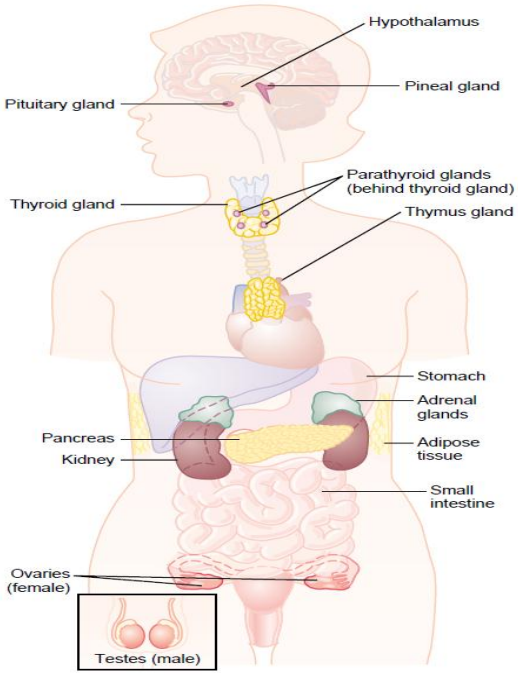
Lecture No . 2

Mechanisms of Action of Hormones



Hormone Receptors and Their Activation

The first step of a hormone's action is to bind to specific *receptors* at the target cell. Cells that lack receptors for the hormones do not respond. Receptors for some hormones are located on the target cell membrane, whereas other hormone receptors are located in the cytoplasm or the nucleus. When the hormone combines with its receptor, this usually initiates a cascade of reactions in the cell, with each stage becoming more powerfully activated so that even small concentrations of the hormone can have a large effect. Hormonal receptors are large proteins, and each cell that is to be stimulated usually has some 2000 to 100,000 receptors. Also, each receptor is usually highly specific for a single hormone; this determines the type of hormone that will act on a particular tissue. The target tissues that are affected by a hormone are those that contain its specific receptors. The locations for the different types of hormone receptors are generally the following:

1. ***In or on the surface of the cell membrane***. The membrane receptors are specific mostly for the protein, peptide, and catecholamine hormones.
2. ***In the cell cytoplasm***. The primary receptors for the different steroid hormones are found mainly in the cytoplasm.

3. ***In the cell nucleus***. The receptors for the thyroid hormones are found in the nucleus and are believed to be located in direct association with one or more of the chromosomes.

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

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

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

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

***G Protein–Linked Hormone Receptors*.**

Many hormones activate receptors that indirectly regulate the activity of target proteins (e.g., enzymes or ion channels) by coupling with groups of cell membrane proteins called *heterotrimeric GTP-binding proteins (G proteins)*. There are more than 1000 known G protein–coupled receptors, all of which have seven transmembrane segments that loop in and out of the cell membrane. Some parts of the receptor that protrude into the cell cytoplasm (especially the cytoplasmic tail of the receptor) are coupled to G proteins that include three (i.e., trimeric) parts—the α, β, and γ subunits. When the ligand (hormone) binds to the extracellular part of the receptor, a conformational change occurs in the receptor that activates the G proteins and induces intracellular signals that either (1) open or close cell membrane ion channels or (2) change the activity of an enzyme in the cytoplasm of the cell. The trimeric G proteins are named for their ability to bind *guanosine nucleotides.* In their inactive state, the α, β, and γ subunits of G proteins form a complex that binds *guanosine diphosphate (GDP)* on the α subunit. When the receptor is activated, it undergoes a conformational change that causes the GDP-bound trimeric G protein to associate with the cytoplasmic part of the receptor and to exchange GDP for *guanosine triphosphate (GTP).* Displacement of GDP by GTP causes the α subunit to dissociate from the trimeric complex and to associate with other intracellular signaling proteins; these proteins, in turn, alter the activity of ion channels or intracellular enzymes such as *adenylyl cyclase* or *phospholipase C*, which alters cell function. The signaling event is rapidly terminated when the hormone is removed and the α subunit inactivates itself by converting its bound GTP to GDP; then the α subunit once again combines with the β and γ subunits to form an inactive, membrane-bound trimeric G protein. Some hormones are coupled to *inhibitory G proteins* (denoted Gi proteins), whereas others are coupled to *stimulatory G proteins* (denoted Gs proteins). Thus, depending on the coupling of a hormone receptor to an inhibitory or stimulatory G protein, a hormone can either increase or decrease the activity of intracellular enzymes. This complex system of cell membrane G proteins provides a vast array of potential cell responses to different hormones in the various target tissues of the body.

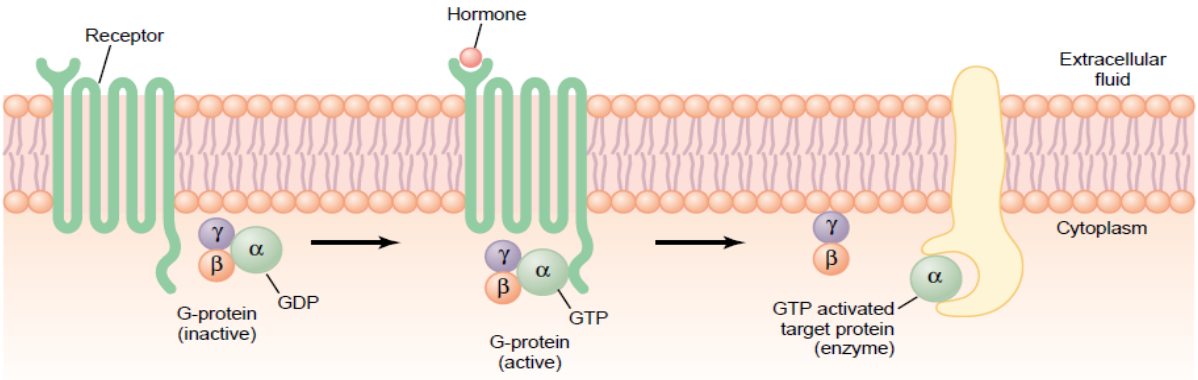


Figure 74–4

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

***Enzyme-Linked Hormone Receptors*.**

Some receptors, when activated, function directly as enzymes or are closely associated with enzymes that they activate. These *enzyme-linked receptors* are proteins that pass through the membrane only once, in contrast to the seven-transmembrane G protein–coupled receptors. Enzyme-linked receptors have their hormone-binding site on the outside of the cell membrane and their catalytic or enzyme-binding site on the inside. When the hormone binds to the extracellular part of the receptor, an enzyme immediately inside the cell membrane is activated (or occasionally inactivated). Although many enzyme-linked receptors have intrinsic enzyme activity, others rely on enzymes that are closely associated with the receptor to produce changes in cell function. One example of an enzyme-linked receptor is the *leptin receptor*. Leptin is a hormone secreted by fat cells and has many physiological effects, but it is especially important in regulating appetite and energy balance . The leptin receptor is a member of a large family of *cytokine receptors* that do not themselves contain enzymatic activity but signal through associated enzymes. In the case of the leptin receptor, one of the signaling pathways occurs through a *tyrosine kinase* of the *janus kinase (JAK)* family, *JAK2*. The leptin receptor exists as a dimer (i.e., in two parts), and binding of leptin to the extracellular part of the receptor alters its conformation, enabling phosphorylation and activation of the intracellular associated JAK2 molecules. The activated JAK2 molecules then phosphorylate other tyrosine residues within the leptin receptor– JAK2 complex to mediate intracellular signaling. The intracellular signals include phosphorylation of *signal transducer and activator of transcription (STAT)* proteins, which activates transcription by leptin target genes to initiate protein

synthesis. Phosphorylation of JAK2 also leads to activation of other intracellular enzyme pathways such as *mitogen-activated protein kinases (MAPK)* and *phosphatidylinositol 3-kinase (PI3K).* Some of the effects of leptin occur rapidly as a result of activation of these intracellular enzymes, whereas other actions occur more slowly and require synthesis of new proteins. Another example, one widely used in hormonal control of cell function, is for the hormone to bind with a special transmembrane receptor, which then becomes the activated enzyme *adenylyl cyclase* at the end that protrudes to the interior of the cell. This cyclase catalyzes the formation of cAMP, which has a multitude of effects inside the cell to control cell activity, as discussed in greater detail later. cAMP is called a *second messenger* because it is not the hormone itself that directly institutes the intracellular changes; instead, the cAMP serves as a second messenger to cause these effects. For a few peptide hormones, such as atrial natriuretic peptide (ANP), *cyclic guanosine monophosphate (cGMP),* which is only slightly different from cAMP, serves in a similar manner as a second messenger.

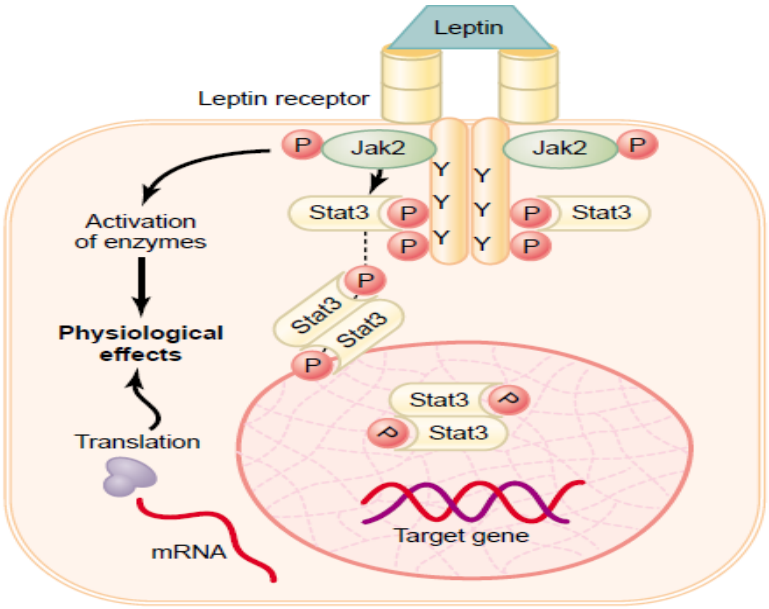


Figure 74–5

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

***Intracellular Hormone Receptors and Activation of Genes*.**

Several hormones, including adrenal and gonadal steroid hormones, thyroid hormones, retinoid hormones, and vitamin D, bind with protein receptors inside the cell rather than in the cell membrane. Because these hormones are lipid soluble, they readily cross the cell membrane and interact with receptors in the cytoplasm or nucleus. The activated hormone receptor complex then binds with a specific regulatory (promoter) sequence of the DNA called the *hormone response element*, and in this manner either activates or represses transcription of specific genes and formation of messenger RNA (mRNA) (Figure 74–6). Therefore, minutes, hours, or even days after the hormone has entered the cell, newly formed proteins appear in the cell and become the controllers of new or altered cellular functions. Many different tissues have identical intracellular hormone receptors, but the genes that the receptors regulate are different in the various tissues. An intracellular receptor can activate a gene response only if the appropriate combination of gene regulatory proteins is present, and many of these regulatory proteins are tissue specific. Thus, the responses of different tissues to a hormone are determined not only by the specificity of the receptors but also by the genes that the receptor regulates.

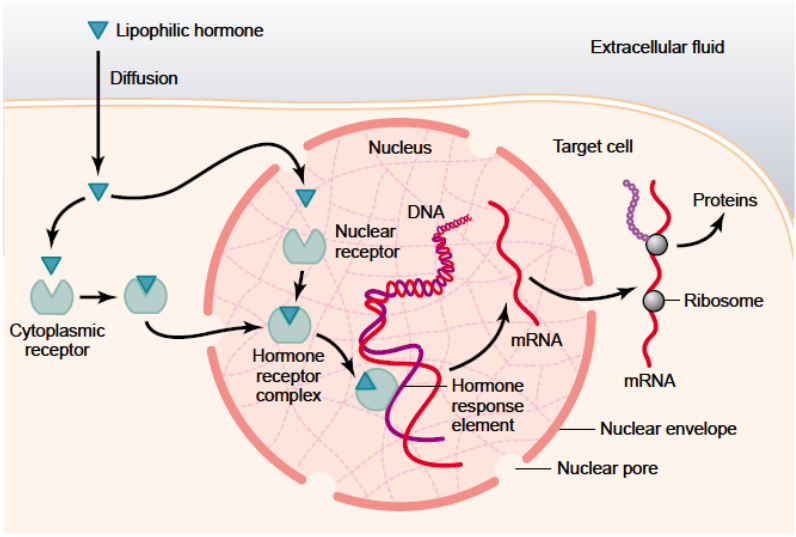


Figure 74–6

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

Second Messenger Mechanisms for Mediating Intracellular Hormonal Functions

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

hormone has on the cell is to activate a single type of membrane receptor. The second messenger does the rest cAMP is not the only second messenger used by the different hormones. Two other especially important ones are (1) calcium ions and associated *calmodulin* and (2) products of membrane phospholipid breakdown.

Measurement of Hormone Concentrations in the Blood

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

Radioimmunoassay

The method of performing radioimmunoassay is as follows. First, an antibody that is highly specific for the hormone to be measured is produced. Second, a small quantity of this antibody is (1) mixed with a quantity of fluid from the animal containing the hormone to be measured and (2) mixed simultaneously with an appropriate amount of purified standard hormone that has been tagged with a radioactive isotope. However, one specific condition must be met:There must be too little antibody to bind completely both the radioactively tagged hormone and the hormone in the fluid to be assayed.Therefore, the natural hormone in the assay fluid and the radioactive standard hormone *compete for the binding sites* of the antibody. In the process of competing, the quantity of each of the two hormones, the natural and the radioactive, that binds is proportional to its concentration in the assay fluid. Third, after binding has reached equilibrium, the antibody-hormone complex is separated from the remainder of the solution, and the quantity of radioactive hormone bound in this complex is measured by radioactive counting techniques. If a large amount of radioactive hormone has bound with the antibody, it is clear that there was only a small amount of natural hormone to compete with the radioactive hormone, and therefore the concentration of the natural hormone in the assayed fluid was small. Conversely, if only a small amount of radioactive hormone has bound, it is clear that there was a large amount of natural hormone to compete for the binding sites. Fourth, to make the assay highly quantitative, the radioimmunoassay procedure is also performed for “standard” solutions of untagged hormone at several concentration levels. Then a “standard curve” is plotted, as shown in Figure 74–9. By comparing the radioactive counts recorded from the “unknown” assay procedures with the standard

curve, one can determine within an error of 10 to 15 per cent the concentration of the hormone in the "unknown" assayed fluid. As little as billionths or even trillionths of a gram of hormone can often be assayed in this way.

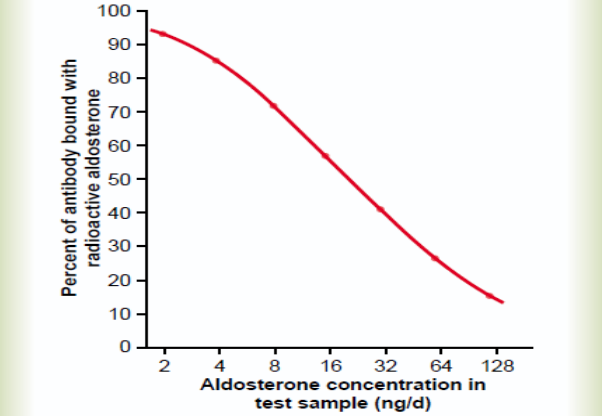


Figure 74–9

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

Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assays (ELISAs) can be used to measure almost any protein, including hormones. This test combines the specificity of antibodies with the sensitivity of simple enzyme assays. Figure 74–10 shows the basic elements of this method, which is often performed on plastic plates that each have 96 small wells. Each well is coated with an antibody (AB1) that is specific for the hormone being assayed. Samples or standards are added to each of the wells, followed by a second antibody (AB2) that is also specific for the hormone but binds to a different site of the hormone molecule.A third antibody (AB3) is added that recognizes AB2 and is coupled to an enzyme that converts a suitable substrate to a product that can be easily detected by colorimetric or fluorescent optical methods. Because each molecule of enzyme catalyzes the formation of many thousands of product molecules, even very small amounts of hormone molecules can be detected. In contrast to competitive radioimmunoassay methods, ELISA methods use excess antibodies so that all hormone molecules are captured in antibody-hormone complexes. Therefore, the amount of hormone present in the sample or in the standard is proportional to the amount of product formed. The ELISA method has become widely used in clinical laboratories because (1) it does not employ radioactive isotopes, (2) much of the assay can be automated using 96-well plates, and (3) it has proved to be a cost-effective and accurate method for assessing hormone levels.

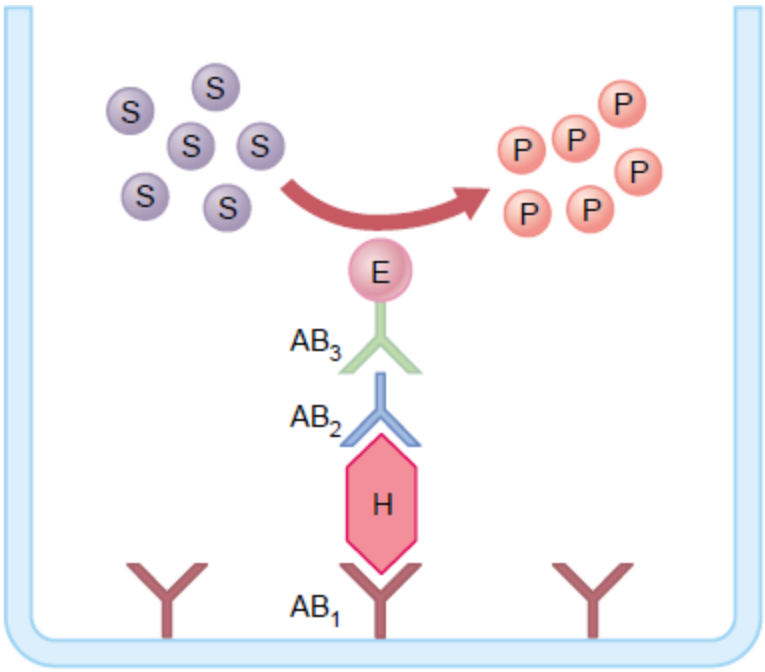


Figure 74–10

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