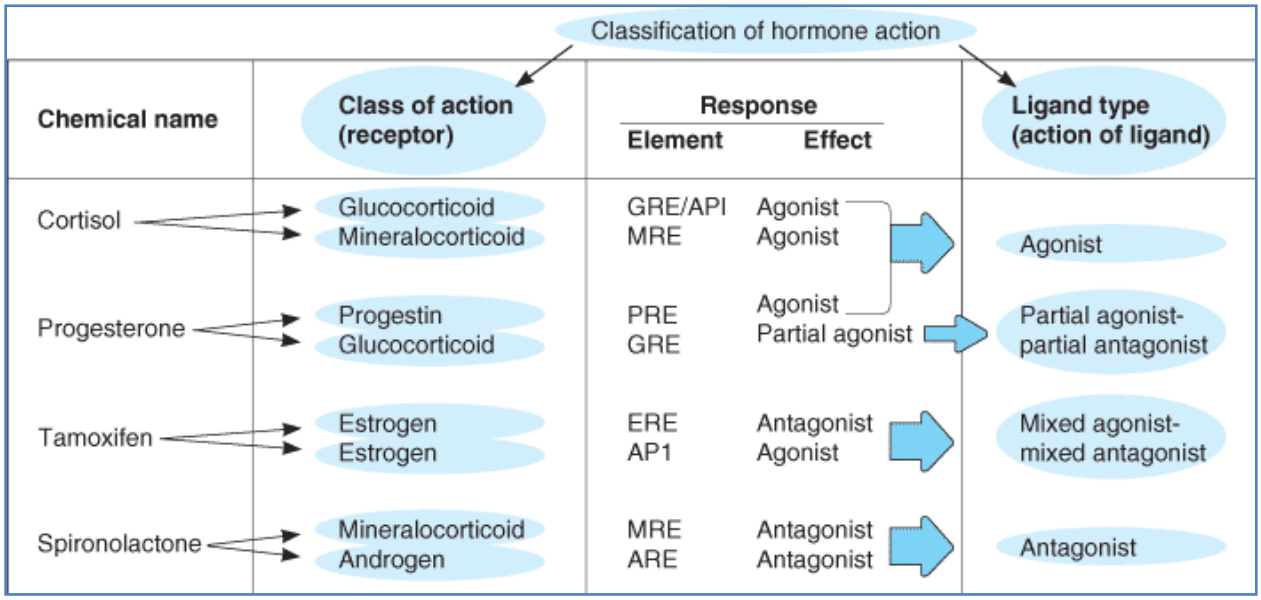


Figure 1-8. Classification of actions of ligands that interact with hormone receptors. Shown are examples of different types of ligands with classification of the type of ligand and the receptors through which they interact. GRE, glucocorticoid response element; AP1, activating protein 1; MRE, mineralocorticoid response element; PRE, progesterone response element; ERE, estrogen response element; ARE, androgen response element.

Classification of Hormone Action by Receptor Type

Traditionally, hormones were classified according to effects they elicited. Glucocorticoids were named for carbohydrate-regulating activities, mineralocorticoids for salt-regulating activities, and pituitary hormones for various tropisms. This nomenclature poses problems. Effects of a particular hormone that are recognized first might represent a subset of its primary effects. For example, glucocorticoids also have anti-inflammatory effects and can influence abdominal fat deposition. Furthermore, several different hormones exert the same effects through the same

receptor (Figures 1–8). For example, both the "glucocorticoid" cortisol and the "mineralocorticoid" aldosterone regulate salt and water metabolism by interactions with mineralocorticoid receptors. Different hormones can also have the same effect through interactions with different receptors. Thus, glucocorticoids and insulin promote glycogen deposition via interactions with their respective receptors. Finally, the same hormone can act through more than one receptor with different physiologic consequences. Prostaglandins, estrogens, and progesterone can act through distinct cell surface and nuclear receptors.

Classification of Hormone Action by Ligand Type

Classification of ligands as agonists, partial agonists-partial antagonists, antagonists, or inactive compounds has been widely utilized.

Inactive Compounds
Inactive compounds do not bind to receptors and have neither agonist nor antagonist activity.

Agonists
Agonists bind to receptors and transforms binding into a response. Most naturally produced ligands are agonists.

Antagonists
Antagonists usually bind to the receptor and thus compete with agonists to block their binding to the receptor, thereby preventing agonist action. Most hormone antagonists bind reversibly in the hormone binding site and are referred to as competitive antagonists. However, other types of antagonists are possible. Noncompetitive antagonists of hormone response can bind receptor in the hormone binding site or some other site and block receptor action.

Natural high-affinity antagonists exist. For example, progesterone can act as a mineralocorticoid or glucocorticoid receptor antagonist but interacts with each of these receptors with low affinity, and normal progesterone concentrations are too low for the steroid to occupy substantial numbers of either receptor. Synthetic receptor hormone antagonists are clinically useful. Examples include the antiestrogens tamoxifen and raloxifene, the antiprogestin and antiglucocorticoid mifepristone (RU 486), the α- and β-adrenergic blockers, and the angiotensin 2 receptor blockers.

Partial Agonist-Partial Antagonists
Partial agonist-antagonists bind to receptors and produce a response that is less than that of a full agonist at saturating ligand concentrations. A partial agonist blocks binding of a full agonist and suppresses receptor activity to the level induced by the partial agonist alone.

Mixed Agonists-Antagonists
These compounds act in different ways through the same receptor type depending on the context (eg, which cells, which promoter). For example, the estrogen "antagonists" tamoxifen and raloxifene act mostly as antagonists in breast but have estrogen agonist actions in bone and uterus. This property has been exploited clinically, because the effects on both breast and bone are useful.

Regulation of Hormone Responsiveness

Responsiveness to hormones is extensively regulated and plays important roles in both physiology and disease. A disease state where this aspect is prominent is type 2 diabetes mellitus, where insulin resistance is a critical component. Continuous hormone stimulation can result in a continuous response, but more commonly hormone responses are self-limiting. The duration of membrane signaling events can have important functional consequences for cell fate. Acute stimulation of MAP kinase cascades in nerve cells can enhance cellular proliferation, whereas chronic stimulation inhibits cellular proliferation and promotes differentiation.

Regulation of Receptor Levels & Activities

Receptor levels and activities are regulated in many different ways (Figure 1–10). Levels of hormone receptors in various tissues influence the extent of response. The homologous hormone commonly regulates receptor levels. This is commonly seen with peptide hormones, where chronic stimulation can decrease the amounts of receptor on the cell surface via internalization and targeting to lysosomes, but it is also observed with some nuclear receptors, where the liganded receptor is degraded more rapidly. Hormone stimulation can result in induction of genes that modulate the levels or activities of nuclear or membrane receptors either positively (feedforward) or negatively (feedback inhibition). Immediate postreceptor signaling events can also exert feedback inhibition of receptor activity. For example, phosphorylation of the β-adrenergic receptor fosters interactions with arrestins that lock the receptor in an unresponsive state. Nuclear receptors can modulate the expression or activities of each other by direct interactions (such as heterodimerization) or by interactions through common cofactors (such as shared coactivators or corepressors). Alternatively, nuclear receptor activities can converge at the level of the same response element, gene promoter, or heterologous transcription factor complexes.

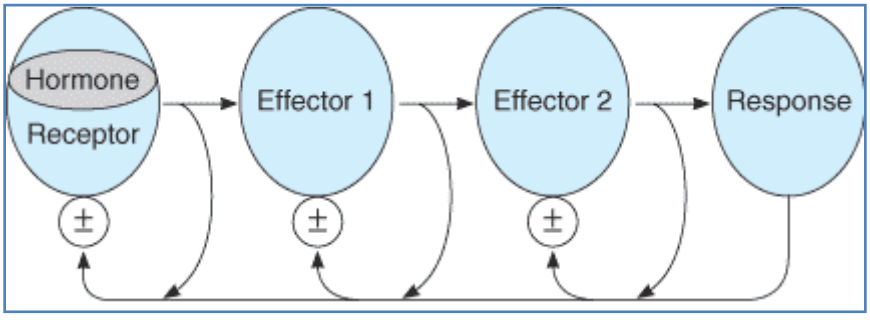


Figure 1-10. Regulation of hormone responsiveness by homologous hormone-receptor complexes can occur at multiple loci. Shown are feedback loops that regulate responsiveness through effects on the receptor, effector, or response limbs in any of the elements of the response network. The plus or minus signs indicate that the influences can be stimulatory or inhibitory.

Hormone Responsiveness Is Regulated by Postreceptor Influences

Postreceptor influences regulate hormone responsiveness (Figure 1–10). Cell surface signals stimulate common or interacting second-messenger systems that modulate downstream signal transduction systems. Cell surface receptors activate second-messenger cascades that lead to

modification of nuclear receptor coactivators and corepressors, or transcription factors that cooperate with the nuclear receptors. Conversely, nuclear receptors regulate expression of genes that regulate downstream signaling pathways. Together, these schemes for cross-talk allow for wide scope in combinatorial interactions between signaling systems. Far downstream effects of hormone action can feed back and influence the response to the original hormone. One example is the effect of insulin on blood sugar levels, which in turn influence insulin action.

Combinatorial Interactions Influence Hormone Signaling in Different Ways

Combinatorial interactions between signaling systems can be synergistic, additive, or antagonistic. Figure 1–11 illustrates a hypothetical synergistic hormone response between different receptors: the response to individual hormones is small, yet the combination produces a response that is greater than additive.

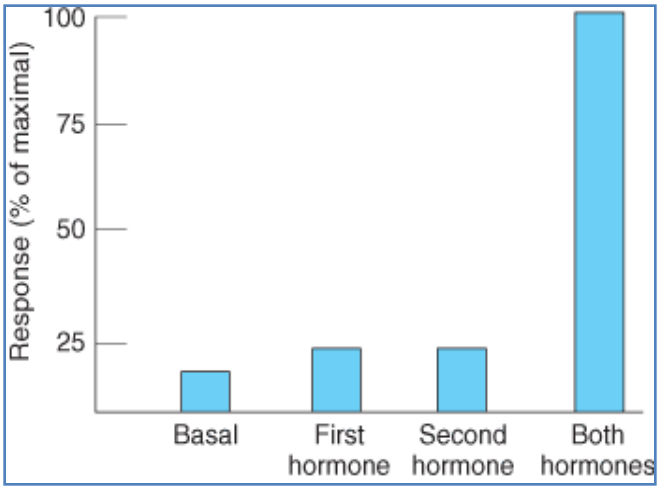


Figure 1-11. Schematic representation of a synergistic hormone response. Note that in this case neither hormone has a major effect alone.

Endocrine & Nervous System Relationships: Neuroendocrinology

Neuroendocrinology deals with interactions between the nervous and endocrine systems. The actions of both systems and their interactions underlie practically every regulatory mechanism in the body.

There are two major mechanisms of neural regulation of endocrine function. The first is neurosecretion, which refers to neurons that secrete hormones into the circulation. The hypothalamus contains neurons that secrete hormones into the general circulation or to blood vessels that communicate with the anterior pituitary. The second is direct autonomic innervation of endocrine tissues, which couples central nervous system signals to hormone release. Examples include innervation of the adrenal medulla, kidney, parathyroid gland, and pancreatic islets and are described in chapters on these organs.

Hypothalamic-Pituitary Relationships

The primary neuroendocrine interface is at the hypothalamus and pituitary, which form a unit that controls peripheral endocrine glands and other physiologic activities. The hypothalamus contains neuronal cells that communicates with other brain regions and regulates many brain functions, including temperature, appetite, thirst, sexual behavior, defensive reactions such as rage and fear, and body rhythms. However, the hypothalamus is also an endocrine gland that releases hormones. The ventral hypothalamus supplies axons and nerve endings to form the posterior pituitary. Neurohypophysial neurons in the posterior pituitary release vasopressin (ADH) and oxytocin into the general circulation. Hypophysiotropic neurons of the hypothalamus

release hormones into the hypothalamic-pituitary blood vessels, which deliver hormones to the anterior pituitary—a major endocrine organ.

Hypothalamic Hormones

The hypophysiotropic neurons of the hypothalamus produce hormones that are secreted into blood vessels, serving the anterior pituitary and regulating hormone release. Stimulating hormones (releasing hormones) include TRH, GnRH, CRH, growth hormone-releasing hormone (GHRH), PRL-releasing factor, and ADH. Inhibitory hormones include somatostatin and dopamine. Some releasing hormones can regulate multiple hormones. For example, TRH stimulates both TSH and PRL release. In contrast, more than one releasing hormone can affect a single pituitary hormone. ACTH release is stimulated both by CRH and by ADH.

In addition to specialized hormones, the hypothalamus produces neurotransmitters, including bioactive amines, peptides, and amino acids. The bioactive amines include dopamine, norepinephrine, epinephrine, serotonin, acetylcholine, gamma-aminobutyric acid (GABA), and histamine. The neuropeptides include VIP, substance P, neurotensin, components of the renin-angiotensin system, cholecystokinin (CCK), opioid peptides, ANP and related peptides, galanin, endothelin, and neuropeptide Y. The amino acids include glutamate and glycine. These neurotransmitters variously affect the anterior and posterior pituitary gland and the central nervous system.

Regulation of Anterior Pituitary Hormone Release

The anterior pituitary produces hormones. It has little innervation, and hormone release is usually regulated by vascular delivery of hypothalamic and peripheral hormones. There are three main patterns of anterior pituitary hormone release (Figure 1–5).

1. Spontaneous brain rhythms promote pulsatile hypothalamic and pituitary hormone release, as illustrated by the patterns of LH and FSH release under control of GnRH from the hypothalamus. The amplitude and frequency of the pulses are governed variously by inputs from the central nervous system and intrinsic properties of the cells. Pulses of LH release can be as frequent as every hour during the follicular phase of the menstrual cycle. Release of other pituitary hormones mostly occurs with rhythmic patterns that vary with different hormones and also can be circadian as is seen with ACTH. Various factors, including food intake, other hormones, and in some cases light can regulate these patterns.

2. Peripheral hormones regulate pituitary hormone release through feedback loops. Thus, cortisol, thyroid hormone, and estrogens inhibit release of their trophic hormones—ACTH, TSH, and LH, respectively. Occasionally, hormones from the target organs exert positive feedback. Contrary to its usual fast-acting negative effect on LH production, sustained estradiol stimulation (48–72 hours) also initiates a preovulatory surge in LH secretion. Feedback influences can be directed at the pituitary or the hypothalamus or at both typically.

3. Intervening factors such as stress, nutritional influences, illnesses, and other hormones affect hormone release. Thus, stress increases the release of ACTH, GH, and PRL; and systemic illness can suppress the hypothalamic-pituitary-thyroid axis and gonadotropin release. Immunomodulators such as interleukin-1 and interleukin-2 and epinephrine increase CRH and ACTH release; and angiotensin II, interleukin-2, CCK, and oxytocin can stimulate ACTH release.

The regulation of PRL and GH is different from that of many other anterior pituitary hormones; neither is subject to the same degree of classic feedback regulatory mechanisms as other hormones, although IGF-I, which is produced in response to GH, can feed back to inhibit GH release. Specific releasing hormones (PRL-releasing factor and GHRH, respectively) and inhibitory hormones (dopamine and somatostatin, respectively) regulate PRL and GH production. The inhibitory hormone is more important for PRL release, whereas the stimulatory hormone is dominant for GH release.

Actions of Hormones

Some of the many effects of hormones are summarized in this section.

Fetal Development

Hormones exert widespread influences on development. Cretinism resulting from severe hypothyroidism, dwarfism resulting from GH deficiency, and inability to develop and survive with a steroid hormone synthesis defect illustrate the profound effects of hormones on development. Hormones influence sexual development, as illustrated by the failure of male sexual development in the androgen-deficient state.

Cell Growth & Cancer

Hormones are important for cell growth. Peptide hormones such as GH, IGF-I, and IGF-II stimulate linear growth and cellular proliferation in other tissues. Other peptides such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and TGF-α and TGF-β are growth factors in multiple tissues and in endocrine glands. Tropic factors regulate growth of target endocrine glands—e.g., ACTH and angiotensin II on the adrenal gland, TSH on the thyroid gland, and LH and FSH on the ovary and testis. Thyroid hormones stimulate growth of several tissues. Steroid hormones can both inhibit and stimulate cell growth. Glucocorticoids inhibit the growth of several cell types and kill some lymphocyte cell types, whereas estradiol, testosterone, and dihydrotestosterone stimulate growth of breast and prostate, respectively.

Hormones influence cancer. Often a hormone influences growth of the cancer cell in the same way that it influences the normal progenitor cells. This is illustrated by the effects of estrogens on breast cancer and androgens on prostate cancer. However, deranged hormone signaling pathways can also cause cancer. Many oncogenes are analogs of growth factors or growth factor receptors, as described above in the section on hormones and oncogenes.

Intermediary Metabolism

Hormones regulate the metabolism of all major classes of macromolecules. Insulin, glucagon, somatostatin, GH, catecholamines (epinephrine, norepinephrine), thyroid hormones, glucocorticoids, and other hormones regulate carbohydrate, fat, protein, amino acid, and nucleic acid metabolism. The hormone signals are coordinated for finely tuned regulation of intermediary metabolism and numerous conditions such as stress or starvation. Insulin is dominant in lowering blood glucose and in stimulating metabolism of glucose and synthesis of fat, proteins, and nucleic acids. In contrast, cortisol, glucagon, catecholamines, and GH elevate blood glucose by diverse mechanisms. However, these hormones differ in their effects on protein, fat, and nucleic acid metabolism. Hormones affect the uptake of glucose, amino acids, nucleosides, and other small molecules. For example, insulin increases glucose uptake by promoting redistribution of glucose transporters to the plasma membrane. Hormones also affect levels and activity of enzymes involved in metabolism, including, among others, those involved in gluconeogenesis, lipolysis, glycogen synthesis, amino acid metabolism and synthesis, and lipid synthesis.

Mineral & Water Metabolism

Hormones affect most aspects of mineral metabolism. Vasopressin regulates serum osmolality and water excretion and has numerous effects in the cardiovascular and central nervous systems. The mineralocorticoid aldosterone regulates sodium and potassium ion balance, and chloride and bicarbonate ion balance to some extent. Other hormones, including ANP, insulin, glucagon, catecholamines, angiotensin II, and PTH, also regulate ionic balance.

Cardiovascular & Renal Function

Hormones profoundly affect the cardiovascular system. These include the renin-angiotensin system, natriuretic peptides, endothelins, catecholamines, steroid hormones, thyroid hormone, prostaglandins, kinins, vasopressin, cytokines, nitric oxide, substance P, and CGRP, and urotensin II. Most of these substances can variously affect heart rate or contractility and constrictor and dilator mechanisms of arteries and veins. The growth regulatory properties of hormones influence cardiovascular development and smooth muscle hyperplasia and hypertrophy and are involved in pathologic processes leading to hypertensive vascular changes, atherosclerosis, heart failure, and cardiac hypertrophy. Hormones influence renal function and blood pressure by regulating renal blood flow; glomerular filtration rate; and the transport of ions, water, and other chemicals. Hormones also regulate active and passive transport processes in kidney through activation, redistribution, and stimulation of the synthesis of channels or by generating energy for active transport. Hormones can have effects on lipoprotein metabolism and on cholesterol transport. Drugs that block the synthesis or actions of many of these hormones, such as converting enzyme inhibitors, β-adrenergic blockers, and mineralocorticoid hormone antagonists, are used extensively in therapy.

Skeletal Function

Bone is constantly being deposited and resorbed. This process is under complex hormonal control. Hormones control growth and mineralization variously through influences on both the matrix and mineral phases of bone. IGF-1 promotes bone growth. Cytokines—particularly TNF—play a major role in bone remodeling. Other hormones that influence bone remodeling include PTH, vitamin D, glucocorticoids, and thyroid hormone. Estrogens promote the accrual of bone matrix at certain points during development and help prevent development of osteoporosis once the skeleton is mature. Excessive amounts of glucocorticoids and thyroid hormone promote bone resorption. PTH stimulates the osteoblast that deposits bone, which, in turn, induces secondary effects on the osteoclast that promote bone resorption. Thus, whereas chronic hyperparathyroidism can result in major bone loss, PTH also is being used in therapy in a more intermittent manner to treat osteoporosis. This illustrates the complex interplay of these hormones and how they can be used to treat disease.

Reproductive Function

Numerous aspects of reproductive function are regulated by hormones. Gonadotropins regulate ovarian and testicular function and the hormone secretion from these organs. Testosterone and DHT regulate development of male sexual characteristics, whereas female sex steroids regulate functions of female reproductive organs. Leptin, secreted from adipose tissue, promotes maturation of the reproductive tract and may trigger the onset of puberty. Pregnancy is regulated by hormones. Progesterone is essential for establishing and maintaining pregnancy in humans. It also decreases myometrial sensitivity to oxytocin, leading to suppression of uterine contractile function. Hormones are critical for egg and sperm development, preparation of the uterus for conception and implantation, and development of the fetus. The placenta produces a number of hormones, some of which are mostly unique (chorionic somatomammotropin, placental lactogen; CS) and others that are also produced abundantly by other endocrine glands (progesterone and other steroid hormones).

Immune System

Hormones have diverse influences on the immune system. As mentioned earlier, the cytokines and other regulators of the immune system are hormones in many contexts and exert other types of paracrine actions. Glucocorticoids blunt immunologic and inflammatory responses; these actions form the basis for the use of glucocorticoids in therapy to suppress these responses when they are excessive. Sex steroids usually suppress the immune response. Estrogens may stimulate antibody production, and females tend to have higher levels of the major immunoglobulin classes under both basal and stimulated conditions. Females tend to have a higher incidence of autoimmune diseases than males and more active cellular and humoral immune responses. These differences are not observed before puberty. Thyroid hormone, GH, catecholamines, PRL, and other hormones influence immunologic or inflammatory functions, but their roles are still being defined.

Pregnancy, with its associated hormonal changes, can ameliorate autoimmune diseases through unknown mechanisms. Pregnancy tends to suppress the cellular but not the humoral immune

responses. This may be critical to prevent maternal rejection of fetal tissues, although susceptibility to a number of viral and fungal diseases is increased. Immunosuppression is most pronounced in the second and third trimesters and rebounds by about 3–6 months postpartum.

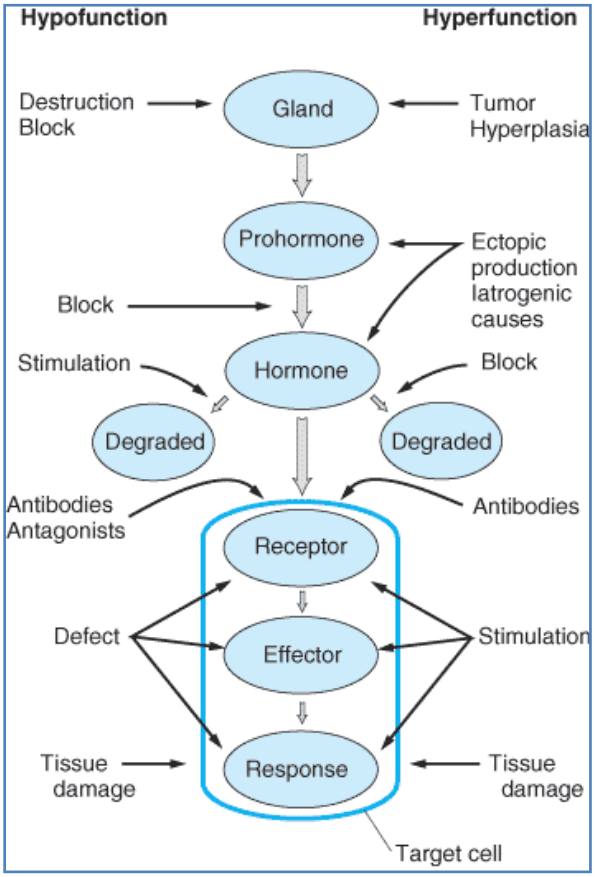
Central Nervous System

The endocrine and nervous systems interact in multiple ways. Hormones regulate behavioral and cognitive functions such as mood, appetite, learning, memory, and sexual activity. Hormones also have secondary influences on the central nervous system through effects on general metabolism. There are a number of examples of mental abnormalities associated with hormone excess or deficiency. These include depressed mental status that can progress to coma with severe hypothyroidism, psychosis that can occur with glucocorticoid excess, and coma that can occur with hypoglycemia due to insulin excess.

Disorders of the Endocrine System

Classic disorders of the endocrine system arise from states of excess or deficiency of hormones, but resistance to hormones also plays a major role in disease. The endocrinologist is also confronted with specific tumors and other problems such as iatrogenic syndromes. The types of abnormalities that in principle can occur are illustrated in Figure 1–13.

Figure 1-13. Causes of hypofunction and hyperfunction of the endocrine system.



Endocrine Hypofunction

Destruction of the Gland

Glandular hypofunction can be caused by destruction of the gland through several different mechanisms. Autoimmune disease is a common cause as with type 1 diabetes mellitus, hypothyroidism, adrenal insufficiency, and gonadal failure. A polyglandular failure syndrome (Schmidt's syndrome) results from autoimmune destruction of several different endocrine glands in the same patient. Destruction of the pituitary is usually due to tumor, hemorrhage, or an infiltrative process. Hypofunction of any of the endocrine glands may result from damage by neoplasms, infection, or hemorrhage.

Extraglandular Disorders

Endocrine hypofunction can be caused by defects outside traditional endocrine glands. In some cases, these are simply due to damage to tissues that produce hormones or convert hormone precursors to active forms. Thus, renal disease can result in defective conversion of 25-OH-cholecalciferol to 1,25-$(OH)_2$-cholecalciferol, with consequent abnormalities in calcium and phosphate balance. Renal disease can also provoke hypoaldosteronism and anemia by damaging the renin-producing juxtaglomerular cells and the erythropoietin-producing cells, respectively.

In some cases, factors that influence hormone degradation or sensitivity can produce or aggravate hormone deficiency when there is insufficient reserve in the endocrine gland. For example, glucocorticoid therapy, which reduces insulin sensitivity, increases the need for insulin and can produce diabetes mellitus or aggravate existing diabetes. Thyroid hormones increase cortisol metabolism, and treatment of hypothyroidism with thyroid hormone can unmask latent adrenal insufficiency.

Defects in Hormone Biosynthesis

Endocrine hypofunction can be due to defects in hormone synthesis. These can be due to defects in genes that: encode hormones; regulate hormone production; encode hormone-producing enzymes; or are involved in hormone metabolism. The 21-hydroxylase deficiency syndrome results in defective cortisol production and is one of the most common genetic diseases. Other adrenal gland defects include 11β-hydroxylase, 17-hydroxylase, and 18-hydroxylase deficiency syndromes. Dietary iodine deficiency results in deficient thyroid hormone biosynthesis and afflicts millions of people worldwide. Mutations in genes encoding polypeptide hormones can decrease hormone production or lead to production of defective hormones. Growth deficiency can result from mutations or deletions in the gene for GH or other genes involved in GH production and signaling pathways. A rare form of diabetes mellitus results from a mutation in the insulin gene, with production of abnormal insulin.

Endocrine Hyperfunction

Hyperfunction of endocrine glands results usually from tumors, hyperplasia, or autoimmune stimulation. Endocrine gland tumors can produce excess hormone. Thus, pituitary tumors can overproduce one of the major classes of pituitary hormones (ACTH, GH, PRL, TSH, LH, and FSH). This leads to stimulation of other glands, as is seen with cortisol excess due to pituitary ACTH-producing tumors or the rare syndrome of hyperthyroidism due to pituitary TSH-

producing tumors. Other examples of tumors in endocrine organs resulting in overproduction of hormones are the following: parathyroid glands, PTH; thyroid parafollicular cells, calcitonin; thyroid follicular cells, thyroglobulin or thyroid hormone; pancreatic islets, insulin, or glucagon; adrenals, cortisol, aldosterone, deoxycorticosterone, androgens, and other steroids; kidney and renin, or erythropoietin. There are also syndromes of multiple endocrine neoplasia (MEN), in which there is a predisposition to develop tumors of several glands. In contrast, most thyroid gland tumors do not overproduce thyroid hormone, and it is rare for ovarian or testicular tumors to overproduce steroids or for posterior pituitary tumors to overproduce oxytocin or vasopressin.

There can also be ectopic production of hormones by tumors. Ectopically produced hormones are usually polypeptide hormones and include ACTH, ADH, and calcitonin. However, other polypeptide hormones such as insulin are rarely if ever expressed ectopically.

Hyperplasia, with increased cellularity and hormone overproduction, can be seen with most endocrine glands. Hyperplasia of the parathyroid glands is seen in renal failure, where low serum calcium and 1,25-$(OH)_2$-cholecalciferol levels stimulate the glands. Hyperplasia is commonly seen in the adrenal glomerulosa, where it results in aldosterone excess and is a major cause of the syndrome of primary aldosteronism. Hyperplasia of the adrenal zonae fasiculata and reticularis results in cortisol excess and Cushing's syndrome and is almost always due to a pituitary ACTH-producing tumor. The cause of hyperplasia of the adrenal zona glomerulosa is not known, and the disorder is, therefore, referred to as idiopathic hyperplasia or idiopathic aldosteronism. Hyperplasia of the thyroid gland is common and may be due to autoimmune stimulation, iodine deficiency with impaired T_4 synthesis and subsequent TSH hypersecretion, or nodular goiter due to genetic biosynthetic abnormalities. Hyperplasia of the ovaries is very common and results in polycystic ovary syndrome, with abnormalities in ovarian steroid production and insulin resistance.

Autoimmune stimulation resulting in hyperfunction is seen most commonly with hyperthyroidism. In this case, antibodies are produced that bind to and activate the TSH receptor. Hyperinsulinism due to autoimmune attack on the pancreatic βcells can be seen transiently early in the course of development of type 1 diabetes mellitus. Otherwise, autoimmune stimulation leading to hyperfunction of endocrine glands is rare.

Defects in Sensitivity to Hormones

Genetic and acquired defects in hormone sensitivity play a crucial role in the pathogenesis of both common and rare disorders. Common disorders include type 2 diabetes mellitus and hypertension. Resistance may be due to a number of different types of defects, for example in the hormone receptor, in functions distal to the receptor, or in functions extrinsic to the receptor-response pathway.

A number of disorders of primary resistance to hormones are due to receptor defects. Genetic defects in receptors that cause resistance syndromes include those for glucocorticoids, thyroid hormones, androgens, vitamin D, leptin, mineralocorticoids, peroxisomal proliferators, ADH, GH, insulin, and TSH. Defects in hormone response that are due to mutations in postreceptor signaling pathways are less well understood. An exception is the syndrome of

pseudohypoparathyroidism, in which mutations occur in the gene encoding the guanyl nucleotide binding protein that links PTH-receptor binding to activation of adenylyl cyclase. Although resistance to thyroid hormone is ordinarily due to mutations in the TR, defects in the receptor were not found in many cases. It is thought that these defects may involve proteins involved in thyroid hormone transport across the membrane or postreceptor loci such as coregulatory proteins.

Hormone resistance that is due to events distal to the ligand-receptor interaction occurs in type 2 diabetes mellitus, the most common form of that disease. Weight reduction and diet can normalize these manifestations in some patients, suggesting that the problem is one of impaired adaptation—perhaps due to excessive down-regulation of responsiveness to stimuli. Syndrome X (metabolic syndrome) is characterized by insulin resistance with overlap into type 2 diabetes mellitus, dyslipidemia with increased triglycerides and elevated LDL and often low high-density lipoprotein cholesterol, hypertension, and polycystic ovarian disease. Although there is debate about this syndrome and its pathogenesis, its manifestations are commonly observed in obese individuals and are associated with much of the hypertension in Western societies. In hypertension, there are variations in sensitivity to salt, to angiotensin II, to release of renin in response to various stimuli, and to other effectors. Although mechanisms for the resistance are poorly understood, insights into disorders such as these should come from a better understanding of the physiologic mechanisms that govern sensitivity to hormones. Figure 1–14 is a schematic representation of resistance to hormones at postreceptor loci.

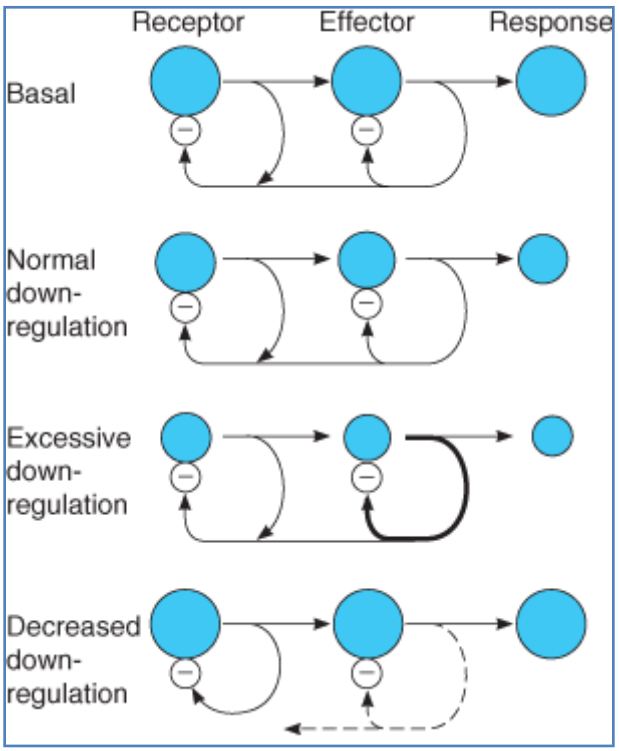


Figure 1-14. Scheme for generation of hyperresponsiveness or hyporesponsiveness to hormones through excessive or impaired down-regulation. The top row indicates the response network before down-regulation occurs. The second row indicates the magnitude of the response network with normal down-regulation. The third row indicates a situation with excessive down-regulation due to a defect in the effector arm of the response with normal receptor function. The sizes of the circles reflect the influence, and the increased size of the arrow reflects the enhanced down-regulation. The fourth row indicates decreased down-regulation at the effector arm of the response. The dotted arrow reflects the decreased down-regulation and the larger response circles, compared with the second panel, reflect the increased response resulting from the decreased down-regulation.

Laboratory Studies

Laboratory tests in endocrine diagnosis are performed to measure hormone levels in body fluids, the effects of the hormone on target cells, or the systemic sequelae of the underlying process. Tests can be done under random or basal conditions, precisely defined conditions, or in response to provocative or suppressive maneuvers. In measuring hormone levels, the sensitivity of the assay refers to the lowest concentration of the hormone that can be accurately detected, and the specificity refers to the extent to which cross-reacting species are scored inappropriately in the assay.

Measurements of Hormone Levels: Basal Levels
Immunoassays are usually used for measurements of hormone levels in body fluids. Most measurements use blood or urine samples and detect active hormone, although there are some instances in which measurement of either a metabolite or precursor of the hormone or a concomitantly released substance sometimes provides the most reliable information. Thus, in assessing vitamin D status, it is usually more informative to measure the precursor hormone, 25-OH-cholecalciferol, even though the final active hormone is 1,25-$(OH)_2$-cholecalciferol.

Plasma & Urine Assays
Although hormone assays reflect levels at the time of sampling, such measurements also provide an integrated assessment of long-term hormonal status for hormones with long half-lives (e.g., T_4). For hormones with shorter half-lives, such as epinephrine or cortisol, the assays provide information relevant only to the time of sampling. Thus, with a pheochromocytoma that episodically releases epinephrine, elevated plasma epinephrine levels would be found only during periods of release and not between them.

Urine assays are generally restricted to measurement of levels of steroid and catecholamine hormones and are not useful for polypeptide hormones. The collection period can be a random sample or, more often, a 24-hour collection. Interpretations of urinary measurements must account for the fact that urinary levels reflect renal handling of the hormone. In the past, urine measurements were utilized more frequently, despite their inconvenience, because larger quantities of the hormone of interest are usually present in urine. However, with modern highly sensitive immunoassays, this advantage is disappearing, and blood measurements are usually preferred.

A further advantage of urinary assays is that they provide an integrated assessment of hormonal status. With cortisol, 1–3% of the hormone released by the adrenal appears in the urine, but measurement of the urine cortisol in a 24-hour "urine free cortisol" sample gives an assessment of integrated cortisol production. This is important; cortisol is released episodically, so a random plasma cortisol sample can be normal in the face of mild to moderate Cushing's disease.

Free Hormone Levels
As discussed earlier, many hormones circulate bound to plasma proteins, and the free hormone fraction is generally that which is biologically relevant. Thus, assessment of free hormone levels is more critical than total hormone levels. Tests that measure free hormone levels can utilize

equilibrium dialysis, ultrafiltration, competitive binding, and other means. Examples of such tests in routine clinical use include plasma free T_4 and free testosterone.

Indirect Measurements of Hormonal Status

Measurement of effects of hormones can be more important diagnostically than measuring the hormone levels and can provide critical complementary information. For example, blood glucose and glycosylated hemoglobin (Hgb_{Alc}) levels are generally more useful than plasma insulin levels in diagnosing and treating diabetes mellitus. Plasma insulin levels can be high in the face of hyperglycemia in type 2 diabetes mellitus, and insulin levels are a less reliable index of diabetic status in type 1 diabetes mellitus than blood glucose and Hgb_{Alc}.

Provocative & Suppression Tests

In many cases, the level of a hormone or parameter affected by a hormone is best interpreted following a dynamic challenge either to provoke or suppress hormone secretion. For example, the pulsatile nature of cortisol release results in fluctuating plasma cortisol levels that must be measured under defined conditions. This problem is bypassed in evaluation of adrenal insufficiency by administering an ACTH analog that maximally stimulates the adrenal and of Cushing's syndrome by administrating dexamethasone at levels capable of suppressing normal ACTH and hence cortisol output.

Imaging Studies

Imaging studies are used in diagnosis and follow-up of endocrine diseases. Magnetic resonance imaging (MRI) and computed tomography (CT) allow visualization of endocrine glands and endocrine tumors at high resolution. These procedures are especially useful for evaluation of tumors of the pituitary and adrenals. Scanning the thyroid gland using radioactive iodine is useful for evaluating the functional status of the thyroid.

Biopsy Procedures

Biopsies are not commonly used for evaluation of endocrine diseases but are occasionally useful in the diagnosis of neoplasia. An exception is use of fine-needle biopsy of the thyroid gland, which has had a major impact on evaluation of thyroid nodules.

Diagnosis of Genetic Disease

Diagnosis of genetic diseases is facilitated greatly using DNA analyses. Thus, DNA can be obtained from peripheral blood cells, the region of interest can be amplified by PCR, and the gene can be rapidly sequenced. In cases where the mutation is known, these procedures can lead to rapid and accurate diagnosis in the general population or in kindreds with known mutations, such as those with maturity-onset diabetes of the young (MODY), or medullary carcinoma of the thyroid.

Large-scale sequencing of genes that contribute to a particular disease phenotype can improve diagnosis. For example, MODY is caused by monogenic defects in several different genes (including glucokinase or pancreatic transcription factors) that lead to glucose intolerance and diabetes-like symptoms. Sequencing of possible MODY genes in patients—along with analysis of other family members—can distinguish subsets of patients with MODY from those with type 1 diabetes and guide treatment.