General physiology
Spring 2024
Physiology lecture 17
Mechanism of Action of Chemical Messengers and Intracellular Signaling

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Lecture objectives

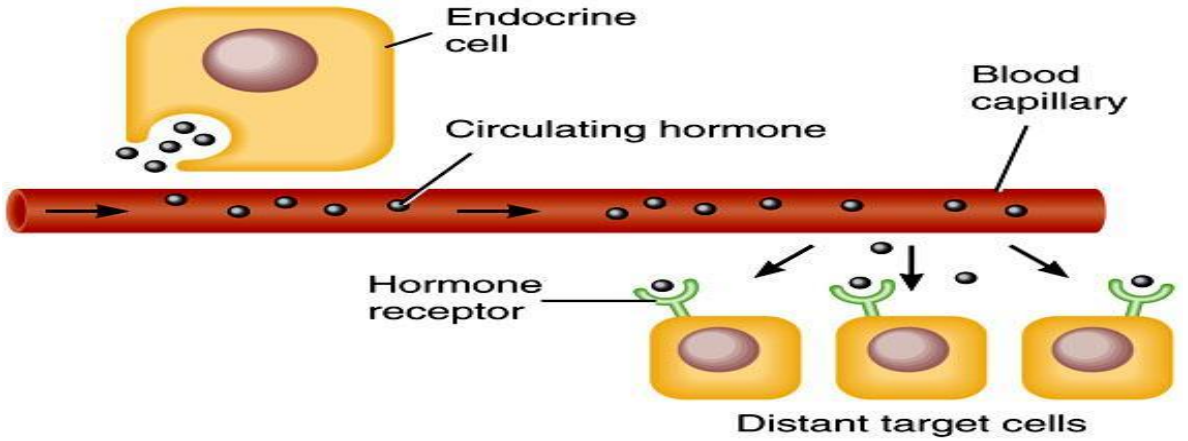
- List different types of chemical messenger and how they coordinate multiple activities of the cells, tissues, and organs of the body
- Review different types of ionic channels and inotropic and metabotropicrecptors
- Define G protein coupled rectors and G proteins and understand their role in ligand gated intracellular transduction
- Understand the role of G protein coupled receptors in intracellular signaling pathways
- Understand the mechanism of intracellular transduction vis tyrosine kinase receptors
- Describe how second messengers (cAMP, cGMP, DAG and IP3 etc) regulate and amplify signal transduction.
- Describe how intracellular calcium concentration is regulated and used in intracellular signal transduction.

Coordination of Body Functions by Chemical Messengers and Intercellular Communication

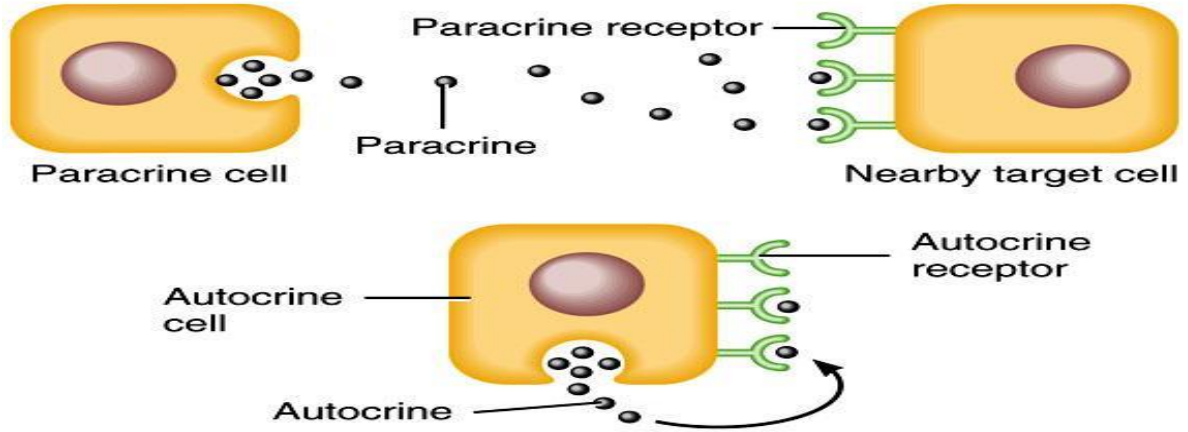
- The multiple activities of the cells, tissues, and organs of the body are coordinated by the interplay of several types of chemical messenger systems:
- Three general types of intercellular communication are mediated by messengers in the ECF:
- N**eural communication,** in which neurotransmitters are released at synaptic junctions from nerve cells and act across a narrow synaptic cleft on a postsynaptic cell;
- **Endocrine communication in which hormones** are released by glands or specialized cells into the circulating blood and influence the function of target cells at another location
- P**aracrine communication,** in which the products of cells diffuse in the ECF to affect neighboring cells that may be some distance away
- **Autocrine communication** cells secrete chemical messengers that in some situations bind to receptors on the same cell, that is, the cell that secreted the messenger
- *Cytokines* are peptides secreted by cells into the extracellular fluid and can function as autocrines, paracrines, or endocrine hormones. Examples of cytokines include the *interleukins* and other *lymphokines* that are secreted by helper cells and act on other cells of the immune system
- Cytokine hormones also known as (e.g., *leptin*) produced by adipocytes

Intercellular Communication

- Circulating hormones
- Paracrine communication a hormone or a substance acts on neighboring cells
- Autocrine substance released by the cell and acts on the same cell that secreted them



(a) Circulating hormones



(b) Local hormones (paracrines and autocrines)

Mechanisms By Which Chemical Messengers (Hormones And Neurotransmitters) Act on Cells and Cause Physiological Effects

- Receptor–ligand interaction is usually just the beginning of the cell response. This event is transduced into secondary responses within the cell that can be divided into four broad categories:
- (1) ion channel activation
- (2) **G proteins** activation
- (3) activation of enzyme activity within the cell
- (4) direct activation of transcription.

Ion Channel–Linked Receptors:

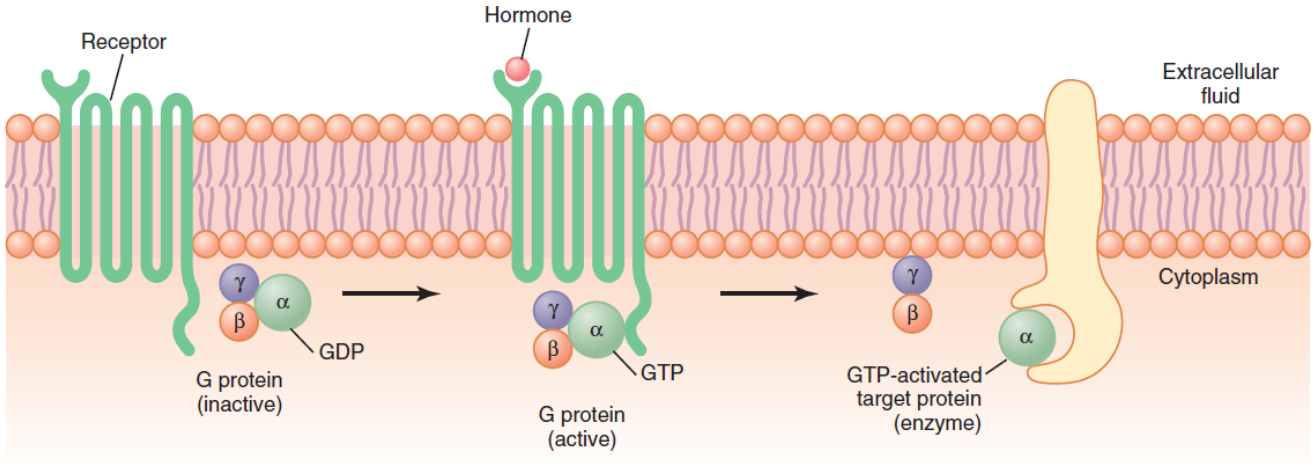
- All the neurotransmitter substances, such as Ach and NE, combine with receptors in the postsynaptic membrane
- This combination usually causes a change in the structure of the receptor, resulting in opening or closing a channel for one or more ions (such as channels for Na^+, K^+, and Ca^{2+}).
  - Inotropic and metabotropic receptors
- Few neurohormones and hormones exert 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.

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 **G proteins** (guanine nucleotide-binding proteins).
- There are more than 1000 G protein–coupled receptors; all have **seven** transmembrane segments that loop in and out of the cell membrane.
- Large G protein = macromolecule composed of **three subunits** (heterotrimeric) made up of alpha (α), beta (β) and gamma (γ) subunits. G proteins are **GTPases** enzymes.
- G proteins have the ability to bind and **hydrolyze** guanosine triphosphate (GTP) to guanosine diphosphate (GDP).

G Protein-Linked Hormone Receptors (cont.):

- The alpha subunit is attached to either a GTP ("on" or active state) or GDP ("off" or inactive state). As such, the alpha subunit serves as an **on-off switch** for the activation of G-protein.



- Activation of the G-protein is achieved by exchanging the GDP on the alpha subunit to GTP. This exchange is mediated by the intracellular G protein-linked receptor domain when a signal molecule binds to the extracellular domain.
- The binding of GTP to the alpha subunit results in a structural change and its dissociation from the rest of the G-protein.
- The alpha subunit binds membrane-bound target proteins (enzymes) that initiate intracellular signals (i.e. downstream signaling cascade).

G Protein-Linked Hormone Receptors (cont.):

- The activation of the G proteins and induction of intracellular signals either:
  1. Open or close cell membrane ion channels.
  2. Change the activity of an enzyme in the cytoplasm of the cell, such as adenylyl cyclase or phospholipase C.
  3. Activate gene transcription.
- The signaling event is terminated when the hormone is removed and the α subunit inactivates itself by converting (hydrolyzing) 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** (Gi), whereas others are coupled to **stimulatory G proteins** (Gs). 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.

Metabotropic Receptors

The second messenger system by which a neurotransmitter can affect the activity of postsynaptic cell

NTR complex are coupled to G protein coupled receptor

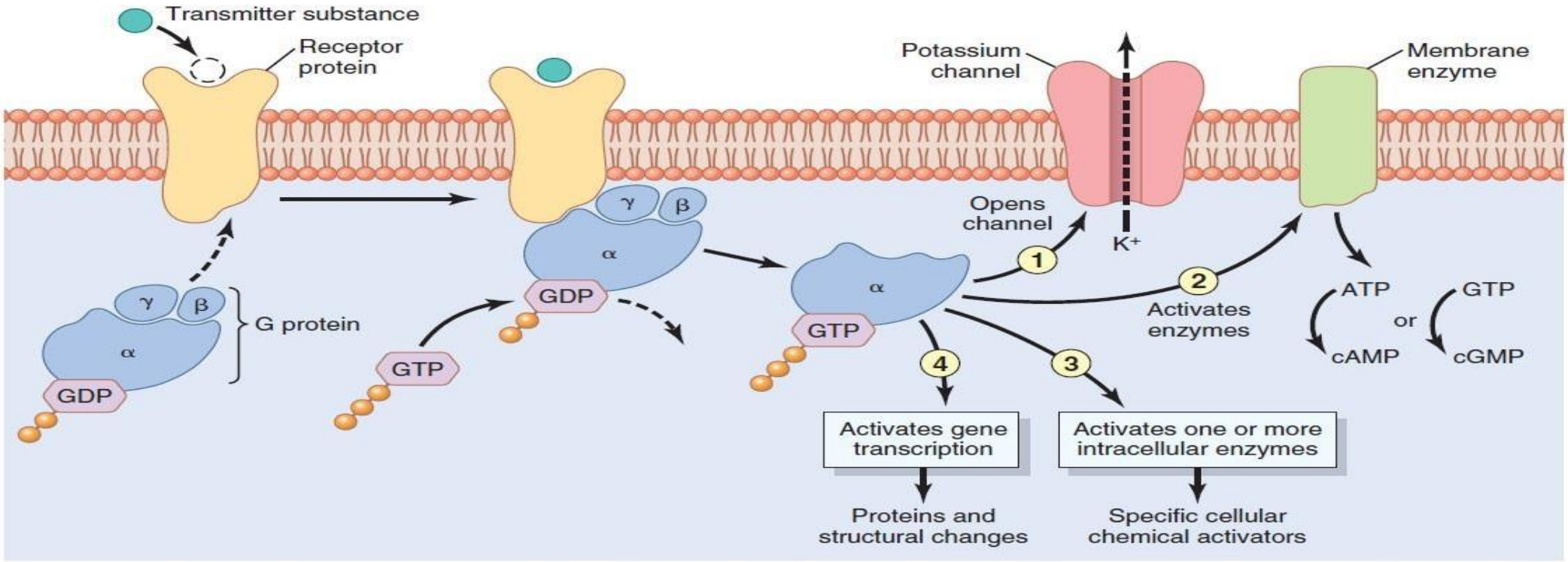


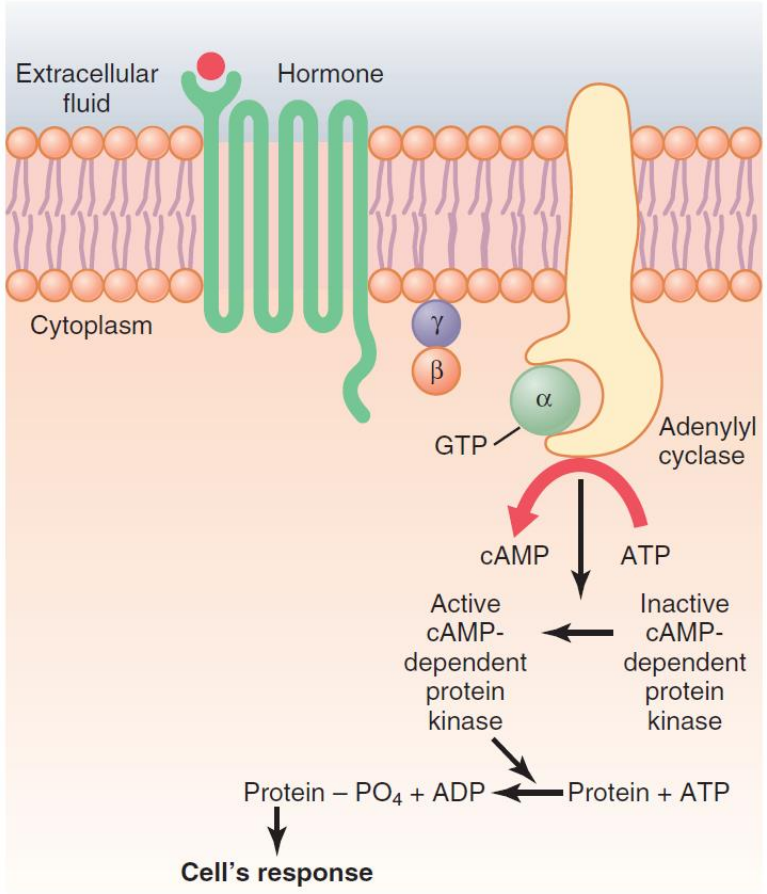
Figure The "second messenger" system by which a transmitter substance from an initial neuron can activate a second neuron by first causing a transformational change in the receptor that releases the activated alpha (α) subunit of the G protein into the second neuron's cytoplasm. Four subsequent possible effects of the G protein are shown, including *1,* opening an ion channel in the membrane of the second neuron; *2,* activating an enzyme system in the neuron's membrane; *3,* activating an intracellular enzyme system; and/or *4,* causing gene transcription in the second neuron. Return of the G protein to the inactive state occurs when guanosine triphosphate *(GTP)* bound to the α subunit is hydrolyzed to guanosine diphosphate *(GDP)* and the β and γ subunits are reattached to the α subunit.

Second Messenger Mechanisms:

- Second messengers are intracellular signaling molecules released by the cell in response to exposure to extracellular signaling molecules (the first messengers such as the hormones).
- The second messenger include (1) cAMP, (2) cGMP, (3) Calcium ions and associated *Calmodulin* and (4) products of membrane phospholipid breakdown.

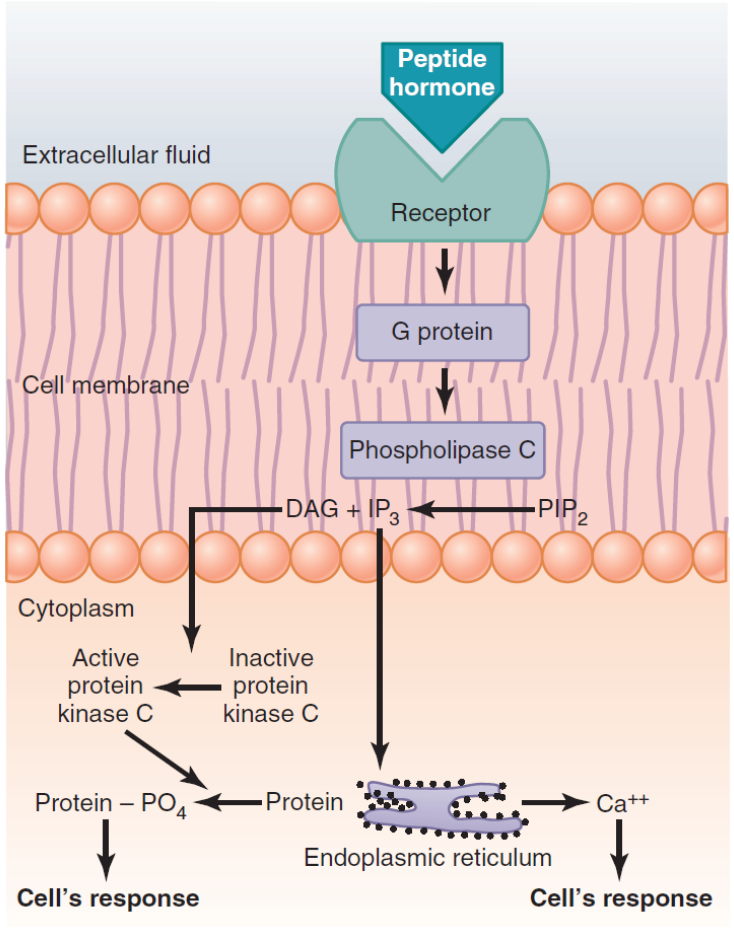
1- Adenylyl Cyclase–cAMP Second Messenger System:

- If the Gs protein stimulates the adenylyl cyclase-cAMP system, adenylyl cyclase catalyzes the conversion of a **small** amount of cytoplasmic adenosine triphosphate (ATP) into cAMP inside the cell.
- This then activates cAMP-dependent protein kinase (also called protein kinase A or PKA). PKA phosphorylates specific cell proteins, triggering biochemical reactions that ultimately lead to the cell's response to the hormone (i.e. downstream signaling **cascade** to greatly **amplify** the strength of the original first messenger signal). Example, the action of **ADH** hormone in the kidney.
- If binding of the hormone to its receptors is coupled to an inhibitory Gi protein, adenylyl cyclase will be inhibited, reducing formation of cAMP and ultimately leading to an inhibitory action in the cell. Example, **somatostatin** hormone.



3- Cell Membrane Phospholipid Second Messenger System:

- Some hormones activate transmembrane receptors that activate the enzyme phospholipase C attached to the inside projections of the receptors.
- Phospholipase C catalyzes the breakdown of some phospholipids in the cell membrane, especially phosphatidylinositol biphosphate (PIP2), into two different second messenger products: inositol triphosphate (**IP3**) and diacylglycerol (**DAG**).
- The IP3 mobilizes calcium ions from mitochondria and the endoplasmic reticulum, and the calcium ions then have their own second messenger effects, such as smooth muscle contraction and changes in cell secretion.
- DAG, the other lipid second messenger, activates the enzyme protein kinase C, which then phosphorylates a large number of proteins, leading to the cell's response.



2- Guanylyl Cyclase–cGMP Second Messenger System:

- The second messenger cGMP is generated by the enzyme guanylyl cyclase (GC).
- There are **two forms of guanylyl cyclase**, a soluble, cytoplasmic form and a membrane-localized form. Soluble GC is a target for the paracrine-signaling molecule nitric oxide (NO).
- It is a cyclic nucleotide derived from guanosine triphosphate (GTP). cGMP acts as a second messenger much like cAMP.
- **Nitric oxide** is a famous stimulator of cGMP synthesis. cGMP relaxes smooth muscle tissues. In blood vessels, relaxation of vascular smooth muscles lead to vasodilation and increased blood flow.
- cGMP is involved in the regulation of some protein-dependent kinases. Example, protein kinase G (PKG). Activation of PKG reduces cytoplasmic Ca^{2+} concentrations, resulting in smooth muscle relaxation.
- The transmembrane form of guanylyl cyclase is a receptor for **atrial natriuretic** (ANP) peptide. Binding of ANP to transmembrane guanylyl cyclase in the kidney increases cGMP, which stimulates Na^{+} excretion to reduce blood volume.

4- Calcium-Calmodulin Second Messenger System:

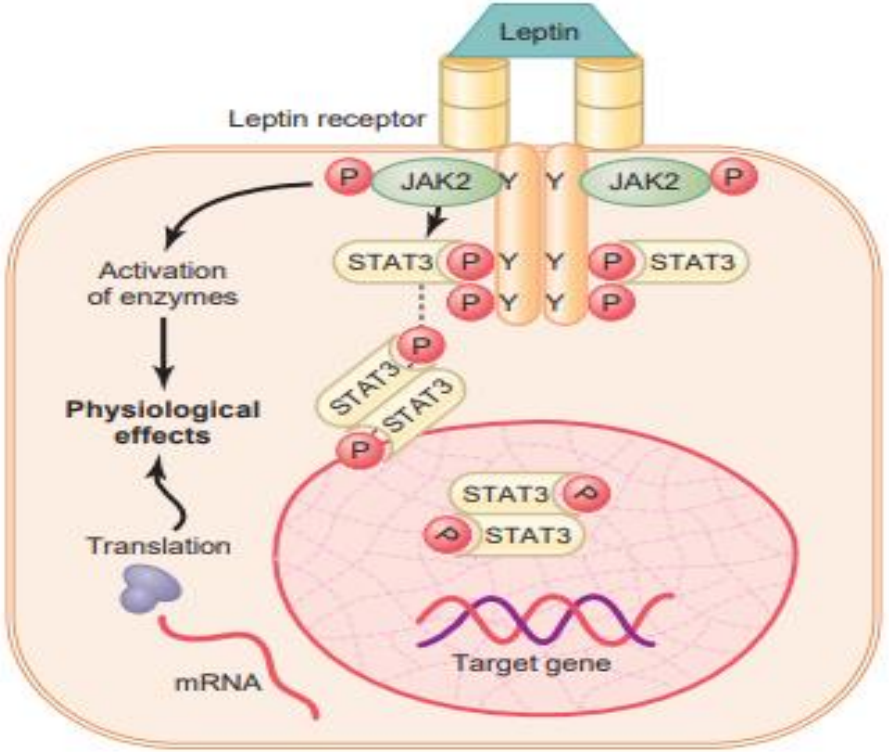
- Calcium influx may be initiated by (1) opening of voltage-gated calcium channels or (2) a hormone interacting with membrane receptors that open calcium channels (ligand-gated).
- Upon entering a cell, calcium ions bind with the protein **calmodulin**.
- Calmodulin has **four calcium sites**, and when three or four of these sites have become bound with calcium, the calmodulin changes its shape and initiates multiple effects inside the cell, including activation or inhibition of protein kinases.
- Example, calmodulin activates myosin light chain kinase, which acts directly on the myosin of smooth muscle to cause smooth muscle contraction.
- **Troponin C** in skeletal and cardiac muscle is similar to calmodulin in both function and protein structure.

Enzyme linked receptors

- Some receptors are enzyme-linked receptors that pass through the membrane **only once**, in contrast to the seven transmembrane G protein-coupled receptors. Example is the **cytokine receptors** such as the **leptin receptor**.
- 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).

An enzyme-linked receptor

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 (P) and activation of the intracellular associated janus kinase 2 (JAK2). This mechanism 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:

- Steroid hormones, thyroid hormones, retinoid hormones, and vitamin D, bind with protein receptors **inside the cell** rather than in the cell membrane.
- The activated hormone-receptor complex binds with a specific regulatory (promoter) sequence of the DNA called the hormone response element, and activates or represses **transcription** of specific genes and formation of **messenger RNA (mRNA)**.
- After certain period following the entrance of the hormone inside the cell, newly formed proteins appear in the cell and become the controllers of new or altered cellular functions.

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 action either activates or inhibits gene transcription, formation of messenger RNA *(mRNA)*, and protein synthesis.