G-Proteins Receptors and 2nd Messenger Mechanism

(A lot of information in this sheet is repeated over and over. In my opinion, this is the easiest lecture, enjoy ☺)

Recap:

Receptors are specific protein molecules. They can be found on the membrane outside, or they can be intracellular. Membrane receptors are for water soluble hormones/substances.
Intra-cellular receptors are for lipid soluble hormones/substances --- > usually work on DNA and gene transcription/protein formation.
Ligands are small molecules that bind to very specific receptors.

Types of Receptors:

1st type of receptors Ionotropic receptors. Receptors are ion channels.

Ionotropics are like ligand gated ion channels of which ligands can bind too. These receptors change permeability of ion channels either by closing or opening channels

Ex. Acetylcholine receptors cause Na+ channels to open--- > depolarization
Neurotransmitter receptor binds to its receptor on membrane--→ changes permeability of channel

For example: Effect of ach.
When the action potential reaches the pre-synaptic neuron, it causes voltage-gated Ca+2 channels to open. Ca+2 ions enter according to the electrochemical gradient difference (so movement of ions from ECF to ICF) The increase in intracellular Ca+2 causes movement of vesicles to the membrane. These vesicles fuse with presynaptic membrane, and release neurotransmitter into cleft. Neurotransmitter is

released, which then binds to the receptors on post-synaptic membrane. Binding of NT to receptor changes permeability of Na+/Ca+2 channels or opens these channels. These kinds of receptors are called ionotropic receptors. NOT TO BE CONFUSED with INOtropic, which is something completely different.

How is the effect of Ach stopped? It is either:
1- broken down by acetylcholinesterase
2- recycled and 3- diffusion of Ach into interstitial fluid.

2nd type of membrane receptor: G-Protein coupled receptor.

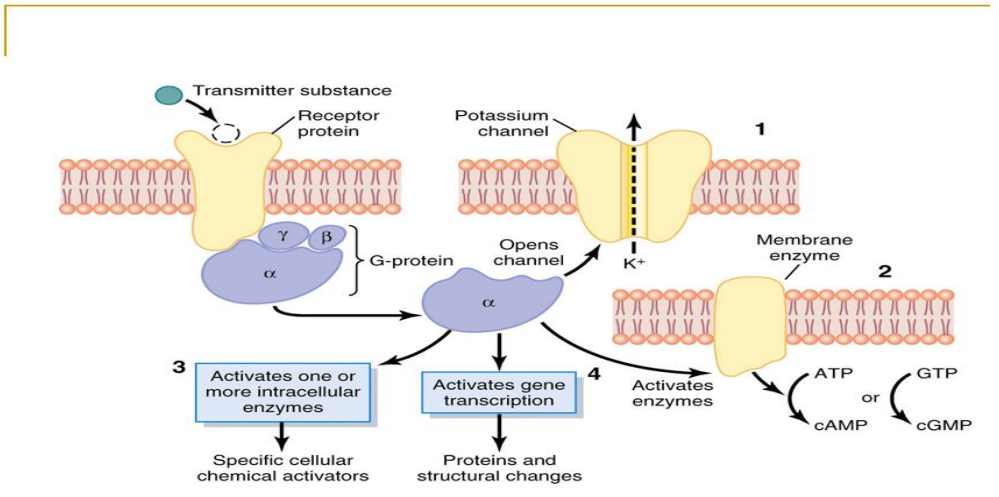
It's called "G"protein because it binds to either Guanosyne tri-phosphate (a compound similar to ATP) or GMP.
The protein is made up of (7) hepta- helices. It is a membranous protein, situated in the membrane. It has an exposed extracellular binding site, and an intracellular active site. It consists of 3 subunits, alpha, beta and gamma. When all of the subunits are together in the membrane and bound to GDP, the protein is said to be INACTIVE.

Why is it called G-Protein?

because it is able to bind GDP or GTP

When does it become activated?

It becomes activated when GDP is exchanged for GTP. When it is activated, it releases the alpha subunit. The alpha subunit has GTP-ase activity. When it is released from the rest of the protein, it is ACTIVE. The beta and gamma subunits STAY in the membrane.



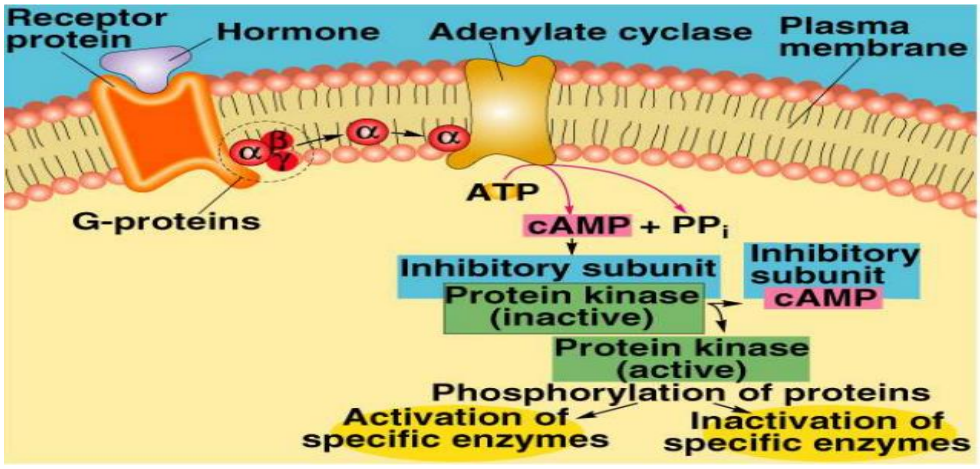
What does the free alpha subunit do? There are many possibilities:

1- It could go to a nearby ion channel and change its permeability. This change in permeability could give various responses like action potential and end-plate potential.
2- It could also go to the DNA and activate the gene-transcription/protein formation.
3- It could activate intracellular enzymes and change the metabolism of the cell
4- The alpha subunit could go to a nearby membrane protein/enzyme, like adenylate cyclase which changes ATP to cyclic AMP. (or its counterpart, guanylate cyclase, which changes GTP to cyclic GMP) This ultimately changes the metabolism of the cell in the long run. The ATP is converted into cyclic AMP, a secondary messenger. This leads to amplification of a message inside the cell and activation of many processes by activating a protein called protein kinase A (or cAMP dependant protein kinase)

   How?

   Well firstly, the cyclic AMP binds to the inhibitory subunit of a protein kinase (remember, kinase means any enzyme that can phosphorylate a substrate). When it binds to the kinase, it activates it. This protein is therefore called: Cyclic-AMP dependant protein kinase, or simply protein kinase A.

   What do protein kinases do? They phosphoralate intracellular proteins/enzymes, either activating it OR deactivating it. It can do both. (phosphorylation is not necessarily activation). Protein kinases generally phosphorylate (take a Pi and give it to a protein.)



So basically:

Hormone ---> receptor --- > G-protein activation -- > enzyme substrate -- > 2nd messenger -->
biological effect <--- phosphorylation of protein/enzyme < -- protein kinase activation

Properties of binding hormones and receptors:

1) Specificity of binding: every hormone has a specific receptor
2) High affinity: the hormone/ligand can work at very little concentration (low affinity means you need a very high concentration for there to be an effect)
3) Saturation: all of the receptors are bound to hormones/ligands (extra ligands are not going to increase activity)
4) Binding is reversible: ligands are recyclable
5) Specialized function model

Types of receptors:

1-Channel linked ----- > Ionotropic receptors
2- Enzyme linked ----- >Protein kinases+phosphorylation (2nd messenger mechanism)
3 –G-protein coupled ----- > Metabotropic receptors
4 - Intracellular receptors: ---- > Lipid soluble hormones (later on this week Inshallah)

2nd messengers:

Protein kinase A

Examples of 2nd messengers: cyclic nucleotides like cAMP and cGMP: target protein kinases

Calcium (Ca+2): targets calmodulin, **protein kinase B**
Diacyl glycerol (DAG) and IP3 (inicitol tri phosphate.)

Protein Kinase C

Targets of 2nd messengers:

1-Enzymes: modulate phosphorylation, which leads to either activation or inactivation

2-Protein kinases: increase phosphorylation

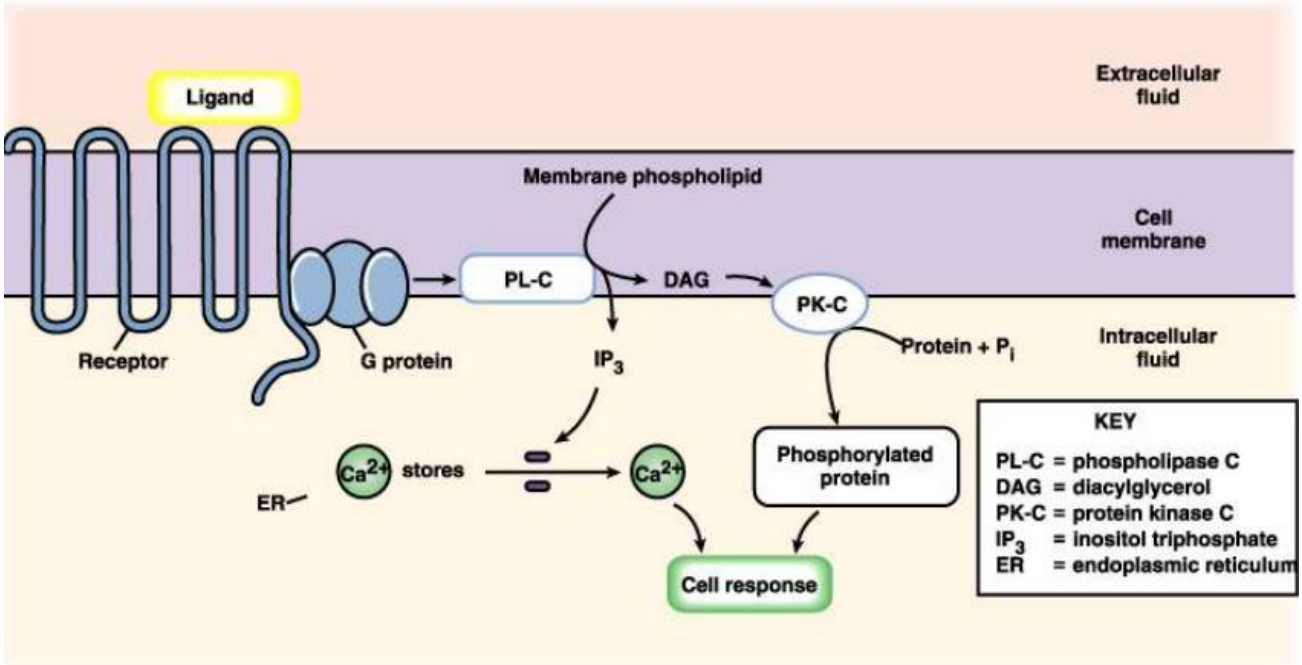
3-Protein phosphatases: decrease phosphorylation.
-activated by Ca+2 and Calmodulin

What does Ca+2 do?

It binds to a protein called calmodulin. (cal—binds calcium. Modulin-modulates actions inside cell). Calmodulin is found in nearly all cells.
When calmodulin is unbound, it is INACTIVE.
In order to become Active, it must bind to atleast one Ca+2 ion, max is 4 Ca+2 ions.
When Ca+2 binds to calmodulin and activates it, they activate Protein Kinase B, called calcium calmodulin dependant protein kinase.
OR!!!! If calmodulin is not present, the Ca+2 ions will bind to DAG, as we will see shortly ;)

DAG and IP3:

Their targets are protein kinase C. The 2nd messenger is DAG and IP3. Binding of ligand to receptor causes the following:
1- activation of G-protein
2- activates membrane phospholipase C.
3- this enzyme breaks down phospholipids in the membrane into DAG and IP3
4- the IP3 goes into the cell to the intracellular ER, the storage site of Ca+2 ions. (or causes Ca+2 channels to open)
5- the Ca+2 ions are released.
6- Ca+2 and DAG activate protein kinase C (PK-C)
7- PK-C phosphorylates specific proteins



*Hormones that use intracellular 2nd messengers are water soluble hormones, whose receptors are membrane bound receptors
ex. Catechol and polypeptides. This leads to transduction.

What is the ultimate goal out of all of this?

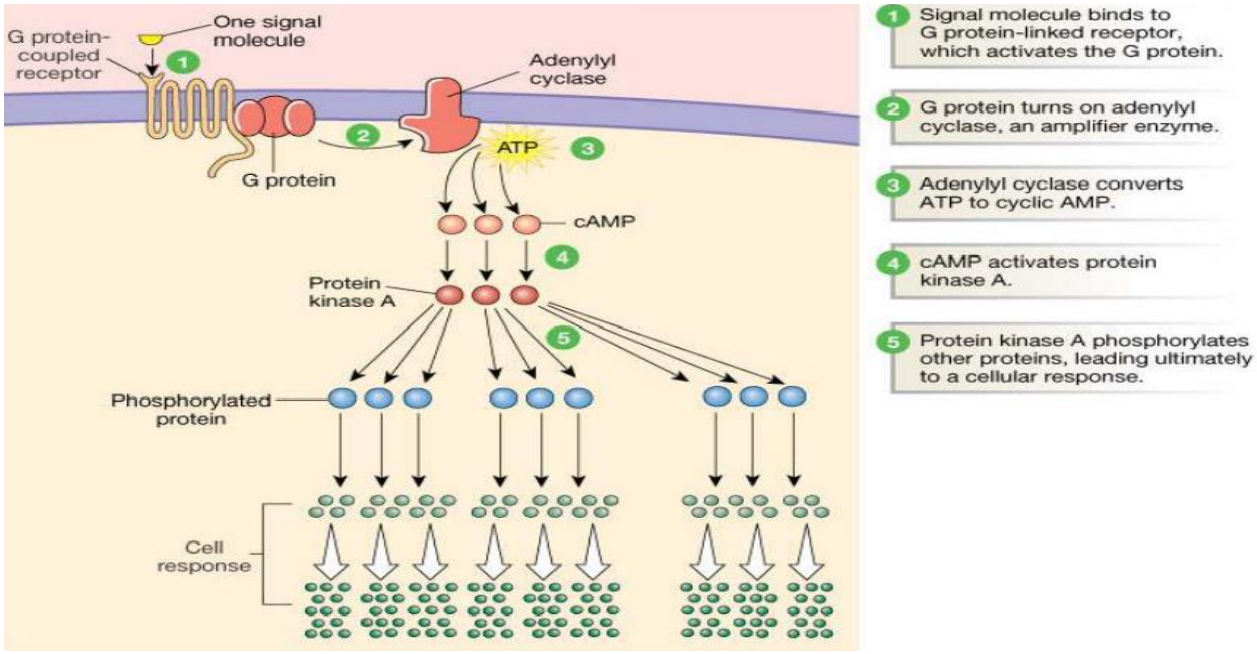
1- Amplification (cascade of events, zay al shalal)
2- Signal Transduction
3- change in metabolism

These events lead to changes in the metabolism of the cell, which is why these receptors are said to be metabotropic.

How is it terminated?

cAMP is converted to AMP by phosphodiesterase in order to stop the cycle. It hydrolyzes cAMP into inactive fragments.

Amplification: Action of 2nd messenger. A cascade of events inside the cell.



Type of 2nd messenger	Activated protein kinase
cAMP	A
Ca+2 and calmodulin	B
Ca+2 and DAG	C

Binding of (ex. Epinephrine- H2O soluble-) to receptor:

The binding of the ligand to the receptor activates the G-protein alpha subunit. This in turn activates phospholipase C. Phospholipase C breaks down phospholipids into IP3 and DAG, which are both 2nd messengers. The IP3 diffuses into the cell, causing Ca+2 to be released from the ER.

Ca+2 and DAG activate protein kinase C (calcium DAG dependant kinase). This protein phosphorylates proteins and enzymes.

IF CALMODULIN IS PRESENT IN CELL, Ca+2 will bind with calmodulin and activate kinase B. Calmodulin is activated when Ca+2 binds to it. This kinase then phosphorylates various enzymes.

H--- > R---> G- protein --- > PL-C --- > IP3 --- > ER --- > Ca+2 release --- >

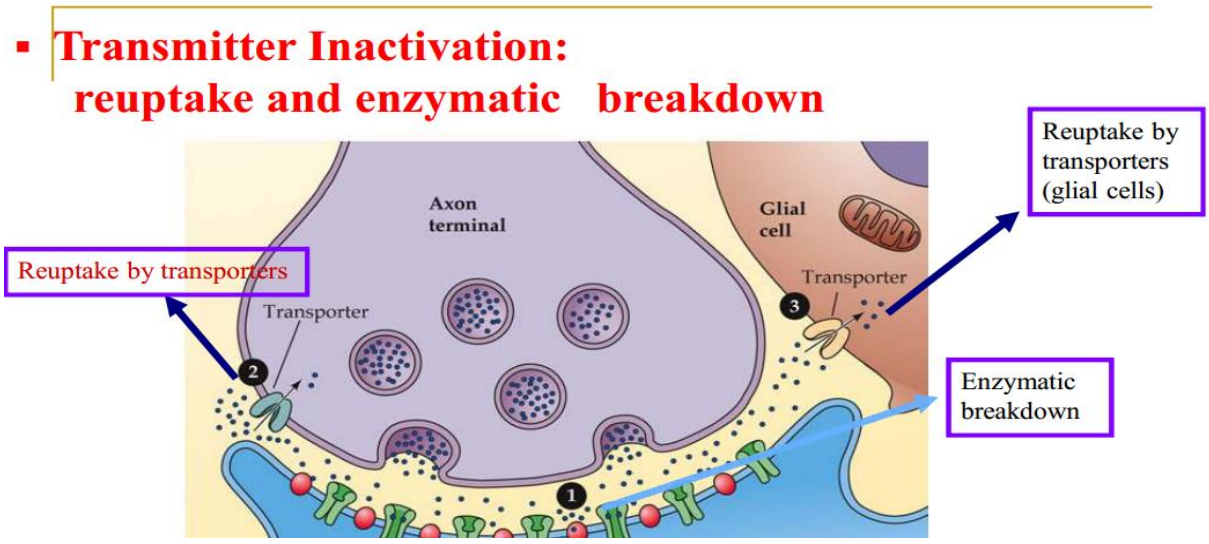
If calmodulin present: Ca+2 binds with it, then activate protein kinase B

If calmodulin not present: Ca+2 binds with DAG, activation protein kinase C

Khalas ifhimto al tab5a :P

Action potential in brief: Action potential arrives at the presynaptic membrane, causing depolarization of this membrane, and opening the voltage gates Ca+2 channels, then Ca+2 enters causing vesicles to fuse and release the NT which binds to its receptors on the postsynaptic membrane which causes opening of ion channels (increasing in the permeability) and the action potential continues.

<u>End of neurotransmitters: after they bind to their receptors, what happens to them?</u>
1- they are broken down, for example the enzyme acetylcholinestrase breaks down the neurotransmitter acetylcholine after it has fulfilled its purpose (monoamine oxidase for epinephrine)
2- they can be reuptaken (recycled) 3- they can diffuse into the interstitial space between cells.



Kinds of Neurotransmitter receptors:

1- ionotropic receptors: receptor itself is an ion channel
2- Metabatropic receptors: works by G-proteins and can

1) change perm. Of ion channel
2) affect DNA and gene transcription
3) affect intracellular enzyme
4) affect membrane enzymes

* Iono tropic receptors are MUCH faster than metabotropic receptors, due to extremely rapid ion permeability change. Since metabotropic receptors involve G-proteins, it takes time going through all the activations and phosphorylations and blah blah blah.

I wish you all the best of luck :D

Your colleague and friend, Hasan Hammo.
Shout outs to Ghassan, Ali, Ali, Asem, Omar, Yazan, Akkawi, Mamoun, Rami, Qusai, and basically the whole duf3a. And thanks

إن الله يحب إذا عمل أحدكم عملاً أن يتقنه