

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

^^ please refer to the slides they have imp information and illustration figures.

^ today we will continue talking about transduction cascade:

^ yesterday we have talked about 7TM protein ; how they activate G-protein and how G-protein activate Adenylate cyclase which produce CAMP, by this process we get transduction(amplification) of the signal, so cellular response occur.

^ today we will look at :

- Phosphoinositide Cascade (another transduction system)
- Calcium ion as a second messenger
- Tyrosine kinase and receptor dimerization

Slide # (2+3)

- Phosphoinositide Cascade

#Used by many hormones (for example Vasopressin; which produced by posterior pituitary gland)

#upon binding of a hormone to 7TM receptor (again similar receptor) → Activation of G-Protein → Activation of Phospholipase C → which will act on membrane inositol 4,5 bisphosphate produce:

- Two messengers
 - » Inositol 1,4,5-trisphosphate (a lot of negative charges (At least 6) so it's hydrophilic and its soluble thus it's diffuses in the cytoplasm)
 - » Diacylglycerol (amphipathic; Stays in the membrane)

on which bond Phospholipase C act ?

>> on diester bond which connect glycerol with phosphate.
phosphatidylinositol 4,5-bisphosphate → DiacylGlycerol + IP3

slide#4

Phospholipase C has many Isoforms, which have different a.a sequence, different structure and different properties and different Kinetics .

Each of these isoenzymes formed from several domains (the domain is part of the protein that full fill an specific function) .

we have beta, alpha, delta and gamma isoforms ; beta isoform is very similar to delta and beta is somehow similar to gamma isoform.

#**The domain structures of the three isoforms of phospholipase C :**

Beta isoform is the **only** one who has G-protein interaction domain; so it's the only isoform that interact with G-protein.

Then we have C2 domain which binds to phospholipid head group in plasma membrane.

#Then catalytic domain (that is similar in the isoforms)

Then EF HANDS domain.

Then pleckstrin homology domain (PH domain) also interact with polar head groups=lipid head group in the plasma membrane.

slide#5

#Binding of G-protein brings the enzyme into a catalytically active form.

#upon stimulation of the G-proteins, alpha subunit is produced, this will interact with G-proteins interacting domain so all the enzymes will be anchored or attracted to the membrane then the enzyme become in close proximity with anterior leaflet of the membrane, after that the catalytic domain become in front of phospholipid which is inositol bisphosphate.

All of this happen after activating G-protein by binding of a Hormone to it's receptor.

slide#6

Effects of Second Messengers

Inositol triphosphate → Opens Calcium Channels → Binds to IP3-gated Channel the binding is Cooperative binding.

- Inositol triphosphate being water soluble diffuse into the membrane it reaches into the ER/SR in SMOOTH MUSCLE cell.
- These SR/ER are intracellular calcium stores, so Ca^{+} is sequestered and cannot be leave this stores until IP3 channels are opened.
- The channels are formed from 4 similar/identical subunits and it needs 3 molecules of IP3 to open.
- The cooperative binding produce sigmoidal shape, So this cooperatively allow the very small change in IP3 to open the channel; the binding of first subunit will make the binding of second subunit more favorable which lead to rapid release of Ca^{+} because of different concentration.

Diacylglycerol (remain in the membrane) → activate protein Kinase C (Ca^{2+} is required; because of this requirement we called it Kinase C In comparison with proteins kinase A which require CAMP) → phosphorylation of many target proteins in the cell .

slide#7

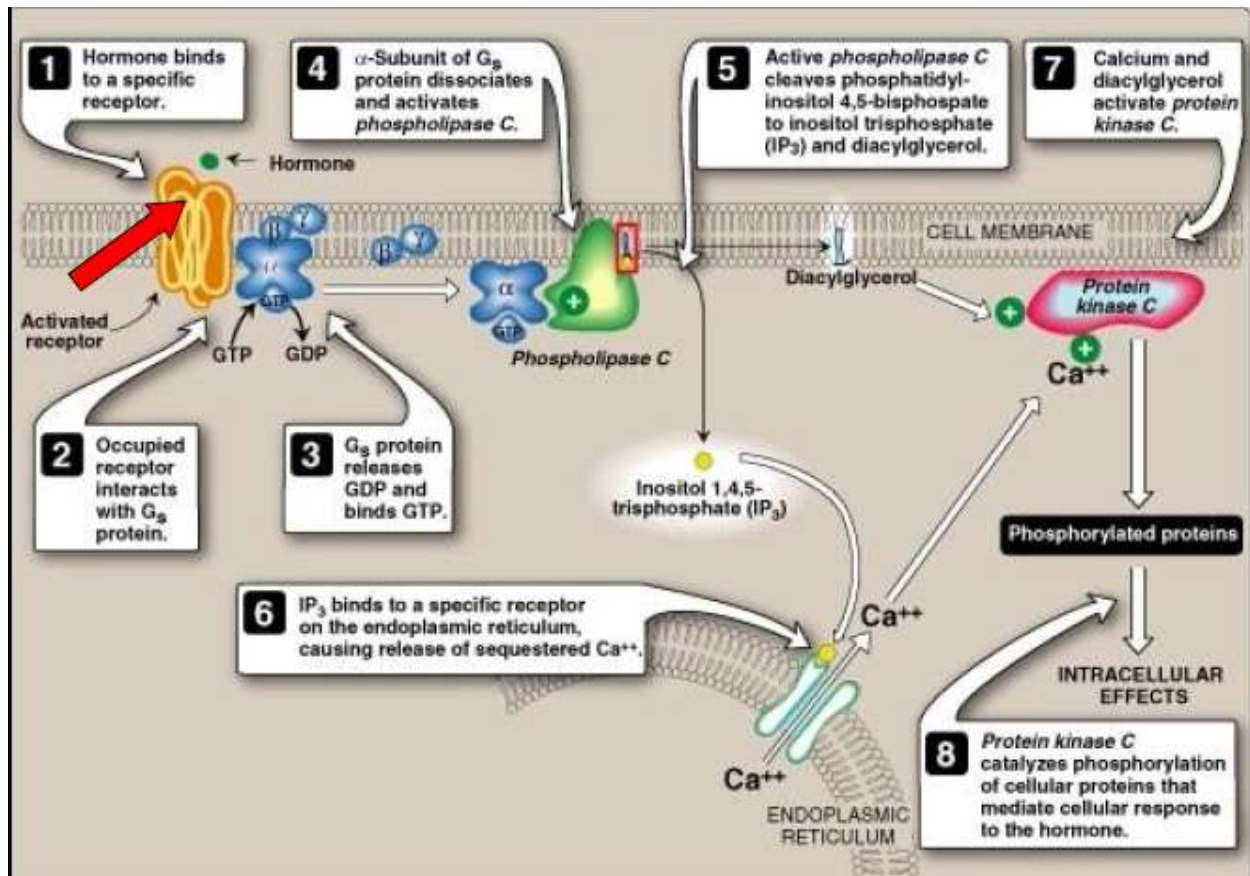
#This picture shows the events:

we have phosphatidylinositol bisphosphate found on the interior leaflet of the membrane, we also have phospholipase C acting upon phosphatidylinositol bisphosphate releasing IP3 that binds to the channel (which is 4 subunits, 3 molecules of IP3 are required) ,after binding the channel is open and Ca^{+} is released from SR/ER (where Ca^{+} concentration is high) after that Ca^{+} will bind to protein kinase C, after binding to Ca^{+} ,protein kinase C can interact with DAG which is found in the phospholipid membrane as a result activation of protein kinase C will phosphorylate many target proteins, which lead to smooth muscle contraction, glycogen breakdown, and vesicle release (many process that require Ca^{+} will be activated by this way.

#the effect of IP3 was discovered by microinjection of IP3 inside the cell after injection the Ca^{+} concentration is increased, by this they have discovered that IP3 is a second messenger .

Slide#8

This picture taken from Lippincott (summarization) :



Slide#9

As phospholipase C has many Isoforms (which main function hydrolysis of Phospholipids) protein kinase dose so (which its main function o phosphorylate proteins) ...please do not mix between them

#we have alpha beta and gamma isoforms.

The domain structure of protein kinase C:

- » the first domain: protein kinase domain (which is found in all of isoforms).
- »The second: C2 domain which is also found in phospholipase C, it interacts with lipids.
- » the third domain: C1A and C1B (these domains binds DAG) and the effect of DAG occurs in these subunits.

››The fourth domain: pseudo-substrate its resembles the substrate Sequence, it contains A-R-K-G-A-L-R-Q-K sequence.

**this enzyme has many isoforms, too.

** A: Alanine ... R:Arginine ... etc.

Slide# (10+11)

the sequence for phosphorylation should be :

X(amino acid)-arginine-x-x-(serine,thyrone)-hydrophobic amino acid-arginine .

There is a Specific sequence, and wherever there is serine or thyrone they will be targets of phosphorylation.

#Pseudo-substrate sequence is almost similar *but instead of serine or threonine there's Alanine*(which does not have OH group, cannot be phosphorylated) so when pseudo substrate bind to active site of protein kinase C but no phosphorylation would occur (competitive inhibitor within the same enzyme) .

#Pseudo -substrate blocks the active site so it's inactivate protein kinase.

upon stimulation binding of C1A or C1B or even binding of C2 to phospholipids (this binding requires the Ca ions) every one of these bindings removes the pseudo substrate from the active site, as a consequence the active site become ready for interaction with target proteins and get phosphorylated.

#the same idea found in protein kinase A but in protein kinase A the regulatory and catalytic subunit are completely separate subunits , but here protein kinase C they are separate domains .

Slide #12

Termination of IP3 signal:

#Because of it's function (IP3 as second messenger requires the immediate activation and deactivation (that's why its short lived messenger).

►How its terminated ?

By dephosphorylation, the 3 groups of phosphate are removed one after the other, once the first group is removed the IP3 is no longer activated.

NOTE: even phosphorylation by 4 phosphate molecules (which becomes in these cases inositol tetraphosphate) is still inactive and it becomes dephosphorylated in different sequence.

#So we have two ways for inactivating the IP3:

- 1- Either by removing one group after the other
- 2- or by addition of 4th group of phosphate then removing them (2 or 4 phosphate group ; both of them are inactive form)

note that when we remove the phosphate group we do not remove the last one added.

►Clinically:

Lithium ions is used in the past for treatment of some psychological disorders (by inhibiting the IP3 recycling, it keeps IP3 in its active form, so Ca⁺ concentration is kept high).

Slide #13

Why Calcium is suitable as second messenger?

◆Ca⁺ cannot form soluble compounds with phosphate and carboxylate, if the concentration of calcium-phosphate salt or carboxylate salt increase precipitation would occur. ← (the first reason) .

as we know phosphate level inside the cell is high and logically Ca⁺ levels must be low(continuously removed) from the cell, so there is a large concentration difference between outside (mM) and inside the cell (μM) ;the difference in Ca. levels is 10,000 times more outside as compared to intracellular Ca⁺ concentration.

#when Ca channels are opened it will rapidly influx the Ca. (Found in red in slide), because any regulatory signal should be easily activated as well as easily terminated.

So how it's terminated??

By calcium pumps which continuously pump Ca⁺ outside of cell, so the overall result is that we can get rapid increase in intra calcium levels and followed by rapid decrease, and this change in concentration can be used to modulate the effect of many enzymes.

Slide #14

◆ Ca⁺ can make coordination binding with up to eight oxygen atoms. ← (the second reason)

these oxygen atoms can come from aspartate side chain from main chain CO, and from water .

this tight binding of this ion (Ca⁺) with many side chains and many atoms, can bring these side chains or atoms closer to each other so it can cause conformational change .

Slide #15

Useful tools in studying the role of Ca⁺⁺.

1- **ionophores:**

#it's a substance that can bind specifically to certain ions; we have ionophores for hydrogen, for K⁺ and for Ca⁺.

It also they have hydrophobic surface.

◆ so they can bind Ca⁺ and can diffuse through the membrane; they can come to the side where Ca⁺ concentration is high and bind to Ca⁺ then go to the side where Ca⁺ is low and the Ca⁺ will disassociate here

,so they can introduce Ca^{2+} inside the cell without opening the channels

.

as a conclusion we can add ionophores for the cells and increase Ca^{2+} concentration inside the cell ; through this we can mimic the action of the hormones which use Ca^{2+} as a second messenger .

2-*Calcium Chelators:*

Examples: egta and edta

#they have the opposite function compared to ionophores; they can bind to Ca^{2+} and inactivate it ; they decrease Ca^{2+} concentration .

3-*Fluorescent Chelators :*

#Fluorescent substance : the substance that can absorb light in certain wave length (UV light) and produce light in different wave length (visible light).

if they are specific to Ca^{2+} , we can use them to measure the Ca^{2+} concentration while the cell is still a live .

Slide #(16+17)

Calcium binding proteins:

Ca^{2+} dose not act alone (its bounded to Ca^{2+} binding protein) and this proteins mediate the action of Ca^{2+} when it binds to enzymes (causing the activation or deactivation).

#There are many binding proteins including:

Calmodulin (the most famous), *Troponin C* and *Parvalbumin*.

#these binding proteins have similar structures, and they are rich in asp. and glu. **Why?**

→Because they contain carboxyl groups which can bind Ca^{2+} .

#they have several alpha segments (its ridge molecule but when it binds Ca^{2+} it causes conformational changes).

#the binding site is formed of helix-loop-helix (they found that all binding proteins are made in this sequence).

Ca⁺ binds to the loop and could make large conformational changes between the 2 helices.

#when this conformation discovered in case of parvalbumin , (helix E) is numbered 5 while (helix F) is numbered 6 because its made up of 6 helices they form EF2 hand .

#EF2 hand are super-secondary structures are found in many binding proteins, usually involved in Ca⁺ binding, when Ca⁺ bind between E and F it produce conformational change .

Slide# (18+19)

Calmodulin:

-Found in all eukaryotic cells.

- protien that has seventeen KD mass.

-consist of 2 globular regions (2 domains).

-connected by flexible region

-each region contains 2 EF hands (so 4 Ca⁺ binding sites found in calmodulin)

#This structure(in the slide) resembles the amino acid sequence of calmodulin and consist of 149 a.a.

it has 4 domains ,which represents that Ca. can bind several A.A. together.

as we said calmodulin is rich in Asp. And Glu. (this is why Ca. can bind to calmodulin)

Slide# (20+21+22)

Calmodulin changes upon binding:

#The inactive calmodulin undergoes changes in the structure after Ca⁺ binding which make it able to bind to inactive enzymes and making them active ones.

What happens actually here is that some hydrophobic residues move from inside to outside of the domain, so once Ca⁺ binds ,it will cause minimal changes leading to expose the hydrophobic residues/ patches

(they were interior and become exterior), and the purpose of this is to allow other proteins to be bound to calmodulin.

So we conclude that a large number of enzymes can bind to calmodulin as a result of exposing the hydrophobic patches caused by Ca^{2+} binding. For example, phosphorylase kinase but the most important ones are:

1-Calmodulin-dependent protein kinase: it can add phosphate groups to a variety of target proteins (including itself).

►How is it activated?

As its name suggests, it binds to calmodulin.

Overall interactions: when there is an increase in Ca^{2+} level, it will bind to calmodulin and in turn, calmodulin will bind to calmodulin-binding protein, and the latter will phosphorylate many target proteins (including itself), meaning it will phosphorylate many other calmodulin.

Q: What's the difference between calmodulin-dependent proteins (phosphorylated and non-phosphorylated ones)??

The phosphorylated form is partially active when Ca^{2+} level is low, so once Ca^{2+} level increases, it will lead to phosphorylation of calmodulin-dependent protein eventually and it will remain active (or phosphorylated) even after the Ca^{2+} level decreases [sort of memory indicates that Ca^{2+} concentration was high].

2-Calcium dependent ATPase pump:

This pump will be activated once Ca^{2+} level increases, which leads to pumping Ca^{2+} ions and lowering the concentration again.

So both of these (calmodulin-dependent protein and Calcium dependent ATPase pump) regulate the concentration of Ca^{2+} .

Slide#23

the Calcium transporters are:

- abundant in sarcoplasmic reticulum.

Single Membrane-Spanning Helix; just one polypeptide forming alpha helix will tell the intracellular domain that the extracellular domain has bound to a hormone .

#monomeric will not bind to a hormone, but dimeric will bind to a hormone.

#the binding of hormone to one subunit in the extracellular domain will make the binding to the second receptor cooperative = more favorable so the two receptors will come close to each other ; they can phosphorylate each other and phosphorylate other target protein as well .

#Note there's a change in quaternary structure instead of tertiary structure, because two molecules will be bounded at the same time.
slide #(30+31)

#each cellular domain is associated with protein kinase called JAK2; the JAK2 has protein kinase domain, protein kinase like domain (similar but inactive one), SH2 domain that bind to phosphorylated tyrosine and ERM which is important in interaction with the membrane .

the name Janus related to Greek idol .

the similarity between the protein and the idol is that both of the the have double headed form.

#hormone that induced the dimerization will bring the two JAK2 together.

by binding to phosphorylated tyrosine SH2 can make JAK2 more closely associated with the receptor.

#These can phosphorylate more target protein where the phosphorylation occur at tyrosine.

#In its monomeric form its inactive but in its dimeric form it's inactive.

slide# (32+33+34)

#JAK2 can phosphorylate other proteins like:

^ **STAT 5**(Signal transducer and activators of transcription) :

- regulate transcription; when its phosphorylation will lead to binding of two tyrosine molecules to it (forming a dimer) which binds to DNA.
- if JAK2 remains active it will produce **Cancer** .
- Phosphorylation of the Receptor itself (wich will make the binding more tight) .

^Activated JAK 2 can phosphorylate other substrates

- Association with JAK2.
 - Association with other proteins in the signal transduction pathway.
- ^^ i try my best to make it clear, forgive me for any mistake .

your fellow : omayma otoom

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((هي إنزعاج القلب لروعة الإنتباه من رقدة الغافلين))