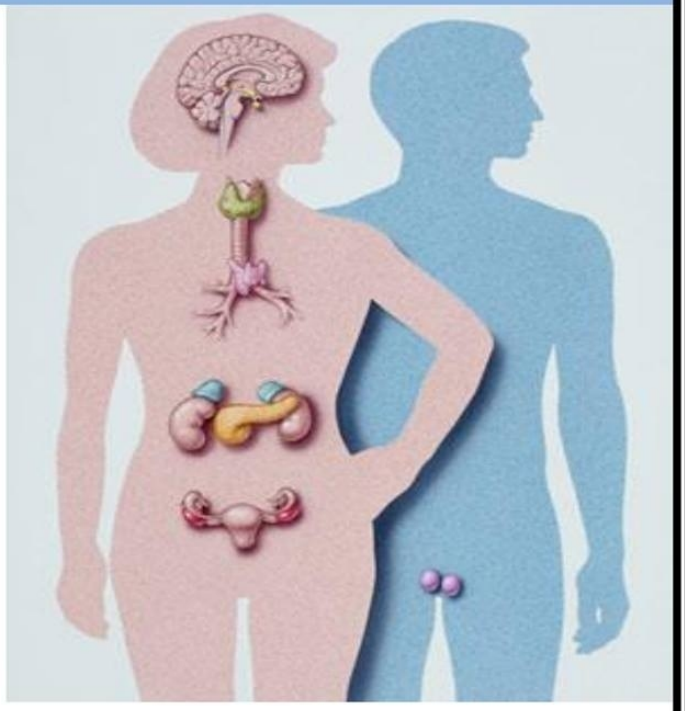
University of Jordan - Faculty of Medicine
(2013-19)

Medical Committee
The University of Jordan

Endocrine System



- ☐ Anatomy/Embryology/Histology
- ■ Biochemistry
- ☐ Physiology
- ☐ Pharmacology
- ☐ Pathology
- ☐ PBL

☐ Slide ■ Sheet ☐ Handout ☐ Other

Lecture #: **2**

Date:

Dr's Name: **Nafeth**

Price:

Written by: **Muhannad Haddadin**
& Mohammad Al-Zghoul

Designed by: Zakaria W. Shkoukani

Hormones & Signal Transduction

In the first lecture, we started taking about hormones and their types. Today we will continue..

What is the hormone?

It is a chemical molecule (organic in its nature), secreted through the blood, then it goes from its source which is a gland to the target tissue, and it makes a response. Hormones are produced in low amounts.

Many types of cells are affected by hormonal action. If we have more than one hormone affecting the same cell, each hormone acts independently on its receptors only **or** there is a sort of integration in between the different hormones**?**

The answer is yes, but how are these hormones integrating with each other in the action on the same cell type?

Each hormone affects the cell by itself. Hormones can integrate with each other and produce different kinds of action (effect) such as:

1) Permissive effect: it means binding of the first hormone will make it easier for the second hormone to bind and will make the response to the second hormone much bigger than if the second hormone binds alone.

Two hormones, each one has its own effect, the first hormone binds, it changes the situation of the cell, then the second one comes and finds that it's easier to bind and its response would be greater.

Example: when estrogen binds to the cell, it up-regulates progesterone receptors in uterus and when progesterone comes, it finds more receptors and eventually will make bigger effect in the cell.

Another example: thyroid hormone binds to the cell and then epinephrine comes. So epinephrine works stronger in the process of breakdown of triglycerides in adipocytes.

2) Integrative effect: (definition from slides: hormones produce complementary effects on different tissues) if both of these hormones are complementing each other in action, the best example on this kind is vitamin D (calcitriol; the active form of vitamin D) with parathyroid hormone. (the two hormones are making the same action, each one alone is doing it, and the effect of them together will not increase). Further explanation: calcitriol uptakes 10 calcium molecules from the intestines for example, and parathyroid hormone does the same thing, so if you have these 2 hormones that will give you 20 molecules of calcium. If the effect was synergistic ,for example, you'll have 100 Ca molecules).

3) Synergistic effect: it means if you have two hormones and each one has its own effect, if you put them together the effect will be much higher than each one alone. Example on this kind is FSH with estrogen will make the process of oocyte development at its best state, they are both needed for normal oocyte development. FSH with testosterone will make the process of spermatogenesis at its best state (each one alone doesn't make the process as efficient as when they work together).

4) Antagonistic effect: which means two hormones are antagonizing each other in action on the cell. Example on this kind: insulin and glucagon (insulin decreases glucose levels while glucagon increases it).

Signal transduction

Signal transduction means what will happen at the cellular level after the hormone binds to the receptors.

Transduction: conversion of one form of a signal to another form so as cells can produce many kinds of responses in different ways

Amplification in the process of hormonal signal: we should have amplification of the hormone in order to increase the response. Amplification is a must. The doctor asked why don't we increase the concentration of the hormones and the answer was in order to save energy. Doctor said if you forget about energy and you increased the

concentration of hormones but you do not have enough receptors on cells. Actually hormones are produced in low amounts, but assuming that we have large concentration of hormones, and in order to keep homeostasis in your body at its best levels and to have a good effect of the hormone but with no amplification you need receptors for that hormone covering the entire surface of the cell, and that may not be efficient.

Explanation: you need amplification not only to save energy, but also because there's no other way of transferring your message except by amplification.

What happens after amplification? You need something to transfer your signal inside the cell. Receptors are found in membranes, how can the massage be transferred from the outside (membrane) to the inside in order to affect DNA and proteins in these cells? By **second messengers**.

The receptors that are found for hormones are called intrinsic receptors. The proteins that are found in the membranes can be either peripheral or integral. Integral membrane proteins can be removed and extracted from the membrane by certain methods without interrupting the membrane. Intrinsic membrane proteins have same features of integral membrane proteins but chemically they cannot be removed from the membrane, the membrane wouldn't be intact after removing these proteins and the only way to extract out these proteins is to degrade the membrane completely because they bind strongly to the membrane.

Signal should bind to receptors that are: 1) intrinsic membrane proteins. 2) Transmembrane which means they are crossing the membrane, as they have a cytoplasmic end and an extracellular end. Extracellular end binds with the hormone and intracellular end transmits the message.

When the hormone binds the receptor you need a second messenger, it will be amplified and it binds to an effector molecule (mainly enzymes) and changes their action. One of the options that the second messengers can do is going to the channels in the membrane, opening certain channels and closing others. These channels may transfer sodium, potassium… etc.

Types of second messengers:

Chemical wise, small molecules such as: cyclic AMP, cyclic GMP & Ca^{+2}. The second messenger can be a kinase that phosphorylates a lot of enzymes and proteins to make them active or inactive. These kinases and their receptors (receptor kinases) work within cells as second messengers.

The problem we are facing when we discuss second messengers is that the number of second messengers is very small compared to the number of hormones that are known which approximate 50 hormones, 30 different types of these hormones use cyclic AMP as second messenger.

How the cells differentiate between different hormones that use the same second messenger?

According to cell type. All the cells have the same DNA but each cell type has its own *protein expression profile*, the gene is found in the DNA, it's transcribed in cell #1 but it's not transcribed in cell #2 and that's what makes the content of one cell different from the other.

After the hormone completes its action, there should be termination of the signal. Why do we need to terminate the signal? Is it important?

To save energy. Termination of signal keeps cells responsive to new signals. Failure of termination may cause problem e.g. GH & cancer.

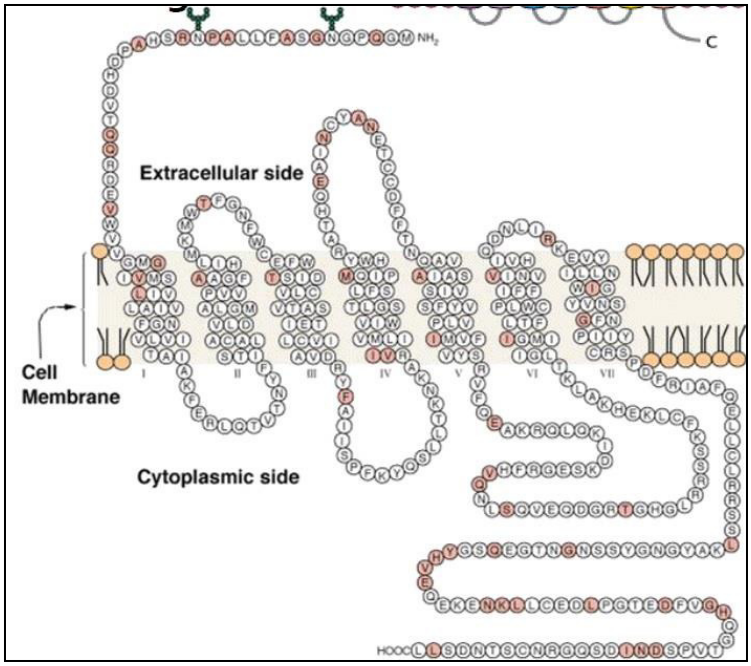
How does the cell terminate the signal?

1) Attachment between hormone and receptor is reversible, because it's non-covalent. When hormone's concentration drops immediately it'll be dissociated from its receptors.

2) Degradation of the second messenger: cAMP and cGMP are destroyed be an enzyme called phosphodiesterase; it destroys the phosphodiester bond.

3) Dephosphorylation by hydrolysis (by phosphatases).

Membrane associated receptors: we are going to discuss only one type of receptors which is 7 transmembrane helix receptors. As its name implies, it's composed of 7 α-helices spanning the membrane, it has an extracellular side where hormone binds, and an intracellular side.



1 Figure

Each small circle in this receptor resembles an amino acid, and if you examined the intracellular side of this receptor, you will find 2 letters; S and T. S stands for serine and T for threonine, these 2 amino acids have a *hydroxyl group* in their side chains, and they can bind **phosphate**. If you want to add a phosphate group to a protein, you add it to serine or threonine and sometimes tyrosine (those amino acids that have oxygen in their side chains).
Kinases can add this phosphate group to the intracellular side of this transmembrane receptor, it's phosphorylated and then its activity increases.

This is the 7 TM helix receptor for rhodopsin, which is a protein that binds to the retina and is responsible for the visual cycle. You can notice the black protein inside the receptor.

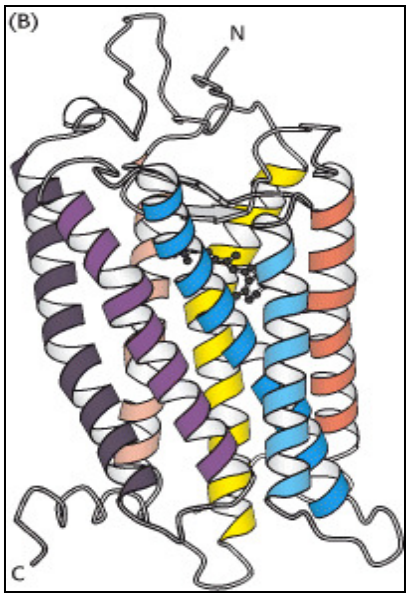


Figure 2

How can you insert 7 α-helices inside a membrane?

α-helix is a secondary structure of globular proteins, its shape is a helix and it maintains this helical formation through hydrogen bonding. But if it has hydrogen bonding it would be hydrophilic, how it would be inserted in the membrane which is hydrophobic? If the α-helices have already create this hydrogen bonding so it can make no more hydrogen bonds with the surroundings, thus it becomes hydrophobic with no ability to create new hydrogen bonds and then it can be inserted within the membrane.

This receptor is rigid due to the extensive hydrogen bonding. Once the hormone binds on the extracellular side of the receptor, this induces a conformational change in this receptor (protein), this changes its shape and then the message continues.

Wide range of biological functions and effects that can be transmitted through these receptors (7TM):

Smell, taste, vision, neurotransmission, hormone Secretion, chemotaxis, exocytosis, cell growth, development & viral infection.

These receptors share the same basic structure; however, they differ in their specificity and effects.

How does the 7TM receptor transmit the message through the cell? By binding to a protein that is free and can move, and this protein produces a second messenger that can be used by cells. All types of 7TM helices are bound to G-protein, that's why these receptors are called G-protein coupled receptors.

G-protein is a trimer; it has 3 subunits; alpha (α), beta (β) and gamma (γ) subunits. GDP & GTP can bind to this protein, α subunit is associated with the

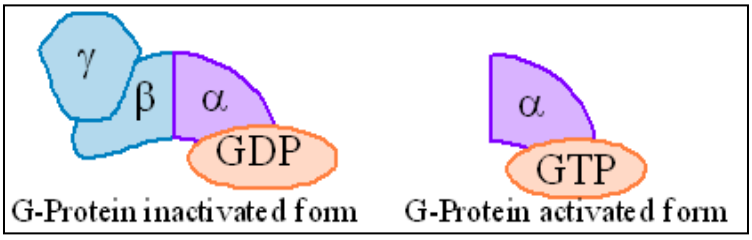


Figure 3

dimer β-γ that do not dissociate form each other, but α dissociates from β and γ when it binds with GTP.

Hormone binds to its receptor, around this receptor is a G-protein, once the hormone is bound to the receptor, it induces a conformational change in the receptor and sometimes it induces phosphorylation because the receptor has Ser and Thr in its cytoplasmic side, this phosphorylation might change the conformation of the receptor. Before this change in the receptor's structure, the affinity between the 7TM protein (receptor) and the G-protein is low, but after the conformational change has occurred due to hormone binding, the affinity toward G-proteins increases, a small change in the structure of the receptor increases its affinity towards G-protein. G-protein was far away from the receptor, once the hormone binds and the structural change occurs, it comes in close relation with the receptor.

We said that G-protein has 3 subunits, most of the time it's inactive where you have α subunit bound to GDP molecule, when exchange occurs (not phosphorylation of GDP to GTP), GDP goes away and GTP comes in, once GTP is bound, G-protein becomes active, α subunit dissociates and goes to another enzyme found within the membrane, it's **adenylate cyclase** which has the capacity to convert ATP into cAMP.

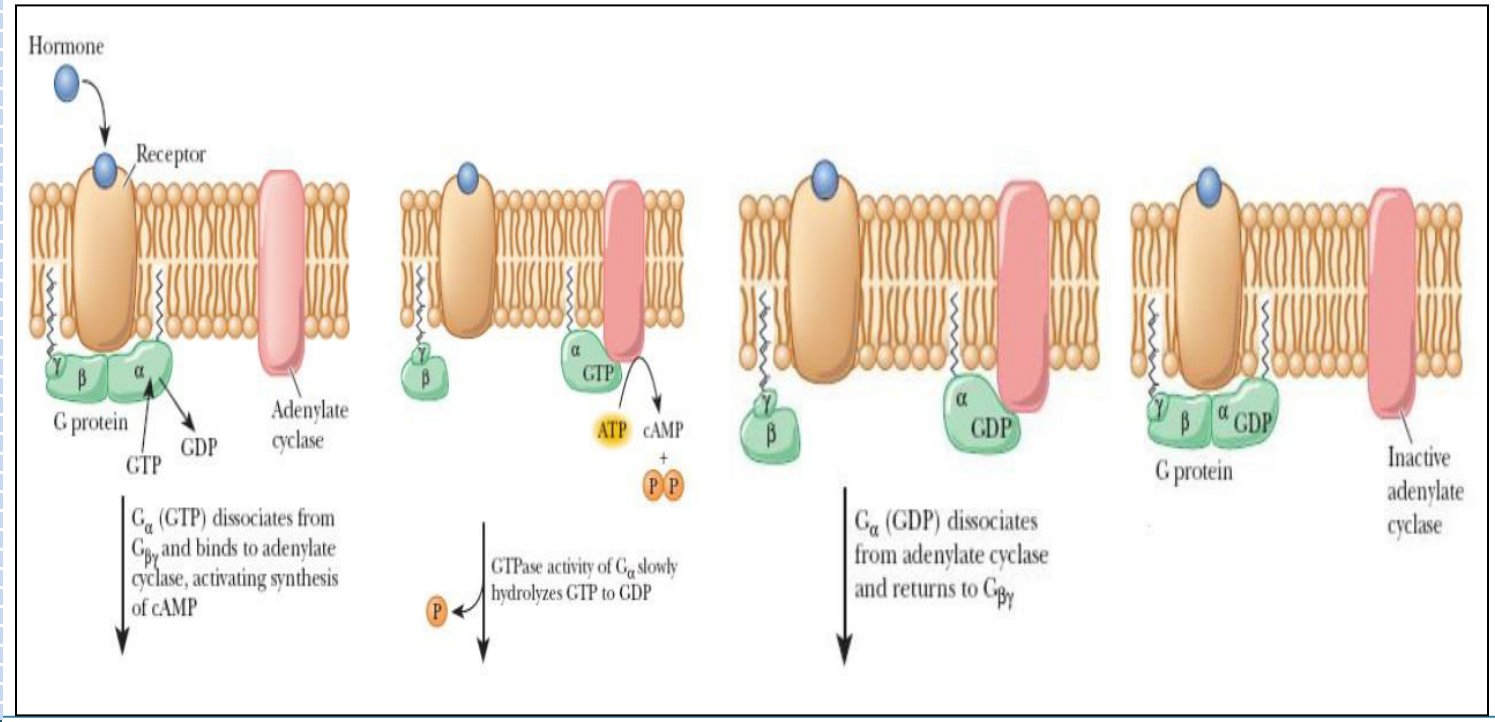


Figure 4

When one hormone binds to one receptor, on average, 100 G-proteins can bind this receptor, each G-protein can induce only one adenylate cyclase. Adenylate cyclase is an effective enzyme; it converts 1000 ATP molecules into 1000 cAMP molecules per second. If one hormone binds to one receptor: 100,000 cAMP per second are produced by adenylate cyclase. This is how amplification occurs.

receptor → 100 G proteins → 100 adenylate cyclase → 100 X 1000 cAMP molecules/sec

Cyclic AMP molecule has the same structure of AMP; adenosine, ribose & one phosphate, however this phosphate is doing a ring with the ribose so we end up with cAMP.

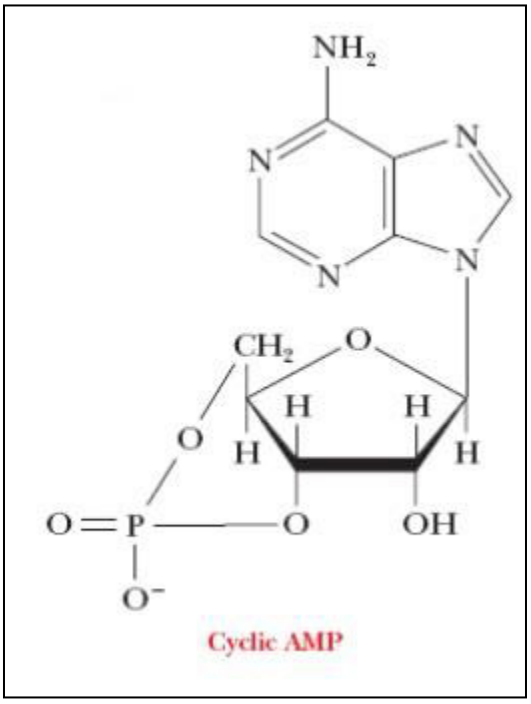


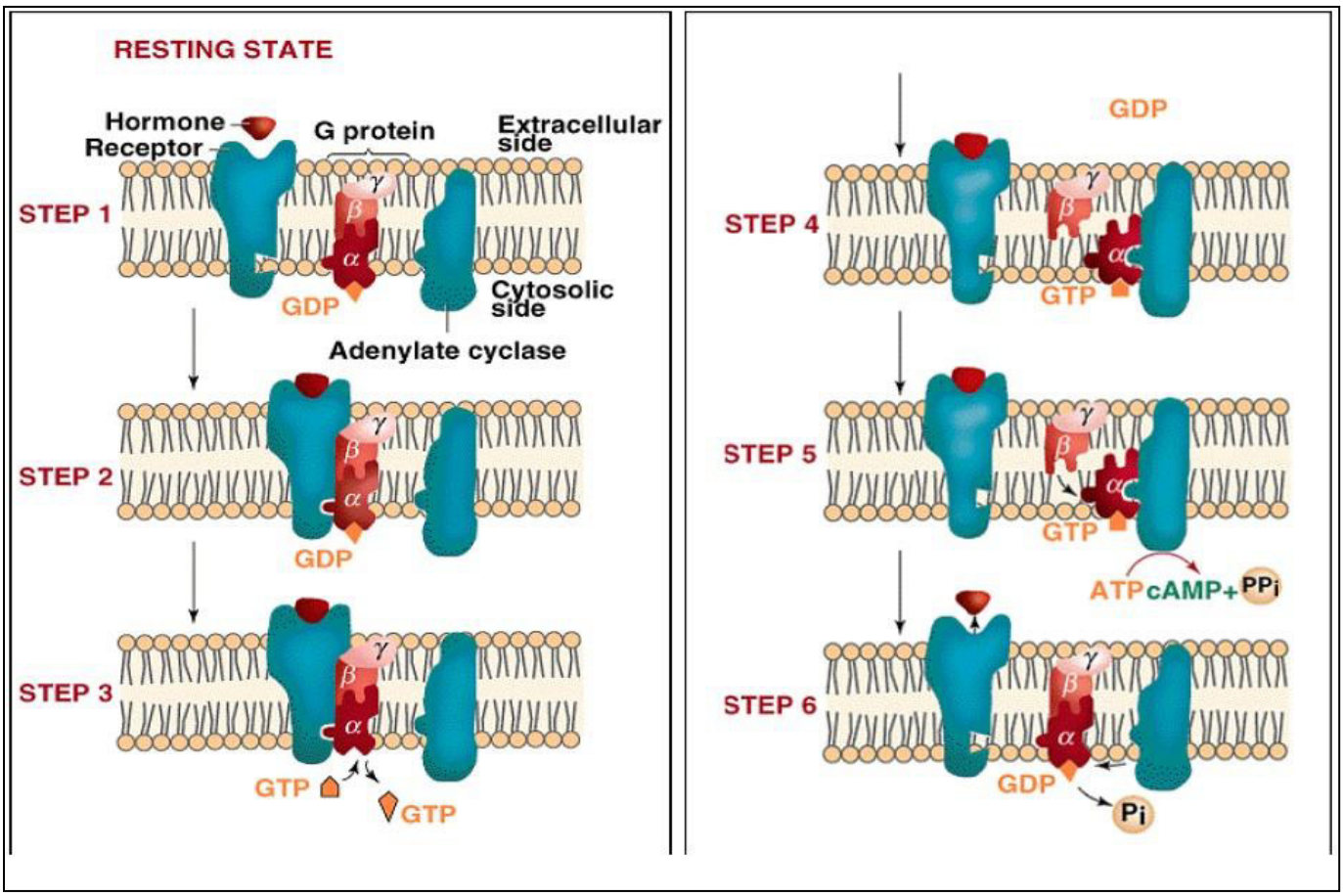
Figure 5

What cAMP does is that it binds to and activates **protein kinase A** which in turn phosphorylates many enzymes and proteins so it mediates different processes within the cell.

After the production of cAMP, what stops this step? **GTPase** activity in G-protein, by hydrolysis of GTP on the α subunit. GTPase is found within α subunit. α subunit is a GTPase but it's slowly hydrolyzing the bound GTP.

So inactivation occurs by dephosphorylation and the activation occurs by exchange.

After the dephosphorylation, GDP is bound to α subunit so it becomes inactive, it dissociates from adenylate cyclase and comes back to associate with β-γ dimer.



Are G-proteins always producing α subunit to stimulate adenylate cyclase?

The hormone binds to the receptor then it stimulates G-protein that will make either activation or inhibition (most of the time it makes activation but sometimes it makes inhibition). Depending on the nature of the α subunit that can be either stimulatory or inhibitory.

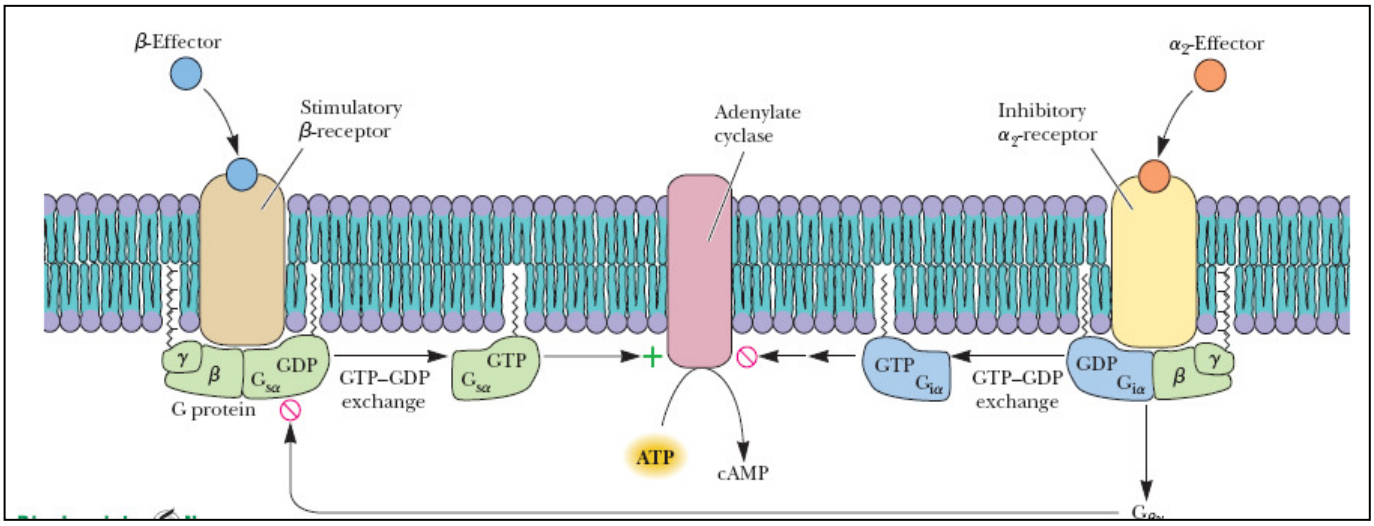
G-α subunit transduces many activities:

1) Gs (stimulatory): ↑ Adenylate Cyclase (it increases adenylate cyclase activity).
2) G_{olf} (olfactory): ↑ Adenylate Cyclase
3) Transducin: (for vision; in retina): ↑ cGMP Phosphodiesterase (Phosphodiesterase destroys GMP so it decreases the effect).
4) Gi (inhibitory): ↓ Adenylate Cyclase

5) Go (open): it opens Ca^{2+} Channels
6) Gq ↑ Phospholipase C

So depending on the nature of the G-α subunit the effect would be either stimulatory or inhibitory.

Another thing that determines if α subunit of G protein is stimulatory or inhibitory is the receptor itself. For example the adrenergic receptors β1 and β2 are stimulatory in their nature and when hormone binds to them (β1 and β2) it will make stimulation while binding of the hormone to the α2 receptors (which are inhibitory in their nature) will produce α subunit which is inhibitory in its nature.



2 things determine whether G-protein will cause stimulation or inhibition:
1) Nature of α subunit (nature of the G-protein itself).

2) Nature of the receptor.

There are nearly 20 G-proteins that are known and the number of receptors that are bound to these G proteins is 100 receptors which are called G-protein coupled receptors.

When the hormone binds to the G-protein coupled receptor, the main action is to stimulate adenylate cyclase and produce cyclic AMP, but to a lesser extent it can stimulate phospholipase C or it can open or close certain channels within the membrane, so it has other effects other than increasing or decreasing the levels of cyclic AMP in the cell.

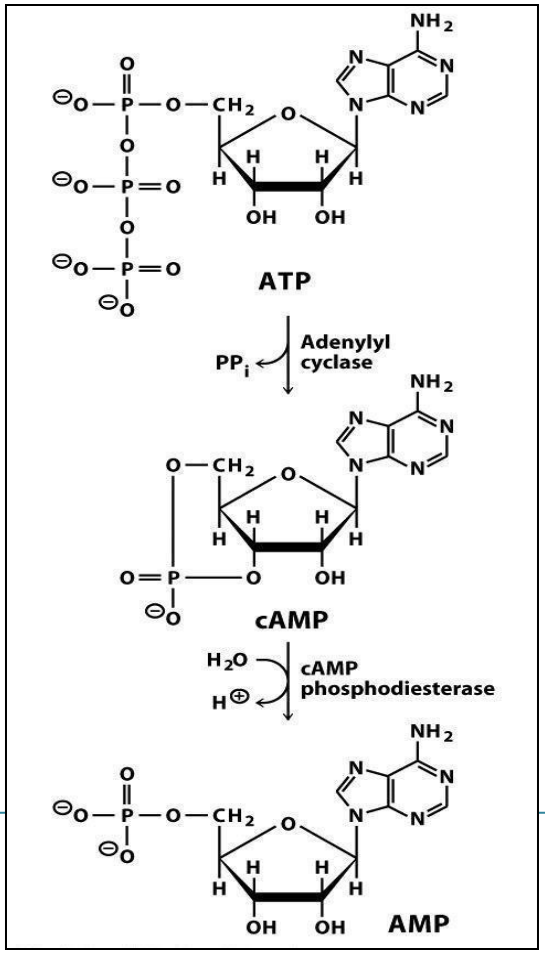
If you notice figure 4 you can see that α (alpha) and γ (gamma) subunits are covalently attached by **fatty acids** in order to keep the G-protein attached to the membrane surface. β (beta) subunit doesn't attach to fatty acids because it's in contact with gamma subunit (they are dimer) so when they are attached from one side it's enough to keep them bound to the membrane (β doesn't dissociate from γ).

α and β-γ can interact with other proteins other that adenylate cyclase but mainly they interact with adenylate cyclase.

All 7TM receptors appear to be coupled to G-proteins; they transmit their messages through G-protein.

Adenylate cyclase which converts ATP into cyclic AMP is a membrane protein with 12 α helices and has two large intracellular domains which are responsible for catalysis and conversion of ATP into cyclic AMP. How ATP is converted to cyclic AMP?

ATP has three phosphates, the oxygen on carbon #3 of the ribose attacks the first phosphate and the other two phosphates are released in the form of pyrophosphate (PPi) out of the reaction producing cyclic AMP.



Breaking the bond that is formed between oxygen and phosphate in the cyclic AMP will transfer it into AMP which can be returned back to ATP and so on.

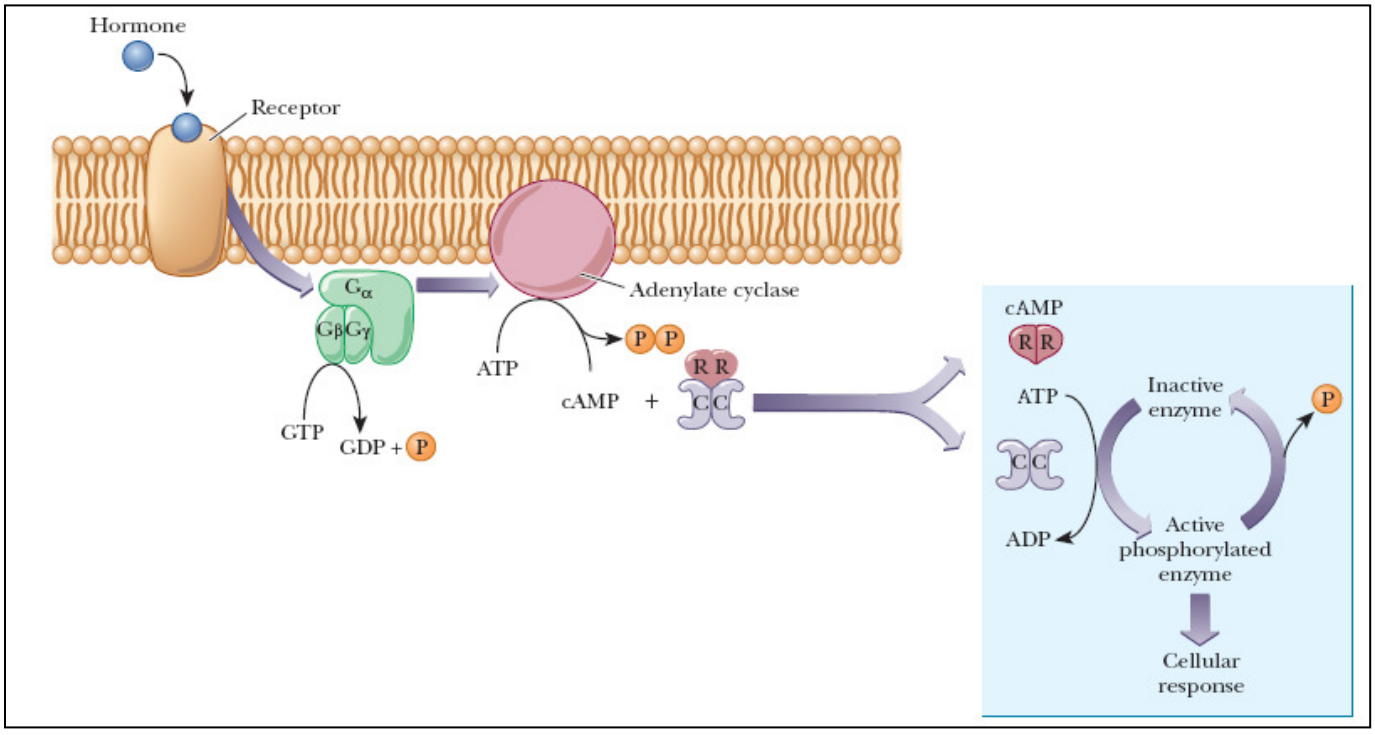
Cyclic AMP can affect a wide range of cellular activities such as: increasing the degradation of storage fuels, increasing the secretion of acids by gastric mucosa, dispersion of melanin pigment in the body, decreasing the aggregation of blood platelets and opening of chloride channels.

Increasing the cyclic AMP in people who have gastric ulcer will worsen their condition,

so anything that increases the cyclic AMP within the body should be stopped in those people. One of the things that increase the cyclic AMP levels is caffeine which blocks the phosphodiesterase (which does the hydrolysis of cyclic AMP) and blocks the site of attachment of adenosine in the brain so adenosine will make more ATP and more cyclic AMP as well.

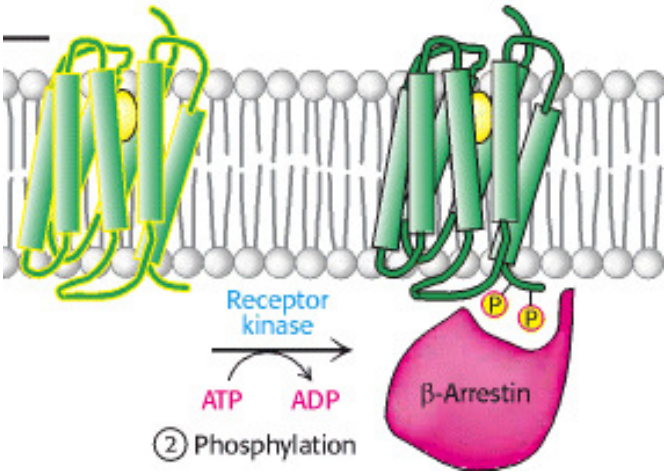
Then what cyclic AMP does is binding to protein kinase A which is a tetramer protein with two regulatory subunits and two catalytic subunits. In inactive state, there's nothing on regulatory subunits. When cyclic AMP comes and binds (we need four molecules of cyclic AMP to bind the regulatory subunits) dissociation of the regulatory subunits from catalytic subunits will occur. Then catalytic subunits will make phosphorylation and we take the phosphate for this reaction from a substrate which is ATP.

Phoshporylation doesn't always lead to activation, sometimes it leads to inhibition as in glycogen synthase. Phosphorylation usually occurs on serine or threonine.



Switching off the signal:

After the hormone complete its action we need to stop the signal. There are many ways that work together to stop the action of the hormone; to stop the process all at once not gradually, such as:1) dissociation of the hormone from the receptor 2) hydrolysis of the second messenger (cAMP) by phosphodiesterase 3) the activated G-protein is inhibited by GTPase activity that is found in α subunit. 4) the last thing to prevent the hormonal action if the hormone is still attached to the receptor is by an internal brake called **β-arrestin**.



How β-arrestin works? After the hormone (yellow) binds to the receptor (7TM) there is something inside the cell called receptor kinase that comes and phosphorylates the receptor itself. Once the receptor is phosphorylated (phosphate is added to serine or threonine) now beta arrestin can bind to the receptor and as the name implies it arrests the action of the receptor. What β-arrestin actually does is binding to the receptor and preventing the G-protein from binding to the receptor so G-protein will not be active.

This means that we have stopped the action of the hormone at the level of: 1) binding: by means of dissociation of the hormone.
2) Transmitting the message to G protein via desensitization (by β-arrestin). 3) adenylate cyclase via GTPase activity. 4) second messenger via hydrolysis of the second messenger itself.

Cholera: a disease that is characterized by severe diarrhea (severe dehydration). It is caused by bacteria called vibrio cholera that secretes the cholera toxin which binds to receptors found on the intestinal epithelial cells, activating G-protein which activates adenylate cyclase to produce cAMP extensively. cAMP will export Cl and Na outside the intestinal cells (active transport of Na^+) in association with water, and this leads to extensive water loss from body fluids to the intestines resulting in severe diarrhea and if not controlled it can lead to death.