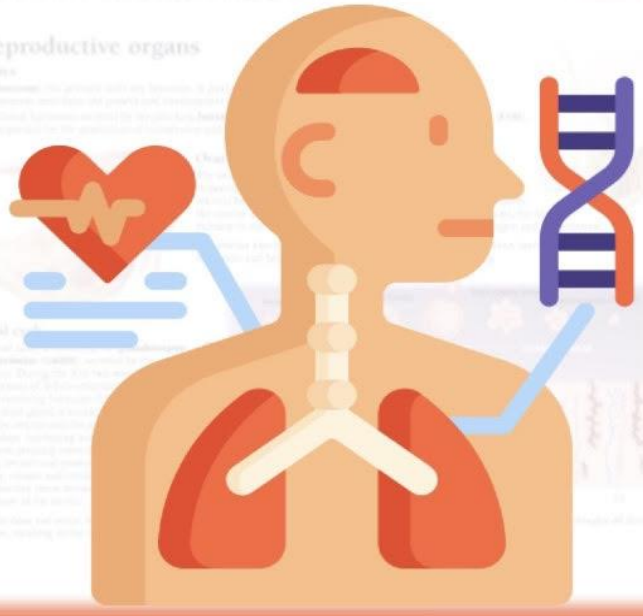
Doctor 022

Physiology

Sheet no.



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Signal Transduction (3rd Lecture)

الكلام باللون الأزرق من السلايدات والكلام اللي بالأسود من شرح الدكتورة وبالتوفيق جميعا.

Introduction: Mechanisms of Hormone Action

- why we are studying classification of hormones:

We all now know that hormones are classified depending on their chemical structure to lipid-soluble and water-soluble hormones, so why is it important to classify hormones according to their chemical structure? It's because cellular hormones which belong to the same chemical class share:

1- similar events that occur in the target cells.

2- similar location of receptors.
(شرحهم بداية الصفحة التالية)

So that's why we must understand and memorize them, because this is the only way to figure out the way by which hormones can affect our cellular function.

- What we need to respond to a hormone:

We need to have a certain physiological concentration of the hormone that can have its affect on cells.

target cell must have specific receptor for that hormone (specificity).

- Hormone exhibition:

Hormones are characterized by certain characteristics and one of them is the affinity.

- Affinity (binding to receptors with high bond strength)

(Affinity depends on the nature of chemical bonds between the ligand and the receptor)

- Saturation (low capacity of receptors)

(Once the hormone is released it will bind to all receptors and any increase in hormone concentration will not give us any stronger physiological response)

- Response depends on both hormone and target cell
  (as we said the chemical nature of the hormone determine the mechanism (the downstream events that will happen)
- Lipid-soluble hormones bind to receptors inside target cells (but still they have the capability to bind to cell surface receptor for specific functions)
- Water-soluble hormones bind to receptors on the plasma membrane.
  - ✓ A series of events will lead to Activating second messenger system and they will make the change which the hormone is responsible for without the entering of the hormone physically into the cell.
  - ✓ Production of second messenger leads to Amplification of original small signal because these second messengers have much higher concentration than the hormone and they affect their effectors for a good period.
- Responsiveness of target cell depends on
  - ✓ Hormone's concentration
  - ✓ Abundance of target cell receptors

Receptors:

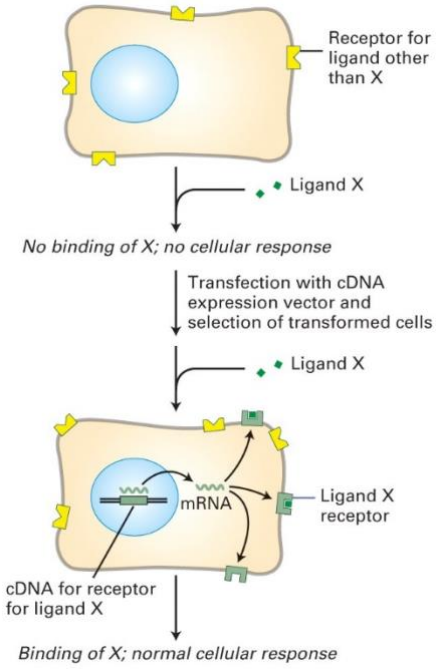
- Receptors are specific membrane proteins, which can recognize and bind to corresponding ligand molecules, become activated, and transduce signal to next signaling molecules. (Glycoprotein or Lipoprotein mostly)
- Specificity and affinity both terms are related to each other, but they have different meaning.
- Specificity: The "specificity" of a ligand for a receptor is a description of how favorable the binding of the ligand for the receptor compared with its possible binding to other types of receptors that may also be present.
  (So even if there is more than one ligand that can bind to one receptor there is one "specific" ligand which have the best binding to that receptor)

- Affinity: "Affinity" simply refers to how strong the binding is (it is judged by K association or K dissociation (kd) and ΔGo (read the note below). “High affinity" refers to a very strong binding. Kd is inversely related to affinity (large negative ΔGo and a very small Kd). The association or dissociation constant is often referred to as the "affinity” or “binding” constant (low affinity means low biding so we need higher concentration to bind to 50% of receptors (definition of Kd) so we need higher kd)

Note: (the doctor mentioned the relation between Kd and affinity only from these terms).

Ligand:

A small molecule that binds specifically to a larger one; for example, a hormone is the ligand for its specific protein receptor.



This experiment shows that if we have the ligand X and a cell that doesn’t have a specific receptor for that ligand nor a receptor that might bind to that ligand (it has other receptors, but they don’t bind to X) because of that; if you treat the cell with X you will not see any response or change.

Transfection: The insertion of a piece of DNA that expresses receptor x inside the cell, so the cell starts responding to X.

This experiment shows the importance of having a specific receptor in order to have the response of the hormone.

Types of receptors:

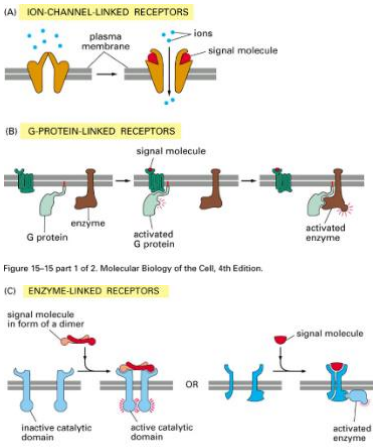
- Membrane receptor (integral protein)
  Membrane Glycoprotein
- Intracellular receptors
  Cytosol or nuclei: DNA binding protein (bind to the ligand from one side and to the DNA from the other side and then it promotes or stops gene expression).

- Three major classes of surface receptors for signaling:

1- Ion-channel linked receptors.

2- protein linked/coupled receptors. (GPCR)

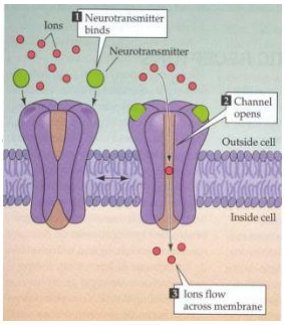
3- Enzyme-linked receptors

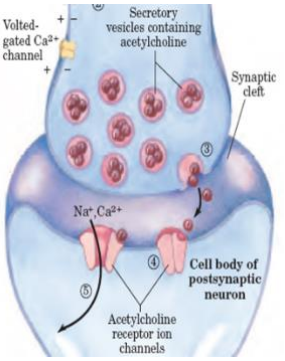


1- Ligand-gated ion channels type

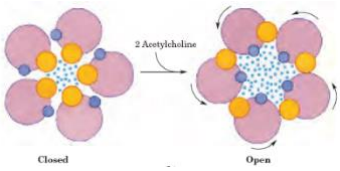
(Cyclic receptors) (ionotropic)

ligand→ receptor→ ion channel opens or closes





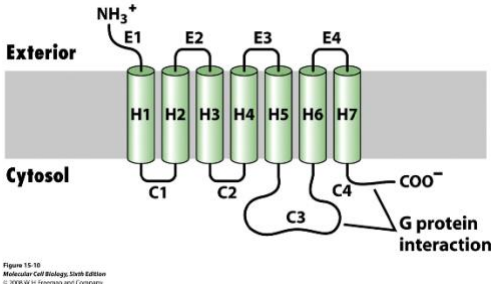
In this example, when the neurotransmitter (acetylcholine) binds to the Ligand-gated ion channel(receptor), it causes an action which can be either opening or closing the channel which allows the ions to flow across the membrane.



2-G protein-coupled receptors (GPRs): largest family of cell surface receptors; present in all eukaryotes; ex: adrenergic receptors, opioid receptors.

1. Overview: common characteristics

a. 7 trans-membrane spanning domains (7 helical domains trans the membrane)



b. Act as receptors for many different ligands including NT, H (N terminal extracellular domain which binds to the ligand and C terminal cytosolic domain which interacts with a complex called G protein complex)

c. Large amount of receptor diversity, but common mechanism of action.

d. Transmit signals to intracellular targets via G proteins.

e. Targets are plasma membrane bounded enzymes or ion channels.

2. Mechanism of Activation of GPRs:

A (alpha) & g (gamma) subunits have covalently attached lipid anchors that bind a G-protein to the plasma membranes cytosolic surface:

a. Binding of ligand to extracellular domain of GPRs induces conformational change that allows cytosolic domain of the receptor to bind to an inactive G protein (in the inactive form the three subunits are bound to each other) at inner face of PM.

b. This interaction activates the G protein, which dissociates from the receptor.

c. Activated G protein α subunit can now bind to a GTP instead of GDP, causing dissociation into activated α vs. β γ subunits. Each of these can go on to activate the target proteins. (In most cases α activate the effector)

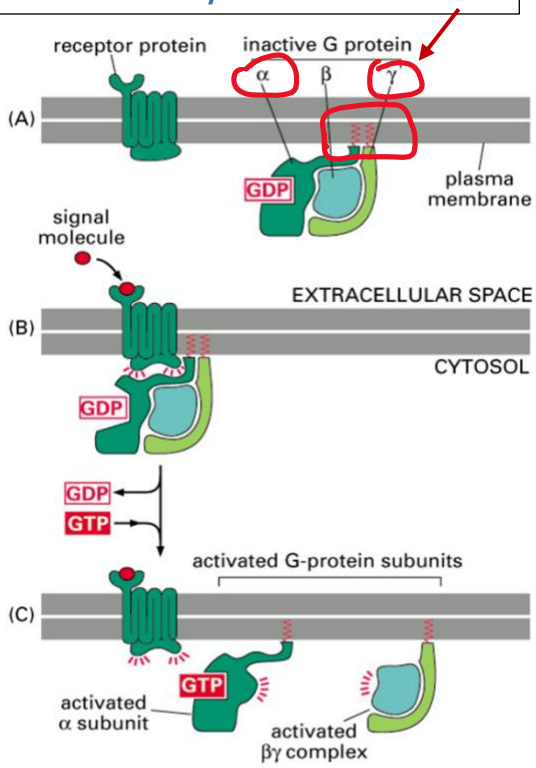
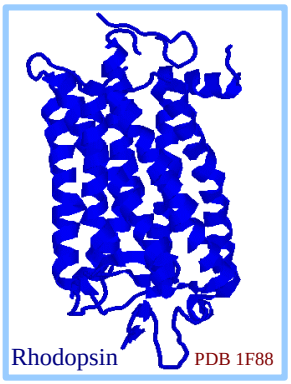
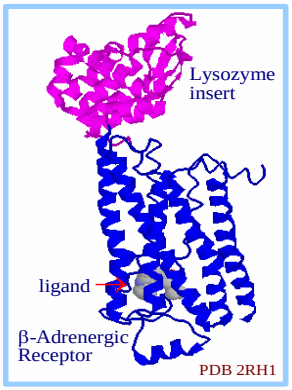


Figure 15–28. Molecular Biology of the Cell, 4th Edition.

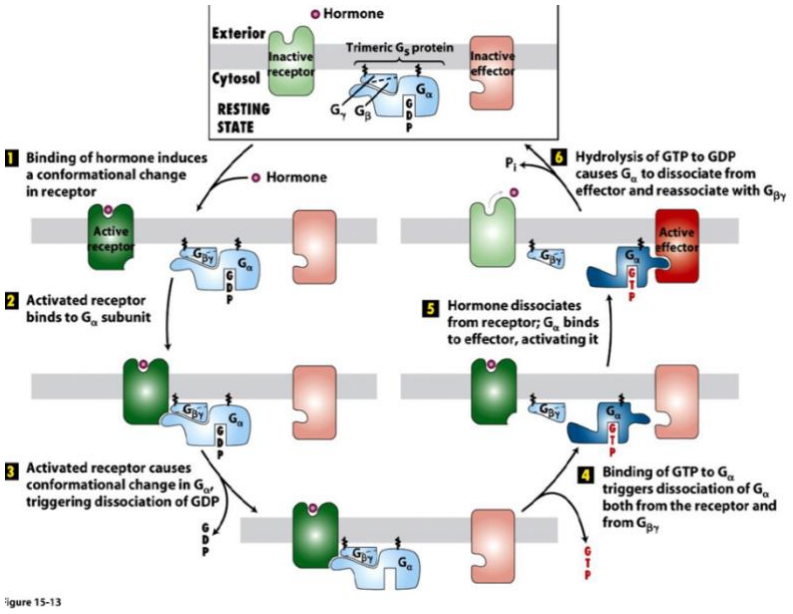
- This large family could be a receptor for many ligands such as neurotransmitters or hormones.
- Rhodopsin was the first of these to have its 7-helix structure confirmed by X-ray crystallography (in retina, low light vision) (it's not activated by a ligand, but it's activated by light). Rhodopsin is unique; It senses light via a bound chromophore, retinal.
- Another example for G protein is β -adrenergic receptor which is activated by epinephrine (adrenaline) & norepinephrine (noradrenaline).

- Approx. 800 different GPCRs are encoded in the human genome.





- The Sequence of activating a G protein coupled receptor (look at the steps in the photo)
- In step 3: the activation involves replacement of GDP with GTP.
- The effector which was activated by α subunit is often an enzyme.
- α subunit has a GTPase activity so it inactivates itself by the replacement of GTP into GDP and then it binds to β and γ subunits.



igure 15-13

- One of the most important functions of β and γ subunits is to inactivate α subunit by binding with it.

- There are 3 types of Gα subunit according to the downstream effect that happened:

  1- A G-protein that activates cyclic-AMP formation within a cell is called a stimulatory G-protein, designated Gs with alpha subunit **Gsα**.
  (it stimulates adenylate cyclase to produce cAMP)
  Adenylate Cyclase (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.

  2- When an inhibitory **Giα** binds to GTP, it inhibits Adenylate cyclase so as a result it will reduce the production of cAMP.
  Different effectors & their receptors induce **Giα** to exchange GDP for GTP than those that activate **Gsα**.

  3- **Gqα** (don't worry about it we will explain it later) it activates phospholipase c which activates the production of DAG (diacyl glycerol) and production of inositol triphosphate which will bind to IP3 receptors on the ER and a series of events happens there.

يجب حفظ هذا الجدول باستثناء الصفوف التي بجانبها ✖

TABLE 15-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors*

G_α CLASS	ASSOCIATED EFFECTOR	2ND MESSENGER	RECEPTOR EXAMPLES
$G_{\alpha s}$	Adenylyl cyclase	cAMP (increased)	β-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin
$G_{\alpha i}$	Adenylyl cyclase K^+ channel ($G_{\beta\gamma}$ activates effector)	cAMP (decreased) Change in membrane potential	α_2-Adrenergic receptor Muscarinic acetylcholine receptor
✖ $G_{\alpha olf}$	Adenylyl cyclase	cAMP (increased)	Odorant receptors in nose
$G_{\alpha q}$	Phospholipase C	IP_3, DAG (increased)	α_1-Adrenergic receptor
$G_{\alpha o}$	Phospholipase C	IP_3, DAG (increased)	Acetylcholine receptor in endothelial cells
✖ $G_{\alpha t}$	cGMP phosphodiesterase	cGMP (decreased)	Rhodopsin (light receptor) in rod cells

*A given G_α subclass may be associated with more than one effector protein. To date, only one major $G_{\alpha s}$ has been identified, but multiple $G_{\alpha q}$ and $G_{\alpha i}$ proteins have been described. Effector proteins commonly are regulated by G_α but in some cases by $G_{\beta\gamma}$ or the combined action of G_α and $G_{\beta\gamma}$.
IP_3 = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.
SOURCES: See L. Birnbaumer, 1992, *Cell* **71**:1069; Z. Farfel et al., 1999, *New Eng. J. Med.* **340**:1012; and K. Pierce et al., 2002, *Nature Rev. Mol. Cell Biol.* **3**:639.

Table 15-1
Molecular Cell Biology, Sixth Edition

ADH

Same ligand and different receptors =different effects.

- This is an example when the ligand of the muscarinic acetylcholine receptor binds to it, this process activates the G- protein and breaks it to βγ subunit and α subunit. In this case βγ subunit (not α subunit) activates the K+ channel (the effector), which leads to hyperpolarization because k+ ions move outside the cell.
- It's inhibitory because it inhibits reserving action potential (it becomes harder because of the hyperpolarization.)

• Summary of hormones and their second messengers:

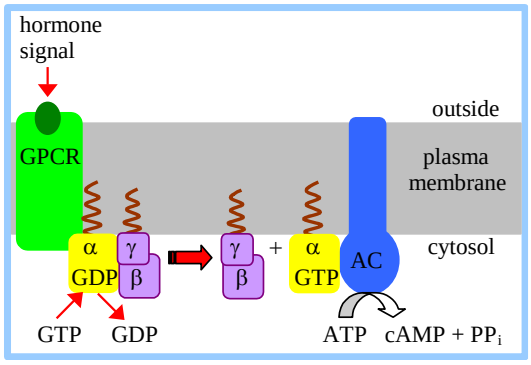
(Some hormones have more than one receptor and a second messenger but for this level this is what you need to know) (we need to memorize it too when we finish its second part).

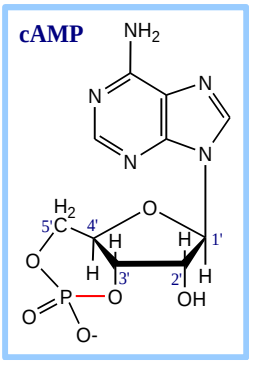
IP_3	cAMP	cGMP	Tyrosine kinase - intrinsic	Tyrosine kinase - receptor associated	Steroid
GnRH	FSH	ANP	Insulin	Prolactin	Glucocorticoid
Gastrin	LH	NO (EDRF)	IGF-1	Cytokines (IL-2,6,8)	Estrogen
Oxytocin	ACTH		FGF	GH	Progesterone
TRH	TSH		PDGF		Testosterone
ADH (V_1)	CRH				Aldosterone
Histamine (H_1)	hCG				Vitamin D
Angiotensin II	PTH				T_3/T_4
	Calcitonin				Cortisol
	Glucagon				
	GHRH (can act via IP_3 as well)				

- Adenylate Cyclase (Adenylyl Cyclase) catalyzes:

ATP ⟶ cAMP + PPi

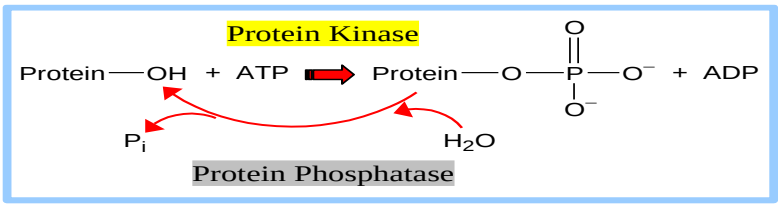
Binding of certain hormones (e.g., epinephrine) to the outer surface of a cell activates Adenylate Cyclase to form cAMP within the cell.





Cyclic AMP is thus considered to be a second messenger.

- Protein Kinase A which is activated by binding to cAMP (kinase means that it phosphorylates the protein which leads to turning it on) (cAMP Dependent Protein Kinase) catalyzes the transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity. (The opposite reaction is removing the phosphate group by hydrolysis reaction by phosphatases which causes turning off the signal.)



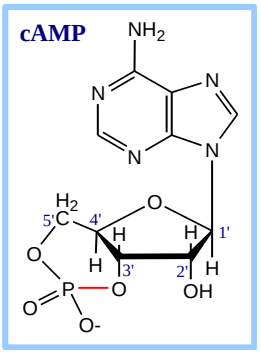
-Protein kinases and phosphatases are regulated by complex signal cascades For example:

- Some protein kinases are activated by Ca++-calmodulin.
- Protein Kinase A is activated by cyclic-AMP (cAMP).

- Turning off the signal:

1- Gα hydrolyzes GTP to GDP + Pi. (GTPase). The presence of GDP on Gα causes it to rebind to the inhibitory βγ complex.
The Adenylate Cyclase is no longer activated.

2- Phosphodiesterases catalyze hydrolysis of
cAMP + H2O → AMP



The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.
Thus, cAMP stimulates its own degradation, leading to rapid turnoff of a cAMP signal.

3- Receptor desensitization varies with the hormone.
In some cases, the activated receptor is phosphorylated via a G-protein Receptor Kinase.
The phosphorylated receptor then may bind to a protein **β-arrestin.**

- (Turning off in the receptor level) β -Arrestin promotes the removal of the receptor from the membrane by **clathrin-mediated endocytosis**.
(Endocytosis of the receptor makes it no longer available for signaling.)

- (Turning off in the enzyme level) β -Arrestin may also bind to a cytosolic Phosphodiesterase, bringing this enzyme close to where cAMP is being produced, contributing to signal turn off.

4- Protein Phosphatase catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein
Kinase A.

5- The complex of Gβγ that is released when Gα binds to GTP is itself an effector that binds to and activates or inhibits several other proteins.
E.g., G βγ inhibits one of several isoforms of Adenylate Cyclase, contributing to rapid signal turnoff in cells that express that enzyme.

"لَا أَبْرَحُ حَتَّى أَبْلُغَ".