The Diversity of the Endocrine System

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ACTH	Adrenocorticotropic hormone
ANF	Atrial natriuretic factor
cAMP	Cyclic adenosine monophosphate
CBG	Corticosteroid-binding globulin
CG	Chorionic gonadotropin
cGMP	Cyclic guanosine monophosphate
CLIP	Corticotropin-like intermediate lobe peptide
DBH	Dopamine β-hydroxylase
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DIT	Diiodotyrosine
DOC	Deoxycorticosterone
EGF	Epidermal growth factor
FSH	Follicle-stimulating hormone
GH	Growth hormone
IGF-I	Insulin-like growth factor-I
LH	Luteotropic hormone
LPH	Lipotropin
MIT	Monoiodotyrosine
MSH	Melanocyte-stimulating hormone
OHSD	Hydroxysteroid dehydrogenase
PNMT	Phenylethanolamine-*N*-methyltransferase
POMC	Pro-opiomelanocortin
SHBG	Sex hormone-binding globulin
StAR	Steroidogenic acute regulatory (protein)
TBG	Thyroxine-binding globulin
TEBG	Testosterone-estrogen-binding globulin
TRH	Thyrotropin-releasing hormone
TSH	Thyrotropin-stimulating hormone

BIOMEDICAL IMPORTANCE

The survival of multicellular organisms depends on their ability to adapt to a constantly changing environment. Intercellular communication mechanisms are necessary requirements for this adaptation. The nervous system and the endocrine system provide this intercellular, organism-wide communication. The nervous system was originally viewed as providing a fixed communication system, whereas the endocrine system supplied hormones, which are mobile messages. In fact, there is a remarkable convergence of these regulatory systems. For example, neural regulation of the endocrine system is important in the production and secretion of some hormones; many neurotransmitters resemble hormones in their synthesis, transport, and mechanism of action; and many hormones are synthesized in the nervous system. The word "hormone" is derived from a Greek term that means to arouse to activity. As classically defined, a hormone is a substance that is synthesized in one organ and transported by the circulatory system to act on another tissue. However, this original description is too restrictive because hormones can act on adjacent cells (paracrine action) and on the cell in which they were synthesized (autocrine action) without entering the systemic circulation. A diverse array of hormones—each with distinctive mechanisms of action and properties of biosynthesis, storage, secretion, transport, and metabolism—has evolved to provide homeostatic responses. This biochemical diversity is the topic of this chapter.

THE TARGET CELL CONCEPT

There are about 200 types of differentiated cells in humans. Only a few produce hormones, but virtually all of the 75 trillion cells in a human are targets of one or more of the over 50 known hormones. The concept of the target cell is a useful way of looking at hormone action. It was thought that hormones affected a single cell type—or only a few kinds of cells—and that a hormone elicited a unique biochemical or physiologic action. We now know that a given hormone can affect several different cell types; that more than one hormone can affect a given cell type; and that hormones can exert many dif-

ferent effects in one cell or in different cells. With the discovery of specific cell-surface and intracellular hormone receptors, the definition of a target has been expanded to include any cell in which the hormone (ligand) binds to its receptor, whether or not a biochemical or physiologic response has yet been determined.

Several factors determine the response of a target cell to a hormone. These can be thought of in two general ways: (1) as factors that affect the concentration of the hormone at the target cell (see Table 42–1) and (2) as factors that affect the actual response of the target cell to the hormone (see Table 42–2).

HORMONE RECEPTORS ARE OF CENTRAL IMPORTANCE

Receptors Discriminate Precisely

One of the major challenges faced in making the hormone-based communication system work is illustrated in Figure 42–1. Hormones are present at very low concentrations in the extracellular fluid, generally in the range of 10^{-15} to 10^{-9} mol/L. This concentration is much lower than that of the many structurally similar molecules (sterols, amino acids, peptides, proteins) and other molecules that circulate at concentrations in the 10^{-5} to 10^{-3} mol/L range. Target cells, therefore, must distinguish not only between different hormones present in small amounts but also between a given hormone and the 10^{6}- to 10^{9}-fold excess of other similar molecules. This high degree of discrimination is provided by cell-associated recognition molecules called receptors. Hormones initiate their biologic effects by binding to specific receptors, and since any effective control system also must provide a means of stopping a response, hormone-induced actions generally terminate when the effector dissociates from the receptor.

A target cell is defined by its ability to selectively bind a given hormone to its cognate receptor. Several biochemical features of this interaction are important in order for hormone-receptor interactions to be physiologically relevant: (1) binding should be specific, ie, displaceable by agonist or antagonist; (2) binding should be saturable; and (3) binding should occur within the concentration range of the expected biologic response.

Table 42–1. Determinants of the concentration of a hormone at the target cell.

The rate of synthesis and secretion of the hormones.
The proximity of the target cell to the hormone source (dilution effect).
The dissociation constants of the hormone with specific plasma transport proteins (if any).
The conversion of inactive or suboptimally active forms of the hormone into the fully active form.
The rate of clearance from plasma by other tissues or by digestion, metabolism, or excretion.

Table 42–2. Determinants of the target cell response.

The number, relative activity, and state of occupancy of the specific receptors on the plasma membrane or in the cytoplasm or nucleus.
The metabolism (activation or inactivation) of the hormone in the target cell.
The presence of other factors within the cell that are necessary for the hormone response.
Up- or down-regulation of the receptor consequent to the interaction with the ligand.
Postreceptor desensitzation of the cell, including down-regulation of the receptor.

Both Recognition & Coupling Domains Occur on Receptors

All receptors have at least two functional domains. A recognition domain binds the hormone ligand and a second region generates a signal that couples hormone recognition to some intracellular function. Coupling (signal transduction) occurs in two general ways. Polypeptide and protein hormones and the catecholamines bind to receptors located in the plasma membrane and thereby generate a signal that regulates various intracellular functions, often by changing the activity of an enzyme. In contrast, steroid, retinoid, and thyroid hormones interact with intracellular receptors, and it is this ligand-receptor complex that directly provides the signal, generally to specific genes whose rate of transcription is thereby affected.

The domains responsible for hormone recognition and signal generation have been identified in the protein polypeptide and catecholamine hormone receptors. Steroid, thyroid, and retinoid hormone receptors have several functional domains: one site binds the hormone; another binds to specific DNA regions; a third is involved in the interaction with other coregulator proteins that result in the activation (or repression) of gene transcription; and a fourth may specify binding to one or more other proteins that influence the intracellular trafficking of the receptor.

The dual functions of binding and coupling ultimately define a receptor, and it is the coupling of hormone binding to signal transduction—so-called **receptor-effector coupling**—that provides the first step in amplification of the hormonal response. This dual purpose also distinguishes the target cell receptor from the

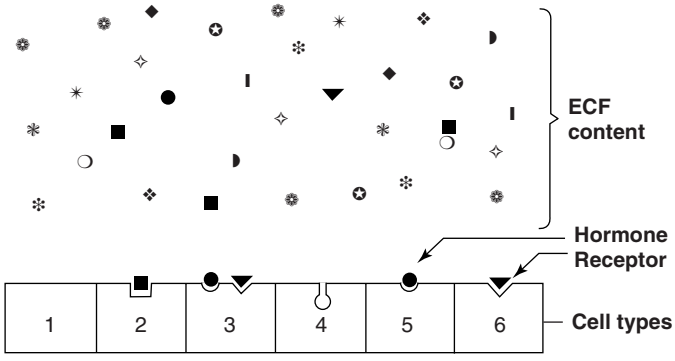


Figure 42–1. Specificity and selectivity of hormone receptors. Many different molecules circulate in the extracellular fluid (ECF), but only a few are recognized by hormone receptors. Receptors must select these molecules from among high concentrations of the other molecules. This simplified drawing shows that a cell may have no hormone receptors (1), have one receptor (2+5+6), have receptors for several hormones (3), or have a receptor but no hormone in the vicinity (4).

plasma carrier proteins that bind hormone but do not generate a signal (see Table 42–6).

Receptors Are Proteins

Several classes of peptide hormone receptors have been defined. For example, the insulin receptor is a heterotetramer ($\alpha_2\beta_2$) linked by multiple disulfide bonds in which the extracellular α subunit binds insulin and the membrane-spanning β subunit transduces the signal through the tyrosine protein kinase domain located in the cytoplasmic portion of this polypeptide. The receptors for insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) are generally similar in structure to the insulin receptor. The growth hormone and prolactin receptors also span the plasma membrane of target cells but do not contain intrinsic protein kinase activity. Ligand binding to these receptors, however, results in the association and activation of a completely different protein kinase pathway, the Jak-Stat pathway. Polypeptide hormone and catecholamine receptors, which transduce signals by altering the rate of production of cAMP through G-proteins, are characterized by the presence of seven domains that span the plasma membrane. Protein kinase activation and the generation of cyclic AMP, (cAMP, 3′5′-adenylic acid; see Figure 20–5) is a downstream action of this class of receptor (see Chapter 43 for further details).

A comparison of several different steroid receptors with thyroid hormone receptors revealed a remarkable conservation of the amino acid sequence in certain regions, particularly in the DNA-binding domains. This led to the realization that receptors of the steroid or thyroid type are members of a large superfamily of nuclear receptors. Many related members of this family have no known ligand at present and thus are called orphan receptors. The nuclear receptor superfamily plays a critical role in the regulation of gene transcription by hormones, as described in Chapter 43.

HORMONES CAN BE CLASSIFIED IN SEVERAL WAYS

Hormones can be classified according to chemical composition, solubility properties, location of receptors, and the nature of the signal used to mediate hormonal action within the cell. A classification based on the last two properties is illustrated in Table 42–3, and general features of each group are illustrated in Table 42–4.

The hormones in group I are lipophilic. After secretion, these hormones associate with plasma transport or carrier proteins, a process that circumvents the problem of solubility while prolonging the plasma half-life of the hormone. The relative percentages of bound and free hormone are determined by the binding affinity and binding capacity of the transport protein. The free hormone, which is the biologically active form, readily traverses the lipophilic plasma membrane of all cells and encounters receptors in either the cytosol or nucleus of target cells. The ligand-receptor complex is assumed to be the intracellular messenger in this group.

The second major group consists of water-soluble hormones that bind to the plasma membrane of the target cell. Hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called **second messengers** (the hormone itself is the first messenger), which are generated as a consequence of the ligand-receptor interaction. The second messenger concept arose from an observation that epinephrine binds to the plasma membrane of certain cells and increases intracellular cAMP. This was followed by a series of experiments in which cAMP was found to mediate the effects of many hormones. Hormones that clearly employ this mechanism are shown in group II.A of Table 42–3. To date, only one hormone, atrial natriuretic factor (ANF), uses cGMP as its second messenger, but other hormones will probably be added to group II.B. Several hormones, many of which were previously thought to affect cAMP, appear to use ionic calcium (Ca^{2+}) or

Table 42–3. Classification of hormones by mechanism of action.

I. Hormones that bind to intracellular receptors
- Androgens
- Calcitriol (1,25[OH]$_2$-D$_3$)
- Estrogens
- Glucocorticoids
- Mineralocorticoids
- Progestins
- Retinoic acid
- Thyroid hormones (T$_3$ and T$_4$)

II. Hormones that bind to cell surface receptors

A. The second messenger is cAMP:
- α_2-Adrenergic catecholamines
- β-Adrenergic catecholamines
- Adrenocorticotropic hormone
- Antidiuretic hormone
- Calcitonin
- Chorionic gonadotropin, human
- Corticotropin-releasing hormone
- Follicle-stimulating hormone
- Glucagon
- Lipotropin
- Luteinizing hormone
- Melanocyte-stimulating hormone
- Parathyroid hormone
- Somatostatin
- Thyroid-stimulating hormone

B. The second messenger is cGMP:
- Atrial natriuretic factor
- Nitric oxide

C. The second messenger is calcium or phosphatidylinositols (or both):
- Acetylcholine (muscarinic)
- α_1-Adrenergic catecholamines
- Angiotensin II
- Antidiuretic hormone (vasopressin)
- Cholecystokinin
- Gastrin
- Gonadotropin-releasing hormone
- Oxytocin
- Platelet-derived growth factor
- Substance P
- Thyrotropin-releasing hormone

D. The second messenger is a kinase or phosphatase cascade:
- Chorionic somatomammotropin
- Epidermal growth factor
- Erythropoietin
- Fibroblast growth factor
- Growth hormone
- Insulin
- Insulin-like growth factors I and II
- Nerve growth factor
- Platelet-derived growth factor
- Prolactin

Table 42–4. General features of hormone classes.

	Group I	Group II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma half-life	Long (hours to days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, metabolites of complex phosphoinositols, kinase cascades

metabolites of complex phosphoinositides (or both) as the intracellular signal. These are shown in group II.C of the table. The intracellular messenger for group II.D is a protein kinase-phosphatase cascade. Several of these have been identified, and a given hormone may use more than one kinase cascade. A few hormones fit into more than one category, and assignments change as new information is brought forward.

DIVERSITY OF THE ENDOCRINE SYSTEM

Hormones Are Synthesized in a Variety of Cellular Arrangements

Hormones are synthesized in discrete organs designed solely for this specific purpose, such as the thyroid (triiodothyronine), adrenal (glucocorticoids and mineralocorticoids), and the pituitary (TSH, FSH, LH, growth hormone, prolactin, ACTH). Some organs are designed to perform two distinct but closely related functions. For example, the ovaries produce mature oocytes and the reproductive hormones estradiol and progesterone. The testes produce mature spermatozoa and testosterone. Hormones are also produced in specialized cells within other organs such as the small intestine (glucagon-like peptide), thyroid (calcitonin), and kidney (angiotensin II). Finally, the synthesis of some hormones requires the parenchymal cells of more than one organ—eg, the skin, liver, and kidney are required for the production of 1,25(OH)$_2$-D$_3$ (calcitriol). Examples of this diversity in the approach to hormone synthesis, each of which has evolved to fulfill a specific purpose, are discussed below.

Hormones Are Chemically Diverse

Hormones are synthesized from a wide variety of chemical building blocks. A large series is derived from cholesterol. These include the glucocorticoids, mineralocorticoids, estrogens, progestins, and $1,25(OH)_2$-D_3 (see Figure 42–2). In some cases, a steroid hormone is the precursor molecule for another hormone. For example, progesterone is a hormone in its own right but is also a precursor in the formation of glucocorticoids, mineralocorticoids, testosterone, and estrogens. Testosterone is an obligatory intermediate in the biosynthesis of estradiol and in the formation of dihydrotestosterone (DHT). In these examples, described in detail below, the final product is determined by the cell type and the associated set of enzymes in which the precursor exists.

The amino acid tyrosine is the starting point in the synthesis of the catecholamines and of the thyroid hormones tetraiodothyronine (thyroxine; T_4) and triiodothyronine (T_3) (Figure 42–2). T_3 and T_4 are unique in that they require the addition of iodine (as I^-) for bioactivity. Because dietary iodine is very scarce in many parts of the world, an intricate mechanism for accumulating and retaining I^- has evolved.

Many hormones are polypeptides or glycoproteins. These range in size from thyrotropin-releasing hormone (TRH), a tripeptide, to single-chain polypeptides like adrenocorticotropic hormone (ACTH; 39 amino acids), parathyroid hormone (PTH; 84 amino acids), and growth hormone (GH; 191 amino acids) (Figure 42–2). Insulin is an AB chain heterodimer of 21 and 30 amino acids, respectively. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and chorionic gonadotropin (CG) are glycoprotein hormones of αβ heterodimeric structure. The α chain is identical in all of these hormones, and distinct β chains impart hormone uniqueness. These hormones have a molecular mass in the range of 25–30 kDa depending on the degree of glycosylation and the length of the β chain.

Hormones Are Synthesized & Modified For Full Activity in a Variety of Ways

Some hormones are synthesized in final form and secreted immediately. Included in this class are the hormones derived from cholesterol. Others such as the catecholamines are synthesized in final form and stored in the producing cells. Others are synthesized from precursor molecules in the producing cell, then are processed and secreted upon a physiologic cue (insulin). Finally, still others are converted to active forms from precursor molecules in the periphery (T_3 and DHT). All of these examples are discussed in more detail below.

MANY HORMONES ARE MADE FROM CHOLESTEROL

Adrenal Steroidogenesis

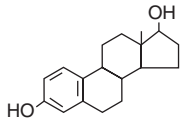
The adrenal steroid hormones are synthesized from cholesterol. Cholesterol is mostly derived from the plasma, but a small portion is synthesized in situ from acetyl-CoA via mevalonate and squalene. Much of the cholesterol in the adrenal is esterified and stored in cytoplasmic lipid droplets. Upon stimulation of the adrenal by ACTH, an esterase is activated, and the free cholesterol formed is transported into the mitochondrion, where a **cytochrome P450 side chain cleavage enzyme (P450scc)** converts cholesterol to pregnenolone. Cleavage of the side chain involves sequential hydroxylations, first at C_{22} and then at C_{20}, followed by side chain cleavage (removal of the six-carbon fragment isocaproaldehyde) to give the 21-carbon steroid (Figure 42–3, top). An ACTH-dependent **steroidogenic acute regulatory (StAR) protein** is essential for the transport of cholesterol to P450scc in the inner mitochondrial membrane.

All mammalian steroid hormones are formed from cholesterol via pregnenolone through a series of reactions that occur in either the mitochondria or endoplasmic reticulum of the adrenal cell. Hydroxylases that require molecular oxygen and NADPH are essential, and dehydrogenases, an isomerase, and a lyase reaction are also necessary for certain steps. There is cellular specificity in adrenal steroidogenesis. For instance, 18-hydroxylase and 19-hydroxysteroid dehydrogenase, which are required for aldosterone synthesis, are found only in the zona glomerulosa cells (the outer region of the adrenal cortex), so that the biosynthesis of this mineralocorticoid is confined to this region. A schematic representation of the pathways involved in the synthesis of the three major classes of adrenal steroids is presented in Figure 42–4. The enzymes are shown in the rectangular boxes, and the modifications at each step are shaded.

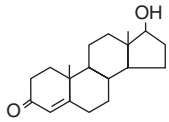
A. Mineralocorticoid Synthesis

Synthesis of aldosterone follows the mineralocorticoid pathway and occurs in the zona glomerulosa. Pregnenolone is converted to progesterone by the action of two smooth endoplasmic reticulum enzymes, **3β-hydroxysteroid dehydrogenase (3β-OHSD)** and **$\Delta^{5,4}$-isomerase.** Progesterone is hydroxylated at the C_{21} position to form 11-deoxycorticosterone (DOC), which is an active (Na^+-retaining) mineralocorticoid. The next hydroxylation, at C_{11}, produces corticosterone, which has glucocorticoid activity and is a weak mineralocorticoid (it has less than 5% of the potency of aldosterone). In some species (eg, rodents), it is the most potent glucocorticoid.

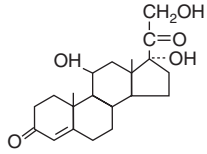
A. CHOLESTEROL DERIVATIVES



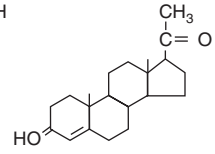
17ß-Estradiol



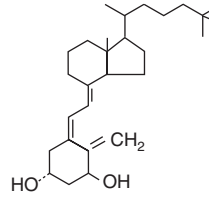
Testosterone



Cortisol

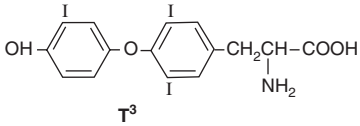


Progesterone

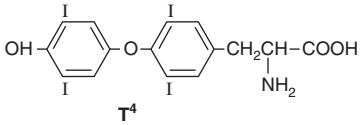


$1,25(OH)_2\text{-}D_3$

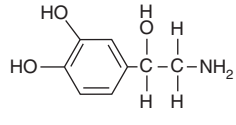
B. TYROSINE DERIVATIVES



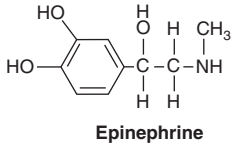
T^3



T^4

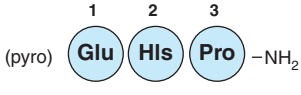


Norepinephrine

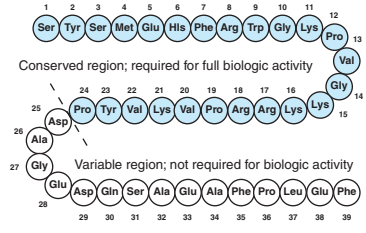


Epinephrine

C. PEPTIDES OF VARIOUS SIZES



TRH



Structure of human ACTH.

ACTH

D. GLYCOPROTEINS (TSH, FSH, LH)

common α subunits

unique β subunits

Figure 42–2. Chemical diversity of hormones. **A.** Cholesterol derivatives. **B.** Tyrosine derivatives. **C.** Peptides of various sizes **D.** Glycoproteins (TSH, FSH, LH) with common α subunits and unique β subunits.

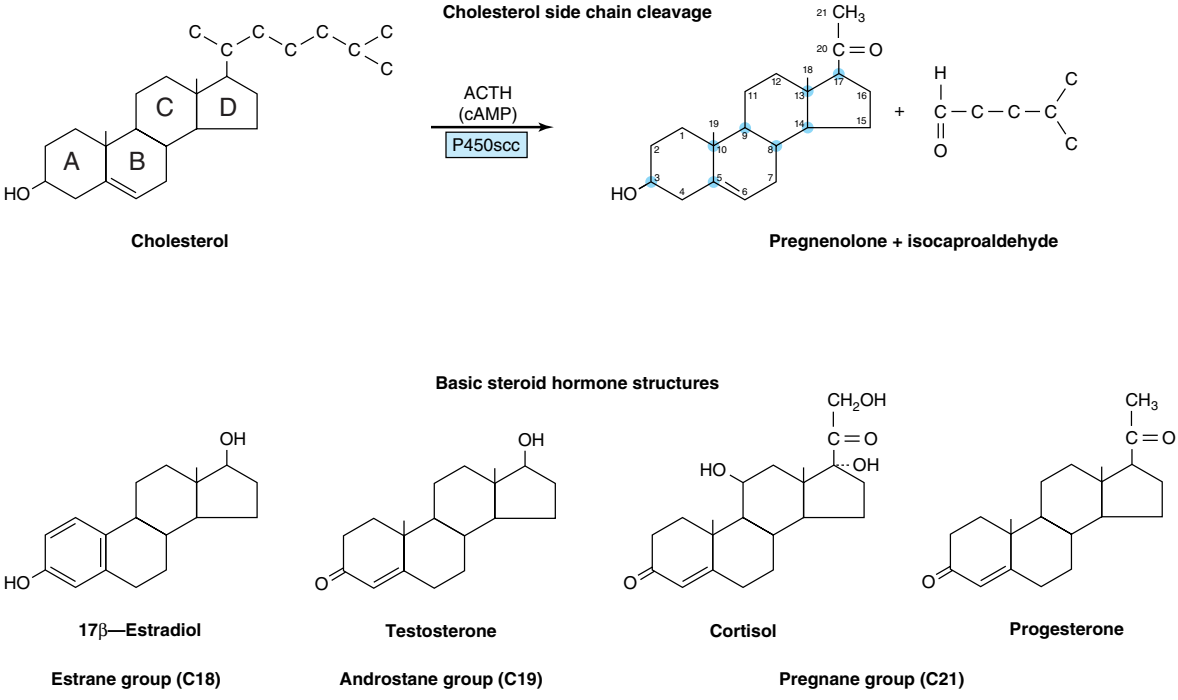


Figure 42–3. Cholesterol side-chain cleavage and basic steroid hormone structures. The basic sterol rings are identified by the letters A–D. The carbon atoms are numbered 1–21 starting with the A ring. Note that the estrane group has 18 carbons (C18), etc.

C_{21} hydroxylation is necessary for both mineralocorticoid and glucocorticoid activity, but most steroids with a C_{17} hydroxyl group have more glucocorticoid and less mineralocorticoid action. In the zona glomerulosa, which does not have the smooth endoplasmic reticulum enzyme 17α-hydroxylase, a mitochondrial 18-hydroxylase is present. The **18-hydroxylase (aldosterone synthase)** acts on corticosterone to form 18-hydroxycorticosterone, which is changed to aldosterone by conversion of the 18-alcohol to an aldehyde. This unique distribution of enzymes and the special regulation of the zona glomerulosa by K^+ and angiotensin II have led some investigators to suggest that, in addition to the adrenal being two glands, the adrenal cortex is actually two separate organs.

B. Glucocorticoid Synthesis

Cortisol synthesis requires three hydroxylases located in the fasciculata and reticularis zones of the adrenal cortex that act sequentially on the C_{17}, C_{21}, and C_{11} positions. The first two reactions are rapid, while C_{11} hydroxylation is relatively slow. If the C_{11} position is hydroxylated first, the action of **17α-hydroxylase** is impeded and the mineralocorticoid pathway is followed (forming corticosterone or aldosterone, depending on the cell type). 17α-Hydroxylase is a smooth endoplasmic reticulum enzyme that acts upon either progesterone or, more commonly, pregnenolone. 17α-Hydroxyprogesterone is hydroxylated at C_{21} to form 11-deoxycortisol, which is then hydroxylated at C_{11} to form cortisol, the most potent natural glucocorticoid hormone in humans. 21-Hydroxylase is a smooth endoplasmic reticulum enzyme, whereas 11β-hydroxylase is a mitochondrial enzyme. Steroidogenesis thus involves the repeated shuttling of substrates into and out of the mitochondria.

C. Androgen Synthesis

The major androgen or androgen precursor produced by the adrenal cortex is dehydroepiandrosterone (DHEA). Most 17-hydroxypregnenolone follows the glucocorticoid pathway, but a small fraction is subjected to oxidative fission and removal of the two-carbon side chain through the action of 17,20-lyase. The lyase activity is actually part of the same enzyme (P450c17) that catalyzes 17α-hydroxylation. This is therefore a **dual function protein.** The lyase activity is important in both the adrenals and

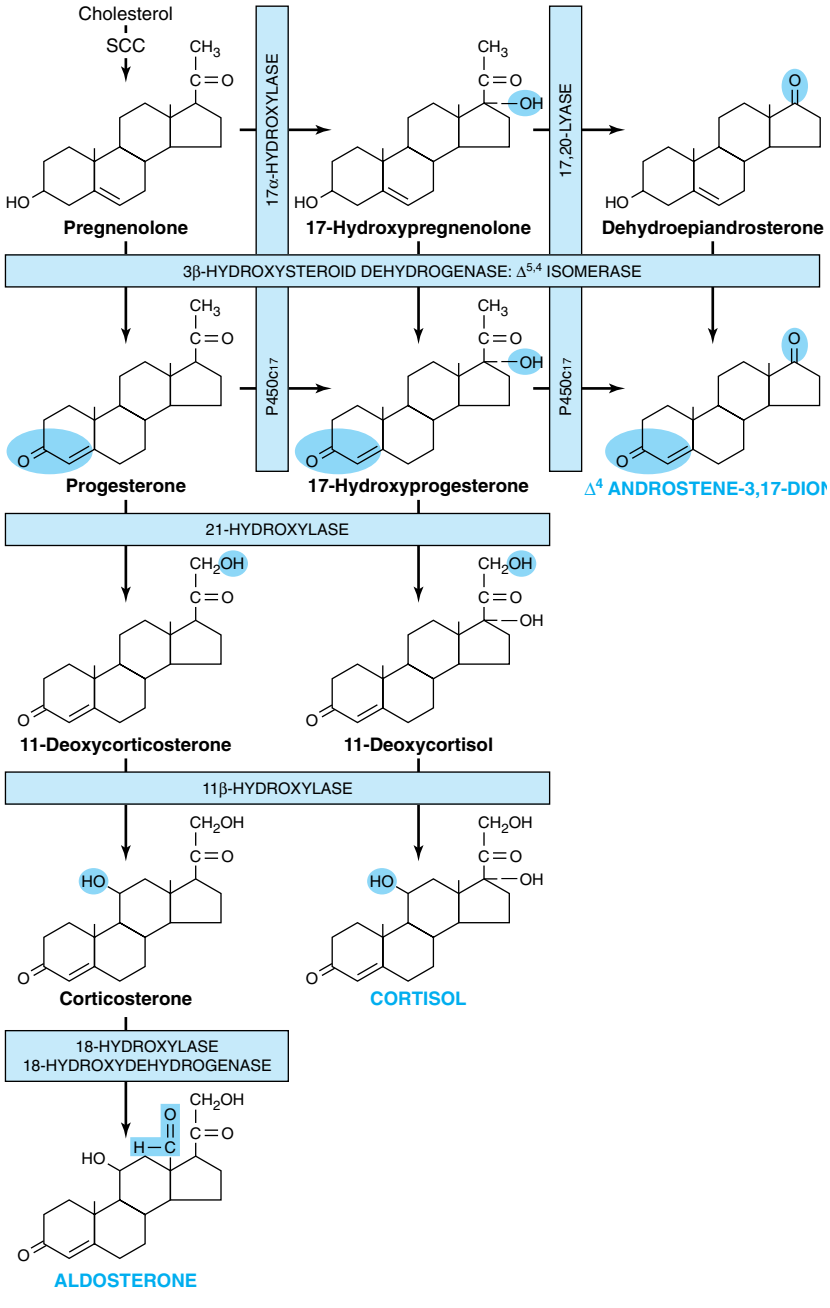


Figure 42–4. Pathways involved in the synthesis of the three major classes of adrenal steroids (mineralocorticoids, glucocorticoids, and androgens). Enzymes are shown in the rectangular boxes, and the modifications at each step are shaded. Note that the 17α-hydroxylase and 17,20-lyase activities are both part of one enzyme, designated P450c17. (Slightly modified and reproduced, with permission, from Harding BW: In: *Endocrinology,* vol 2. DeGroot LJ [editor]. Grune & Stratton, 1979.)

the gonads and acts exclusively on 17α-hydroxy-containing molecules. Adrenal androgen production increases markedly if glucocorticoid biosynthesis is impeded by the lack of one of the hydroxylases **(adrenogenital syndrome).** DHEA is really a prohormone, since the actions of 3β-OHSD and $\Delta^{5,4}$-isomerase convert the weak androgen DHEA into the more potent **androstenedione.** Small amounts of androstenedione are also formed in the adrenal by the action of the lyase on 17α-hydroxyprogesterone. Reduction of androstenedione at the C_{17} position results in the formation of **testosterone,** the most potent adrenal androgen. Small amounts of testosterone are produced in the adrenal by this mechanism, but most of this conversion occurs in the testes.

Testicular Steroidogenesis

Testicular androgens are synthesized in the interstitial tissue by the Leydig cells. The immediate precursor of the gonadal steroids, as for the adrenal steroids, is cholesterol. The rate-limiting step, as in the adrenal, is delivery of cholesterol to the inner membrane of the mitochondria by the transport protein StAR. Once in the proper location, cholesterol is acted upon by the side chain cleavage enzyme P450scc. The conversion of cholesterol to pregnenolone is identical in adrenal, ovary, and testis. In the latter two tissues, however, the reaction is promoted by LH rather than ACTH.

The conversion of pregnenolone to testosterone requires the action of five enzyme activities contained in three proteins: (1) 3β-hydroxysteroid dehydrogenase (3β-OHSD) and $\Delta^{5,4}$-isomerase; (2) 17α-hydroxylase and 17,20-lyase; and (3) 17β-hydroxysteroid dehydrogenase (17β-OHSD). This sequence, referred to as the **progesterone (or Δ^4) pathway,** is shown on the right side of Figure 42–5. Pregnenolone can also be converted to testosterone by the **dehydroepiandrosterone (or Δ^5) pathway,** which is illustrated on the left side of Figure 42–5. The Δ^5 route appears to be most used in human testes.

The five enzyme activities are localized in the microsomal fraction in rat testes, and there is a close functional association between the activities of 3β-OHSD and $\Delta^{5,4}$-isomerase and between those of a 17α-hydroxylase and 17,20-lyase. These enzyme pairs, both contained in a single protein, are shown in the general reaction sequence in Figure 42–5.

Dihydrotestosterone Is Formed From Testosterone in Peripheral Tissues

Testosterone is metabolized by two pathways. One involves oxidation at the 17 position, and the other involves reduction of the A ring double bond and the 3-ketone. Metabolism by the first pathway occurs in many tissues, including liver, and produces 17-ketosteroids that are generally inactive or less active than the parent compound. Metabolism by the second pathway, which is less efficient, occurs primarily in target tissues and produces the potent metabolite dihydrotestosterone (DHT).

The most significant metabolic product of testosterone is DHT, since in many tissues, including prostate, external genitalia, and some areas of the skin, this is the active form of the hormone. The plasma content of DHT in the adult male is about one-tenth that of testosterone, and approximately 400 μg of DHT is produced daily as compared with about 5 mg of testosterone. About 50–100 μg of DHT are secreted by the testes. The rest is produced peripherally from testosterone in a reaction catalyzed by the NADPH-dependent **5α-reductase** (Figure 42–6). Testosterone can thus be considered a prohormone, since it is converted into a much more potent compound (dihydrotestosterone) and since most of this conversion occurs outside the testes. Some estradiol is formed from the peripheral aromatization of testosterone, particularly in males.

Ovarian Steroidogenesis

The estrogens are a family of hormones synthesized in a variety of tissues. 17β-Estradiol is the primary estrogen of ovarian origin. In some species, estrone, synthesized in numerous tissues, is more abundant. In pregnancy, relatively more estriol is produced, and this comes from the placenta. The general pathway and the subcellular localization of the enzymes involved in the early steps of estradiol synthesis are the same as those involved in androgen biosynthesis. Features unique to the ovary are illustrated in Figure 42–7.

Estrogens are formed by the aromatization of androgens in a complex process that involves three hydroxylation steps, each of which requires O_2 and NADPH. The **aromatase enzyme complex** is thought to include a P450 monooxygenase. Estradiol is formed if the substrate of this enzyme complex is testosterone, whereas estrone results from the aromatization of androstenedione.

The cellular source of the various ovarian steroids has been difficult to unravel, but a transfer of substrates between two cell types is involved. Theca cells are the source of androstenedione and testosterone. These are converted by the aromatase enzyme in granulosa cells to estrone and estradiol, respectively. Progesterone, a precursor for all steroid hormones, is produced and secreted by the corpus luteum as an end-product hormone because these cells do not contain the enzymes necessary to convert progesterone to other steroid hormones (Figure 42–8).

Significant amounts of estrogens are produced by the peripheral aromatization of androgens. In human males, the peripheral aromatization of testosterone to estradiol (E_2) accounts for 80% of the production of the latter. In females, adrenal androgens are important

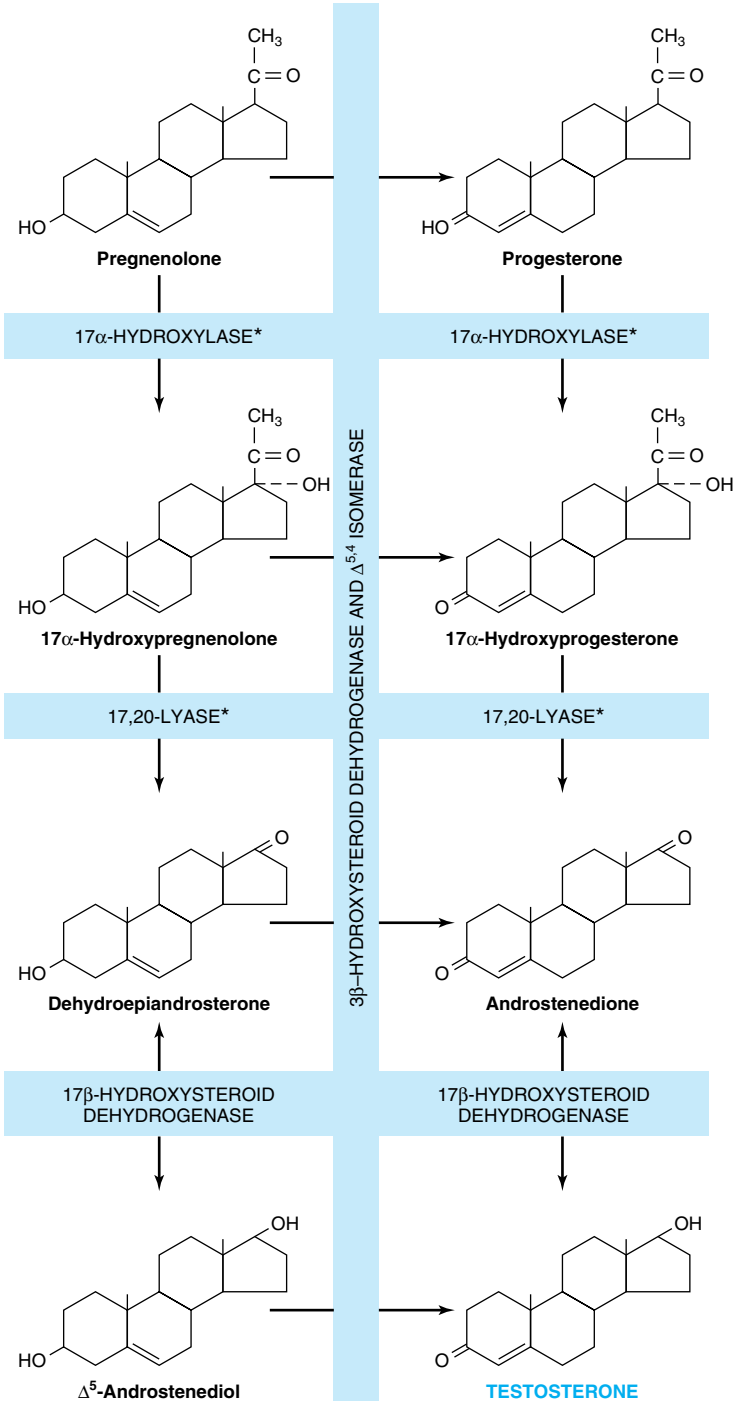


Figure 42–5. Pathways of testosterone biosynthesis. The pathway on the left side of the figure is called the Δ^5 or dehydroepiandrosterone pathway; the pathway on the right side is called the Δ^4 or progesterone pathway. The asterisk indicates that the 17α-hydroxylase and 17,20-lyase activities reside in a single protein, P450c17.

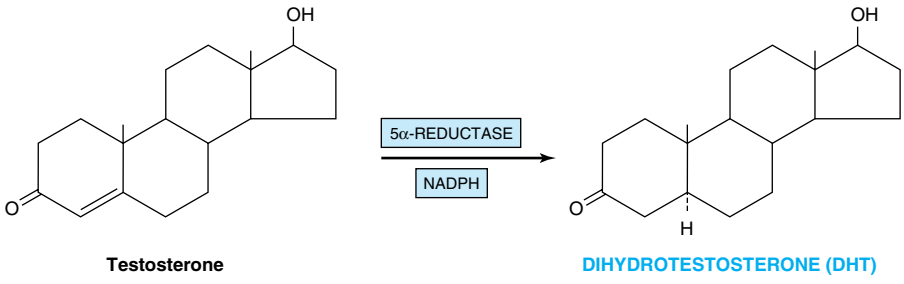


Figure 42–6. Dihydrotestosterone is formed from testosterone through action of the enzyme 5α-reductase.

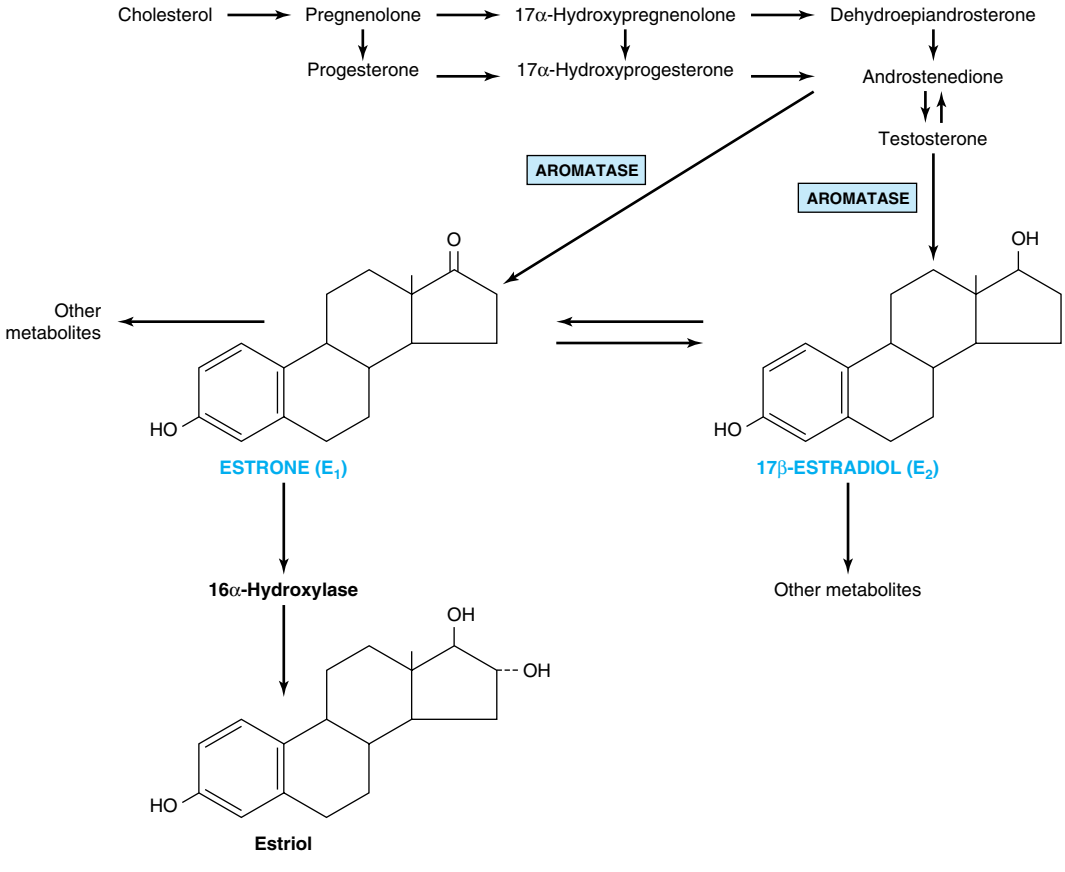


Figure 42–7. **Biosynthesis of estrogens.** (Slightly modified and reproduced, with permission, from Ganong WF: *Review of Medical Physiology,* 20th ed. McGraw-Hill, 2001.)

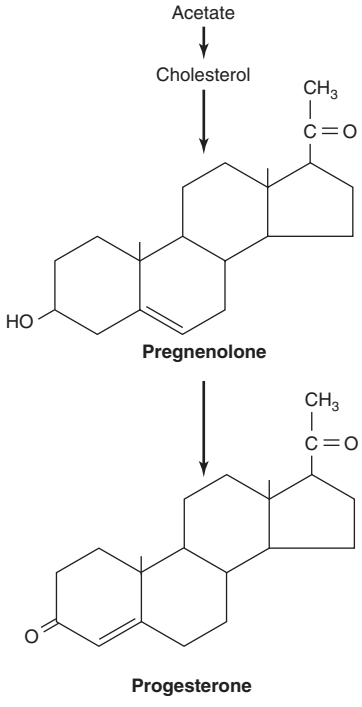


Figure 42–8. Biosynthesis of progesterone in the corpus luteum.

substrates, since as much as 50% of the E_2 produced during pregnancy comes from the aromatization of androgens. Finally, conversion of androstenedione to estrone is the major source of estrogens in postmenopausal women. Aromatase activity is present in adipose cells and also in liver, skin, and other tissues. Increased activity of this enzyme may contribute to the "estrogenization" that characterizes such diseases as cirrhosis of the liver, hyperthyroidism, aging, and obesity.

1,25(OH)$_2$-D$_3$ (Calcitriol) Is Synthesized From a Cholesterol Derivative

1,25(OH)$_2$-D$_3$ is produced by a complex series of enzymatic reactions that involve the plasma transport of precursor molecules to a number of different tissues (Figure 42–9). One of these precursors is vitamin D—really not a vitamin, but this common name persists. The active molecule, 1,25(OH)$_2$-D$_3$, is transported to other organs where it activates biologic processes in a manner similar to that employed by the steroid hormones.

A. Skin

Small amounts of the precursor for 1,25(OH)$_2$-D$_3$ synthesis are present in food (fish liver oil, egg yolk), but most of the precursor for 1,25(OH)$_2$-D$_3$ synthesis is produced in the malpighian layer of the epidermis from 7-dehydrocholesterol in an ultraviolet light-mediated, nonenzymatic **photolysis** reaction. The extent of this conversion is related directly to the intensity of the exposure and inversely to the extent of pigmentation in the skin. There is an age-related loss of 7-dehydrocholesterol in the epidermis that may be related to the negative calcium balance associated with old age.

B. Liver

A specific transport protein called the **vitamin D-binding protein** binds vitamin D$_3$ and its metabolites and moves vitamin D$_3$ from the skin or intestine to the liver, where it undergoes 25-hydroxylation, the first obligatory reaction in the production of 1,25(OH)$_2$-D$_3$. 25-Hydroxylation occurs in the endoplasmic reticulum in a reaction that requires magnesium, NADPH, molecular oxygen, and an uncharacterized cytoplasmic factor. Two enzymes are involved: an NADPH-dependent cytochrome P450 reductase and a cytochrome P450. This reaction is not regulated, and it also occurs with low efficiency in kidney and intestine. The 25(OH)$_2$-D$_3$ enters the circulation, where it is the major form of vitamin D found in plasma, and is transported to the kidney by the vitamin D-binding protein.

C. Kidney

25(OH)$_2$-D$_3$ is a weak agonist and must be modified by hydroxylation at position C$_1$ for full biologic activity. This is accomplished in mitochondria of the renal proximal convoluted tubule by a three-component monooxygenase reaction that requires NADPH, Mg^{2+}, molecular oxygen, and at least three enzymes: (1) a flavoprotein, renal ferredoxin reductase; (2) an iron sulfur protein, renal ferredoxin; and (3) cytochrome P450. This system produces 1,25(OH)$_2$-D$_3$, which is the most potent naturally occurring metabolite of vitamin D.

CATECHOLAMINES & THYROID HORMONES ARE MADE FROM TYROSINE

Catecholamines Are Synthesized in Final Form & Stored in Secretion Granules

Three amines—dopamine, norepinephrine, and epinephrine—are synthesized from tyrosine in the chromaffin cells of the adrenal medulla. The major product of the adrenal medulla is epinephrine. This compound constitutes about 80% of the catecholamines in the medulla, and it is not made in extramedullary tissue. In contrast, most of the norepinephrine present in organs innervated by sympathetic nerves is made in situ (about 80% of the total), and most of the rest is made in other nerve endings and reaches the target sites via the circu-

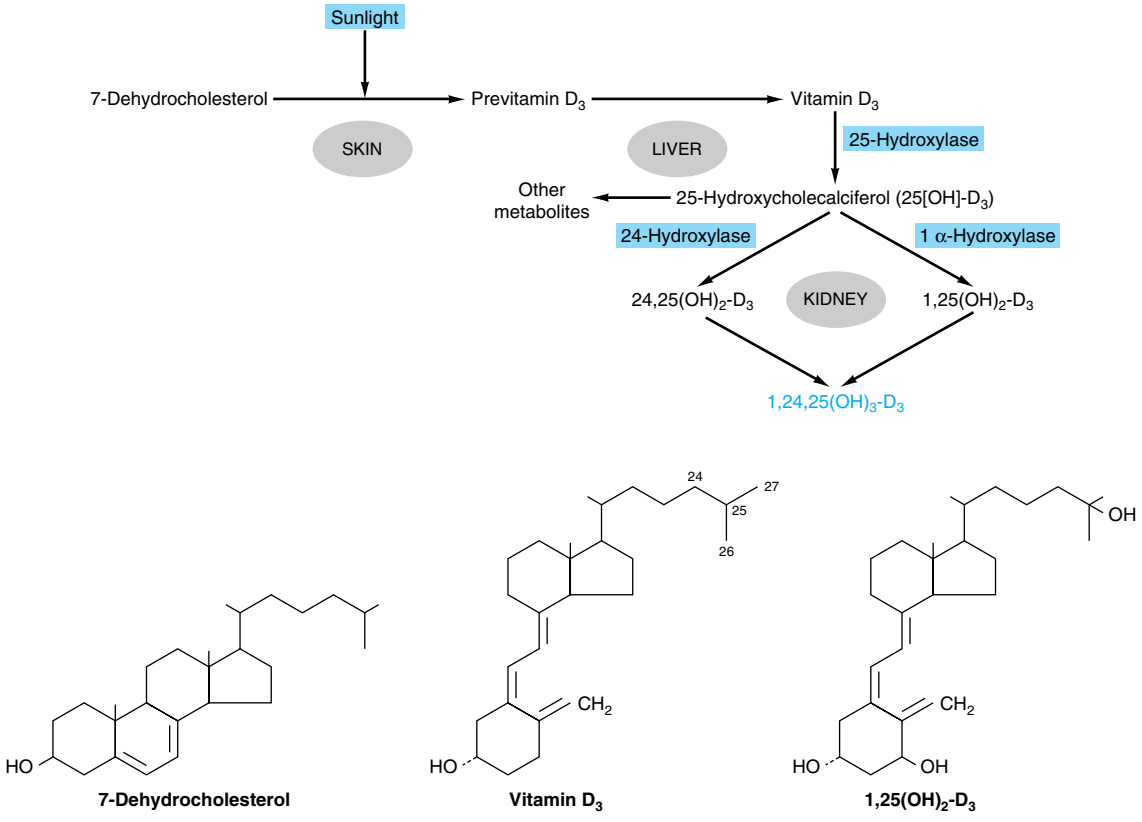


Figure 42–9. Formation and hydroxylation of vitamin D_3. 25-Hydroxylation takes place in the liver, and the other hydroxylations occur in the kidneys. $25,26(OH)_2$-D_3 and $1,25,26(OH)_3$-D_3 are probably formed as well. The formulas of 7-dehydrocholesterol, vitamin D_3, and $1,25(OH)_2$-D_3 are also shown. (Modified and reproduced, with permission, from Ganong WF: *Review of Medical Physiology,* 20th ed. McGraw-Hill, 2001.)

lation. Epinephrine and norepinephrine may be produced and stored in different cells in the adrenal medulla and other chromaffin tissues.

The conversion of tyrosine to epinephrine requires four sequential steps: (1) ring hydroxylation; (2) decarboxylation; (3) side chain hydroxylation to form norepinephrine; and (4) N-methylation to form epinephrine. The biosynthetic pathway and the enzymes involved are illustrated in Figure 42–10.

A. Tyrosine Hydroxylase Is Rate-Limiting for Catecholamine Biosynthesis

Tyrosine is the immediate precursor of catecholamines, and **tyrosine hydroxylase** is the rate-limiting enzyme in catecholamine biosynthesis. Tyrosine hydroxylase is found in both soluble and particle-bound forms only in tissues that synthesize catecholamines; it functions as an oxidoreductase, with tetrahydropteridine as a cofactor, to convert L-tyrosine to L-dihydroxyphenylalanine **(L-dopa).** As the rate-limiting enzyme, tyrosine hydroxylase is regulated in a variety of ways. The most important mechanism involves feedback inhibition by the catecholamines, which compete with the enzyme for the pteridine cofactor. Catecholamines cannot cross the blood-brain barrier; hence, in the brain they must be synthesized locally. In certain central nervous system diseases (eg, Parkinson's disease), there is a local deficiency of dopamine synthesis. L-Dopa, the precursor of dopamine, readily crosses the blood-brain barrier and so is an important agent in the treatment of Parkinson's disease.

B. Dopa Decarboxylase Is Present in All Tissues

This soluble enzyme requires pyridoxal phosphate for the conversion of L-dopa to 3,4-dihydroxyphenylethylamine **(dopamine).** Compounds that resemble L-dopa, such as α-methyldopa, are competitive inhibitors of this reaction. α-Methyldopa is effective in treating some kinds of hypertension.

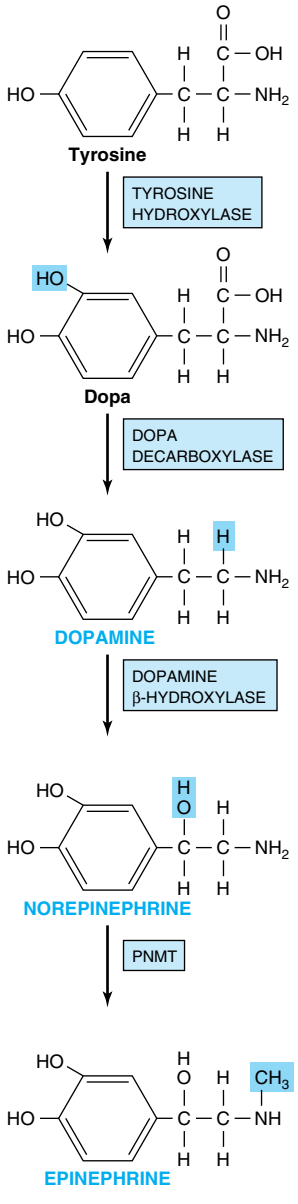


Figure 42–10. Biosynthesis of catecholamines. (PNMT, phenylethanolamine-*N*-methyltransferase.)

C. Dopamine β-Hydroxylase (DBH) Catalyzes the Conversion of Dopamine to Norepinephrine

DBH is a monooxygenase and uses ascorbate as an electron donor, copper at the active site, and fumarate as modulator. DBH is in the particulate fraction of the medullary cells, probably in the secretion granule; thus, the conversion of dopamine to **norepinephrine** occurs in this organelle.

D. Phenylethanolamine-*N*-Methyltransferase (PNMT) Catalyzes the Production of Epinephrine

PNMT catalyzes the N-methylation of norepinephrine to form **epinephrine** in the epinephrine-forming cells of the adrenal medulla. Since PNMT is soluble, it is assumed that norepinephrine-to-epinephrine conversion occurs in the cytoplasm. The synthesis of PNMT is induced by glucocorticoid hormones that reach the medulla via the intra-adrenal portal system. This special system provides for a 100-fold steroid concentration gradient over systemic arterial blood, and this high intra-adrenal concentration appears to be necessary for the induction of PNMT.

T_3 & T_4 Illustrate the Diversity in Hormone Synthesis

The formation of **triiodothyronine (T_3)** and **tetraiodothyronine (thyroxine; T_4)** (see Figure 42–2) illustrates many of the principles of diversity discussed in this chapter. These hormones require a rare element (iodine) for bioactivity; they are synthesized as part of a very large precursor molecule (thyroglobulin); they are stored in an intracellular reservoir (colloid); and there is peripheral conversion of T_4 to T_3, which is a much more active hormone.

The thyroid hormones T_3 and T_4 are unique in that iodine (as iodide) is an essential component of both. In most parts of the world, iodine is a scarce component of soil, and for that reason there is little in food. A complex mechanism has evolved to acquire and retain this crucial element and to convert it into a form suitable for incorporation into organic compounds. At the same time, the thyroid must synthesize thyronine from tyrosine, and this synthesis takes place in thyroglobulin (Figure 42–11).

Thyroglobulin is the precursor of T_4 and T_3. It is a large iodinated, glycosylated protein with a molecular mass of 660 kDa. Carbohydrate accounts for 8–10% of the weight of thyroglobulin and iodide for about 0.2–1%, depending upon the iodine content in the diet. Thyroglobulin is composed of two large subunits. It contains 115 tyrosine residues, each of which is a potential site of iodination. About 70% of the iodide in thyroglobulin exists in the inactive precursors, **monoiodotyrosine (MIT)** and **diiodotyrosine (DIT),** while 30% is in the **iodothyronyl residues,** T_4 and T_3. When iodine supplies are sufficient, the T_4:T_3 ratio is about 7:1. In **iodine deficiency,** this ratio decreases, as does the DIT:MIT ratio. Thyroglobulin, a large molecule of about 5000 amino acids, provides the confor-

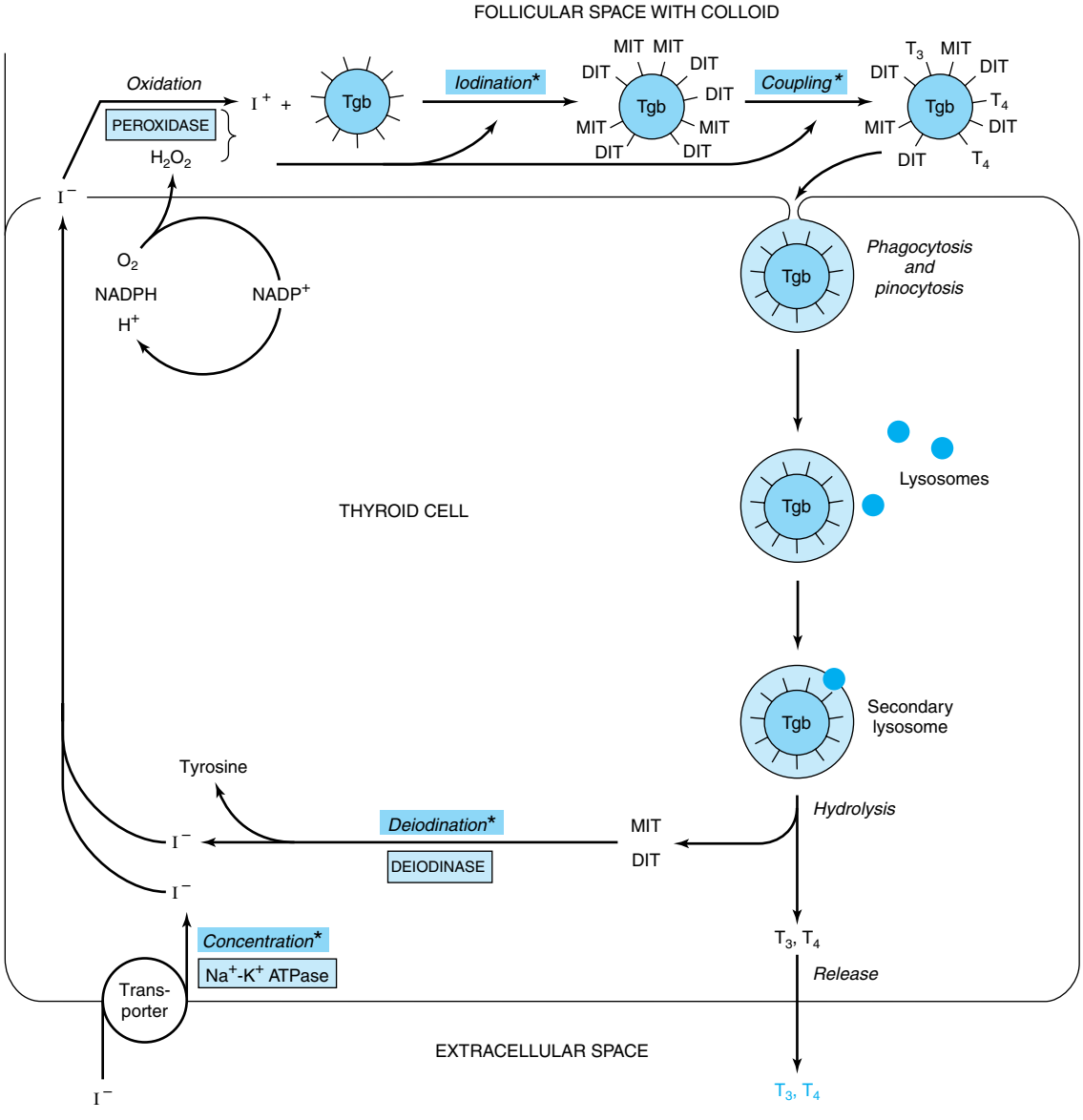


Figure 42–11. Model of iodide metabolism in the thyroid follicle. A follicular cell is shown facing the follicular lumen (top) and the extracellular space (at bottom). Iodide enters the thyroid primarily through a transporter (bottom left). Thyroid hormone synthesis occurs in the follicular space through a series of reactions, many of which are peroxidase-mediated. Thyroid hormones, stored in the colloid in the follicular space, are released from thyroglobulin by hydrolysis inside the thyroid cell. (Tgb, thyroglobulin; MIT, monoiodotyrosine; DIT, diiodotyrosine; T_3, triiodothyronine; T_4, tetraiodothyronine.) Asterisks indicate steps or processes that are inherited enzyme deficiencies which cause congenital goiter and often result in hypothyroidism.

mation required for tyrosyl coupling and iodide organification necessary in the formation of the diaminoacid thyroid hormones. It is synthesized in the basal portion of the cell and moves to the lumen, where it is a storage form of T_3 and T_4 in the colloid; several weeks' supply of these hormones exist in the normal thyroid. Within minutes after stimulation of the thyroid by TSH, colloid reenters the cell and there is a marked increase of phagolysosome activity. Various acid proteases and peptidases hydrolyze the thyroglobulin into its constituent amino acids, including T_4 and T_3, which are discharged from the basal portion of the cell (see Figure 42–11). Thyroglobulin is thus a very large prohormone.

Iodide Metabolism Involves Several Discrete Steps

The thyroid is able to concentrate I^- against a strong electrochemical gradient. This is an energy-dependent process and is linked to the Na^+-K^+ ATPase-dependent thyroidal I^- transporter. The ratio of iodide in thyroid to iodide in serum (T:S ratio) is a reflection of the activity of this transporter. This activity is primarily controlled by TSH and ranges from 500:1 in animals chronically stimulated with TSH to 5:1 or less in hypophysectomized animals (no TSH). The T:S ratio in humans on a normal iodine diet is about 25:1.

The thyroid is the only tissue that can oxidize I^- to a higher valence state, an obligatory step in I^- organification and thyroid hormone biosynthesis. This step involves a heme-containing peroxidase and occurs at the luminal surface of the follicular cell. Thyroperoxidase, a tetrameric protein with a molecular mass of 60 kDa, requires hydrogen peroxide as an oxidizing agent. The H_2O_2 is produced by an NADPH-dependent enzyme resembling cytochrome *c* reductase. A number of compounds inhibit I^- oxidation and therefore its subsequent incorporation into MIT and DIT. The most important of these are the thiourea drugs. They are used as antithyroid drugs because of their ability to inhibit thyroid hormone biosynthesis at this step. Once iodination occurs, the iodine does not readily leave the thyroid. Free tyrosine can be iodinated, but it is not incorporated into proteins since no tRNA recognizes iodinated tyrosine.

The coupling of two DIT molecules to form T_4—or of an MIT and DIT to form T_3—occurs within the thyroglobulin molecule. A separate coupling enzyme has not been found, and since this is an oxidative process it is assumed that the same thyroperoxidase catalyzes this reaction by stimulating free radical formation of iodotyrosine. This hypothesis is supported by the observation that the same drugs which inhibit I^- oxidation also inhibit coupling. The formed thyroid hormones remain as integral parts of thyroglobulin until the latter is degraded, as described above.

A deiodinase removes I^- from the inactive mono- and diiodothyronine molecules in the thyroid. This mechanism provides a substantial amount of the I^- used in T_3 and T_4 biosynthesis. A peripheral deiodinase in target tissues such as pituitary, kidney, and liver selectively removes I^- from the 5′ position of T_4 to make T_3 (see Figure 42–2), which is a much more active molecule. In this sense, T_4 can be thought of as a prohormone, though it does have some intrinsic activity.

SEVERAL HORMONES ARE MADE FROM LARGER PEPTIDE PRECURSORS

Formation of the critical disulfide bridges in insulin requires that this hormone be first synthesized as part of a larger precursor molecule, proinsulin. This is conceptually similar to the example of the thyroid hormones, which can only be formed in the context of a much larger molecule. Several other hormones are synthesized as parts of large precursor molecules, not because of some special structural requirement but rather as a mechanism for controlling the available amount of the active hormone. PTH and angiotensin II are examples of this type of regulation. Another interesting example is the POMC protein, which can be processed into many different hormones in a tissue-specific manner. These examples are discussed in detail below.

Insulin Is Synthesized as a Preprohormone & Modified Within the β Cell

Insulin has an AB heterodimeric structure with one intrachain (A6–A11) and two interchain disulfide bridges (A7–B7 and A20–B19) (Figure 42–12). The A and B chains could be synthesized in the laboratory, but attempts at a biochemical synthesis of the mature insulin molecule yielded very poor results. The reason for this became apparent when it was discovered that insulin is synthesized as a **preprohormone** (molecular weight approximately 11,500), which is the prototype for peptides that are processed from larger precursor molecules. The hydrophobic 23-amino-acid pre-, or leader, sequence directs the molecule into the cisternae of the endoplasmic reticulum and then is removed. This results in the 9000-MW proinsulin molecule, which provides the conformation necessary for the proper and efficient formation of the disulfide bridges. As shown in Figure 42–12, the sequence of proinsulin, starting from the amino terminal, is B chain—connecting (C) peptide—A chain. The proinsulin molecule undergoes a series of site-specific peptide cleavages that result in the formation of equimolar amounts of mature insulin and C peptide. These enzymatic cleavages are summarized in Figure 42–12.

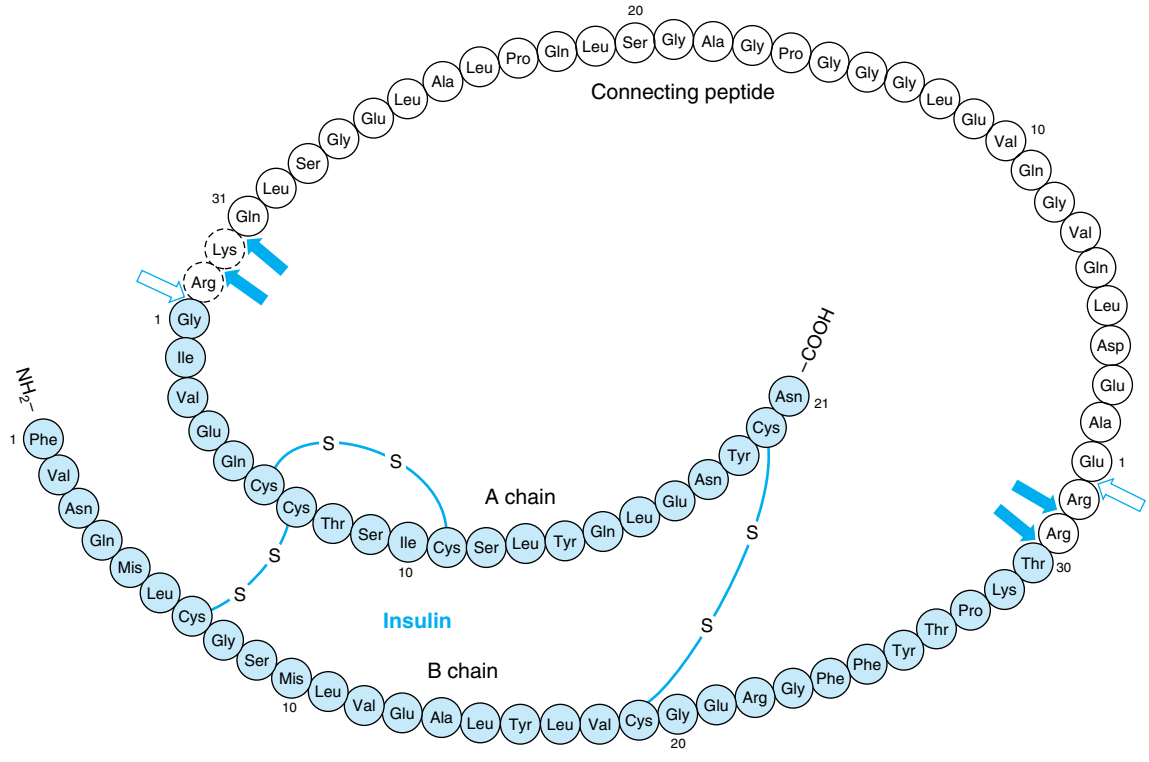


Figure 42–12. Structure of human proinsulin. Insulin and C-peptide molecules are connected at two sites by dipeptide links. An initial cleavage by a trypsin-like enzyme (open arrows) followed by several cleavages by a carboxypeptidase-like enzyme (solid arrows) results in the production of the heterodimeric (AB) insulin molecule (light blue) and the C-peptide.

Parathyroid Hormone (PTH) Is Secreted as an 84-Amino-Acid Peptide

The immediate precursor of PTH is **proPTH,** which differs from the native 84-amino-acid hormone by having a highly basic hexapeptide amino terminal extension. The primary gene product and the immediate precursor for proPTH is the 115-amino-acid **preproPTH.** This differs from proPTH by having an additional 25-amino-acid amino terminal extension that, in common with the other leader or signal sequences characteristic of secreted proteins, is hydrophobic. The complete structure of preproPTH and the sequences of proPTH and PTH are illustrated in Figure 42–13. $PTH_{1–34}$ has full biologic activity, and the region 25–34 is primarily responsible for receptor binding.

The biosynthesis of PTH and its subsequent secretion are regulated by the plasma ionized calcium (Ca^{2+}) concentration through a complex process. An acute decrease of Ca^{2+} results in a marked increase of PTH mRNA, and this is followed by an increased rate of PTH synthesis and secretion. However, about 80–90% of the proPTH synthesized cannot be accounted for as intact PTH in cells or in the incubation medium of experimental systems. This finding led to the conclusion that most of the proPTH synthesized is quickly degraded. It was later discovered that this rate of degradation decreases when Ca^{2+} concentrations are low, and it increases when Ca^{2+} concentrations are high. Very specific fragments of PTH are generated during its proteolytic digestion (Figure 42–13). A number of proteolytic enzymes, including cathepsins B and D, have been identified in parathyroid tissue. Cathepsin B cleaves PTH into two fragments: $PTH_{1–36}$ and $PTH_{37–84}$. $PTH_{37–84}$ is not further degraded; however, $PTH_{1–36}$ is rapidly and progressively cleaved into di- and tripeptides. Most of the proteolysis of PTH occurs within the gland, but a number of studies confirm that PTH, once secreted, is proteolytically degraded in other tissues, especially the liver, by similar mechanisms.

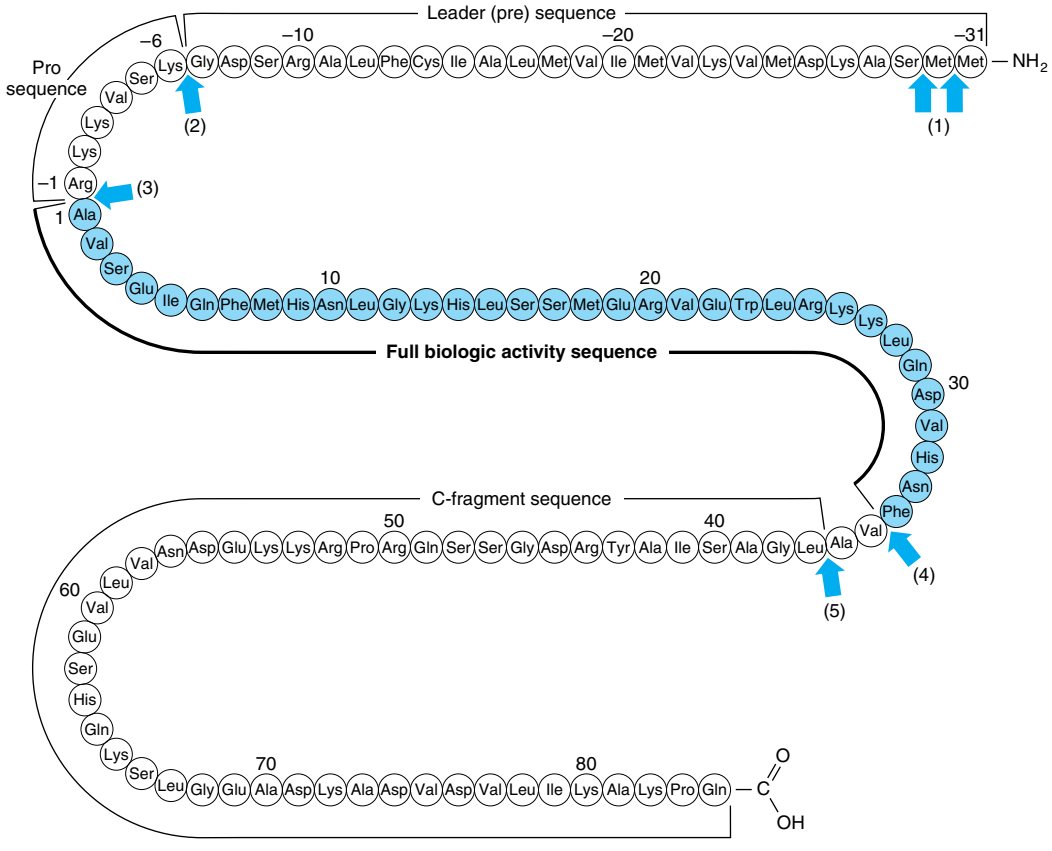


Figure 42–13. Structure of bovine preproparathyroid hormone. Arrows indicate sites cleaved by processing enzymes in the parathyroid gland (1–5) and in the liver after secretion of the hormone (4–5). The biologically active region of the molecule is flanked by sequence not required for activity on target receptors. (Slightly modified and reproduced, with permission, from Habener JF: Recent advances in parathyroid hormone research. Clin Biochem 1981;14:223.)

Angiotensin II Is Also Synthesized From a Large Precursor

The renin-angiotensin system is involved in the regulation of blood pressure and electrolyte metabolism (through production of aldosterone). The primary hormone involved in these processes is angiotensin II, an octapeptide made from angiotensinogen (Figure 42–14). Angiotensinogen, a large α_2-globulin made in liver, is the substrate for renin, an enzyme produced in the juxtaglomerular cells of the renal afferent arteriole. The position of these cells makes them particularly sensitive to blood pressure changes, and many of the physiologic regulators of renin release act through renal baroreceptors. The juxtaglomerular cells are also sensitive to changes of Na^+ and Cl^- concentration in the renal tubular fluid; therefore, any combination of factors that decreases fluid volume (dehydration, decreased blood pressure, fluid or blood loss) or decreases NaCl concentration stimulates renin release. Renal sympathetic nerves that terminate in the juxtaglomerular cells mediate the central nervous system and postural effects on renin release independently of the baroreceptor and salt effects, a mechanism that involves the β-adrenergic receptor. Renin acts upon the substrate angiotensinogen to produce the decapeptide angiotensin I.

Angiotensin-converting enzyme, a glycoprotein found in lung, endothelial cells, and plasma, removes two carboxyl terminal amino acids from the decapeptide angiotensin I to form angiotensin II in a step that is not thought to be rate-limiting. Various nonapeptide analogs of angiotensin I and other compounds act as competitive inhibitors of converting enzyme and are used to treat renin-dependent hypertension. These are

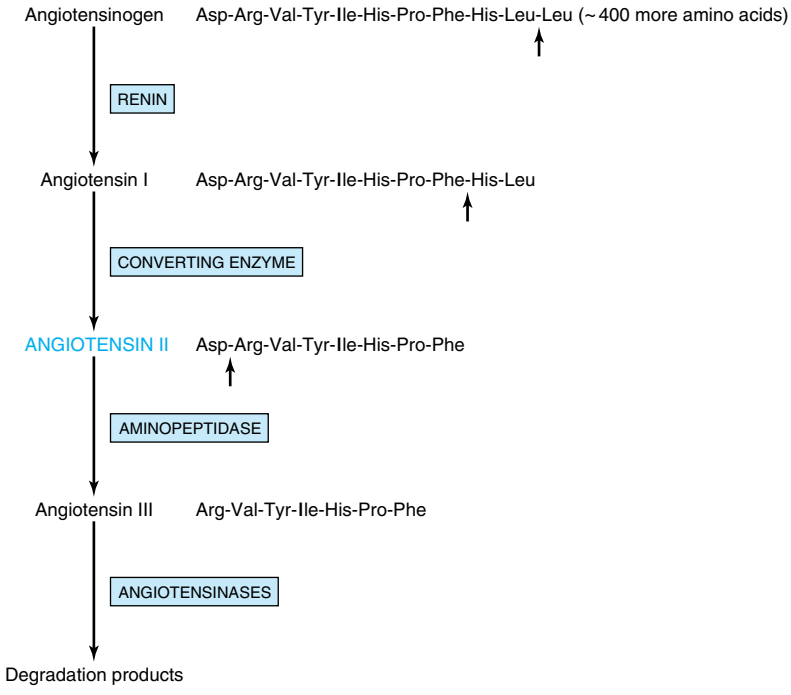


Figure 42–14. Formation and metabolism of angiotensins. Small arrows indicate cleavage sites.

referred to as **angiotensin-converting enzyme (ACE) inhibitors.** Angiotensin II increases blood pressure by causing vasoconstriction of the arteriole and is a very potent vasoactive substance. It inhibits renin release from the juxtaglomerular cells and is a potent stimulator of aldosterone production. This results in Na^+ retention, volume expansion, and increased blood pressure.

In some species, angiotensin II is converted to the heptapeptide angiotensin III (Figure 42–14), an equally potent stimulator of aldosterone production. In humans, the plasma level of angiotensin II is four times greater than that of angiotensin III, so most effects are exerted by the octapeptide. Angiotensins II and III are rapidly inactivated by angiotensinases.

Angiotensin II binds to specific adrenal cortex glomerulosa cell receptors. The hormone-receptor interaction does not activate adenylyl cyclase, and cAMP does not appear to mediate the action of this hormone. The actions of angiotensin II, which are to stimulate the conversion of cholesterol to pregnenolone and of corticosterone to 18-hydroxycorticosterone and aldosterone, may involve changes in the concentration of intracellular calcium and of phospholipid metabolites by mechanisms similar to those described in Chapter 43.

Complex Processing Generates the Pro-opiomelanocortin (POMC) Peptide Family

The POMC family consists of peptides that act as hormones (ACTH, LPH, MSH) and others that may serve as neurotransmitters or neuromodulators (endorphins) (see Figure 42–15). POMC is synthesized as a precursor molecule of 285 amino acids and is processed differently in various regions of the pituitary.

The POMC gene is expressed in the anterior and intermediate lobes of the pituitary. The most conserved sequences between species are within the amino terminal fragment, the ACTH region, and the β-endorphin region. POMC or related products are found in several other vertebrate tissues, including the brain, placenta, gastrointestinal tract, reproductive tract, lung, and lymphocytes.

The POMC protein is processed differently in the anterior lobe than in the intermediate lobe. The intermediate lobe of the pituitary is rudimentary in adult humans, but it is active in human fetuses and in pregnant women during late gestation and is also active in many animal species. Processing of the POMC protein in the peripheral tissues (gut, placenta, male reproductive tract) resem-

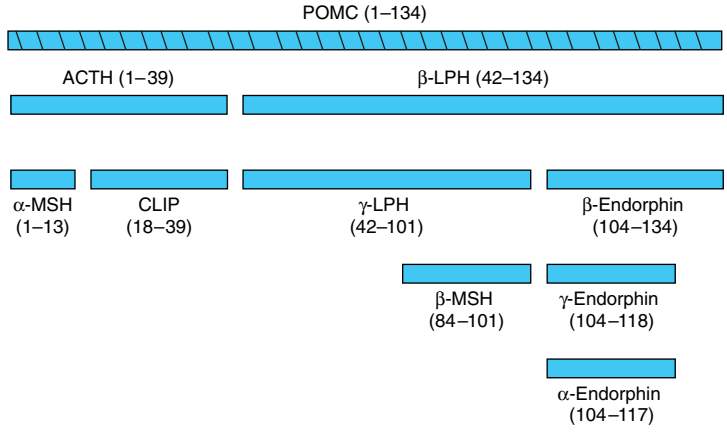


Figure 42–15. Products of pro-opiomelanocortin (POMC) cleavage. (MSH, melanocyte-stimulating hormone; CLIP, corticotropin-like intermediate lobe peptide; LPH, lipotropin.)

bles that in the intermediate lobe. There are three basic peptide groups: (1) ACTH, which can give rise to α-MSH and corticotropin-like intermediate lobe peptide (CLIP); (2) β-lipotropin (β-LPH), which can yield γ-LPH, β-MSH, and β-endorphin (and thus α- and γ-endorphins); and (3) a large amino terminal peptide, which generates γ-MSH. The diversity of these products is due to the many dibasic amino acid clusters that are potential cleavage sites for trypsin-like enzymes. Each of the peptides mentioned is preceded by Lys-Arg, Arg-Lys, Arg-Arg, or Lys-Lys residues. After the prehormone segment is cleaved, the next cleavage, in both anterior and intermediate lobes, is between ACTH and β-LPH, resulting in an amino terminal peptide with ACTH and a β-LPH segment (Figure 42–15). $ACTH_{1–39}$ is subsequently cleaved from the amino terminal peptide, and in the anterior lobe essentially no further cleavages occur. In the intermediate lobe, $ACTH_{1–39}$ is cleaved into α-MSH (residues 1–13) and CLIP (18–39); β-LPH (42–134) is converted to γ-LPH (42–101) and β-endorphin (104–134). β-MSH (84–101) is derived from γ-LPH.

There are extensive additional tissue-specific modifications of these peptides that affect activity. These modifications include phosphorylation, acetylation, glycosylation, and amidation.

THERE IS VARIATION IN THE STORAGE & SECRETION OF HORMONES

As mentioned above, the steroid hormones and $1,25(OH)_2\text{-}D_3$ are synthesized in their final active form. They are also secreted as they are made, and thus there is no intracellular reservoir of these hormones. The catecholamines, also synthesized in active form, are stored in granules in the chromaffin cells in the adrenal medulla. In response to appropriate neural stimulation, these granules are released from the cell through exocytosis, and the catecholamines are released into the circulation. A several-hour reserve supply of catecholamines exists in the chromaffin cells.

Parathyroid hormone also exists in storage vesicles. As much as 80–90% of the proPTH synthesized is degraded before it enters this final storage compartment, especially when Ca^{2+} levels are high in the parathyroid cell (see above). PTH is secreted when Ca^{2+} is low in the parathyroid cells, which contain a several-hour supply of the hormone.

The human pancreas secretes about 40–50 units of insulin daily, which represents about 15–20% of the hormone stored in the B cells. Insulin and the C-peptide (see Figure 42–12) are normally secreted in equimolar amounts. Stimuli such as glucose, which provokes insulin secretion, therefore trigger the processing of proinsulin to insulin as an essential part of the secretory response.

A several-week supply of T_3 and T_4 exists in the thyroglobulin that is stored in colloid in the lumen of the thyroid follicles. These hormones can be released upon stimulation by TSH. This is the most exaggerated example of a prohormone, as a molecule containing approximately 5000 amino acids must be first synthesized, then degraded, to supply a few molecules of the active hormones T_4 and T_3.

The diversity in storage and secretion of hormones is illustrated in Table 42–5.

Table 42–5. Diversity in the storage of hormones.

Hormone	Supply Stored in Cell
Steroids and 1,25(OH)$_2$-D$_3$	None
Catecholamines and PTH	Hours
Insulin	Days
T$_3$ and T$_4$	Weeks

SOME HORMONES HAVE PLASMA TRANSPORT PROTEINS

The class I hormones are hydrophobic in chemical nature and thus are not very soluble in plasma. These hormones, principally the steroids and thyroid hormones, have specialized plasma transport proteins that serve several purposes. First, these proteins circumvent the solubility problem and thereby deliver the hormone to the target cell. They also provide a circulating reservoir of the hormone that can be substantial, as in the case of the thyroid hormones. Hormones, when bound to the transport proteins, cannot be metabolized, thereby prolonging their plasma half-life ($t_{1/2}$). The binding affinity of a given hormone to its transporter determines the bound versus free ratio of the hormone. This is important because only the free form of a hormone is biologically active. In general, the concentration of free hormone in plasma is very low, in the range of 10^{-15} to 10^{-9} mol/L. It is important to distinguish between plasma transport proteins and hormone receptors. Both bind hormones but with very different characteristics (Table 42–6).

The hydrophilic hormones—generally class II and of peptide structure—are freely soluble in plasma and do not require transport proteins. Hormones such as insulin, growth hormone, ACTH, and TSH circulate in the free, active form and have very short plasma half-lives. A notable exception is IGF-I, which is transported bound to members of a family of binding proteins.

Table 42–6. Comparison of receptors with transport proteins.

Feature	Receptors	Transport Proteins
Concentration	Very low (thousands/cell)	Very high (billions/µL)
Binding affinity	High (pmol/L to nmol/L range)	Low (µmol/L range)
Binding specificity	Very high	Low
Saturability	Yes	No
Reversibility	Yes	Yes
Signal transduction	Yes	No

Thyroid Hormones Are Transported by Thyroid-Binding Globulin

Many of the principles discussed above are illustrated in a discussion of thyroid-binding proteins. One-half to two-thirds of T$_4$ and T$_3$ in the body is in an extrathyroidal reservoir. Most of this circulates in bound form, ie, bound to a specific binding protein, **thyroxine-binding globulin (TBG).** TBG, a glycoprotein with a molecular mass of 50 kDa, binds T$_4$ and T$_3$ and has the capacity to bind 20 µg/dL of plasma. Under normal circumstances, TBG binds—noncovalently—nearly all of the T$_4$ and T$_3$ in plasma, and it binds T$_4$ with greater affinity than T$_3$ (Table 42–7). The plasma half-life of T$_4$ is correspondingly four to five times that of T$_3$. The small, unbound (free) fraction is responsible for the biologic activity. Thus, in spite of the great difference in total amount, the free fraction of T$_3$ approximates that of T$_4$, and given that T$_3$ is intrinsically more active than T$_4$, most biologic activity is attributed to T$_3$. TBG does not bind any other hormones.

Glucocorticoids Are Transported by Corticosteroid-Binding Globulin

Hydrocortisone (cortisol) also circulates in plasma in protein-bound and free forms. The main plasma binding protein is an α-globulin called **transcortin,** or **corticosteroid-binding globulin (CBG).** CBG is produced in the liver, and its synthesis, like that of TBG, is increased by estrogens. CBG binds most of the hormone when plasma cortisol levels are within the normal range; much smaller amounts of cortisol are bound to albumin. The avidity of binding helps determine the biologic half-lives of various glucocorticoids. Cortisol binds tightly to CBG and has a $t_{1/2}$ of 1.5–2 hours, while corticosterone, which binds less tightly, has a $t_{1/2}$ of less than 1 hour (Table 42–8). The unbound (free) cortisol constitutes about 8% of the total and represents the biologically active fraction. Binding to CBG is not restricted to glucocorticoids. Deoxycorticosterone and

Table 42–7. Comparison of T$_4$ and T$_3$ in plasma.

Total Hormone (µg/dL)		Free Hormone: Percent of Total	Free Hormone: ng/dL	Free Hormone: Molarity	$t_{1/2}$ in Blood (days)
T$_4$	8	0.03	~2.24	3.0×10^{-11}	6.5
T$_3$	0.15	0.3	~0.4	$\sim 0.6 \times 10^{-11}$	1.5

Table 42–8. Approximate affinities of steroids for serum-binding proteins.

	SHBG[1]	CBG[1]
Dihydrotestosterone	1	> 100
Testosterone	2	> 100
Estradiol	5	> 10
Estrone	> 10	> 100
Progesterone	> 100	~ 2
Cortisol	> 100	~ 3
Corticosterone	> 100	~ 5

[1]Affinity expressed as K_d (nmol/L).

progesterone interact with CBG with sufficient affinity to compete for cortisol binding. Aldosterone, the most potent natural mineralocorticoid, does not have a specific plasma transport protein. Gonadal steroids bind very weakly to CBG (Table 42–8).

Gonadal Steroids Are Transported by Sex Hormone-Binding Globulin

Most mammals, humans included, have a plasma β-globulin that binds testosterone with specificity, relatively high affinity, and limited capacity (Table 42–8). This protein, usually called **sex hormone-binding globulin (SHBG)** or testosterone-estrogen-binding globulin (TEBG), is produced in the liver. Its production is increased by estrogens (women have twice the serum concentration of SHBG as men), certain types of liver disease, and hyperthyroidism; it is decreased by androgens, advancing age, and hypothyroidism. Many of these conditions also affect the production of CBG and TBG. Since SHBG and albumin bind 97–99% of circulating testosterone, only a small fraction of the hormone in circulation is in the free (biologically active) form. The primary function of SHBG may be to restrict the free concentration of testosterone in the serum. Testosterone binds to SHBG with higher affinity than does estradiol (Table 42–8). Therefore, a change in the level of SHBG causes a greater change in the free testosterone level than in the free estradiol level.

Estrogens are bound to SHBG and progestins to CBG. SHBG binds estradiol about five times less avidly than it binds testosterone or DHT, while progesterone and cortisol have little affinity for this protein (Table 42–8). In contrast, progesterone and cortisol bind with nearly equal affinity to CBG, which in turn has little avidity for estradiol and even less for testosterone, DHT, or estrone.

These binding proteins also provide a circulating reservoir of hormone, and because of the relatively large binding capacity they probably buffer against sudden changes in the plasma level. Because the metabolic clearance rates of these steroids are inversely related to the affinity of their binding to SHBG, estrone is cleared more rapidly than estradiol, which in turn is cleared more rapidly than testosterone or DHT.

SUMMARY

- The presence of a specific receptor defines the target cells for a given hormone.
- Receptors are proteins that bind specific hormones and generate an intracellular signal (receptor-effector coupling).
- Some hormones have intracellular receptors; others bind to receptors on the plasma membrane.
- Hormones are synthesized from a number of precursor molecules, including cholesterol, tyrosine per se, and all the constituent amino acids of peptides and proteins.
- A number of modification processes alter the activity of hormones. For example, many hormones are synthesized from larger precursor molecules.
- The complement of enzymes in a particular cell type allows for the production of a specific class of steroid hormone.
- Most of the lipid-soluble hormones are bound to rather specific plasma transport proteins.

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Hormone Action & Signal Transduction

43

Daryl K. Granner, MD

BIOMEDICAL IMPORTANCE

The homeostatic adaptations an organism makes to a constantly changing environment are in large part accomplished through alterations of the activity and amount of proteins. Hormones provide a major means of facilitating these changes. A hormone-receptor interaction results in generation of an intracellular signal that can either regulate the activity of a select set of genes, thereby altering the amount of certain proteins in the target cell, or affect the activity of specific proteins, including enzymes and transporter or channel proteins. The signal can influence the location of proteins in the cell and can affect general processes such as protein synthesis, cell growth, and replication, perhaps through effects on gene expression. Other signaling molecules—including cytokines, interleukins, growth factors, and metabolites—use some of the same general mechanisms and signal transduction pathways. Excessive, deficient, or inappropriate production and release of hormones and of these other regulatory molecules are major causes of disease. Many pharmacotherapeutic agents are aimed at correcting or otherwise influencing the pathways discussed in this chapter.

HORMONES TRANSDUCE SIGNALS TO AFFECT HOMEOSTATIC MECHANISMS

The general steps involved in producing a coordinated response to a particular stimulus are illustrated in Figure 43–1. The stimulus can be a challenge or a threat to the organism, to an organ, or to the integrity of a single cell within that organism. Recognition of the stimulus is the first step in the adaptive response. At the organismic level, this generally involves the nervous system and the special senses (sight, hearing, pain, smell, touch). At the organismic or cellular level, recognition involves physicochemical factors such as pH, O_2 tension, temperature, nutrient supply, noxious metabolites, and osmolarity. Appropriate recognition results in the release of one or more hormones that will govern generation of the necessary adaptive response. For purposes of this discussion, the hormones are categorized as described in Chapter 42, ie, based on the location of their specific cellular receptors and the type of signals generated. Group I hormones interact with an intracellular receptor and group II hormones with receptor recognition sites located on the extracellular surface of the plasma membrane of target cells. The cytokines, interleukins, and growth factors should also be considered in this latter category. These molecules, of critical importance in homeostatic adaptation, are hormones in the sense that they are produced in specific cells, have the equivalent of autocrine, paracrine, and endocrine actions, bind to cell surface receptors, and activate many of the same signal transduction pathways employed by the more traditional group II hormones.

SIGNAL GENERATION

The Ligand-Receptor Complex Is the Signal for Group I Hormones

The lipophilic group I hormones diffuse through the plasma membrane of all cells but only encounter their specific, high-affinity intracellular receptors in target cells. These receptors can be located in the cytoplasm or in the nucleus of target cells. The hormone-receptor complex first undergoes an **activation reaction.** As shown in Figure 43–2, receptor activation occurs by at least two mechanisms. For example, glucocorticoids diffuse across the plasma membrane and encounter their cognate receptor in the cytoplasm of target cells. Ligand-receptor binding results in the dissociation of heat shock protein 90 (hsp90) from the receptor. This step appears to be necessary for subsequent nuclear localization of the glucocorticoid receptor. This receptor also contains nuclear localization sequences that assist in the translocation from cytoplasm to nucleus. The now activated receptor moves into the nucleus (Figure 43–2) and binds with high affinity to a specific DNA sequence called the **hormone response element (HRE).** In the case illustrated, this is a glucocorticoid response element, or GRE. Consensus sequences for HREs are shown in Table 43–1. The DNA-bound, liganded receptor serves as a high-affinity binding site for

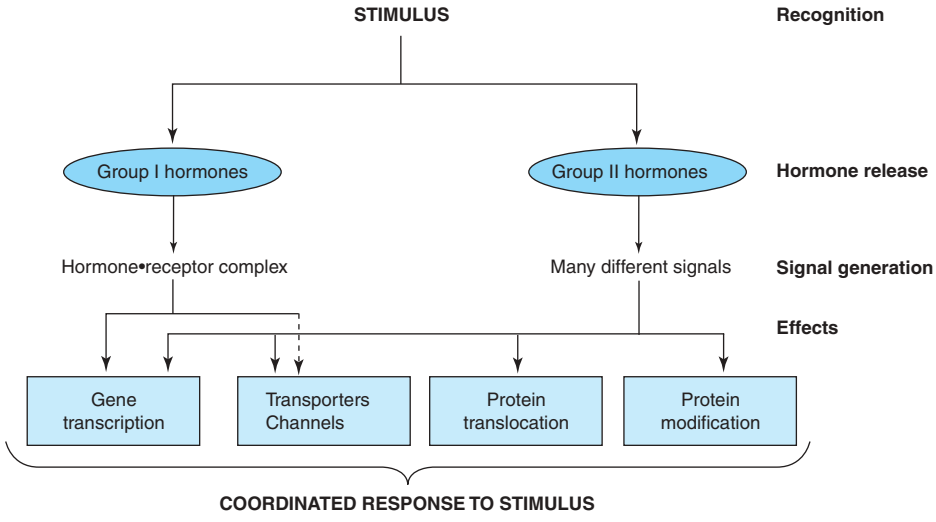

Figure 43–1. Hormonal involvement in responses to a stimulus. A challenge to the integrity of the organism elicits a response that includes the release of one or more hormones. These hormones generate signals at or within target cells, and these signals regulate a variety of biologic processes which provide for a coordinated response to the stimulus or challenge. See Figure 43–8 for a specific example.

one or more coactivator proteins, and accelerated gene transcription typically ensues when this occurs. By contrast, certain hormones such as the thyroid hormones and retinoids diffuse from the extracellular fluid across the plasma membrane and go directly into the nucleus. In this case, the cognate receptor is already bound to the HRE (the thyroid hormone response element [TRE], in this example). However, this DNA-bound receptor fails to activate transcription because it is complexed with a corepressor. Indeed, this receptor-corepressor complex serves as an active repressor of gene transcription. The association of ligand with these receptors results in dissociation of the corepressor. The liganded receptor is now capable of binding one or more coactivators with high affinity, resulting in the activation of gene transcription. The relationship of hormone receptors to other nuclear receptors and to coregulators is discussed in more detail below.

By selectively affecting gene transcription and the consequent production of appropriate target mRNAs, the amounts of specific proteins are changed and metabolic processes are influenced. The influence of each of these hormones is quite specific; generally, the hormone affects less than 1% of the genes, mRNA, or proteins in a target cell; sometimes only a few are affected. The nuclear actions of steroid, thyroid, and retinoid hormones are quite well defined. Most evidence suggests that these hormones exert their dominant effect on modulating gene transcription, but they—and many of the hormones in the other classes discussed below—can act at any step of the "information pathway" illustrated in Figure 43–3. Direct actions of steroids in the cytoplasm and on various organelles and membranes have also been described.

GROUP II (PEPTIDE & CATECHOLAMINE) HORMONES HAVE MEMBRANE RECEPTORS & USE INTRACELLULAR MESSENGERS

Many hormones are water-soluble, have no transport proteins (and therefore have a short plasma half-life), and initiate a response by binding to a receptor located in the plasma membrane (see Tables 42–3 and 42–4). The mechanism of action of this group of hormones can best be discussed in terms of the **intracellular signals** they generate. These signals include cAMP (cyclic AMP; 3′,5′-adenylic acid; see Figure 18–5), a nucleotide derived from ATP through the action of adenylyl cyclase; cGMP, a nucleotide formed by guanylyl cyclase; Ca^{2+}; and phosphatidylinositides. Many of these second messengers affect gene transcription, as described in the previous paragraph; but they also influ-

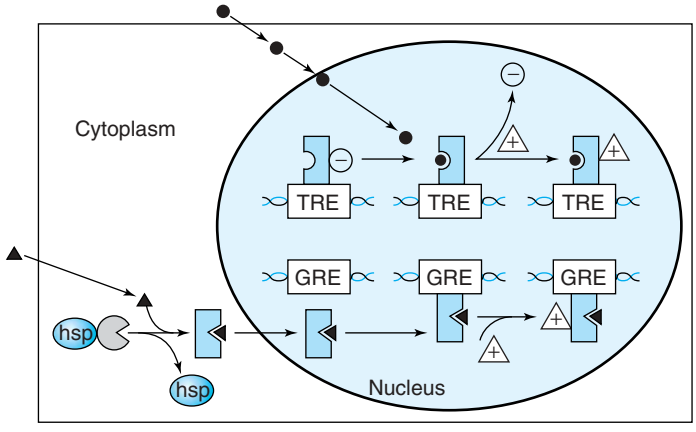

Figure 43–2. Regulation of gene expression by class I hormones. Steroid hormones readily gain access to the cytoplasmic compartment of target cells. Glucocorticoid hormones (solid triangles) encounter their cognate receptor in the cytoplasm, where it exists in a complex with heat shock protein 90 (hsp). Ligand binding causes dissociation of hsp and a conformational change of the receptor. The receptor•ligand complex then traverses the nuclear membrane and binds to DNA with specificity and high affinity at a glucocorticoid response element (GRE). This event triggers the assembly of a number of transcription coregulators (△), and enhanced transcription ensues. By contrast, thyroid hormones and retinoic acid (●) directly enter the nucleus, where their cognate receptors are already bound to the appropriate response elements with an associated transcription repressor complex (⊖). This complex, which consists of molecules such as N-CoR or SMRT (see Table 43–6) in the absence of ligand, actively inhibits transcription. Ligand binding results in dissociation of the repressor complex from the receptor, allowing an activator complex to assemble. The gene is then actively transcribed.

ence a variety of other biologic processes, as shown in Figure 43–1.

G Protein-Coupled Receptors (GPCR)

Many of the group II hormones bind to receptors that couple to effectors through a GTP-binding protein intermediary. These receptors typically have seven hydrophobic plasma membrane-spanning domains. This is illustrated by the seven interconnected cylinders extending through the lipid bilayer in Figure 43–4. Receptors of this class, which signal through guanine nucleotide-bound protein intermediates, are known as **G protein-coupled receptors,** or **GPCRs.** To date, over 130 G protein-linked receptor genes have been cloned from various mammalian species. A wide variety of responses are mediated by the GPCRs.

cAMP Is the Intracellular Signal for Many Responses

Cyclic AMP was the first intracellular signal identified in mammalian cells. Several components comprise a system for the generation, degradation, and action of cAMP.

A. Adenylyl Cyclase

Different peptide hormones can either stimulate (s) or inhibit (i) the production of cAMP from adenylyl cy-

Table 43–1. The DNA sequences of several hormone response elements (HREs).[1]

Hormone or Effector	HRE	DNA Sequence
Glucocorticoids Progestins Mineralocorticoids Androgens	GRE PRE MRE ARE	GGTACA NNN TGTTCT
Estrogens	ERE	AGGTCA --- TGA/TCCT
Thyroid hormone Retinoic acid Vitamin D	TRE RARE VDRE	AGGTCA N3,4,5, AGGTCA
cAMP	CRE	TGACGTCA

[1]Letters indicate nucleotide; N means any one of the four can be used in that position. The arrows pointing in opposite directions illustrate the slightly imperfect inverted palindromes present in many HREs; in some cases these are called "half binding sites" because each binds one monomer of the receptor. The GRE, PRE, MRE, and ARE consist of the same DNA sequence. Specificity may be conferred by the intracellular concentration of the ligand or hormone receptor, by flanking DNA sequences not included in the consensus, or by other accessory elements. A second group of HREs includes those for thyroid hormones, estrogens, retinoic acid, and vitamin D. These HREs are similar except for the orientation and spacing between the half palindromes. Spacing determines the hormone specificity. VDRE (N=3), TRE (N=4), and RARE (N=5) bind to direct repeats rather than to inverted repeats. Another member of the steroid receptor superfamily, the retinoid X receptor (RXR), forms heterodimers with VDR, TR, and RARE, and these constitute the *trans*-acting factors. cAMP affects gene transcription through the CRE.

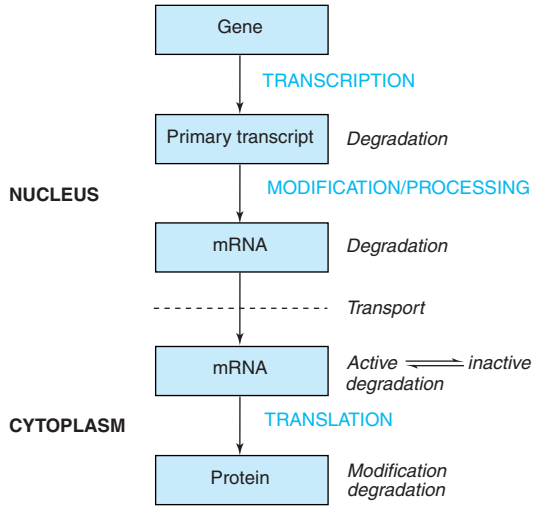

Figure 43–3. The "information pathway." Information flows from the gene to the primary transcript to mRNA to protein. Hormones can affect any of the steps involved and can affect the rates of processing, degradation, or modification of the various products.

clase, which is encoded by at least nine different genes (Table 43–2). Two parallel systems, a stimulatory (s) one and an inhibitory (i) one, converge upon a single catalytic molecule (C). Each consists of a receptor, R_s or R_i, and a regulatory complex, G_s and G_i. G_s and G_i are each trimers composed of α, β, and γ subunits. Because the α subunit in G_s differs from that in G_i, the proteins, which are distinct gene products, are designated α_s and α_i. The α subunits bind guanine nucleotides. The β and γ subunits are always associated (βγ) and appear to function as a heterodimer. The binding of a hormone to R_s or R_i results in a receptor-mediated activation of G, which entails the exchange of GDP by GTP on α and the concomitant dissociation of βγ from α.

The α_s protein has intrinsic GTPase activity. The active form, α_s•GTP, is inactivated upon hydrolysis of the GTP to GDP; the trimeric G_s complex ($\alpha\beta\gamma$) is then re-formed and is ready for another cycle of activation. Cholera and pertussis toxins catalyze the ADP-ribosylation of α_s and $\alpha_{i\text{-}2}$ (see Table 43–3), respectively. In the case of α_s, this modification disrupts the intrinsic GTP-ase activity; thus, α_s cannot reassociate with βγ and is therefore irreversibly activated. ADP-ribosylation of $\alpha_{i\text{-}2}$ prevents the dissociation of $\alpha_{i\text{-}2}$ from βγ, and free $\alpha_{i\text{-}2}$ thus cannot be formed. α_s activity in such cells is therefore unopposed.

There is a large family of G proteins, and these are part of the superfamily of GTPases. The G protein family is classified according to sequence homology into four subfamilies, as illustrated in Table 43–3. There are 21 α, 5 β, and 8 γ subunit genes. Various combinations of these subunits provide a large number of possible αβγ and cyclase complexes.

The α subunits and the βγ complex have actions independent of those on adenylyl cyclase (see Figure 43–4 and Table 43–3). Some forms of α_i stimulate K^+ channels and inhibit Ca^{2+} channels, and some α_s molecules have the opposite effects. Members of the G_q family activate the phospholipase C group of enzymes. The βγ complexes have been associated with K^+ channel stimulation and phospholipase C activation. G proteins are involved in many important biologic processes in addition to hormone action. Notable examples include olfaction (α_{OLF}) and vision (α_t). Some examples are listed in Table 43–3. GPCRs are implicated in a number of diseases and are major targets for pharmaceutical agents.

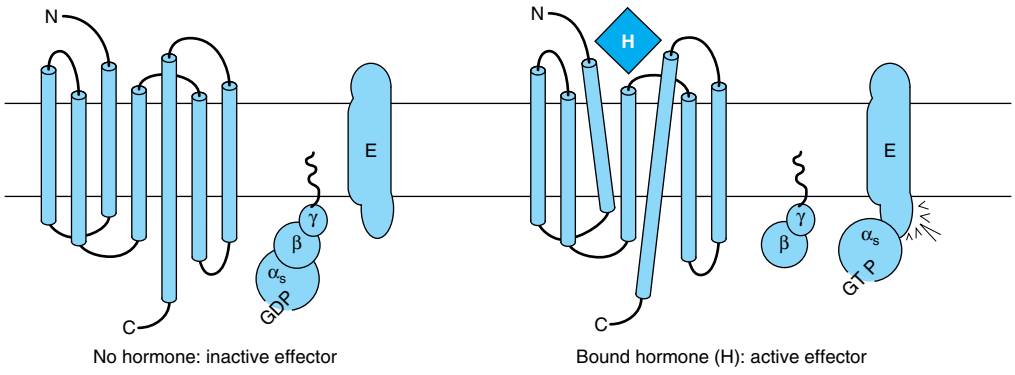

Figure 43–4. Components of the hormone receptor–G protein effector system. Receptors that couple to effectors through G proteins (GPCR) typically have seven membrane-spanning domains. In the absence of hormone (left), the heterotrimeric G-protein complex (α, β, γ) is in an inactive guanosine diphosphate (GDP)-bound form and is probably not associated with the receptor. This complex is anchored to the plasma membrane through prenylated groups on the βγ subunits (wavy lines) and perhaps by myristoylated groups on α subunits (not shown). On binding of hormone (H) to the receptor, there is a presumed conformational change of the receptor—as indicated by the tilted membrane spanning domains—and activation of the G-protein complex. This results from the exchange of GDP with guanosine triphosphate (GTP) on the α subunit, after which α and βγ dissociate. The α subunit binds to and activates the effector (E). E can be adenylyl cyclase, Ca^{2+}, Na^+, or Cl^- channels (α_s), or it could be a K^+ channel (α_i), phospholipase Cβ (α_q), or cGMP phosphodiesterase (α_t). The βγ subunit can also have direct actions on E. (Modified and reproduced, with permission, from Granner DK in: *Principles and Practice of Endocrinology and Metabolism,* 3rd ed. Becker KL [editor]. Lippincott, 2000.)

Table 43–2. Subclassification of group II.A hormones.

Hormones That Stimulate Adenylyl Cyclase (H_s)	Hormones That Inhibit Adenylyl Cyclase (H_i)
ACTH	Acetylcholine
ADH	α_2-Adrenergics
β-Adrenergics	Angiotensin II
Calcitonin	Somatostatin
CRH	
FSH	
Glucagon	
hCG	
LH	
LPH	
MSH	
PTH	
TSH	

B. Protein Kinase

In prokaryotic cells, cAMP binds to a specific protein called catabolite regulatory protein (CRP) that binds directly to DNA and influences gene expression. In eukaryotic cells, cAMP binds to a protein kinase called **protein kinase A (PKA)** that is a heterotetrameric molecule consisting of two regulatory subunits (R) and two catalytic subunits (C). cAMP binding results in the following reaction:

$$4\ \text{cAMP} + R_2C_2 \rightleftharpoons R_2 \cdot (4\ \text{cAMP}) + 2C$$

The R_2C_2 complex has no enzymatic activity, but the binding of cAMP by R dissociates R from C, thereby activating the latter (Figure 43–5). The active C subunit catalyzes the transfer of the γ phosphate of ATP to a serine or threonine residue in a variety of proteins. The consensus phosphorylation sites are -Arg-Arg/Lys-X-Ser/Thr- and -Arg-Lys-X-X-Ser-, where X can be any amino acid.

Protein kinase activities were originally described as being "cAMP-dependent" or "cAMP-independent." This

Table 43–3. Classes and functions of selected G proteins.[1,2]

Class or Type	Stimulus	Effector	Effect
G_s			
α_s	Glucagon, β-adrenergics	↑ Adenylyl cyclase ↑ Cardiac Ca^{2+}, Cl^-, and Na^+ channels	Gluconeogenesis, lipolysis, glycogenolysis
α_{olf}	Odorant	↑ Adenylyl cyclase	Olfaction
G_i			
$\alpha_{i\text{-}1,2,3}$	Acetylcholine, α_2-adrenergics M_2 cholinergics	↓ Adenylyl cyclase ↑ Potassium channels ↓ Calcium channels	Slowed heart rate
α_0	Opioids, endorphins	↑ Potassium channels	Neuronal electrical activity
α_t	Light	↑ cGMP phosphodiesterase	Vision
G_q			
α_q	M_1 cholinergics α_1-Adrenergics	↑ Phospholipase C-β1	↑ Muscle contraction and ↑ Blood pressure
α_{11}	α_1-Adrenergics	↑ Phospholipase c-β2	
G_{12}			
α_{12}	?	Cl^- channel	?

[1]Modified and reproduced, with permission, from Granner DK in: *Principles and Practice of Endocrinology and Metabolism,* 3rd ed. Becker KL (editor). Lippincott, 2000.

[2]The four major classes or families of mammalian G proteins (G_s, G_i, G_q, and G_{12}) are based on protein sequence homology. Representative members of each are shown, along with known stimuli, effectors, and well-defined biologic effects. Nine isoforms of adenylyl cyclase have been identified (isoforms I–IX). All isoforms are stimulated by α_s; α_i isoforms inhibit types V and VI, and α_0 inhibits types I and V. At least 16 different α subunits have been identified.

classification has changed, as protein phosphorylation is now recognized as being a major regulatory mechanism. Several hundred protein kinases have now been described. The kinases are related in sequence and structure within the catalytic domain, but each is a unique molecule with considerable variability with respect to subunit composition, molecular weight, autophosphorylation, K_m for ATP, and substrate specificity.

C. Phosphoproteins

The effects of cAMP in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues. The control of any of the effects of cAMP, including such diverse processes as steroidogenesis, secretion, ion transport, carbohydrate and fat metabolism, enzyme induction, gene regulation, synaptic transmission, and cell growth and replication, could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phosphorylation. These substrates help define a target tissue and are involved in defining the extent of a particular response within a given cell. For example, the effects of cAMP on gene transcription are mediated by the protein **cyclic AMP response element binding protein (CREB).** CREB binds to a cAMP responsive element (CRE) (see Table 43–1) in its nonphosphorylated state and is a weak activator of transcription. When phosphorylated by PKA, CREB binds the coactivator **CREB-binding protein CBP/p300** (see below) and as a result is a much more potent transcription activator.

D. Phosphodiesterases

Actions caused by hormones that increase cAMP concentration can be terminated in a number of ways, including the hydrolysis of cAMP to 5′-AMP by phosphodiesterases (see Figure 43–5). The presence of these hydrolytic enzymes ensures a rapid turnover of the signal (cAMP) and hence a rapid termination of the biologic process once the hormonal stimulus is removed. There are at least 11 known members of the phosphodiesterase family of enzymes. These are subject to regulation by their substrates, cAMP and cGMP; by hormones; and by intracellular messengers such as calcium, probably acting through calmodulin. Inhibitors of phosphodiesterase, most notably methylated xanthine derivatives such as caffeine, increase intracellular cAMP and mimic or prolong the actions of hormones through this signal.

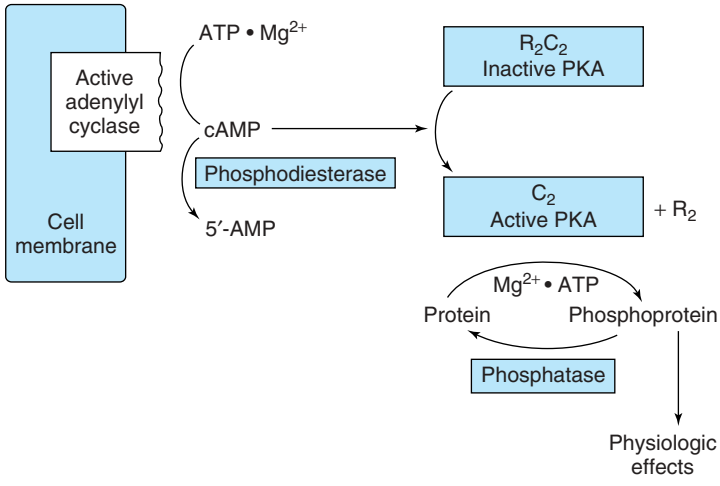

Figure 43–5. Hormonal regulation of cellular processes through cAMP-dependent protein kinase (PKA). PKA exists in an inactive form as an R_2C_2 heterotetramer consisting of two regulatory and two catalytic subunits. The cAMP generated by the action of adenylyl cyclase (activated as shown in Figure 43–4) binds to the regulatory (R) subunit of PKA. This results in dissociation of the regulatory and catalytic subunits and activation of the latter. The active catalytic subunits phosphorylate a number of target proteins on serine and threonine residues. Phosphatases remove phosphate from these residues and thus terminate the physiologic response. A phosphodiesterase can also terminate the response by converting cAMP to 5′-AMP.

E. Phosphoprotein Phosphatases

Given the importance of protein phosphorylation, it is not surprising that regulation of the protein dephosphorylation reaction is another important control mechanism (see Figure 43–5). The phosphoprotein phosphatases are themselves subject to regulation by phosphorylation-dephosphorylation reactions and by a variety of other mechanisms, such as protein-protein interactions. In fact, the substrate specificity of the phosphoserine-phosphothreonine phosphatases may be dictated by distinct regulatory subunits whose binding is regulated hormonally. The best-studied role of regulation by the dephosphorylation of proteins is that of glycogen metabolism in muscle. Two major types of phosphoserine-phosphothreonine phosphatases have been described. Type I preferentially dephosphorylates the β subunit of phosphorylase kinase, whereas type II dephosphorylates the α subunit. Type I phosphatase is implicated in the regulation of glycogen synthase, phosphorylase, and phosphorylase kinase. This phosphatase is itself regulated by phosphorylation of certain of its subunits, and these reactions are reversed by the action of one of the type II phosphatases. In addition, two heat-stable protein inhibitors regulate type I phosphatase activity. Inhibitor-1 is phosphorylated and activated by cAMP-dependent protein kinases; and inhibitor-2, which may be a subunit of the inactive phosphatase, is also phosphorylated, possibly by glycogen synthase kinase-3.

cGMP Is Also an Intracellular Signal

Cyclic GMP is made from GTP by the enzyme guanylyl cyclase, which exists in soluble and membrane-bound forms. Each of these isozymes has unique physiologic properties. The atriopeptins, a family of peptides produced in cardiac atrial tissues, cause natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion. These peptides (eg, atrial natriuretic factor) bind to and activate the membrane-bound form of guanylyl cyclase. This results in an increase of cGMP by as much as 50-fold in some cases, and this is thought to mediate the effects mentioned above. Other evidence links cGMP to vasodilation. A series of compounds, including nitroprusside, nitroglycerin, nitric oxide, sodium nitrite, and sodium azide, all cause smooth muscle re-

laxation and are potent vasodilators. These agents increase cGMP by activating the soluble form of guanylyl cyclase, and inhibitors of cGMP phosphodiesterase (the drug sildenafil [Viagra], for example) enhance and prolong these responses. The increased cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates a number of smooth muscle proteins. Presumably, this is involved in relaxation of smooth muscle and vasodilation.

Several Hormones Act Through Calcium or Phosphatidylinositols

Ionized calcium is an important regulator of a variety of cellular processes, including muscle contraction, stimulus-secretion coupling, the blood clotting cascade, enzyme activity, and membrane excitability. It is also an intracellular messenger of hormone action.

A. Calcium Metabolism

The extracellular calcium (Ca^{2+}) concentration is about 5 mmol/L and is very rigidly controlled. Although substantial amounts of calcium are associated with intracellular organelles such as mitochondria and the endoplasmic reticulum, the intracellular concentration of free or ionized calcium (Ca^{2+}) is very low: 0.05–10 μmol/L. In spite of this large concentration gradient and a favorable transmembrane electrical gradient, Ca^{2+} is restrained from entering the cell. A considerable amount of energy is expended to ensure that the intracellular Ca^{2+} is controlled, as a prolonged elevation of Ca^{2+} in the cell is very toxic. A Na^+/Ca^{2+} exchange mechanism that has a high capacity but low affinity pumps Ca^{2+} out of cells. There also is a Ca^{2+}/proton ATPase-dependent pump that extrudes Ca^{2+} in exchange for H^+. This has a high affinity for Ca^{2+} but a low capacity and is probably responsible for fine-tuning cytosolic Ca^{2+}. Furthermore, Ca^{2+} ATPases pump Ca^{2+} from the cytosol to the lumen of the endoplasmic reticulum. There are three ways of changing cytosolic Ca^{2+}: (1) Certain hormones (class II.C, Table 42–3) by binding to receptors that are themselves Ca^{2+} channels, enhance membrane permeability to Ca^{2+} and thereby increase Ca^{2+} influx. (2) Hormones also indirectly promote Ca^{2+} influx by modulating the membrane potential at the plasma membrane. Membrane depolarization opens voltage-gated Ca^{2+} channels and allows for Ca^{2+} influx. (3) Ca^{2+} can be mobilized from the endoplasmic reticulum, and possibly from mitochondrial pools.

An important observation linking Ca^{2+} to hormone action involved the definition of the intracellular targets of Ca^{2+} action. The discovery of a Ca^{2+}-dependent regulator of phosphodiesterase activity provided the basis for a broad understanding of how Ca^{2+} and cAMP interact within cells.

B. Calmodulin

The calcium-dependent regulatory protein is calmodulin, a 17-kDa protein that is homologous to the muscle protein troponin C in structure and function. Calmodulin has four Ca^{2+} binding sites, and full occupancy of these sites leads to a marked conformational change, which allows calmodulin to activate enzymes and ion channels. The interaction of Ca^{2+} with calmodulin (with the resultant change of activity of the latter) is conceptually similar to the binding of cAMP to PKA and the subsequent activation of this molecule. Calmodulin can be one of numerous subunits of complex proteins and is particularly involved in regulating various kinases and enzymes of cyclic nucleotide generation and degradation. A partial list of the enzymes regulated directly or indirectly by Ca^{2+}, probably through calmodulin, is presented in Table 43–4.

In addition to its effects on enzymes and ion transport, Ca^{2+}/calmodulin regulates the activity of many structural elements in cells. These include the actin-myosin complex of smooth muscle, which is under β-adrenergic control, and various microfilament-mediated processes in noncontractile cells, including cell motility, cell conformation changes, mitosis, granule release, and endocytosis.

C. Calcium Is a Mediator of Hormone Action

A role for Ca^{2+} in hormone action is suggested by the observations that the effect of many hormones is (1) blunted by Ca^{2+}-free media or when intracellular calcium is depleted; (2) can be mimicked by agents that increase cytosolic Ca^{2+}, such as the Ca^{2+} ionophore A23187; and (3) influences cellular calcium flux. The regulation of glycogen metabolism in liver by vasopressin and α-adrenergic catecholamines provides a good example. This is shown schematically in Figures 18–6 and 18–7.

Table 43–4. Enzymes and proteins regulated by calcium or calmodulin.

Adenylyl cyclase
Ca^{2+}-dependent protein kinases
Ca^{2+}-Mg^{2+} ATPase
Ca^{2+}-phospholipid-dependent protein kinase
Cyclic nucleotide phosphodiesterase
Some cytoskeletal proteins
Some ion channels (eg, L-type calcium channels)
Nitric oxide synthase
Phosphorylase kinase
Phosphoprotein phosphatase 2B
Some receptors (eg, NMDA-type glutamate receptor)

A number of critical metabolic enzymes are regulated by Ca^{2+}, phosphorylation, or both, including glycogen synthase, pyruvate kinase, pyruvate carboxylase, glycerol-3-phosphate dehydrogenase, and pyruvate dehydrogenase.

D. Phosphatidylinositide Metabolism Affects Ca^{2+}-Dependent Hormone Action

Some signal must provide communication between the hormone receptor on the plasma membrane and the intracellular Ca^{2+} reservoirs. This is accomplished by products of phosphatidylinositol metabolism. Cell surface receptors such as those for acetylcholine, antidiuretic hormone, and α_1-type catecholamines are, when occupied by their respective ligands, potent activators of phospholipase C. Receptor binding and activation of phospholipase C are coupled by the G_q isoforms (Table 43–3 and Figure 43–6). Phospholipase C catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol trisphosphate (IP_3) and 1,2-diacylglycerol (Figure 43–7). Diacylglycerol is itself capable of activating **protein kinase C (PKC),** the activity of which also depends upon Ca^{2+}. IP_3, by interacting with a specific intracellular receptor, is an effective releaser of Ca^{2+} from

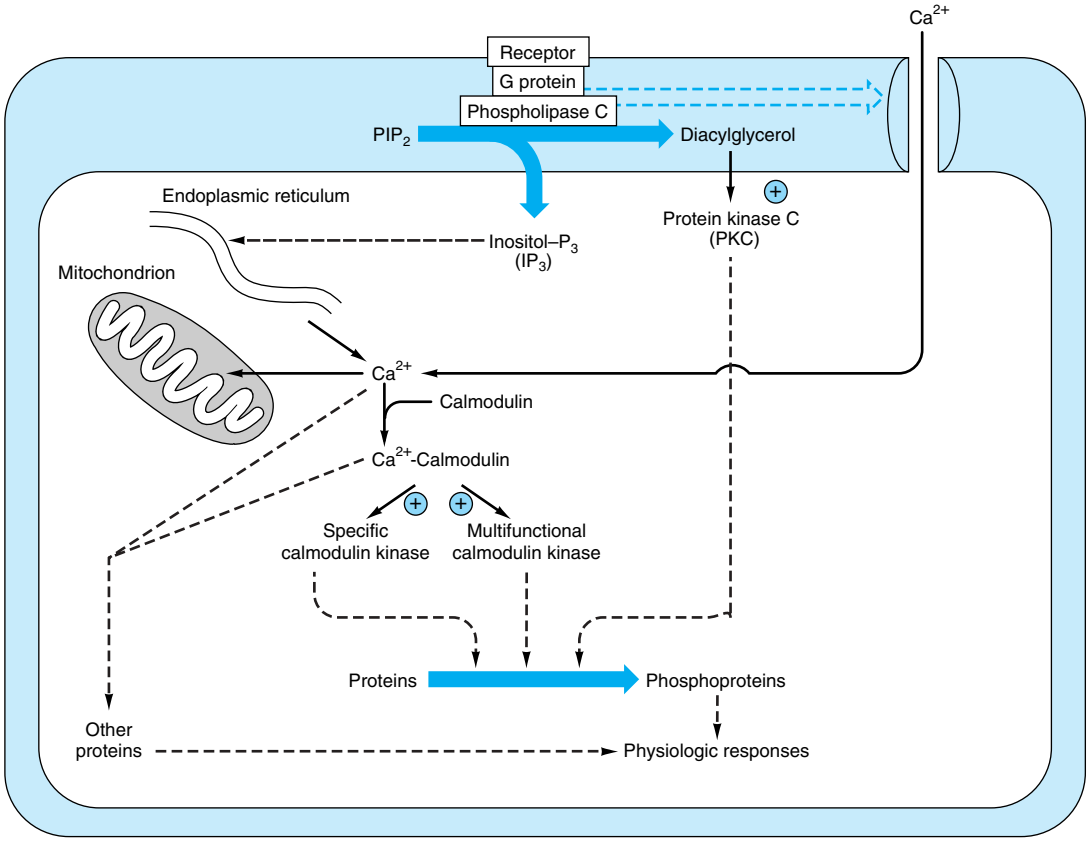

Figure 43–6. Certain hormone-receptor interactions result in the activation of phospholipase C. This appears to involve a specific G protein, which also may activate a calcium channel. Phospholipase C results in generation of inositol trisphosphate (IP_3), which liberates stored intracellular Ca^{2+}, and diacylglycerol (DAG), a potent activator of protein kinase C (PKC). In this scheme, the activated PKC phosphorylates specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases, two of which are shown here. These actions result in phosphorylation of substrates, and this leads to altered physiologic responses. This figure also shows that Ca^{2+} can enter cells through voltage- or ligand-gated Ca^{2+} channels. The intracellular Ca^{2+} is also regulated through storage and release by the mitochondria and endoplasmic reticulum. (Courtesy of JH Exton.)

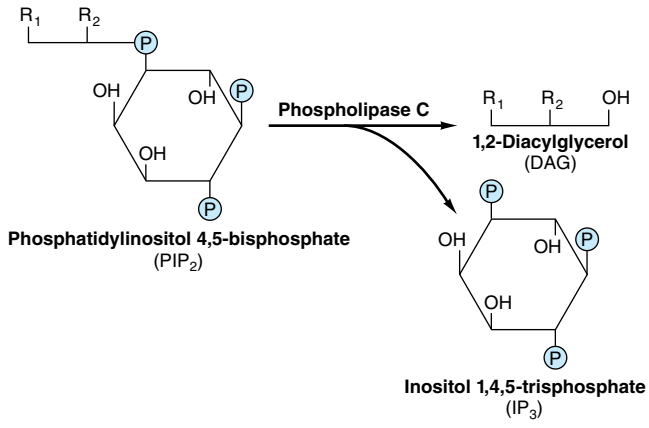

Figure 43–7. Phospholipase C cleaves PIP_2 into diacylglycerol and inositol trisphosphate. R_1 generally is stearate, and R_2 is usually arachidonate. IP_3 can be dephosphorylated (to the inactive I-1,4-P_2) or phosphorylated (to the potentially active I-1,3,4,5-P_4).

intracellular storage sites in the endoplasmic reticulum. Thus, the hydrolysis of phosphatidylinositol 4,5-bisphosphate leads to activation of PKC and promotes an increase of cytoplasmic Ca^{2+}. As shown in Figure 43–4, the activation of G proteins can also have a direct action on Ca^{2+} channels. The resulting elevations of cytosolic Ca^{2+} activate Ca^{2+}–calmodulin-dependent kinases and many other Ca^{2+}–calmodulin-dependent enzymes.

Steroidogenic agents—including ACTH and cAMP in the adrenal cortex; angiotensin II, K^+, serotonin, ACTH, and cAMP in the zona glomerulosa of the adrenal; LH in the ovary; and LH and cAMP in the Leydig cells of the testes—have been associated with increased amounts of phosphatidic acid, phosphatidylinositol, and polyphosphoinositides (see Chapter 14) in the respective target tissues. Several other examples could be cited.

The roles that Ca^{2+} and polyphosphoinositide breakdown products might play in hormone action are presented in Figure 43–6. In this scheme the activated protein kinase C can phosphorylate specific substrates, which then alter physiologic processes. Likewise, the Ca^{2+}-calmodulin complex can activate specific kinases. These then modify substrates and thereby alter physiologic responses.

Some Hormones Act Through a Protein Kinase Cascade

Single protein kinases such as PKA, PKC, and Ca^{2+}-calmodulin (CaM)-kinases, which result in the phosphorylation of serine and threonine residues in target proteins, play a very important role in hormone action. The discovery that the EGF receptor contains an intrinsic tyrosine kinase activity that is activated by the binding of the ligand EGF was an important breakthrough. The insulin and IGF-I receptors also contain intrinsic ligand-activated tyrosine kinase activity. Several receptors—generally those involved in binding ligands involved in growth control, differentiation, and the inflammatory response—either have intrinsic tyrosine kinase activity or are associated with proteins that are tyrosine kinases. Another distinguishing feature of this class of hormone action is that these kinases preferentially phosphorylate tyrosine residues, and tyrosine phosphorylation is infrequent (< 0.03% of total amino acid phosphorylation) in mammalian cells. A third distinguishing feature is that the ligand-receptor interaction that results in a tyrosine phosphorylation event initiates a cascade that may involve several protein kinases, phosphatases, and other regulatory proteins.

A. Insulin Transmits Signals by Several Kinase Cascades

The insulin, epidermal growth factor (EGF), and IGF-I receptors have intrinsic protein tyrosine kinase activities located in their cytoplasmic domains. These activities are stimulated when the receptor binds ligand. The receptors are then autophosphorylated on tyrosine residues, and this initiates a complex series of events (summarized in simplified fashion in Figure 43–8). The phosphorylated insulin receptor next phosphorylates insulin receptor substrates (there are at least four of these molecules, called IRS 1–4) on tyrosine residues. Phosphorylated IRS binds to the Src homology 2 (SH2) domains of a variety of proteins that are directly involved in mediating different effects of insulin. One of these proteins, PI-3 kinase, links insulin receptor activation to insulin action through activation of a number of molecules, including the kinase PDK1 (phosphoinositide-dependent kinase-1). This enzyme propagates the signal through several other kinases, including PKB (akt), SKG, and aPKC (see legend to Figure 43–8 for definitions and expanded abbreviations). An alternative

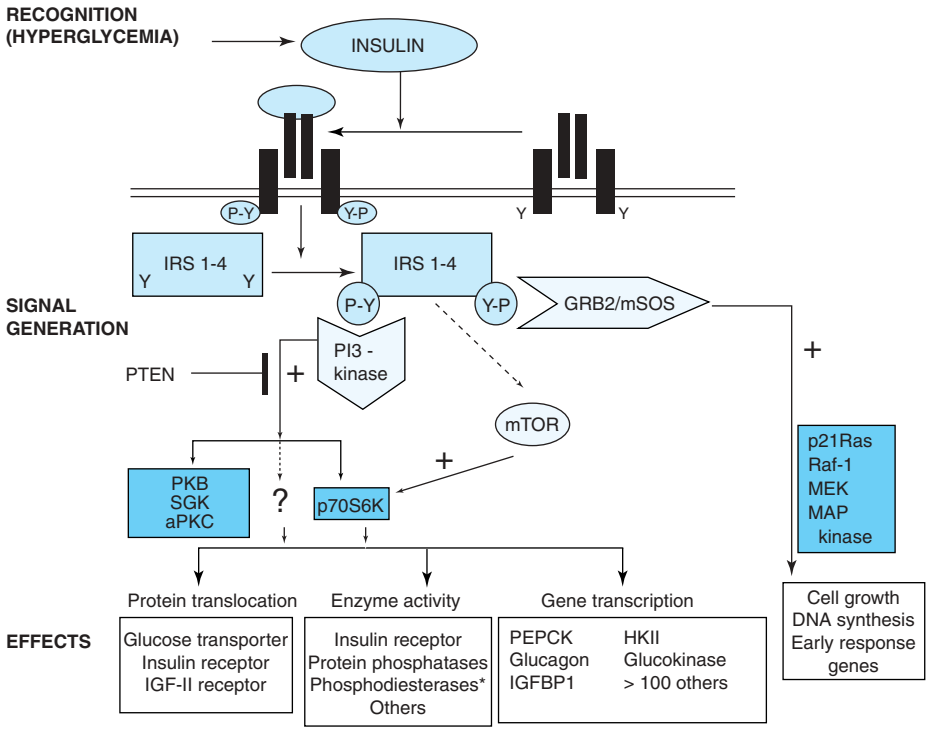

Figure 43–8. Insulin signaling pathways. The insulin signaling pathways provide an excellent example of the "recognition → hormone release → signal generation → effects" paradigm outlined in Figure 43–1. Insulin is released in response to hyperglycemia. Binding of insulin to a target cell-specific plasma membrane receptor results in a cascade of intracellular events. Stimulation of the intrinsic tyrosine kinase activity of the insulin receptor marks the initial event, resulting in increased tyrosine (Y) phosphorylation (Y → Y-P) of the receptor and then one or more of the insulin receptor substrate molecules (IRS 1–4). This increase in phosphotyrosine stimulates the activity of many intracellular molecules such as GTPases, protein kinases, and lipid kinases, all of which play a role in certain metabolic actions of insulin. The two best-described pathways are shown. First, phosphorylation of an IRS molecule (probably IRS-2) results in docking and activation of the lipid kinase, PI-3 kinase, which generates novel inositol lipids that may act as "second messenger" molecules. These, in turn, activate PDK1 and then a variety of downstream signaling molecules, including protein kinase B (PKB or akt), SGK, and aPKC. An alternative pathway involves the activation of p70S6K and perhaps other as yet unidentified kinases. Second, phosphorylation of IRS (probably IRS-1) results in docking of GRB2/mSOS and activation of the small GTPase, p21RAS, which initiates a protein kinase cascade that activates Raf-1, MEK, and the p42/p44 MAP kinase isoforms. These protein kinases are important in the regulation of proliferation and differentiation of several cell types. The mTOR pathway provides an alternative way of activating p70S6K and appears to be involved in nutrient signaling as well as insulin action. Each of these cascades may influence different physiologic processes, as shown. Each of the phosphorylation events is reversible through the action of specific phosphatases. For example, the lipid phosphatase PTEN dephosphorylates the product of the PI-3 kinase reaction, thereby antagonizing the pathway and terminating the signal. Representative effects of major actions of insulin are shown in each of the boxes. The asterisk after phosphodiesterase indicates that insulin indirectly affects the activity of many enzymes by activating phosphodiesterases and reducing intracellular cAMP levels. (IGFBP, insulin-like growth factor binding protein; IRS 1–4, insulin receptor substrate isoforms 1–4); PI-3 kinase, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PKD1, phosphoinositide-dependent kinase; PKB, protein kinase B; SGK, serum and glucocorticoid-regulated kinase; aPKC, atypical protein kinase C; p70S6K, p70 ribosomal protein S6 kinase; mTOR, mammalian target of rapamycin; GRB2, growth factor receptor binding protein 2; mSOS, mammalian son of sevenless; MEK, MAP kinase kinase and ERK kinase; MAP kinase, mitogen-activated protein kinase.)

pathway downstream from PKD1 involves p70S6K and perhaps other as yet unidentified kinases. A second major pathway involves mTOR. This enzyme is directly regulated by amino acids and insulin and is essential for p70S6K activity. This pathway provides a distinction between the PKB and p70S6K branches downstream from PKD1. These pathways are involved in protein translocation, enzyme activity, and the regulation, by insulin, of genes involved in metabolism (Figure 43–8). Another SH2 domain-containing protein is GRB2, which binds to IRS-1 and links tyrosine phosphorylation to several proteins, the result of which is activation of a cascade of threonine and serine kinases. A pathway showing how this insulin-receptor interaction activates the mitogen-activated protein (MAP) kinase pathway and the anabolic effects of insulin is illustrated in Figure 43–8. The exact roles of many of these docking proteins, kinases, and phosphatases remain to be established.

B. The Jak/STAT Pathway Is Used by Hormones and Cytokines

Tyrosine kinase activation can also initiate a phosphorylation and dephosphorylation cascade that involves the action of several other protein kinases and the counterbalancing actions of phosphatases. Two mechanisms are employed to initiate this cascade. Some hormones, such as growth hormone, prolactin, erythropoietin, and the cytokines, initiate their action by activating a tyrosine kinase, but this activity is not an integral part of the hormone receptor. The hormone-receptor interaction promotes binding and activation of **cytoplasmic protein tyrosine kinases,** such as **Tyk-2, Jak1,** or **Jak2.** These kinases phosphorylate one or more cytoplasmic proteins, which then associate with other docking proteins through binding to SH2 domains. One such interaction results in the activation of a family of cytosolic proteins called **signal transducers and activators of transcription (STATs).** The phosphorylated STAT protein dimerizes and translocates into the nucleus, binds to a specific DNA element such as the interferon response element, and activates transcription. This is illustrated in Figure 43–9. Other SH2 docking events may result in the activation of PI 3-kinase, the MAP kinase pathway (through SHC or GRB2), or G protein-mediated activation of phospholipase C (PLCγ) with the attendant production of diacylglycerol and activation of protein kinase C. It is apparent that there is a potential for "cross-talk" when different hormones activate these various signal transduction pathways.

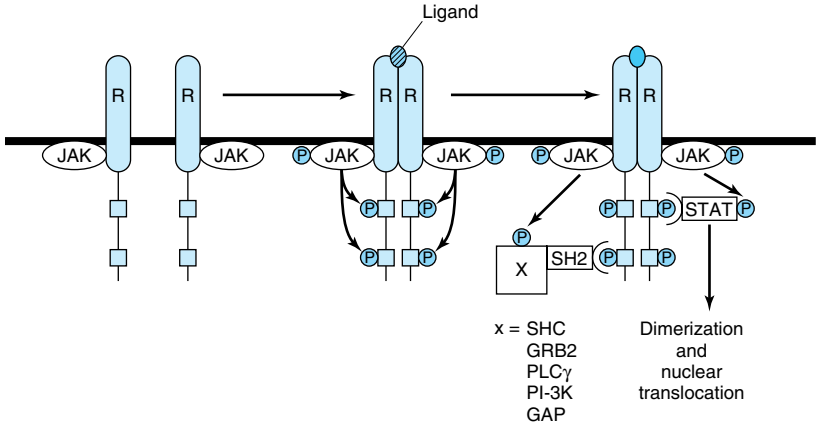

Figure 43–9. Initiation of signal transduction by receptors linked to Jak kinases. The receptors (R) that bind prolactin, growth hormone, interferons, and cytokines lack endogenous tyrosine kinase. Upon ligand binding, these receptors dimerize and an associated protein (Jak1, Jak2, or TYK) is phosphorylated. Jak-P, an active kinase, phosphorylates the receptor on tyrosine residues. The STAT proteins associate with the phosphorylated receptor and then are themselves phosphorylated by Jak-P. STATⓅ dimerizes, translocates to the nucleus, binds to specific DNA elements, and regulates transcription. The phosphotyrosine residues of the receptor also bind to several SH2 domain-containing proteins. This results in activation of the MAP kinase pathway (through SHC or GRB2), PLCγ, or PI-3 kinase.

C. The NF-κB Pathway Is Regulated by Glucocorticoids

The transcription factor NF-κB is a heterodimeric complex typically composed of two subunits termed p50 and p65 (Figure 43–10). Normally, NF-κB is kept sequestered in the cytoplasm in a transcriptionally inactive form by members of the inhibitor of NF-κB (IκB) family. Extracellular stimuli such as proinflammatory cytokines, reactive oxygen species, and mitogens lead to activation of the IκB kinase complex, IKK, which is a heterohexameric structure consisting of α, β, and γ subunits. IKK phosphorylates IκB on two serine residues, and this targets IκB for ubiquitination and subsequent degradation by the proteasome. Following IκB degradation, free NF-κB can now translocate to the nucleus, where it binds to a number of gene promoters and activates transcription, particularly of genes involved in the **inflammatory response.** Transcriptional regulation by NF-κB is mediated by a variety of coactivators such as CREB binding protein (CBP), as described below (Figure 43–13).

Glucocorticoid hormones are therapeutically useful agents for the treatment of a variety of inflammatory and immune diseases. Their anti-inflammatory and immunomodulatory actions are explained in part by the inhibition of NF-κB and its subsequent actions. Evidence for three mechanisms for the inhibition of NF-κB by glucocorticoids has been presented: (1) Glucocorticoids increase IκB mRNA, which leads to an increase of IκB protein and more efficient sequestration of NF-κB in the cytoplasm. (2) The glucocorticoid receptor competes with NF-κB for binding to coactivators. (3) The glucocorticoid receptor directly binds to the p65 subunit of NF-κB and inhibits its activation (Figure 43–10).

HORMONES CAN INFLUENCE SPECIFIC BIOLOGIC EFFECTS BY MODULATING TRANSCRIPTION

The signals generated as described above have to be translated into an action that allows the cell to effectively adapt to a challenge (Figure 43–1). Much of this

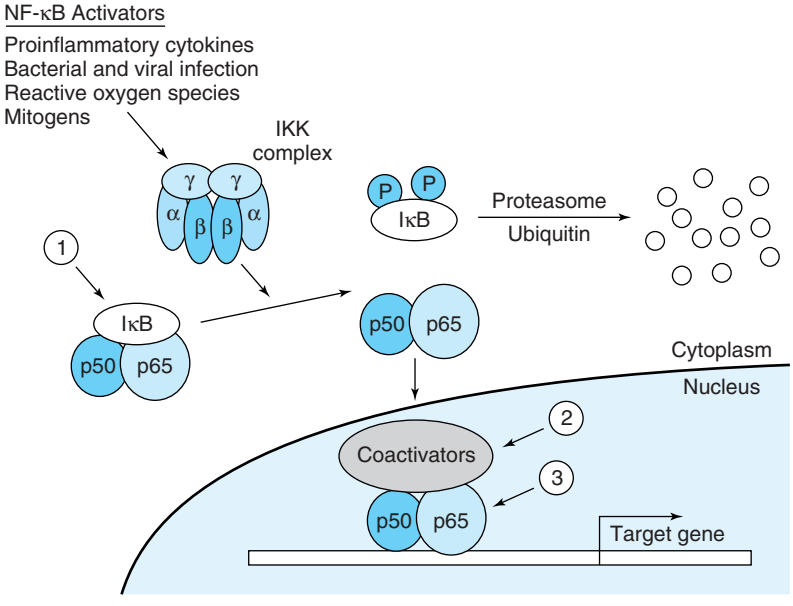

Figure 43–10. Regulation of the NF-κB pathway. NF-κB consists of two subunits, p50 and p65, which regulate transcription of many genes when in the nucleus. NF-κB is restricted from entering the nucleus by IκB, an inhibitor of NF-κB. IκB binds to—and masks—the nuclear localization signal of NF-κB. This cytoplasmic protein is phosphorylated by an IKK complex which is activated by cytokines, reactive oxygen species, and mitogens. Phosphorylated IκB can be ubiquitinylated and degraded, thus releasing its hold on NF-κB. Glucocorticoids affect many steps in this process, as described in the text.

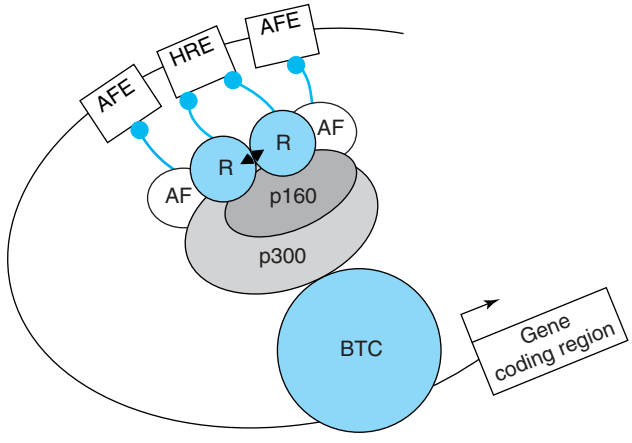

Figure 43–11. The hormone response transcription unit. The hormone response transcription unit is an assembly of DNA elements and bound proteins that interact, through protein-protein interactions, with a number of coactivator or corepressor molecules. An essential component is the hormone response element which binds the ligand (▲)-bound receptor (R). Also important are the accessory factor elements (AFEs) with bound transcription factors. More than two dozen of these accessory factors (AFs), which are often members of the nuclear receptor superfamily, have been linked to hormone effects on transcription. The AFs can interact with each other, with the liganded nuclear receptors, or with coregulators. These components communicate with the basal transcription complex through a coregulator complex that can consist of one or more members of the p160, corepressor, mediator-related, or CBP/p300 families (see Table 43–6).

adaptation is accomplished through alterations in the rates of transcription of specific genes. Many different observations have led to the current view of how hormones affect transcription. Some of these are as follows: (1) Actively transcribed genes are in regions of "open" chromatin (defined by a susceptibility to the enzyme DNase I), which allows for the access of transcription factors to DNA. (2) Genes have regulatory regions, and transcription factors bind to these to modulate the frequency of transcription initiation. (3) The hormone-receptor complex can be one of these transcription factors. The DNA sequence to which this binds is called a hormone response element (HRE; see Table 43–1 for examples). (4) Alternatively, other hormone-generated signals can modify the location, amount, or activity of transcription factors and thereby influence binding to the regulatory or response element. (5) Members of a large superfamily of nuclear receptors act with—or in a manner analogous to—hormone receptors. (6) These nuclear receptors interact with another large group of coregulatory molecules to effect changes in the transcription of specific genes.

Several Hormone Response Elements (HREs) Have Been Defined

Hormone response elements resemble enhancer elements in that they are not strictly dependent on position and location. They generally are found within a few hundred nucleotides upstream (5′) of the transcription initiation site, but they may be located within the coding region of the gene, in introns. HREs were defined by the strategy illustrated in Figure 39–11. The consensus sequences illustrated in Table 43–1 were arrived at through analysis of several genes regulated by a given hormone using simple, heterologous reporter systems (see Figure 39–10). Although these simple HREs bind the hormone-receptor complex more avidly than surrounding DNA—or DNA from an unrelated source—and confer hormone responsiveness to a reporter gene, it soon became apparent that the regulatory circuitry of natural genes must be much more complicated. Glucocorticoids, progestins, mineralocorticoids, and androgens have vastly different physiologic actions. How could the specificity required for these effects be achieved through regulation of gene expression by the same HRE (Table 43–1)? Questions like this have led to experiments which have allowed for elaboration of a very complex model of transcription regulation. For example, the HRE must associate with other DNA elements (and associated binding proteins) to function optimally. The extensive sequence similarity noted between steroid hormone receptors, particularly in their DNA-binding domains, led to discovery of the **nuclear receptor superfamily** of proteins. These—and a large number of **coregulator proteins**—allow for a wide variety of DNA-protein and protein-protein interactions and the specificity necessary for highly regulated physiologic control. A schematic of such an assembly is illustrated in Figure 43–11.

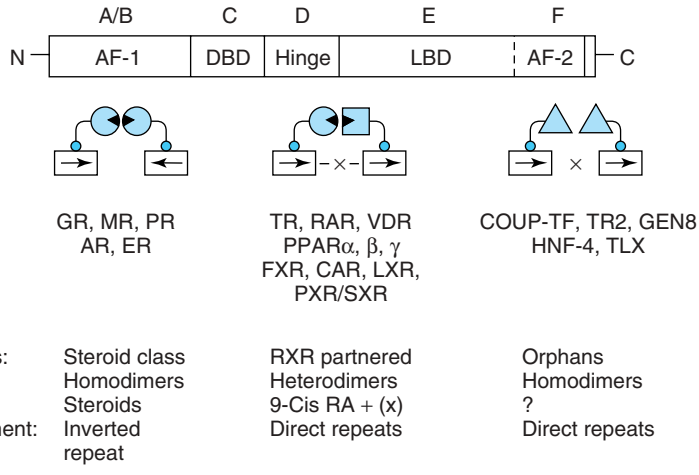

Figure 43–12. The nuclear receptor superfamily. Members of this family are divided into six structural domains (A–F). Domain A/B is also called AF-1, or the modulator region, because it is involved in activating transcription. The C domain consists of the DNA-binding domain (DBD). The D region contains the hinge, which provides flexibility between the DBD and the ligand-binding domain (LBD, region E). The amino (N) terminal part of region E contains AF-2, another domain important for transactivation. The F region is poorly defined. The functions of these domains are discussed in more detail in the text. Receptors with known ligands, such as the steroid hormones, bind as homodimers on inverted repeat half-sites. Other receptors form heterodimers with the partner RXR on direct repeat elements. There can be nucleotide spacers of one to five bases between these direct repeats (DR1–5). Another class of receptors for which ligands have not been determined (orphan receptors) bind as homodimers to direct repeats and occasionally as monomers to a single half-site.

There Is a Large Family of Nuclear Receptor Proteins

The nuclear receptor superfamily consists of a diverse set of transcription factors that were discovered because of a sequence similarity in their DNA-binding domains. This family, now with more than 50 members, includes the nuclear hormone receptors discussed above, a number of other receptors whose ligands were discovered after the receptors were identified, and many putative or orphan receptors for which a ligand has yet to be discovered.

These nuclear receptors have several common structural features (Figure 43–12). All have a centrally located **DNA-binding domain (DBD)** that allows the receptor to bind with high affinity to a response element. The DBD contains two zinc finger binding motifs (see Figure 39–14) that direct binding either as homodimers, as heterodimers (usually with a retinoid X receptor [RXR] partner), or as monomers. The target response element consists of one or two half-site consensus sequences arranged as an inverted or direct repeat. The spacing between the latter helps determine binding specificity. Thus, a direct repeat with three, four, or five nucleotide spacer regions specifies the binding of the vitamin D, thyroid, and retinoic acid receptors, respectively, to the same consensus response element (Table 43–1). A multifunctional **ligand-binding domain (LBD)** is located in the carboxyl terminal half of the receptor. The LBD binds hormones or metabolites with selectivity and thus specifies a particular biologic response. The LBD also contains domains that mediate the binding of heat shock proteins, dimerization, nuclear localization, and transactivation. The latter function is facilitated by the carboxyl terminal transcription activation function **(AF-2 domain),** which forms a surface required for the interaction with

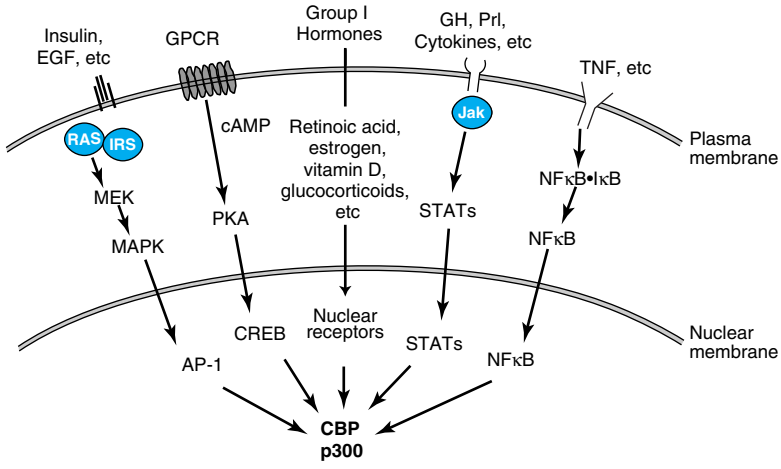

Figure 43–13. Several signal transduction pathways converge on CBP/p300. Ligands that associate with membrane or nuclear receptors eventually converge on CBP/p300. Several different signal transduction pathways are employed. EGF, epidermal growth factor; GH, growth hormone; Prl, prolactin; TNF, tumor necrosis factor; other abbreviations are expanded in the text.

coactivators. A highly variable **hinge region** separates the DBD from the LBD. This region provides flexibility to the receptor, so it can assume different DNA-binding conformations. Finally, there is a highly variable amino terminal region that contains another transactivation domain referred to as **AF-1.** Less well defined, the AF-1 domain may provide for distinct physiologic functions through the binding of different coregulator proteins. This region of the receptor, through the use of different promoters, alternative splice sites, and multiple translation initiation sites, provides for receptor isoforms that share DBD and LBD identity but exert different physiologic responses because of the association of various coregulators with this variable amino terminal AF-1 domain.

It is possible to sort this large number of receptors into groups in a variety of ways. Here they are discussed according to the way they bind to their respective DNA elements (Figure 43–12). Classic hormone receptors for glucocorticoids (GR), mineralocorticoids (MR), estrogens (ER), androgens (AR), and progestins (PR) bind as homodimers to inverted repeat sequences. Other hormone receptors such as thyroid (TR), retinoic acid (RAR), and vitamin D (VDR) and receptors that bind various metabolite ligands such as PPAR α β, and γ, FXR, LXR, PXR/SXR, and CAR bind as heterodimers, with retinoid X receptor (RXR) as a partner, to direct repeat sequences (see Figure 43–12 and Table 43–5). Another group of orphan receptors that as yet have no known ligand bind as homodimers or monomers to direct repeat sequences.

As illustrated in Table 43–5, the discovery of the nuclear receptor superfamily has led to an important understanding of how a variety of metabolites and xenobiotics regulate gene expression and thus the metabolism, detoxification, and elimination of normal body products and exogenous agents such as pharmaceuticals. Not surprisingly, this area is a fertile field for investigation of new therapeutic interventions.

A Large Number of Nuclear Receptor Coregulators Also Participate in Regulating Transcription

Chromatin remodeling, transcription factor modification by various enzyme activities, and the communication between the nuclear receptors and the basal transcription apparatus are accomplished by protein-protein interactions with one or more of a class of coregulator molecules. The number of these coregulator molecules now exceeds 100, not counting species variations and splice variants. The first of these to be described was the **CREB-binding protein, CBP.** CBP, through an amino terminal domain, binds to phosphorylated serine 137 of CREB and mediates transactivation in response to cAMP. It thus is described as a coactivator. CBP and

Table 43–5. Nuclear receptors with special ligands.[1]

Receptor		Partner	Ligand	Process Affected
Peroxisome Proliferator-activated	PPARα	RXR (DR1)	Fatty acids	Peroxisome proliferation
	PPARβ		Fatty acids	
	PPARγ		Fatty acids Eicosanoids, thiazolidinediones	Lipid and carbohydrate metabolism
Farnesoid X	FXR	RXR (DR4)	Farnesol, bile acids	Bile acid metabolism
Liver X	LXR	RXR (DR4)	Oxysterols	Cholesterol metabolism
Xenobiotic X	CAR	RXR (DR5)	Androstanes Phenobarbital Xenobiotics	Protection against certain drugs, toxic metabolites, and xenobiotics
	PXR	RXR (DR3)	Pregnanes Xenobiotics	

[1]Many members of the nuclear receptor superfamily were discovered by cloning, and the corresponding ligands were then identified. These ligands are not hormones in the classic sense, but they do have a similar function in that they activate specific members of the nuclear receptor superfamily. The receptors described here form heterodimers with RXR and have variable nucleotide sequences separating the direct repeat binding elements (DR1–5). These receptors regulate a variety of genes encoding cytochrome p450s (CYP), cytosolic binding proteins, and ATP-binding cassette (ABC) transporters to influence metabolism and protect cells against drugs and noxious agents.

its close relative, p300, interact directly or indirectly with a number of signaling molecules, including activator protein-1 (AP-1), signal transducers and activators of transcription (STATs), nuclear receptors, and CREB (Figure 39–11). **CBP/p300** also binds to the p160 family of coactivators described below and to a number of other proteins, including viral transcription factor Ela, the $p90^{rsk}$ protein kinase, and RNA helicase A. It is important to note that **CBP/p300 also has intrinsic histone acetyltransferase (HAT) activity.** The importance of this is described below. Some of the many actions of CBP/p300, which appear to depend on intrinsic enzyme activities and its ability to serve as a scaffold for the binding of other proteins, are illustrated in Figure 43–11. Other coregulators may serve similar functions.

Several other families of coactivator molecules have been described. Members of the **p160 family of coactivators,** all of about 160 kDa, include (1) SRC-1 and NCoA-1; (2) GRIP 1, TIF2, and NCoA-2; and (3) p/CIP, ACTR, AIB1, RAC3, and TRAM-1 (Table 43–6). The different names for members within a subfamily often represent species variations or minor splice variants. There is about 35% amino acid identity between members of the different subfamilies. The p160 coactivators share several properties. They (1) bind nuclear receptors in an agonist and AF-2 transactivation domain-dependent manner; (2) have a conserved amino terminal basic helix-loop-helix (bHLH) motif (see Chapter 39); (3) have a weak carboxyl terminal transactivation domain and a stronger amino terminal

Table 43–6. Some mammalian coregulator proteins.

I.	**300-kDa family of coactivators**	
	CBP	CREB-binding protein
	p300	Protein of 300 kDa
II.	**160-kDa family of coactivators**	
	A. SRC-1	Steroid receptor coactivator 1
	NCoA-1	Nuclear receptor coactivator 1
	B. TIF2	Transcriptional intermediary factor 2
	GRIP1	Glucocorticoid receptor-interacting protein
	NCoA-2	Nuclear receptor coactivator 2
	C. p/CIP	p300/CBP cointegrator-associated protein 1
	ACTR	Activator of the thyroid and retinoic acid receptors
	AIB	Amplified in breast cancer
	RAC3	Receptor-associated coactivator 3
	TRAM-1	TR activator molecule 1
III.	**Corepressors**	
	NCoR	Nuclear receptor corepressor
	SMRT	Silencing mediator for RXR and TR
IV.	**Mediator-related proteins**	
	TRAPs	Thyroid hormone receptor-associated proteins
	DRIPs	Vitamin D receptor-interacting proteins
	ARC	Activator-recruited cofactor

transactivation domain in a region that is required for the CBP/p16O interaction; (4) contain at least three of the **LXXLL motifs** required for protein-protein interaction with other coactivators; and (5) often have HAT activity. The role of HAT is particularly interesting, as mutations of the HAT domain disable many of these transcription factors. Current thinking holds that these HAT activities acetylate histones and result in remodeling of chromatin into a transcription-efficient environment; however, other protein substrates for HAT-mediated acetylation have been reported. Histone acetylation/deacetylation is proposed to play a critical role in gene expression.

A small number of proteins, including NCoR and SMRT, comprise the **corepressor family.** They function, at least in part, as described in Figure 43–2. Another family includes the TRAPs, DRIPs, and ARC (Table 43–6). These so-called **mediator-related proteins** range in size from 80 kDa to 240 kDa and are thought to be involved in linking the nuclear receptor-coactivator complex to RNA polymerase II and the other components of the basal transcription apparatus.

The exact role of these coactivators is presently under intensive investigation. Many of these proteins have intrinsic enzymatic activities. This is particularly interesting in view of the fact that acetylation, phosphorylation, methylation, and ubiquitination—as well as proteolysis and cellular translocation—have been proposed to alter the activity of some of these coregulators and their targets.

It appears that certain combinations of coregulators—and thus different combinations of activators and inhibitors—are responsible for specific ligand-induced actions through various receptors.

SUMMARY

- Hormones, cytokines, interleukins, and growth factors use a variety of signaling mechanisms to facilitate cellular adaptive responses.
- The ligand-receptor complex serves as the initial signal for members of the nuclear receptor family.
- Class II hormones, which bind to cell surface receptors, generate a variety of intracellular signals. These include cAMP, cGMP, Ca^{2+}, phosphatidylinositides, and protein kinase cascades.
- Many hormone responses are accomplished through alterations in the rate of transcription of specific genes.
- The nuclear receptor superfamily of proteins plays a central role in the regulation of gene transcription.
- These receptors, which may have hormones, metabolites, or drugs as ligands, bind to specific DNA elements as homodimers or as heterodimers with RXR. Some—orphan receptors—have no known ligand but bind DNA and influence transcription.
- Another large family of coregulator proteins remodel chromatin, modify other transcription factors, and bridge the nuclear receptors to the basal transcription apparatus.

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