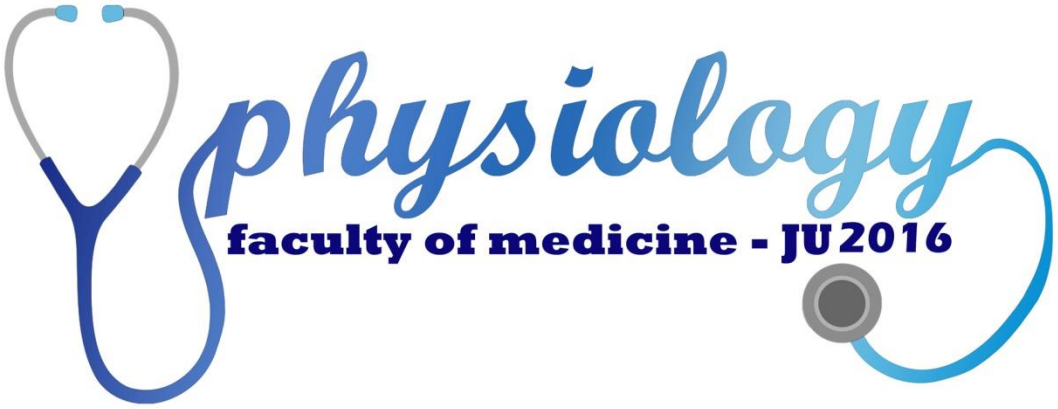

Sheet Slides

Number

22

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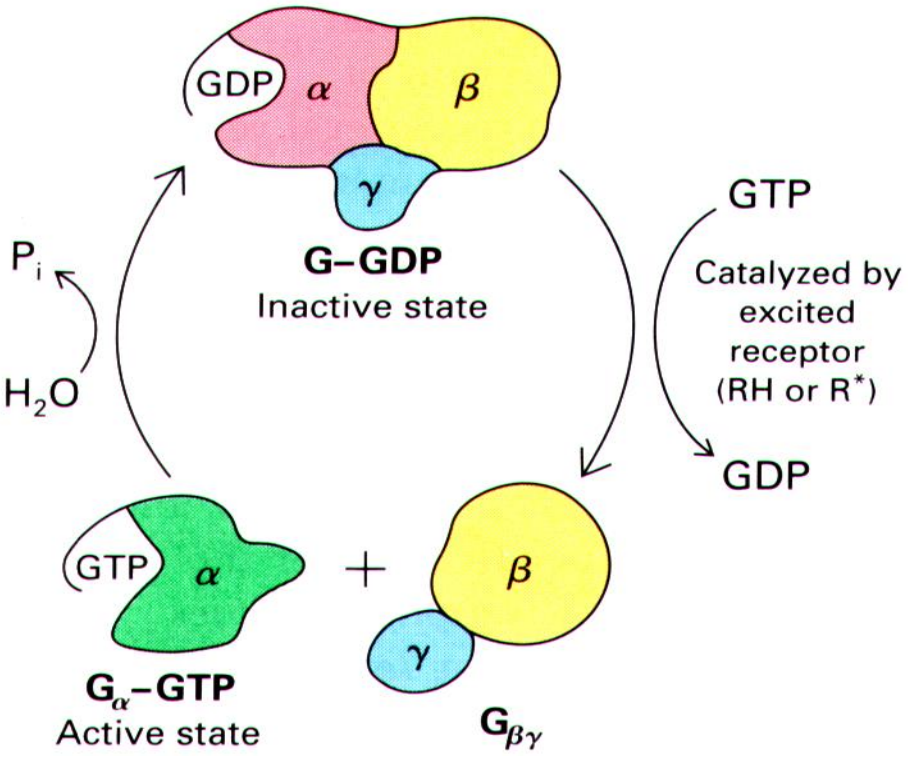
Receptors can be membrane proteins (for water-soluble hormones/ligands) or intracellular (found in the cytosol or nucleus and bind to DNA, for lipid-soluble hormones/ligands).

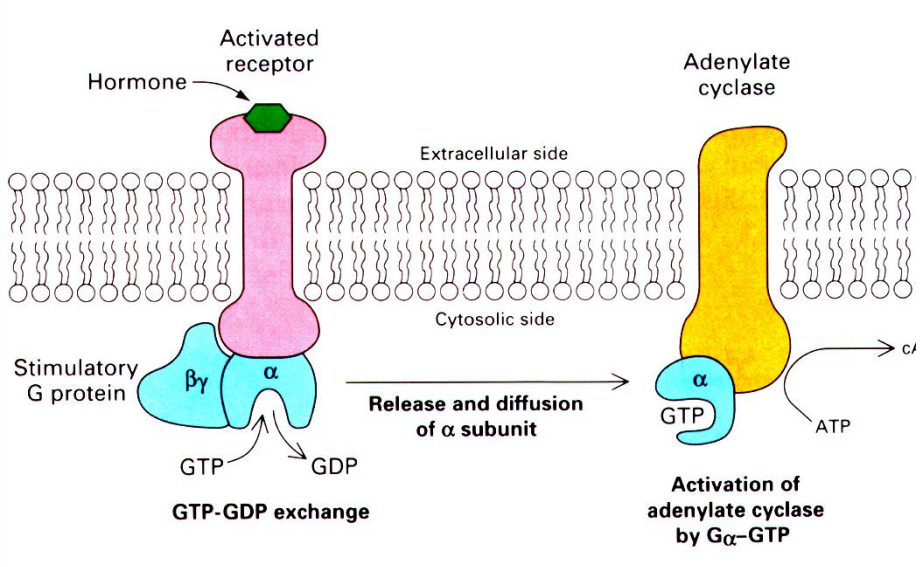
Receptors can also be classified as:

1) Ionotropic: coupled to ion channels (K^+/Na^+/Cl^- …etc) that open/close as a result of binding of the hormone to the receptor eg: acetylcholine. This opening/closing of channels may alter the membrane potential or generate electrical signal that triggers cellular response.

2) Metabotropic: G-protein receptors. G-proteins are transmembrane proteins. There are 2 sides; an extracellular side which binds to ligands, and an intracellular side which binds to GTP/GDP. G-proteins are heterotrimeric (made up of 3 subunits: alpha beta & gamma). *G-protein refers to any protein that binds to GTP/GDP and acts as a signal transducer. When bound to GDP they are inactive, but once a hormone/ligand binds to the receptor, it's activated (GDP is replaced by GTP). The alpha subunit bounded to GTP dissociates and does its action. This can be changing the permeability of any channel directly or activating a second messenger system; it activates another membrane enzyme like Guanylate Cyclase or Adenylate Cyclase…etc.



- Adenylate Cyclase catalyses the conversion of ATP into cAMP. The cAMP produced acts as a second messenger and activates Protein Kinase A (cAMP-dependant protein kinase) which phosphorylates substrates/functional proteins causing a biological effect.



- Guanylate Cyclase catalyses the conversion of GTP to cGMP, which is another important second messenger.

Binding of a hormone/ligand to its receptor changes the behaviour of the cell; this change is called transduction and could be either inhibition or activation.

Properties of the binding of hormones to receptors:

1) High specificity- hormones are specific to certain receptors.

2) High affinity- which is the ability of a hormone to bind to a receptor. Hormones are needed in adequate amounts; sometimes very low concentrations of hormone are not enough to act on the receptor.

3) Saturation

4) Binding is reversible- reversibility depends on the concentration of the hormone present; if there's a high concentration of hormones, they unbind to receptors.

5) Binding of the hormone to its receptor has a special function model.

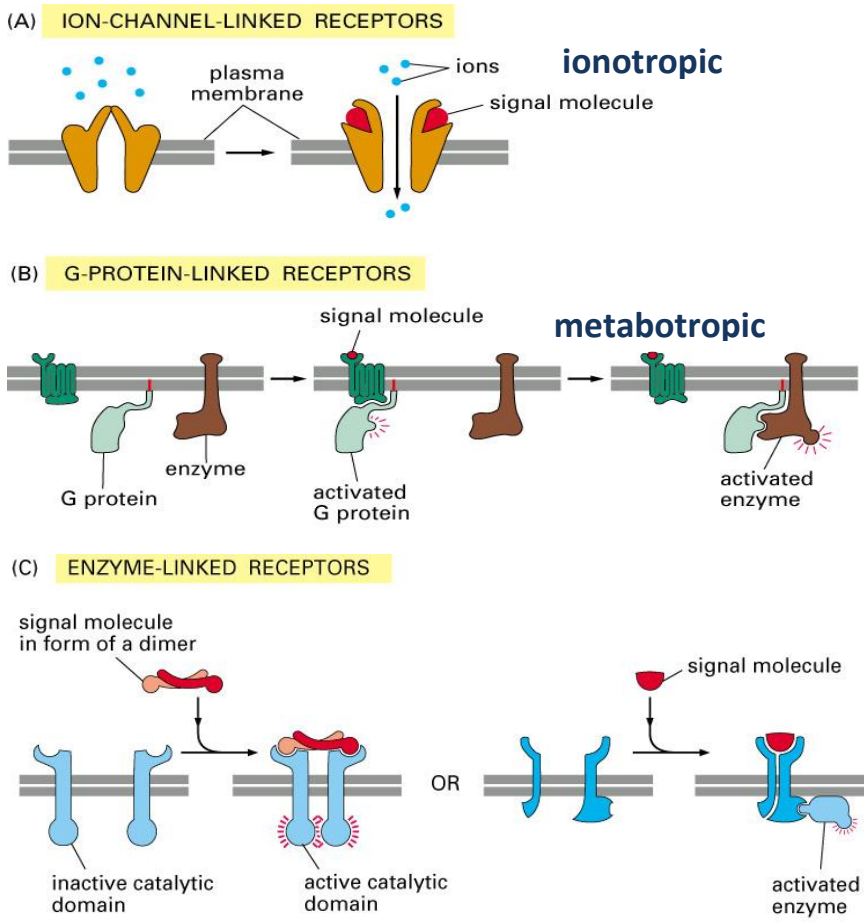
Types of receptors:

1) Channel-linked (ionotropic)

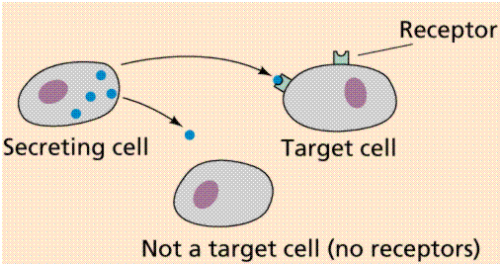
2) Enzyme-linked (involve neurotrophins and phosphorylation by protein kinases) eg: tyrosine kinase

3) G-protein coupled (metabotropic)

4) Intracellular (involve activation by cell-permeant signals)



Hormones only bind to specific receptors. Sometimes a hormone can slightly interact with a receptor for a hormone very similar to it in structure. Therefore, receptors determine response; if there's no receptor, there won't be a response.



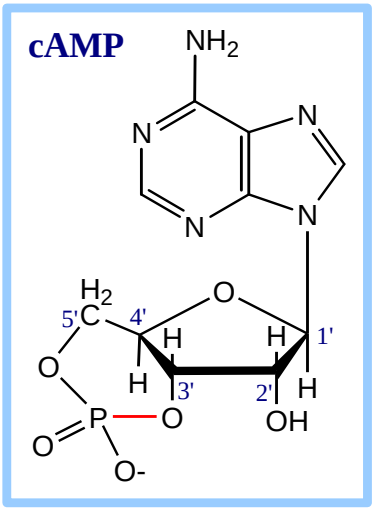
***G-protein coupled receptors' signal cascade**

Signal is passed from GPCR (G-protein coupled receptors) which are seven-helices receptors. There are many different types of GPCRs (around 800 in humans). Binding of a ligand may cause GPCRs to dimerize, forming oligomeric complexes across the membrane which would affect the activity of the receptor.

GPCRs not only react with heterotrimeric G-proteins, but can also react with certain proteins called GIPs (GPCR-interacting proteins). These GIPs modulate receptor functions and may have several effects:

- Altered affinity for the ligand
- Receptor dimerization/oligomerization
- Control the localization of receptors, including their transfer or removal from plasma membrane
- Promoting close association with other signal proteins

Activation of cAMP signaling (wasn't explained in detail in the lecture; from slides)**:**



- A G-protein that activates cAMP formation within a cell is called a stimulatory G-protein G_s, with the alpha subunit $G_{s\alpha}$.
- G_s is activated by receptors for epinephrine or glucagon.
- The GPCR for epinephrine is β_2-adrenergic receptor.
- The G-protein is bound to the cytosolic surface of the plasma membrane by lipid anchors from alpha & gamma subunits of the G-protein.
- Adenylate Cyclase is a transmembrane protein with cytosolic domains which form the catalytic site for the conversion of ATP to cAMP and PPi_2 (2 phosphate). It is activated when a certain hormone (e.g: epinephrine) binds to its receptor on the outer surface of the cell.
- cAMP acts as a second messenger

Sequence of events:

1) Initially, G_α is bounded to GDP and the alpha, beta and gamma subunits are complexed together. $G_{\beta\gamma}$ (the complex formed by beta and gamma subunits) inhibits G_α.

2) A hormone binds to the extracellular domain of a GPCR causing a change in the conformation of the receptor. This change is transmitted to a heterotrimeric G-protein

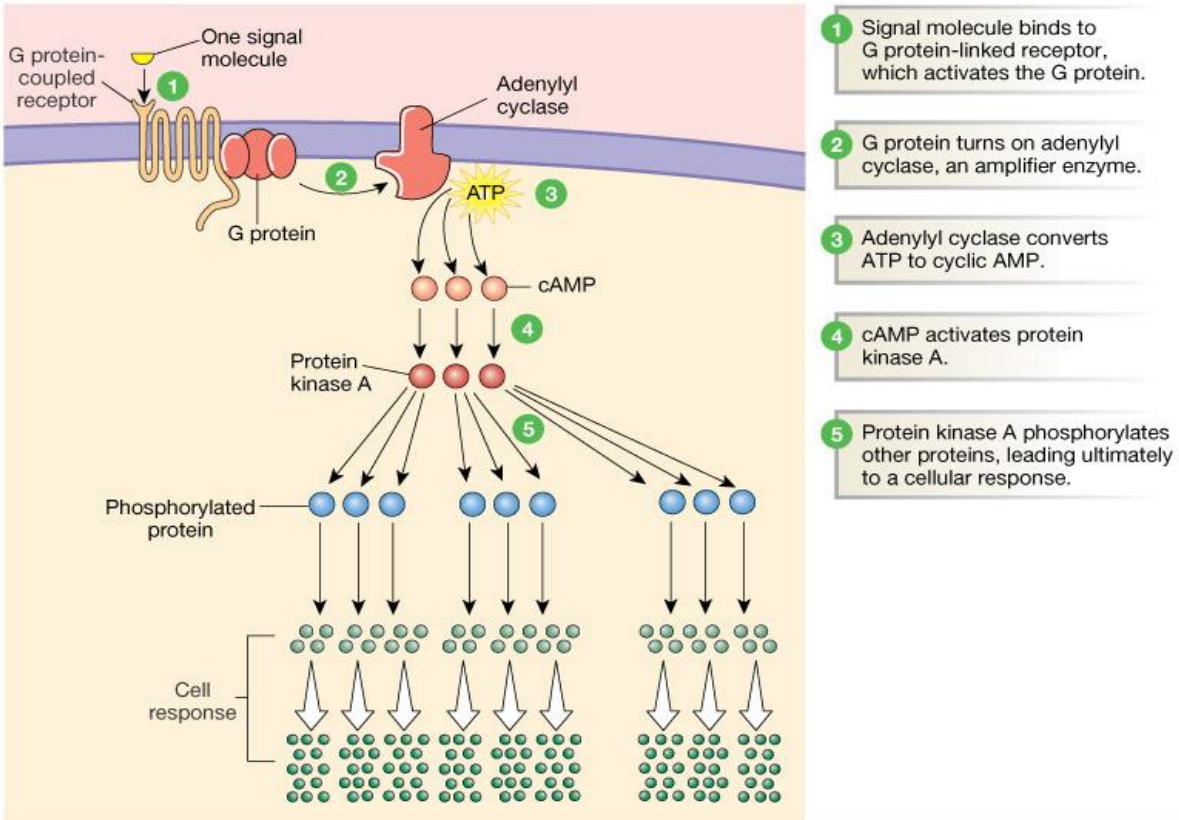
on the cytosolic side of the membrane. The nucleotide-binding site on $G_{s\alpha}$ becomes more accessible to the cytosol. Inside the cytosol, the concentration of GTP is much greater than that of GDP, so $G_{s\alpha}$ releases GDP and replaces it with GTP (GDP-GTP exchange).

3) This substitution of GDP for GTP causes another conformational change in $G_{s\alpha}$, causing the $G_{s\alpha}$-GTP complex to dissociate from the inhibitory $G_{\beta\gamma}$ complex. $G_{s\alpha}$-GTP complex can now bind to Adenylate Cyclase and activate it.

4) Due to its activation, Adenylate Cyclase catalyses the synthesis of cAMP.

5) The increasing concentration of cAMP formed activates Protein Kinase A (cAMP-dependant Protein Kinase), which phosphorylates intracellular substrates (e.g: enzymes or serine/threonine residues of various cellular proteins), it modulates the activity of enzymes present inside the cell and alters the metabolism of the cell

*The phosphorylation of enzymes could either activate or inactivate them (some enzymes get activated when phosphorylated and others may get deactivated).



Turning off the signal:

1) $G_{s\alpha}$ hydrolyses GTP to GDP+P_i (acts as GTPase). The presence of GDP on $G_{s\alpha}$ causes it to bind back to the beta and gamma inhibitory complex.

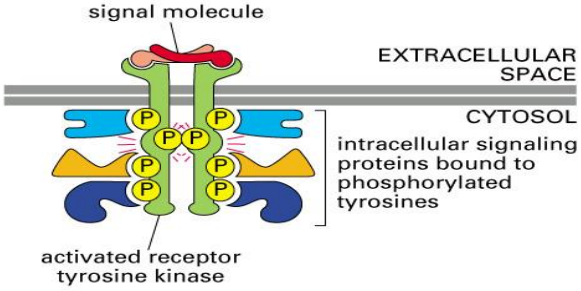
2) cAMP-dependant phosphodiesterase enzymes catalyse the conversion of cAMP to AMP. This phosphodiesterase enzyme is activated by phosphorylation (which was catalyzed by Protein Kinase A). We can see that cAMP stimulates its own degradation. When there's no more cAMP, there's no more stimulation.

*Phosphodiesterases are very specific, and there are many different types corresponding to different substrates.

***Enzyme-linked receptors**

The best example is tyrosine kinase-linked receptors. They're made up of alpha and beta subunits, with the alpha subunits exposed to the extracellular side. They include receptors for insulin and most growth factors (nerve growth factor NGF, epidermal growth factor EGF, platelet-derived growth factor PDGF, vascular endothelial growth factor VEGF…etc.). These receptors can either be single polypeptides or dimers (usually dimers).

When an insulin ligand binds to this receptor via the alpha subunit, it causes the alpha and beta subunits to dimerise (becoming 2 alpha & 2 beta subunits). The binding of insulin and alpha subunits brings about autophosphorylation of the beta subunits. This causes the structure to act as an enzyme (tyrosine kinase), when the activity of tyrosine kinase increases, it phosphorylates intracellular substrates or it creates new specific binding sites to which proteins can bind, transmitting intracellular signals. Phosphorylation of intracellular substrates can open channels, or they may go to DNA to increase protein synthesis, usually glucose transporter proteins. The glucose transporters that are formed are translocated to the plasma membrane where they are needed. They increase glucose uptake into the cell, therefore decrease concentration of glucose in the plasma. As we can see, the binding of insulin lowers blood glucose. Glucose that enters the cell is converted to glycogen, lipids…etc.



As we saw earlier, second messengers may target:

- Enzymes- they modulate phosphorylation which causes activation/inactivation of the enzymes.
- Protein Kinases- they increase phosphorylation.
- Protein Phosphatases- they decrease phosphorylation (cause dephosphorylation). Can be activated by Ca^{2+}/calmodulin.

*Calmodulin (cal- from calcium & modulin- from modulation) is an intracellular protein that is found in almost every cell, it binds Ca^{2+} and regulates cell activities. The equivalent to calmodulin that's found only in muscles is called troponin.

Once the concentration of intracellular Ca^{2+} increases, it binds to calmodulin. Each calmodulin molecule binds 4 Ca^{2+}. This binding exhibits the positive co-operativity phenomenon, where binding of the first molecule is the most difficult, but it makes it easier for the second molecule, which makes it easier for the third etc… (like in voltage gated channels; opening of the first channel is the hardest but it gets easier). Once calmodulin binds Ca^{2+}, it activates Calcium-calmodulin dependant Protein Kinase (Protein Kinase B; depends on Ca^{2+} bound to calmodulin).

Another type of second messengers are cyclic nucleotides like cAMP (which targets Protein Kinase A) & cGMP (which target Protein Kinase G).

We also have lipids like DAG (diacylglycerol) and IP_3 (inositol trisphosphate) acting as second messengers. DAG and IP_3 are formed when Phospholipase C enzymes break down one of the fatty acids of a phospholipid molecule called PIP_2, leaving behind a molecule consisting of 2 fatty acids+glycerol (DAG) and an IP_3 molecule.

$$PIP_2 \longrightarrow DAG + IP_3$$

DAG stays in the cytosol and the IP_3 formed goes to the smooth endoplasmic reticulum (the SER stores Ca^{2+}) and stimulates the release of Ca^{2+} by opening ligand-gated ion channels. The increased concentration of Ca^{2+} in the cytosol and the presence of DAG together activate Protein Kinase C (Ca^{2+}-phospholipid dependant Protein Kinase), which in turn activates proteins inside the cell by phosphorylation.

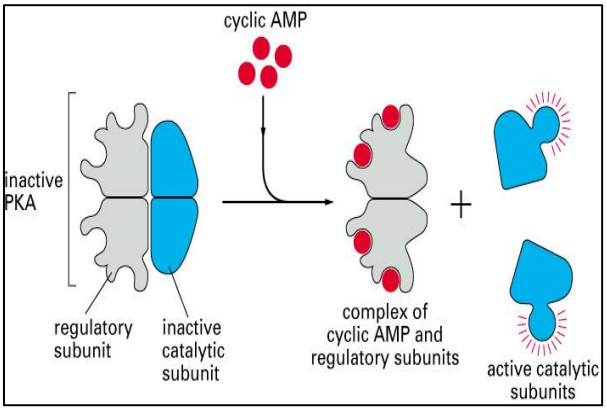
*The 3 types of Protein Kinases we took so far:

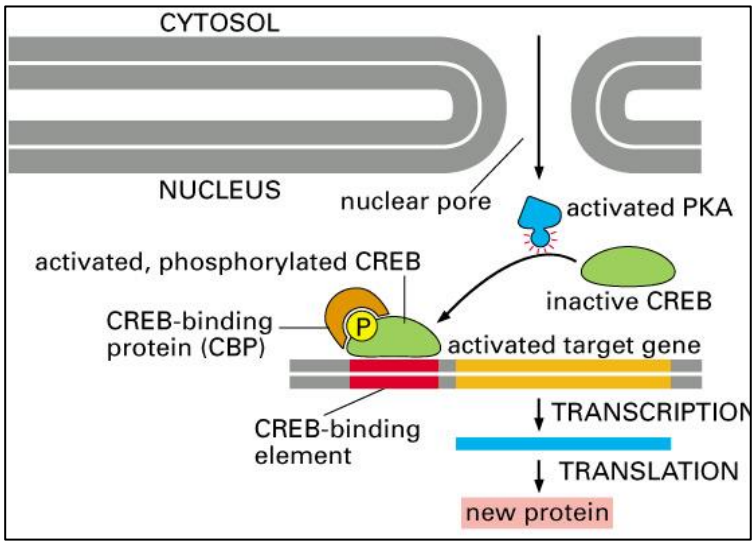
- Protein Kinase A: cAMP dependant
- Protein Kinase B: Ca^{2+}-calmodulin dependant
- Protein Kinase C: Ca^{2+}-phospholipid dependant

Some hormones like catecholamines (epinephrine and norepinephrine), polypeptides and glycoproteins cannot through the plasma membrane, but bind to receptor proteins on the target plasma membrane. These extracellular hormones are transduced into intracellular second messengers (their actions are mediated by second messengers).

Adenylate Cyclase, cAMP and Protein Kinase A:

Adenylate Cyclase converts ATP to cAMP which activates Protein Kinase A (PKA). PKA is a tetramer (4 parts), consisting of 2 regulatory and 2 catalytic subunits. Before the binding of cAMP, the catalytic subunit is inactive, so the PKA is inactive. When cAMP binds to the regulatory subunit, this causes the dissociation of the catalytic subunit, which is released to phosphorylate target proteins in the cytoplasm. The catalytic subunit can go the nucleus, where it phosphorylates CREB proteins (catalytic regulatory element binding proteins). CREB proteins bind to the CRE (cAMP response element). CRE is a regulatory sequence of DNA that is associated with specific genes. This binding to CRE causes transcription of these genes and translation to form new protein. This pathway is the slowest of all.



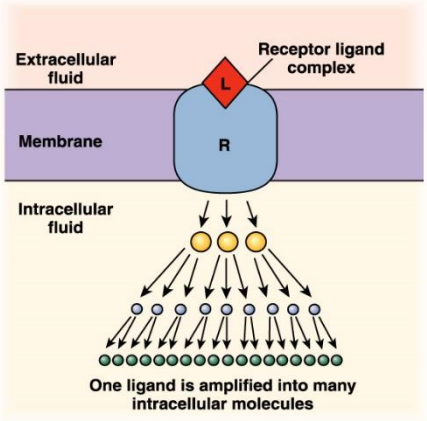


Mentioned only in the slides:

The action of Adenylate Cyclase is regulated by G-proteins: G_s (stimulatory) & G_i (inhibitory).

Pathogens can alter cAMP production: the cholera toxin is made up of many subunits; one of its subunits catalyses the transfer of ADP ribose from intracellular NAD to the α subunit of $G_{s\alpha}$ causing the G-protein to be continuously active. This stimulates adenylyl cyclase indefinitely, causing ion channels that export Cl^- to produce a net efflux of Cl^- and water, leading to severe diarrhea, which is a characteristic of cholera.

The enzyme Guanylate Cyclase converts GTP to cGMP, which activates cGMP-dependant kinases or other targets eg: G-protein coupled rhodopsin photoreceptors in rod cells in the retina.



One of the characteristics of metabotropic receptor signalling is that second messengers amplify the signal at each step of the cascade.

*Notes from past few lectures:

<u>Neurotransmitter release (exocytosis and endocytosis):</u>

1. Transmitter is synthesized and stored
2. Action Potential
3. Depolarization: opening of voltage-gated Ca^{2+} channels
4. Ca^{2+} enters the cell
5. Ca^{2+} causes vesicles to fuse with membrane
6. Neurotransmitter is released (exocytosis)
7. Neurotransmitter binds to postsynaptic receptors
8. Opening or closing of postsynaptic channels

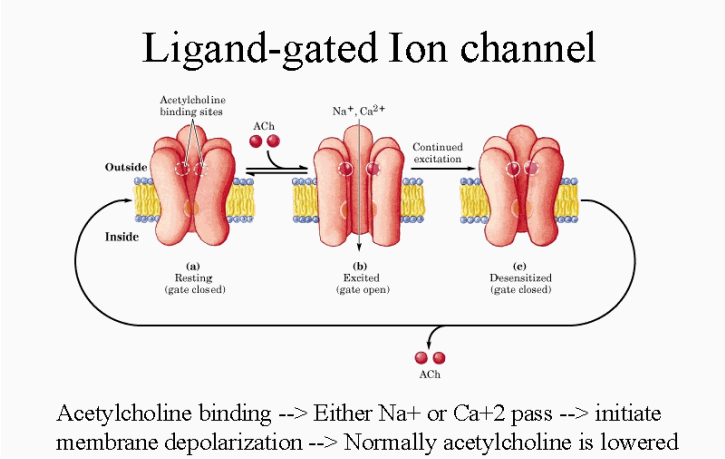
9. Postsynaptic current excites or inhibits postsynaptic potential to change the excitability of cell
10. Retrieval of vesicles from plasma membrane (endocytosis)

Receptors to which neurotransmitters (NT) bind are large, dynamic proteins that exist along and within the cell membrane. They're dynamic– they can increase in number and avidity for their neurotransmitter according to the circumstances.

There are two types of post-synaptic receptors:

1) Ionotropic receptors: NT binding results in the direct opening of specific ion channels.

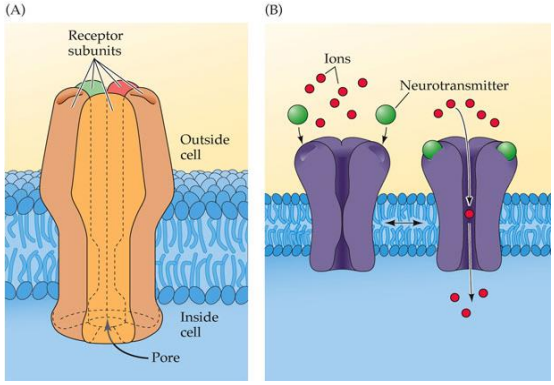
2) Metabotropic receptors: the binding of NT initiates a sequence of internal molecular events, which in turn open specific ion channels.



Binding of NT causes a response in membrane potential

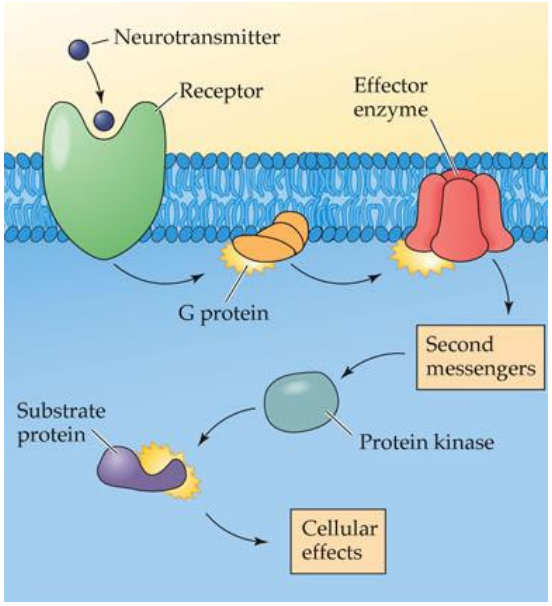
<u>Ionotropic receptors:</u>

They work very fast and are important in fast neurotransmission. Each receptor is made of several subunits, which together form the complete receptor. At the center of receptors is a channel or a pore to allow flow of ions. At rest, receptor channels are closed but when a neurotransmitter binds, the channels immediately open. When the ligand leaves the binding site, the channel quickly closes.



Metabotropic receptors:

They work by activating other proteins called G-proteins. Each receptor is made up of several transmembrane regions. They work more slowly than ionotropic receptors. They stimulate/inhibit the opening of ion channels in the cell membrane or stimulate/inhibit certain effector enzymes. Most of the effector enzymes that are controlled by G-proteins are involved in the synthesis of second messengers. Though it takes longer for postsynaptic cells to respond, the response is somewhat longer-lasting than in ionotropic receptor signalling. The receptors comprise of a single protein subunit, winding back-and-forth through cell membrane seven times (transmembrane domains) and don't possess a channel or pore.



GOOD LUCK 😊