physiology

premed 2018 - JU

◉ Sheet ○ Slides

Number

18

Done by:

Rama Abbady

Corrected by:

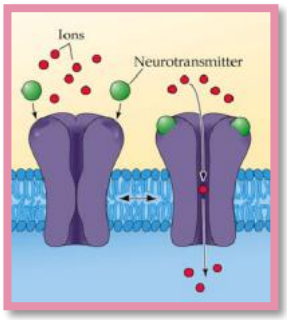
Ameen Alsaras

Doctor

Ebaa Alzayadne

➢ Hormone Receptors and their Activation

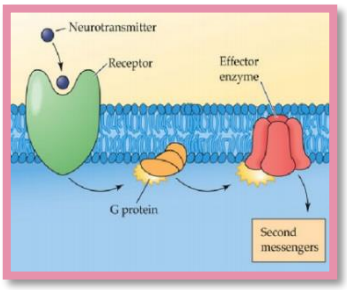
- The first step of a hormone's action is to bind to specific receptor at the target cell.
- Cells that lack receptors for the hormones don't respond. If a cell doesn't express a receptor for a certain hormone, it won't be stimulated by the hormone even if the hormone is secreted in large concentrations. In order for this cell to produce a response, a gene coding for this receptor must be transferred into the cell so that it can express it **(DNA → mRNA→ hormone receptor)**, translocate it into the plasma membrane so that the hormone can bind to it and alter the cell activity.
- Each hormone receptor has different signaling pathway.
- When the hormone combines with its receptor, this action usually initiates a cascade of reactions in the cell, with each stage becoming more powerfully activated ***(Amplification)*** so that even small concentrations of the hormone can have a large effect.
- The locations of the different types of hormone receptors are generally the following:
  a) **Membrane receptors**: membrane glycoproteins.
  b) **Intracellular receptors**: found in the cell cytoplasm or nucleus (DNA binding proteins).
- Based on their structural and functional characteristics, receptors are classified into two broad categories:



  a) **Ionotropic receptors:**
  - ✓ Work very **fast**; important role in fast neurotransmission.
  - ✓ Each receptor is made of several subunits and together they form the complete receptor.
  - ✓ At the center of receptors there is a channel or a pore to allow the flow of ions.
  - ✓ At rest, receptor channels are inactive (closed), they get activated by the binding of a ligand to the receptor causing the opening of these channels. When the ligand leaves the binding site the channel quickly goes back to its inactive state and closes.

  b) **Metabotropic receptors:**
  - ✓ Work more **slowly** than ionotropic receptors but lasts **longer**.
  - ✓ They work by activating other proteins called G proteins.
  - ✓ Each receptor is made of one subunit but has several transmembrane regions.

- ✓ They stimulate or inhibit the opening of ion channels in the cell membrane by stimulating or inhibiting certain effector enzymes.
- ✓ Most effector enzymes controlled by G proteins are involved in synthesis of second messengers.

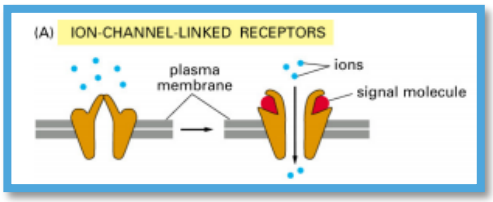


*First messenger→: ligand. *Second messenger→ produced by effector enzyme.

Characteristic	Ionotropic	Metabotropic
Structure	4 or 5 subunits that assemble in the cell membrane.	1 subunit.
Mechanism of action	Contains an intrinsic ion channel that opens in response to ligand binding.	Activates G-proteins in response to ligand binding.
Coupled to second messengers?	No.	Yes.
Speed of action	Faster.	Slower.
Lasting effect	Shorter-lasting effect.	Longer-lasting effect.

- There are three major classes of membrane receptors:
  1) **Ion-channel-linked receptors (ionotropic).**
  2) **Protein-linked receptors (G-protein coupled receptors).**
  3) **Enzyme-linked receptors.**

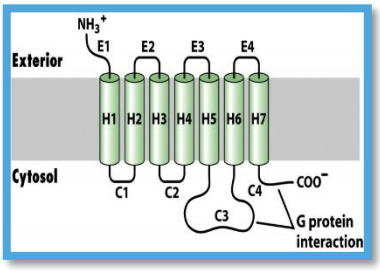
➢ Ion-Channel-linked receptors:



- ✓ ligand gated channels (ionotropic).
- ✓ **E.g**: Acetylcholine binds to Na^+-ligand gated channels causing conformational change which leads to the opening of these channels and the influx of Na^+ into the cell.
- ✓ A few hormones may exert some of their actions through activation of ionotropic receptors. Most hormones that open or close ion channels do this indirectly by binding to G protein-linked or enzyme-linked receptors, as discussed next.

➢ G-Protein Coupled Receptors:

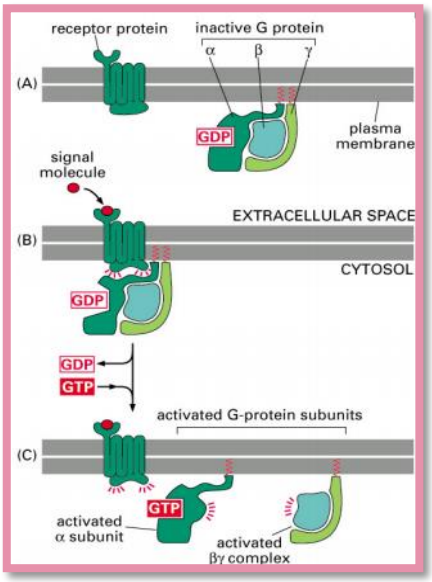
- ✓ largest family of cell surface receptors. They're present in all eukaryotes. **E.g**: adrenergic receptors, opioid receptors.
- ✓ 800 different GPCRs are encoded in the human genome.
- ✓ Structure:
  - One subunit with 7 helices spanning the membrane. (7 trans-membrane spanning domains).
  - The N-terminus and C-terminus differ between different GPCR.
  - The N-terminus (extracellular part) contains the ligand binding domain.
  - The cytoplasmic tail of the receptor is in close proximity to the G-protein.



- ✓ Large amount of receptor diversity. Different GPCR bind to different ligands.
- ✓ The binding of a hormone to a GPCR doesn't always have a stimulatory effect. Whether it'll cause inhibition or stimulation depends on the type of G-protein (G_α) coupled to the receptor.
- ✓ The signal is usually passed from a 7-helix receptor to an intracellular G-protein.
- ✓ G protein coupled receptors have common mechanism of action. They transmit signals to intracellular targets via G proteins.
- ✓ G-proteins are heterotrimeric, with 3 subunits α, β, γ.
- ✓ G-proteins could target plasma membrane bound enzymes or ion channels.

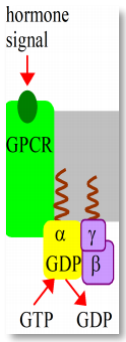
➢ Mechanism of Activation:

1. Binding of a ligand to the extracellular domain of GPCRs induces conformational change that allows cytosolic domain of the receptor to attract the inactive G protein making it even closer and binding to it at the inner face of the plasma membrane.
2. This interaction activates the G protein, causing it to exchange GDP for GTP.
3. Once the α subunit is bound to GTP, the G-protein dissociates into activated G_α and $G_{\beta\gamma}$ complex.
4. Each of these subunits can go on to activate target proteins and alter cell function.



- ✓ Activity of G_{α} is much higher than the activity of $G_{\beta\gamma}$ complex.
- ✓ Activated G_{α} subunit could alter the activity of ion channels or intracellular enzymes such as adenylyl cyclase or phospholipase C which alter cell function.
- ✓ $G_{\beta\gamma}$ complex could also play a role in the signaling pathway by causing activation or inhibition of a certain effector. However, its main role is to bind with the inactivated G_{α} after the signal is turned off to form the G-protein again.

➢ **G-proteins:**



- ✓ G-proteins are heterotrimeric, with 3 subunits α, β, γ.
- ✓ The α subunit (G_{α}) binds GTP and can hydrolyze it to GDP + P_i.
- ✓ α & γ subunits have covalently attached lipid anchors that bind a G-protein to the plasma membrane cytosolic surface.

▪ There are 6 major classes of G-proteins. However, only 3 classes are included with us:

1) **Stimulatory G-protein ($G_{\alpha s}$):** A G-protein that activates cyclic-AMP formation by binding to adenylyl cyclase and activating it.
   - ✓ Stimulated by:
     a) β-adrenergic receptor which binds to epinephrine.
     b) Receptors for glucagon, serotonin, vasopressin.
   - ✓ Associated effector: Adenylyl cyclase↑
   - ✓ Induces the production of the second messenger cAMP. ↑

2) **Inhibitory G-protein ($G_{\alpha i}$):** It binds to adenylyl cyclase and inhibits its activity reducing cAMP production.
   - ✓ Stimulated by:
     a) α2-adrenergic receptor which binds to epinephrine and norepinephrine.
        -When epinephrine or norepinephrine bind to this receptor, **$G_{\alpha i}$** binds to Adenylyl cyclase and inhibits its activity reducing c-AMP production. ↓
     b) Muscarinic acetylcholine receptor.
        -When acetylcholine binds to muscarinic receptor, it causes the opening of K^+ channels leading to the efflux of K^+ out of the cell → hyperpolarization. As a result, the membrane potential becomes far from threshold so a stronger stimulus is needed to generate action potential (inhibition).

- ✓ Associated effector: -Adenylyl cyclase ↓
  -K^+ Channels ↑
- ✓ Effect and second messenger: -C-AMP ↓
  -Membrane potential (hyperpolarization).

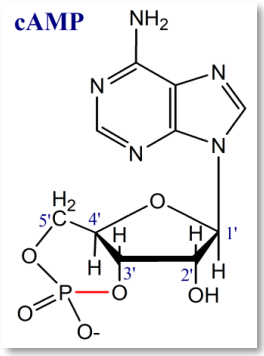
3) **$G_{\alpha q}$** : This G protein is linked to phospholipase C which catalyzes the breakdown of some phospholipids in the plasma membrane producing two different second messengers: IP3 and DAG.
   - ✓ Stimulated by: α1-adrenergic receptor which binds to epinephrine and norepinephrine.
   - ✓ Associated effector: Phospholipase C ↑
   - ✓ Second messenger: -IP3: Inositol 1,4,5-triphosphate ↑
     -DAG: Diacylglycerol ↑

- *This table shows hormones that use each of the following second messenger systems:*

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

-The doctor corrected this table and added two new columns.

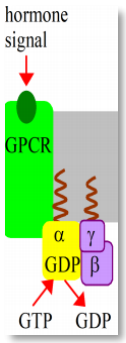
Adenylate Cyclase (Adenylyl Cyclase)



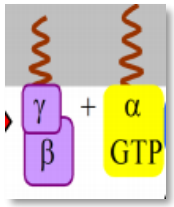
- ✓ Adenylate Cyclase (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.
- ✓ It catalyzes the conversion of ATP to cAMP + PP_i(pyrophosphate).
- ✓ In AMP the phosphate group is bonded to the 5' carbon of the sugar, but in cyclic-AMP, it's bonded to both the 3' and the 5' carbons of the sugar.
- ✓ Binding of certain hormones (e.g., epinephrine) to a receptor found on 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.

G-protein Signal Cascade

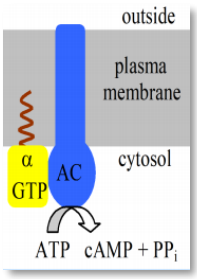
- The sequence of events by which a hormone activates cAMP signaling are as the following:



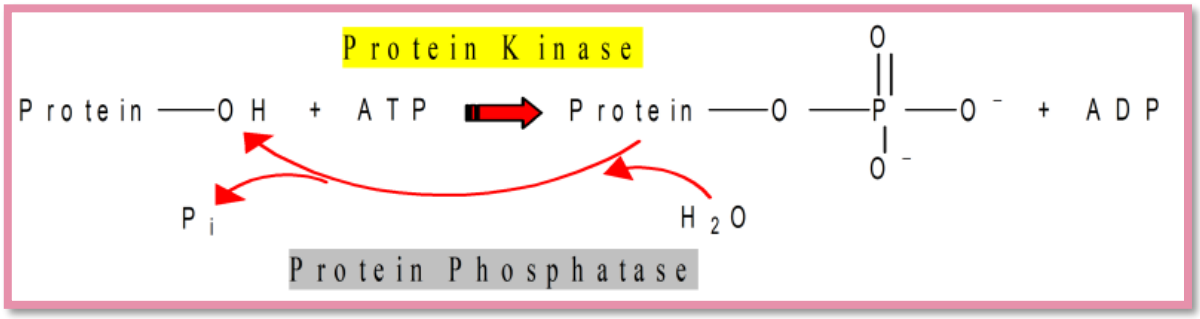
- ✓ Before the binding of a hormone to the receptor, the G protein is inactive since G_α has bound GDP. The α,β,γ subunits are all assembled together forming the G protein. The binding of $G_{\beta\gamma}$ complex to the G_α subunits inhibits the activity of the G_α subunit.
- ✓ When the hormone binds, usually to an extracellular domain of a 7-helix receptor (GPCR), it causes a conformational change in the receptor causing it to become closer to a G-protein on the cytosolic side of the membrane. The nucleotide-binding site on G_α becomes more accessible to the cytosol, where the concentration of [GTP] is higher than [GDP] and that's why G_α releases GDP & binds to GTP (exchange).



- ✓ Substitution of GTP for GDP causes another conformational change in G_α; G_α -GTP dissociates from the inhibitory $G_{\beta\gamma}$ complex and can now bind to and activate Adenylate Cyclase.
- ✓ Adenylate Cyclase, activated by the stimulatory G_α -GTP, catalyzes synthesis of cAMP.
- ✓ cAMP activates Protein Kinase A.
- ✓ Protein Kinase A catalyzes transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity.



- ✓ Some signals turn on phosphatases. Phosphatases do the opposite of Protein Kinase A. They remove phosphate groups from molecules.



➢ Turning off the signal

- After the desired response occurs, the signal is turned off by different mechanisms:

1) G_α hydrolyzes GTP to GDP + P_i (GTPase)

- ✓ Gα has the ability to inactivate itself by hydrolyzing the bound GTP to GDP + P_i.
- ✓ The presence of GDP on G_α causes it to rebind to the inhibitory $G_{\beta\gamma}$ complex.
- ✓ Consequently, Adenylate Cyclase is no longer activated.

2) Activating Phosphodiesterases

- ✓ Phosphodiesterases are enzymes that inactivate cAMP by converting it to AMP. **cAMP + H_2O → AMP.**
- ✓ Phosphodiesterases are activated by phosphorylation catalyzed by Protein Kinase A which was activated by cAMP.
- ✓ Thus cAMP stimulates its own degradation (Negative Feedback), leading to rapid turnoff of a cAMP signal.

3) Receptor desensitization

- ✓ When a ligand binds to its receptor it activates the receptor and as long as the ligand is bound, the receptor remains activated. Therefore, desensitization occurs.
- ✓ Desensitization is the degradation of the receptor.

  - The activated receptor is phosphorylated by the G protein receptor kinases that were activated during the signaling pathway.
  - The phosphorylated receptor attracts a protein called β-arrestin.

- β-arrestin does one of the following:

a) It promotes the removal of the receptor from the cell membrane by clathrin-mediated endocytosis.
-Part of the cell membrane which has the receptor embedded in it become enclosed by a vesicle and it enters the cell (endocytosis).
-The vesicle then fuses with a lysosome and the receptor is degraded.

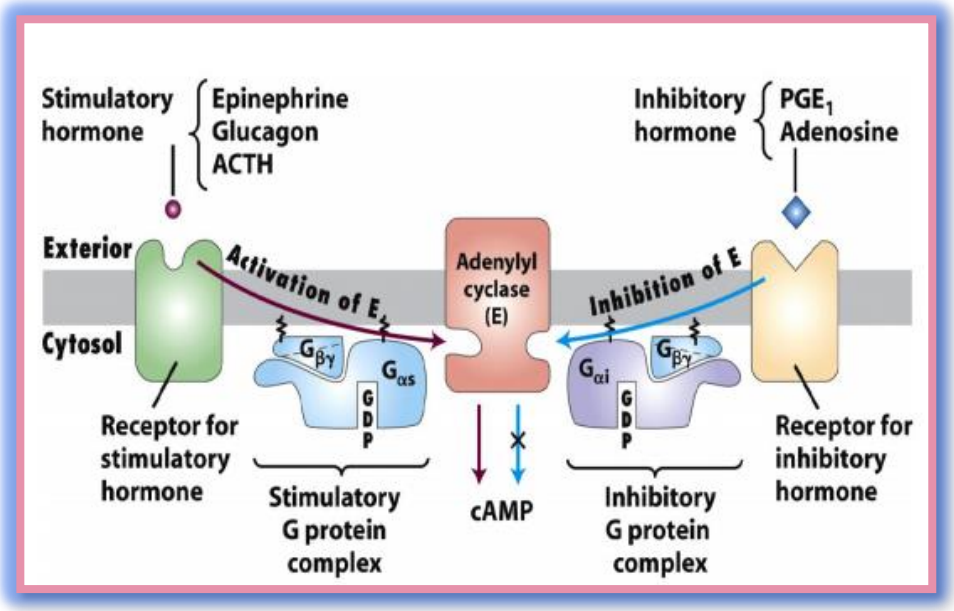
Remember! Clathrin is a coat protein that lines the part of the plasma membrane from which vesicles emerge.

b) β-arrestin may also bind a cytosolic Phosphodiesterase, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.

4) Activation of Protein Phosphatases

- ✓ Protein phosphatases catalyzes the removal of the phosphate groups that were attached to proteins via Protein Kinase A.
- ✓ Consequently, the enzymes that were turned on by the signal are turned off → the effect on the target is turned off.

- **NOTE!** The complex of $G_{\beta\gamma}$ that is released when G_{α} binds GTP is itself an effector that binds to and activates or inhibits several other proteins. For example : $G_{\beta\gamma}$ inhibits one of several isoforms of Adenylate Cyclase, contributing to rapid signal turnoff in cells that express that enzyme.
- This figure illustrates how Adenylyl Cyclase could be affected by more than one hormone (stimulatory, inhibitory) simultaneously.

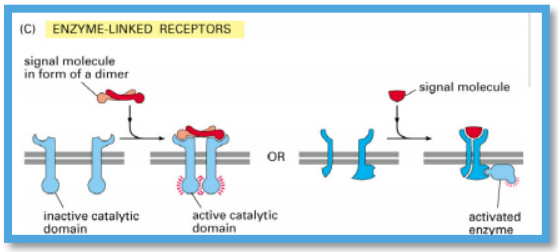


➢ {EXTRA INFO}: Other molecules needed in other receptors

- Proteins called small GTP-binding proteins (different than Gα) might be needed in certain signaling pathways. They have an inactive form (when bound to GDP) and an active form (when bound to GTP). These proteins have different roles and functions.
- GEF (Guanine exchange factor): stimulates the release of GDP and allows the binding of GTP.
- GAP (GTPase-activating proteins): Induces GTP hydrolysis to GDP + P_i.

➢ Enzyme-Linked Receptors

- ✓ Some receptors, when activated, function directly as enzymes or are closely associated with enzymes that they activate.
- ✓ These enzyme-linked receptor pass through the membrane only once in contrast to seven-transmembrane GPCRs.



- ✓ One of the enzyme-linked receptors is Tyrosine Kinase. As said before, kinase is either part of the receptor (Receptor Tyrosine Kinase) or is non-covalently associated with it (Non-receptor Tyrosine Kinase) . When the receptor gets activated, Tyrosine Kinase is activated as well.
- ✓ Examples of Receptor Tyrosine Kinase: Insulin receptors and growth factors receptors.

➢ Mechanism of Activation (RTK)

- ✓ The receptors are found in the plasma membrane as single molecules before the binding of Insulin (Inactive form).
- ✓ When Insulin binds to one receptors, it causes the dimerization of this receptor with another receptor. Once dimerized, the receptors are activated, and the kinases within these receptors are activated as well.
- ✓ Each receptor phosphorylates the tyrosine of the other receptor in the dimer. This is known as auto-phosphorylation.
- ✓ This will lead to the activation of downstream molecules found near the receptors until the desired response occurs.

The doctor talked briefly about Enzyme-linked receptors in this lecture so for more details, wait for the next sheet ☺.

Past Papers Questions

1) One of the following is false about intracellular receptors:
   a) They take part in gene expression.
   b) They're found in the cytoplasm and nucleus.
   c) They have an extracellular part.
   d) They're receptors of lipophilic hormones.

2) An inhibition of GTP might affect the action of:
   a) Ionotropic channels.
   b) G-protein coupled receptors.
   c) Receptor Tyrosine Kinase.

3) You made a drug that bind to G protein coupled receptors to treat the increased production of cAMP. What method is used in this drug?
   a) Causes irreversible binding of GTP to $G_{\beta\gamma}$ complex.
   b) Causes irreversible binding of GTP to G_{α} subunit.
   c) Causes irreversible binding of GDP to $G_{\beta\gamma}$ complex.
   d) Causes irreversible binding of GDP to G_{α} subunit.

4) All of the following are second messengers except for:
   a) Adenylyl cyclase.
   b) c-AMP.
   c) Inositol 1,4,5 triphosphates.
   d) Diacylglycerol.

1	2	3	4
c	b	d	a