***Today's lecture is from chapter 15 from a book called Stryer the doctor gave us the website:**

You can Google it (Pubmed) or www.ncbi.nlm.nih.gov/books/NBK21205/ ,the book also has lots of medical articles that you can refer to in the future ^^

***as the doctor said from the first lecture there is no need to buy the book slides+sheets and records will be enough**

Now let's start:

Slide#1:

Today we will talk about transduction of hormonal signals, detection and amplification of external signals and how cellular responses are generated.

Slide#2: Overview of the signal transduction pathways:

***as we know that there should be some sort of communication between cells either through the CNS or the endocrine system ,these communications are presented as signals that are received by cells**

***the binding of the signal to the cell receptor is not sufficient because it will produce very little change in conformation, so there must be another way to convey this information?**

***transduction is the conversion of one form of a signal to another form (EX. electrical-----> chemical signals) so the cell can produce many kinds of responses in different ways.**

***through the process of transduction there is also what is known as amplification where many molecules of the second messengers are produced. This will amplify the signal. The cell will also respond in a negative feedback mechanism ,in which the cellular response by itself will affect the transduction and reception for the sake of regulating the response of the cell.**

Slide#3: membrane associated receptor transfer the information, why?

-because most signals (neurotransmitters)are very polar and very large, only very few are non-polar so they aren't able to penetrate the cell membrane by diffusion, there must be a transport protein.

-in order for the response to happen the signals should bind to receptors that span the membrane, they are intrinsic membrane proteins (meaning it is impossible to isolate them from the plasma membrane unless the membranes are destroyed. Unlike integral proteins, which can be removed).

-these receptors consist of 2 domains:

1-extracellular domain: where the interaction of the signal occur, then this information (signal) will be transferredd as structural or conformational changes and then transmitted to.

The binding sends a message that there is a signal. This singal is a small conformational change in the cell receptor.

2-the intracellular domain

Slide#4: second messengers relay information:

***why is the second messenger important?**

Because the change in the conformation usually is very little (a few angstroms, 10^-10) so it's not enough to produce all the responses of the body

***with that we have achieved the amplification of the signal because the second messenger is produced by:**

1-enzyme activation: the enzyme is able to catalyze the reaction again and again so it speeds up the reaction to produce large numbers of second messengers made by just 1 single enzyme

2-membrane channels: for instance opening of Ca+2 channels numbers of ions will enter this channel

***so to sum up, the number of secondary messengers that are produced is very large when compared with the cellular conformational changes that occur in the receptor.**

***secondary messengers are able to diffuse to other cellular compartments**

***some second messengers are common in multiple signaling pathways --→ almost 30 hormones will use the same second messenger which is CAMP so how can the cell differentiate which response is for which hormone?**

It can differentiate with the help of the receptors. If the receptor of the hormone is there that means that the cell will respond to that hormone.

But sometimes the same cell can respond to 2 hormones that use the same second messenger how can we tell?

This happens through fine tunning, every hormone has its own degree of effect (as if you were fixing the radio on a certain channel frequency) although it can pose problems.

Slide#5: protein phosphorylation is common means of information transfer.

***protein phosphorylation requires a protein kinase, the P group will be added to specific serine,tyrosine or threonine residues (all of which have OH groups that can form an ester bond with the P group). The hydroxyl group is bound there covalently**

***this will lead to conformational changes ,because the presence of the negative charge that will attract some + charges. This will also induce conformational changes due to change of biding between different proteins.**

***phosphorylation lasts longer than changes in the concentration of the second messenger (few minutes-few seconds respectively). The presence of a phosphorylated protein indicated previous presence of a second messanger.**

***the process of phosphorylation should be reversible, easily done and easily removed by specific protein phosphatases**

Slide#6: termination of the signal:

***why is it important?**

1-keeps cells responsive to new signals. Ex. If the retina senses light changes, it should be able to respond.

2-failure of termination may cause problem such as →Let us suppose that a certain hormone induces growth in cells. Uncontrolled cell growth which may lead to cancer

***termination occurs by 2 ways:**

1) Degradation of the second messenger

2) dephospharylation by hydrolysis

Slide#: Seven-Transmembrane helix receptor(7TM)

***a group of receptors**

***it goes in and out through the membrane 7 times**

***at each time it passes as alpha-helix (a secondary structure),all H-donating and H-accepting groups are involved in stabilized H bonds,so all the functional groups that are able to form H bonds have already formed H-bonds with each other**

***because of large numbers of H-bonds it is very rigid, the change in the conformation in one part can be transmitte across the membrane.**

***Q: is alpha-helix in the receptor hydrophilic or hydrophobic in this case?**

It is hydrophobic; all potential H-bonds have been formed that is why it can be found within the membrane.

***the second illustration shows a 3D structure of the receptor where the ligand can be seen in the center(the doctor said that whether the ligand is present or not it will lead to very little conformational changes but enough to indicate that there is a ligand outside). The illustrated receptor is a rodopsin receptor (found in retina)**

Slide #7: biological function mediated by 7TM :

1-vision

2-smell, taste

3-nuerotransmition

4-hormone secretion

5-chemotaxis

6-exocytosis

7-cell growth development

8-viral infection

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

Slide#8:*shows the primary structure of beta-adrenergic receptor, this is an adrenaline type beta receptor

***you can notice that there are many serine threonine residues in the intracellular domain which are the sites of phosphorylation,so the receptor itself can be phosphorylated.**

Slide#9:cAMP and G proteins : many hormones use cAMP as a secondary messanger.

***cAMP is a small, heat stable molecule, and there is no need for the hormone to enter the cell**

***an EX.if we take liver cells and crush them until there are no intact cells left and then add the hormone on them then, the cAMP is produced, and if we destroy this system by heat you can still isolate this cAMP by heat and after taking it and adding it to another system then it can cause a typical response→ conclusion: hormones actually work by increasing the level of cAMP**

After that, using such systems (intact cells)-→they found out that GTP is important for hormonal response, so if we add GTP and then the hormone GTP is hydrolyzed into GDP,this encouraged scientists to purify the G protein and they were able to discover it.

***as the image shows that the activated receptors (ligand+receptor)-→stimulate the G protein-→which activates adenylate cyclase-→production of cAMP-→produce a biochemical response.**

Slide#10:G protein cycles between 2 forms:

Inactive	Active
Bound to GDP	Bound to GTP
Heterotrimer 3 different subunits alpha-beta-gamma	Disassociated alpha+(beta – gamma) dimer

The inactive form is transformed into the active form by replacing the GDP with a GTP.

Slide#11:

The diagram shows:

Step1:the hormone is not bound to the receptor

Step2:once the hormone is bound to the receptor it will move to the G protein and it binds to it 'Only if it is attached to the hormone'

Step3:note that GDP not GTP leaves and GTP will bind , the binding site for guanylate when binding to the receptor will open and once it does GDP will leave because binding is reversible and the [GDP] is less.

Slide#12:

Step 4:

GTP binds because the receptor has high affinity to it this will lead to conformational changes and so the disassociation of the alpha subunit

Step5:the alpha subunit is able to bind to adenylate cyclase and stimulates it to convert ATP--→cAMP +PPi and large #of CAMP are produced (amplification)

Step6:alpha subunit has intrinsic GTPase activity (an enzyme that catalyzes the hydrolysis of GTP->GDP,so it reverses the signal intrinsically

Slide#13:

It shows:

1-"off position" inactive form ,then the binding of the hormone to the receptor will lead to disassociation of GDP and replased by GTP →leads to disassociation of beta-gamma dimer

2-on position

3-gradually GTP is hydrolyzed to GDP and the G protein goes back to its origanl state.

Slide#14: G protein (con.)

***alpha and gamma subunits have covalently attached fatty acids why? Fatty acids are nonpolar these proteins have to remain anchored in the membrane which is also hydrophobic in nature.**

***alpha and (beta-gamma) subunits can interact with other proteins**

***many G protein molecules can be activated by 1 signal bound receptor and it is not permanently bound to the receptor (amplification)**

***so if each receptor activates 100's of G protein molecules, and each G protein activates 1adenylate cyclase which will produce 1000's molecules /sec of second messengers.**

***all 7tM receptors act by activating G proteins with the help of G protein coupled receptors GPCR**

***many different G proteins exist but with different combinations of alpha, beta and gamma subunits**

Slide#15: G proteins transducer many activities:

The doctor read the slide but G(olf) is for olfactory and transducin refers to vision

The presence of covalently bound fatty acids obligate the G protein to remain in the membrane.

Slide#16:adenylate cyclase:

***membrane protein *12 helices**

***2 large intracellular domains**

***it is activated by G protein**

Slide#17: synthesis and degradation of cAMP:

1-ATP is abundant in the cell,that is why it is the regulatory precursor for synthesis of second messengers. The precursors of second messangers should be widely abundant or can be easily obtained.

2-adenylate cyclase is activated so the hydroxyl group at c#3 will attack the phosphate leading to the removal of the pyrophosphate and the phosphate is bound now to 2 ester bounds C#5 and C#3 that's why it's called 3'5' CAMP and its called cyclic because it made a ring structure

3-CAMP does not have any other functions other than stimulation of protein kinase (only as a second messenger nothing else),and it is easily built and easily degraded by an enzyme called phosphodiesterase ,which hydrolyzes the diester bond at C#3 so we get AMP ,which is not able to work instead of CAMP,at the end it will be converted back to ATP and so on

Note:caffeine is a phosphdiesterase inhibitor ,if u take it it will increase the CAMP due to decreased degradation.

Slide#18:CAMP can affect a wide range of cellular processes:

The doctor read the slide but he added that cAMP can increase the secretion of acid by gastric mucosa that is why patients who suffer from gastric ulcer are advised not to drink caffeine.

Slide#19: most effects of cAMP are mediated by activation of a single enzyme which is protein kinase A which can exist in 2 forms:

1-inactive: tetramer

2-active : dimer

Binding of cAMP disassociates the tetramer and activates it,and it is a reversible process. The tetramer is made of two catalytic subunits and two regulatory subunits. The cAMP will bind to regulatory subunits.

Slide#20:cAMP can affect gene expression:

***phosphorylation of transcriptional factors :synthesis of mRNA in many proteins requires this transcriptional factor, if its phosphorylated it is active and it is called CREB(cyclic response element binding protein) which moves to the nucleus and binds to CRE region in the DNA (cyclic response element)**

***in this case phosphorylation may last for hours not minutes**

Slide#21:switching off the signal initiated by hormone binding to 7TM:

***any regulatory signal should be easily done and easily switched off by one of these mechanisms:**

1-dissaciation of the hormone

2- phosphorylation of the hormone-bound receptor followed by binding to arrestin

3-GTPase activity of G alpha subunit

4- hydrolysis of cAMP

Slide#22:

We will talk about the first 2 mechanisms mentioned before:

1-dissociation of the hormone:as we know, binding of the protein to its receptors depends on the[hormone]→if the [hormone] decreases dissociation will occur because binding is reversible and weak

2-if the receptor was continuously bound to the hormone would it stay in its active state? No ,after a while there is something called receptor kinase,this receptor kinase can add P groups to the specific serine and threonine residue in the receptor (becomes phosphorylated) once the receptor is phosphorylated it binds to a protein called beta-arrestin,which will stop the activity of the activated recptor and it will no longer be able to stimulate G proteins

^^remember that the receptor kinase can only phosphorylate receptors that are bound to the hormone.

***Conclusion: if the [hormone] is continuously at the same level that will lead to no cellular response,that is why hormones are secreted in a pulsatile nature. When secreted in a pulsatile form, hormones can cause responses due to varying levels over time. However, if the concentration of the hormone stays the same, the receptors will be desensitized. This type of desensitization happens such as the smell sensation that is regulated by a 7TMR→if you were exposed to a certain odor you will adapt to it and no longer be able to smell it and feel that it has that unique odor.**

Slide#23: GTPase activity of Galpha subunit: it was mentioned before.

#Sorry for any mistakes but the slides were not with me when I wrote it I referred to last years slides,best of luck,

Sura Aldgheim