Tecjan 2022

PHYSIOLOGY

SHEET NO.

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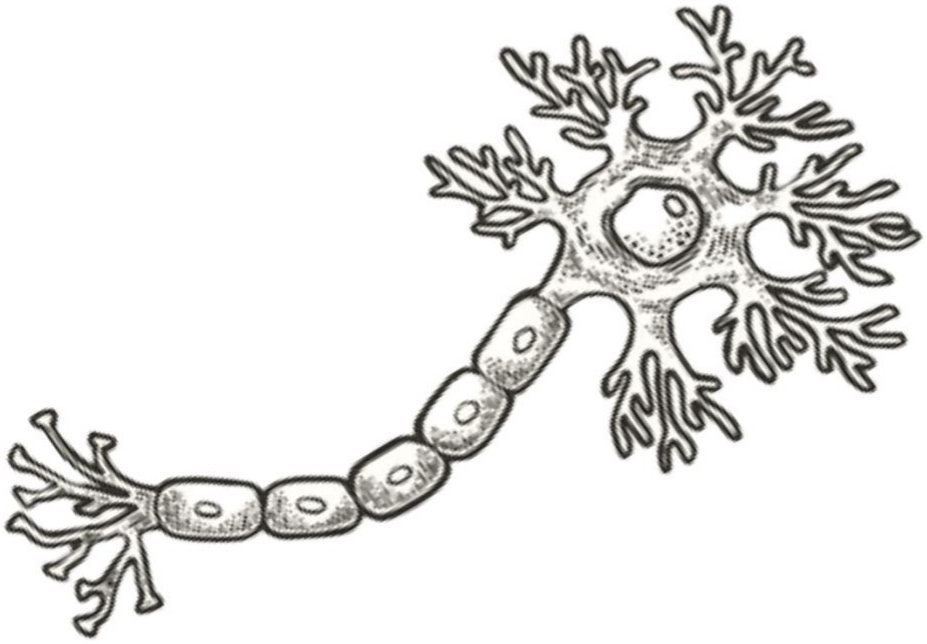
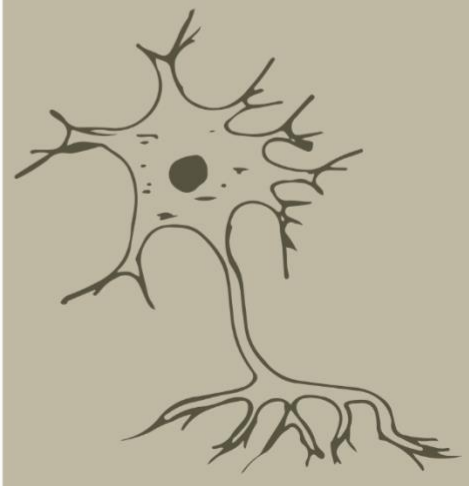


WRITTEN BY: **Ahmad Alkashef**

EDITED BY: **Aya Natour**

DOCTOR: **Dr.Ebaa Zayadneh**



Mechanisms Of Hormone Action

How to make the mechanisms happen?

We need a hormone and a receptor ….hmm…how we get them?

1) To get the hormone to have a response we need to have a stimulation of the gland or the cells that secret it to increase the concentration to reach a physiological level.
2) Also, we need a receptor in the cell or in the tissue in order to have the effect in the cell.

Ligand (hormone)

A small molecule that binds specifically to a larger one; for example, a hormone is the ligand for its specific receptor. There is a binding site make unlocking and when it binds with receptor this will cause a conformational change of the receptor which will activate or effect binding and relationship with other molecular enzymes.

But, hormones of the same chemical class have similar mechanisms of action, similarities include:

1) Location of the cellular receptor proteins depends on the chemical nature of the hormones.
2) Events that occur in the target cells.

Like the water soluble hormone and the lipid soluble hormone they completely differentiate in the way they enter the cell and some other mechanisms.

To respond to a hormone target cell must have specific receptors for that hormone (specificity), hormone exhibit:

1. Affinity (bind to receptors with high bond strength).

   Each hormone binding to a receptor they have what is called "affinity" and describe the binding between the hormone and the receptor and actually it is the strength bond between the ligand and the receptor.

2. Saturation (low capacity of receptors).
   It will bind to the receptor but if we increase the concentration it won't make it more stronger effect, because it has limited number of receptors and it is saturated.

Types of hormones

1. Lipid soluble hormone.
   It bind to receptors inside target cells and they have the capability to enter the cell and bind the inter cytosolic receptors and can directly affect the DNA expression e.g :- all the activity of the synthesis of the enzymes or molecules.
2. Water soluble hormone.
   It bind to receptors on the surface of the plasma membrane and it has more functions, likewise:
   a) Activates second messenger system.
   b) Amplification of original system.
   c) They transduce the signal by producing the second messenger and these messengers have more concentration than the hormone and it will effect more stronger.
   d) response depends on both hormone and target cell and the responsiveness of target cell depends on the hormone's concentration and the abundance of target cell receptors.

Receptor

Receptors are specific membrane proteins, which are able to recognize and bind to corresponding ligand molecules, become activated, and transduce signal to next signaling molecules.

Note: cell membrane receptor is glycoprotein and the lipoproteins, which those are integral proteins,they signal or transduce the signal to intercellular targets or signaling molecules by transducing the signal by activating or stimulating different targets or enzymes resulting generation of second messengers or enzymatic reaction lead to binding interaction of other molecules.

We mentioned the specificity and the affinity ….so what is the difference?

- Specificity: the “specificity” (the favorability) of a ligand for a receptor is a description of how favorable the binding of the ligand for the receptor is compared with its possible binding to other types of receptors that may also be present. Imagine the hormone(ligand) like a magnet if it is strong, it will make a strong attachment.

  Note: For a receptor, "specificity" describes how much the receptor favors a particular ligand relative to the other ligands that may also be present. In real biological systems the specificity of either ligands or receptors is rarely 100%- this is one of the reasons why drugs tend to have side effects. For example, it is

well known that most proteins that bind a given nucleotide (like ATP) are not completely specific for ATP, but can bind a variety of ATP analogs like thio-ATP, AMPPCP, or even GTP.

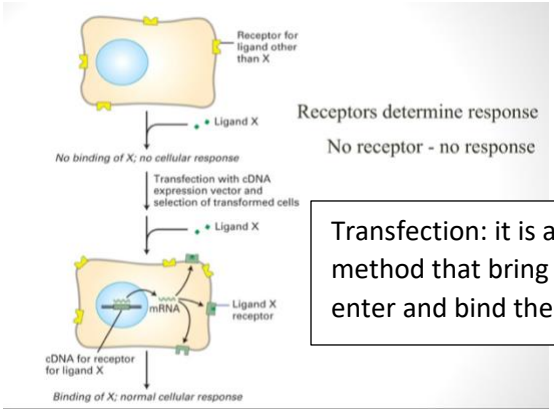
External Note:

a) **Thio-ATP is a modified form of adenosine triphosphate (ATP) that contains a sulfur atom, often used in biochemical studies to investigate ATP-dependent reactions.**

b) **MPPCP, or adenylyl-imidodiphosphate, is a non-hydrolyzable analog of ATP frequently used in biochemical research to study ATP-binding proteins and ATP-dependent reactions.**

- Affinity: "Affinity" simply refers to how strong the binding is (as judged by K association or K dissociation and ΔGo). "High affinity" refers to very strong binding (large negative ΔGo and a very small Kd). The association or dissociation constant is often referred to as the "affinity" or "binding" constant. If the it is higher affinity it is lower concentration ligand to have the effect because we don't have a lot to keep the ligand bind to receptor.

Note:- the dissociation constant inversely related to the affinity. The higher Kd the lower affinity because we need a high concentration to make a good binding and for sure we depend on the free energy how the binding is favorable in ligand with the receptor, so if the free energy increases the affinity will increase.



Transfection: it is a laboratory method that bring a DNA to enter and bind the cell's DNA.

- This is an experiment it shows that if the cell doesn't express a certain receptor (ex;receptor x) so if you treat the cell with hormone x you will not get a response ,but if you transfect the same cell then the DNA of the cell start synthesis receptor x once the receptor x is expressed at the membrane now if you treat the cell with hormone x you will get a response

Types of receptors

1) **Membrane receptors**
   Mostly are Membrane Glycoprotein
2) **Intracellular receptors**
   They maybe in the Cytosol or Nuclei and It is DNA binding protein: they bind a hormone and DNA at the same time to promote expression or inhibition.

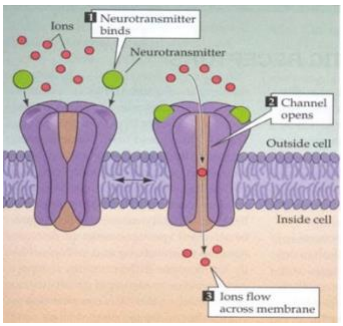
1. Membrane Receptor

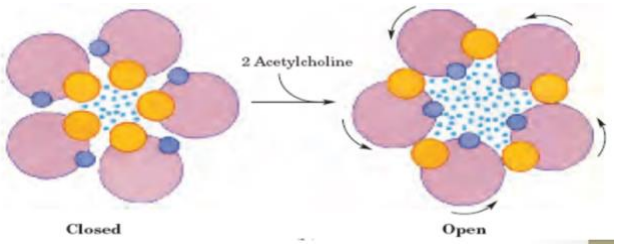
Three major classes of surface receptors for signaling:

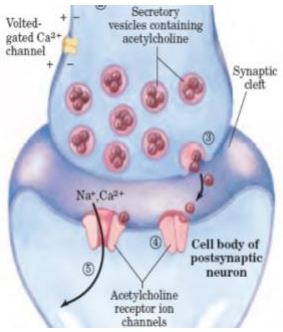
a) Ligand-gate ion channels type

It is a channel that has a receptor site, once bound to the ligand that opens up and allows a certain ions and change membrane potential. It is also called (Cyclic receptor).

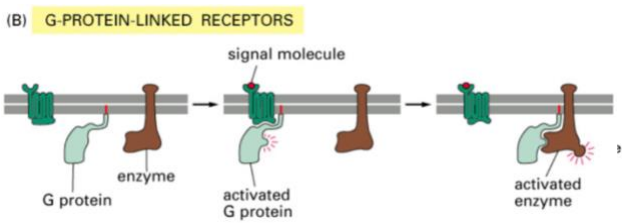
Ligand—> receptor—> ion channel (open or close)



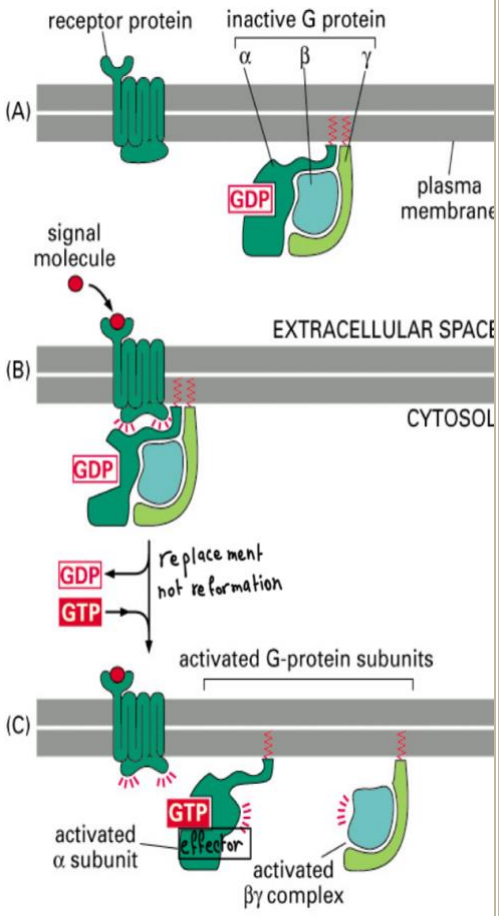




b) G-protein coupled receptor



G protein-coupled receptors (GPRs): largest family of cell surface receptors; present in all eukaryotes; ex: adrenergic receptors, opioid receptors and approx 800 different GPCRs are encoded in the human genes.



1. Overview:

a. 7 helical trans-membrane spanning domains.

b. Act as receptors for many different ligands including NT, H.

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

d. Transmit signals to intracellular targets via G-proteins.

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

2. Mechanism of Activation of GPRs:

a. Binding of ligand to extracellular domain of GPRs induces conformational change that allows cytosolic domain of the receptor to bind to inactive G protein at inner face of PM.

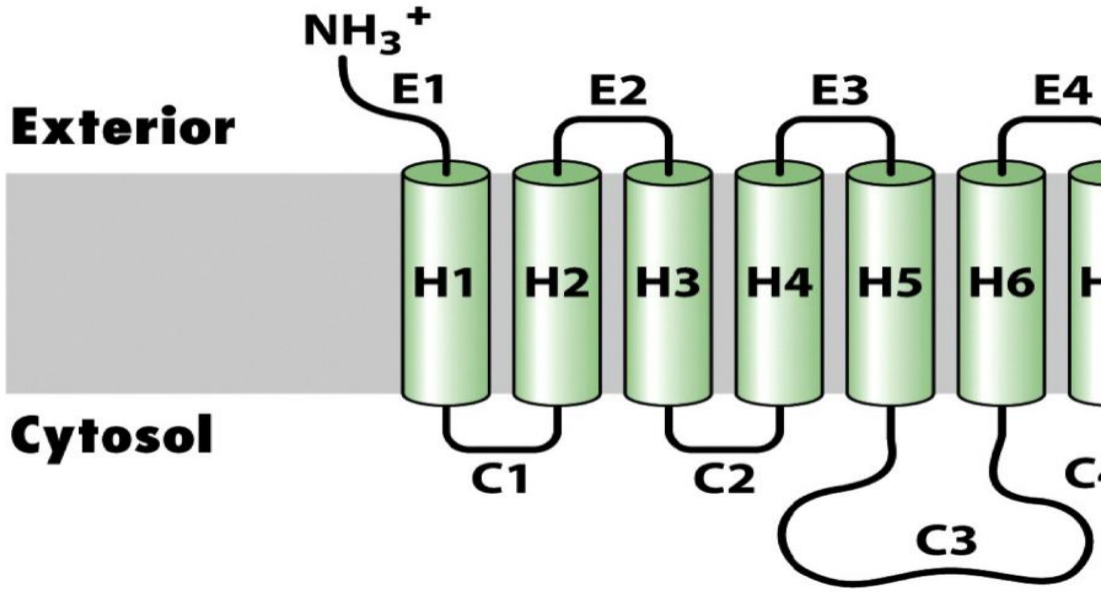
b. This interaction activates the G-protein, which dissociates from the receptor and activate enzyme that is mostly produce 2nd messengers

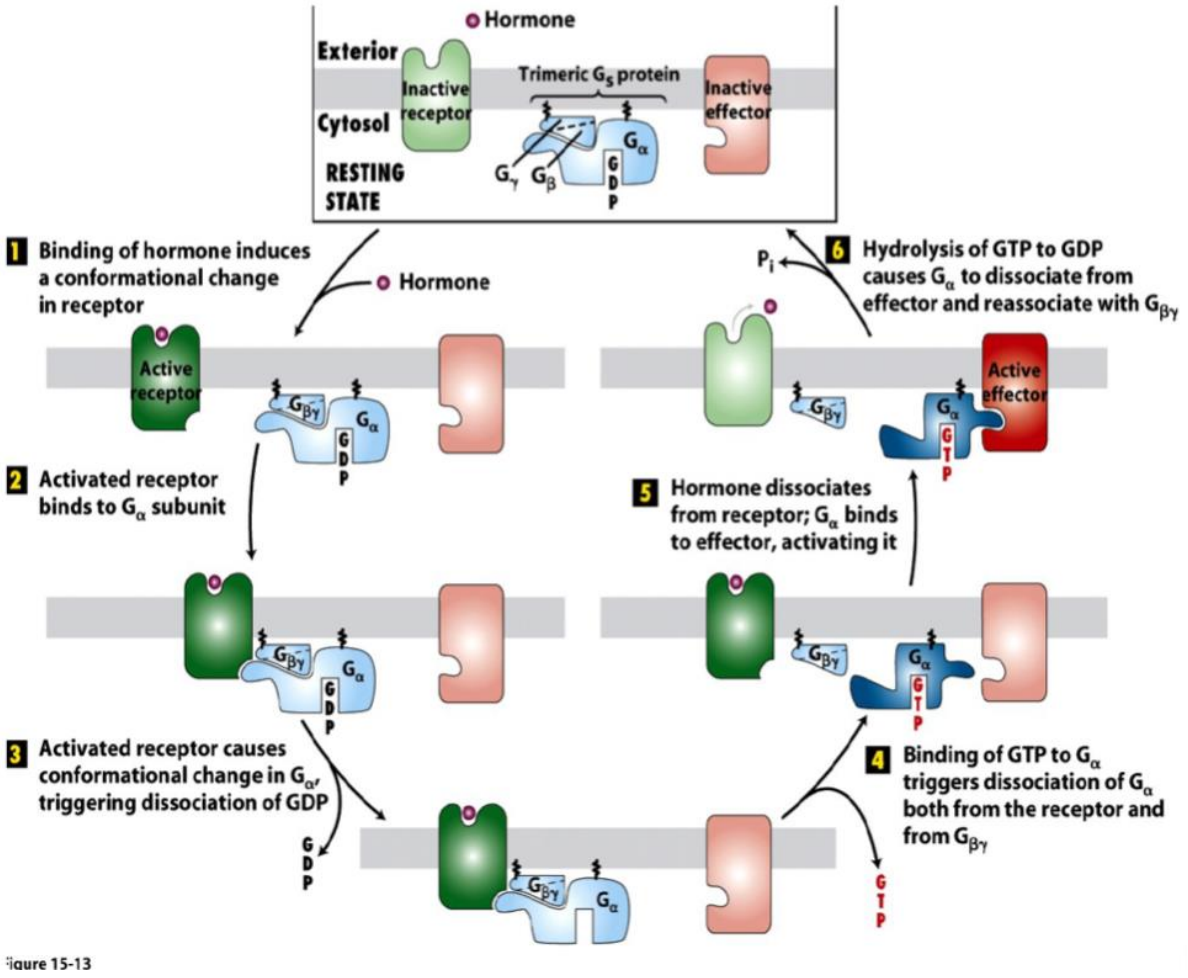
c. Activated G protein alpha subunit can now bind GTP instead of GDP, causing dissociation into alpha vs ßy subunits. Each of these can go on to activate target proteins.

G Protein Signal Cascade

- G-proteins are heterotrimeric, with 3 subunits alpha, ß, y.
- A G-protein that activates cyclic-AMP formation within a cell is called a stimulatory G-protein, designed Gs with alpha subunit Gs alpha.
- Gs is activated, e.g.,by receptors for the hormones epinephrine and glucagon.
- The ß-adrenergic receptor is the GPCR for epinephrine.
- The alpha subunit of a G-protein (G alpha) binds GTP & can hydrolyze it to GDP+Pi.
- Alpha & y subunits have covalently attached lipid anchors that bind a G-protein to the plasma membrane cytosolic surface.

Note: N-terminal extracellular and the C-terminal intercellular. N-terminal is a different binding site depends on the ligand, so it will change the structure. C-terminal will make interactions with G-protein complex this receptor could be neurotransmitters or hormones.

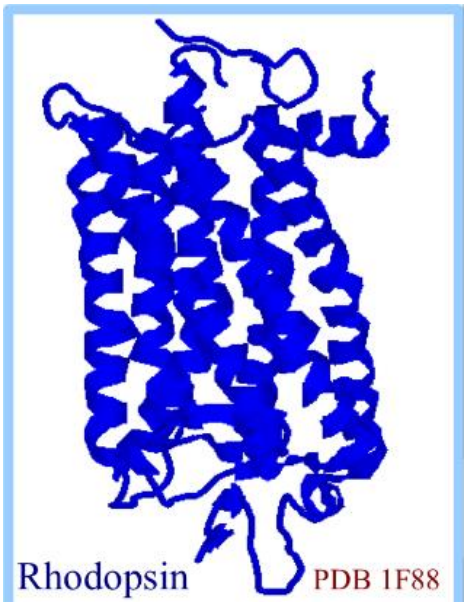




igure 15-13

- Different isoforms of G alpha have different signal roles. E.g.:
- The stimulatory Gs alpha When it binds GTP, activates Adenylate cyclase.
- An inhibitory Gi alpha when it binds GTP, inhibits Adenylate cyclase.
  Different effectors & their receptors induce Gi alpha to exchange GDP for TP than those that activate Gs alpha.
- The complex of Gß,y that is released when G alpha binds GTP is itself an effector that binds to and activates or inhibits several other proteins.
  E.g.: Gß,y inhibits one of several isoforms of Adenylate Cyclase, contributing to rapid signal turnoff in cells that express that enzyme.

G Protein Signal Cascade



Most signal molecules targeted to a cell bind at the cell surface to receptors embedded in the plasma membrane.

Only signal molecules able to cross the plasma membrane (e.g., steroid hormones) interact with intracellular receptors.

A large family of cell surface receptors have a common structural motif, 7 transmembrane alpha-helices.

Rhodopsin was the first of these to have its 7-helix structure confirmed by X-ray crystallography (in retina, low light vision).

- Rhodopsin is unique.

It senses light, via a bound chromophore, retinal.

• Most 7-helix receptors have domains facing the extracellular side of the plasma membrane that recognize & bind signal molecules (ligands).

E.g., the ß-adrenergic receptor is activated by epinephrine & norepinephrine.

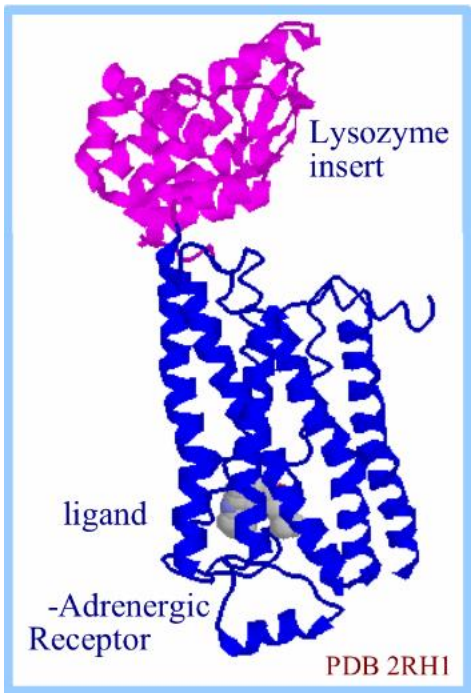


TABLE 15-1 Major Classes of Mammalian Trimeric G Proteins and Their Effectors*

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

A quick hint ;)

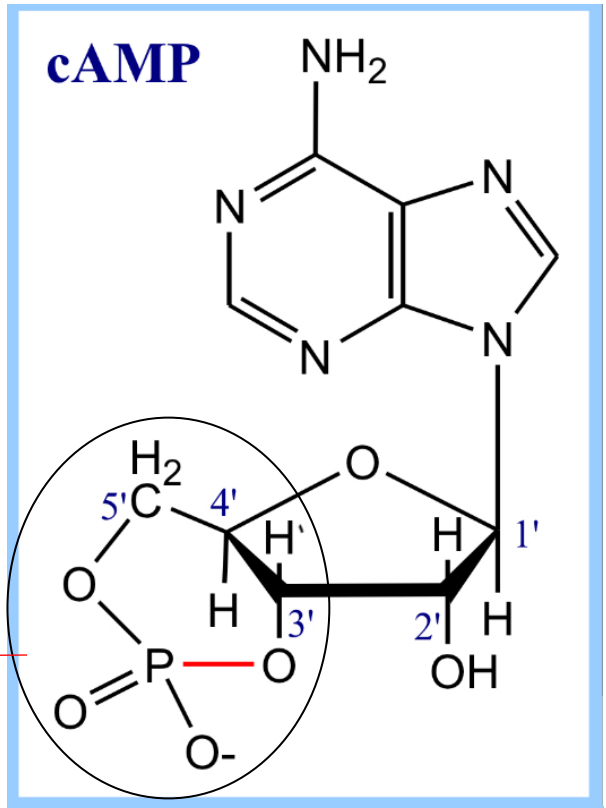
Gi alpha —> i—> eye—>👀—> 2—> alpha 2-adrenergic receptor.

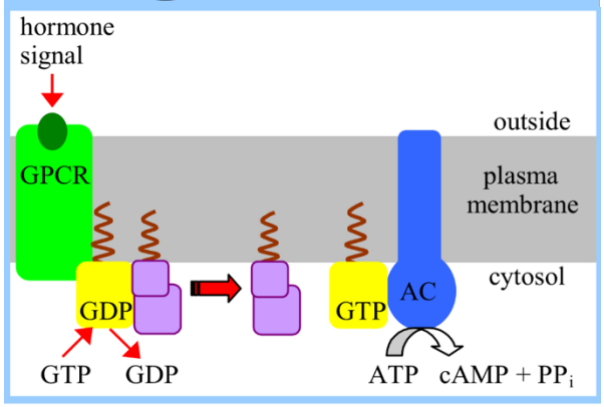
Gq alpha—>q—>queue—> 🧍🧍🧍🧍 —> one row—> alpha 1-adrenergic receptor.

Note : when the Gq associated with phospholipase c to make IP3 and DAG and then by IP3 to release the Ca++ from the stores, so all IP3,DAG and the Ca++ are 2nd messengers activated by Gq alpha.

Adenylate cyclase

- Adenylate cyclase (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.
- Adenylate cyclase (adenylyl cyclase) catalyzes:
  ATP ➡ cAMP+Pi
- 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 2nd messengers.
- Adenylate Cyclase activated by the stimulatory G alpha – GTP, catalyzes synthesis of cAMP.
- If this cycle is removed then it won't be 2nd messenger.

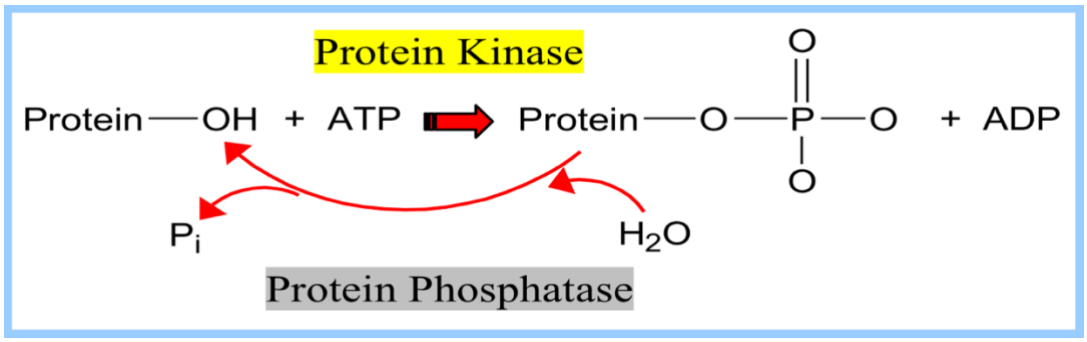




Protein kinase A

(cAMP dependent protein kinase)

Catalyzes transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity.



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

• Some protein kinases are activated by Ca++-calmodulin.

• Protein Kinase A is activated by cyclic-AMP (cAMP).

PKA transfers Pi from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence.

The cell has 2 subunits regulatory and catalytic and this make the cell determines when to turn on and when to turn off, so Protein Kinase A in the resting state is a complex of:

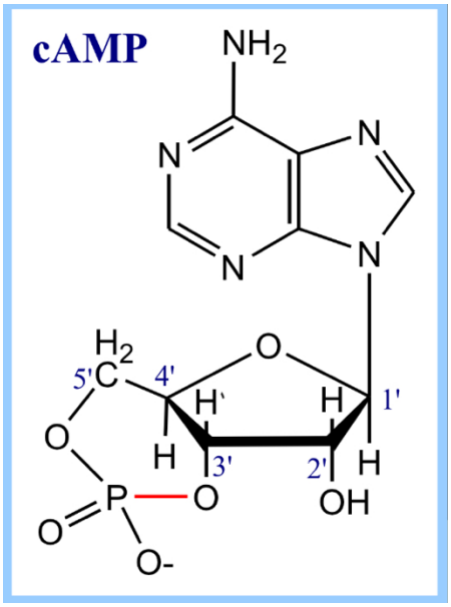
• 2 catalytic subunits (C)

• 2 regulatory subunits (R).

R2C2: When each (R) binds 2 cAMP, a conformational change causes (R) to release (C).

The catalytic subunits can then catalyze phosphorylation of Ser or Thr on target proteins.

PKIs, Protein Kinase Inhibitors, modulate activity of the catalytic subunits



(C).Phosphodiesterase

It enzymes catalyze:

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.

Turn off of the signal:

1. **G alpha hydrolyzes GTP to GDP + Pi. (GTPase).**
   The presence of GDP on G alpha causes it to rebind to the inhibitory By complex.
   [Adenylate Cyclase is no longer activated.]
2. **Phosphodiesterases** catalyze hydrolysis **of cAMP —> AMP.**
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 B-arrestin.**
   - **B-Arrestin promotes removal of the receptor from the membrane by clathrin-mediated endocytosis.**
   - **B-Arrestin may also bind a cytosolic Phosphodiesterase, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.**
4. **Protein Phosphatase catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A.**
5. **. Different isoforms of Ga have different signal roles. E.g.:**

a. The stimulatory Gsa , when it binds GTP, activates
b. Adenylate cyclase
c. An inhibitory Gia, when it binds GTP, inhibits Adenylate cyclase.
d. Different effectors & their receptors induce Gia to exchange GDP for GTP than those that activate Gsa.
e. The complex of GB,y that is released when Ga binds GTP is itself an effector that binds to and activates or inhibits several other proteins.
f. E.g., GB,Y inhibits one of several isoforms of Adenylate Cyclase, contributing to rapid signal turnoff in cells that express that enzyme.

The End