Endocrine system 3

Biochemistry

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Notes:

1- **At** the end of this sheet, there is a brief summary for the whole lecture. If you don't have time, just go through it.
2- **There** are some points present in the slides but the doctor never gave them that much of a credit (just mentioned them rapidly or didn't say anything). Throughout this sheet, these points will be underlined, put in *italic* and put between double quotation marks. ''*Just like this*''**[Everything is included, no need for slide**]
3- **This** sheet was written based on Section 2 recording and it starts from slide 35.

Good luck

- ✓ Last lecture we went through the definition of hormones, classification according to effect, interaction with the nervous system, biochemical problems posed on the endocrine system, Kd, receptors, domains, signal amplification, loops to control hormone action, chemical structure classification and mechanism of action, synthesis of protein hormones, hormonal interaction, signal transduction and 2nd messengers and signal termination. We finished by talking about the types of receptors –including seven transmembrane receptor and its functions. ***Make sure you are familiar with all of the above mentioned.***

- ✓ Each 7-transmembrane receptor present on the cell membrane is attached to ***at least*** one G protein. The number is variable, and you may find around 100 G proteins (each consisting of alpha, beta and gamma subunits) attached to the same receptor. This type of receptor is also called G protein coupled receptor.

  ******There are around 20 different types of G proteins discovered, and around 100 G protein coupled receptors.

- ✓ Each 7-transmembrane receptor present on the cell membrane is **attached** to G protein on the cytosolic side of the membrane. What makes the G protein attached to the membrane and to the receptor in this way is the presence of fatty acids attached with the G protein. Since it's a fatty acid (hydrophobic), it will be attached/embedded in the membrane. The alpha and the gamma subunits each has a fatty acid covalently associated with it, beta doesn't (because beta is associated with gamma subunit making a dimer, they are attached as one unit, while the alpha subunit –which can dissociate from the beta-gamma complex needs its own attachment).

  The G protein is attached to the receptor in a way that, when the hormone binds and causes conformational change in the receptor, the G protein alpha subunit

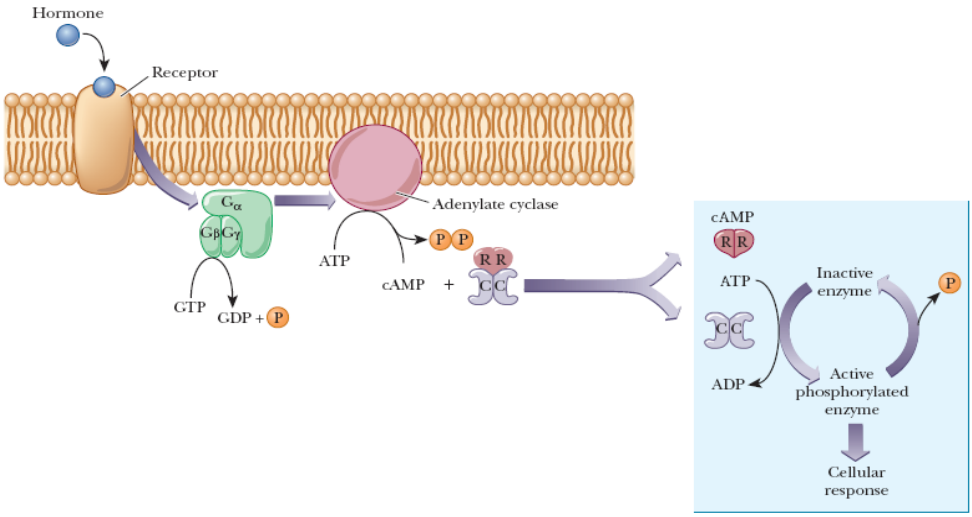
will also undergo a conformational change (due to the close proximity to the receptor). Now, the change in the alpha subunit results inlow GDP affinity, plus high GTP affinity and replacement occurs. The alpha subunit loses its already attached GDP to attach to a new GTP molecule. Once the alpha is bound to GTP, the presence of this new phosphate in GTP causes a conformational change in the alpha causing it to detach from the beta-gamma dimer.

- ✓ The alpha is now active. It will head towards a certain target. Note that there are more than one targets, one of them (the most common target) is the enzyme Adenylatecyclase

- ✓ This enzyme is a membrane enzyme, which consists of 12 alpha helices that span the membrane, and two intracellular domains (attached to the membrane). . This enzyme acts on ATP to produce cAMP ‘’*small and heat stable molecule’’*

- ✓ Since many G proteins can be attached to the same receptor, this causes ***signal amplification***. Each activated alpha subunit (of the various G proteins attached to the same receptor) will target a Adenylatecyclase in the membrane, and each Adenylatecyclase will produce high amounts of cAMP per second, so first we started with one molecule conveying the signal (the hormone molecule) then the number increased (the number of alpha subunits activated) then it further increased (the number of cAMP)→Amplified signal.

- ✓ cAMP then targets Protein kinase A[kinase= it phosphorylates other proteins, A= because it is activated by cyclic ***Adenosine*** monophosphate]. It is composed of two catalytic subunits and two regulatory subunits, which contain 4 binding sites to cAMP. When 4 cAMP molecules bind, the regulatory subunits detach from the catalytic ones, which are now able to phosphorylate other proteins. ‘’*Usually, it happens on Ser/Thr residues’’.*



*Phosphorylation does NOT always lead to activation of the phosphorylated protein. A famous example is the enzyme Glycogen Synthase, which gets inhibited by phosphorylation (the signal here is due to Glucagon hormone or Epinephrine, both are secreted to increase blood glucose level, so it is not the right time to build glycogen, so it's only logical to inhibit such enzyme).

*''G proteins can be activated by combinations of hormones. For example, Glucagon and epinephrine act via a stimulatory G protein in liver cells''

Back to G protein, we said that the active alpha subunit targets Adenylatecyclaseenzyme, but does it always have to involve activation of this enzyme? NO, the type of the pathway (stimulatory or inhibitory in its nature) depends on the nature of the ***alpha subunit*** itself (some alpha subunits are stimulatory by nature, some are inhibitory and are called **Gαi** or **Gi**) and it also depends on the ***receptor*** itself. There are stimulatory receptors ''*such as β1 orβ2 receptors''* and there are inhibitory receptors ''*such as α2 receptors''*

'' So there are different types Gα: some stimulatory and some inhibitory

Gs ……..↑ AdenylateCyclase
Golf…… ↑ AdenylateCyclase
Transducin ……..↑ cGMPPhosphodiesterase
Gi…….. ↓ AdenylateCyclase
Go…….. Ca2+ Channels
Gq…….. ↑ Phospholipase C''

- ✓ After cAMP does its function, it is broken down by the enzyme ***Phosphodiesterase.***

****

The target of the alpha subunit is not always Adenylatecyclase. It may be the enzyme ***Phospholipase C***, or be a membrane ion channel such as chloride or potassium channels ''*can open or close the channels''*.

''*Functions of cAMP (mainly physiology, should be familiar):*

↑ degradation of storage fuels
↑ secretion of acid by gastric mucosa
Dispersion of melanin pigment granules
↓ aggregation of blood platelets
Opening of chloride channels ''

- The full cascade is the following:
  Hormone binding to receptor→ activate G protein→ activate Adenylate cyclase→ produce cAMP→ activation of protein kinase A→ phosphorylation.

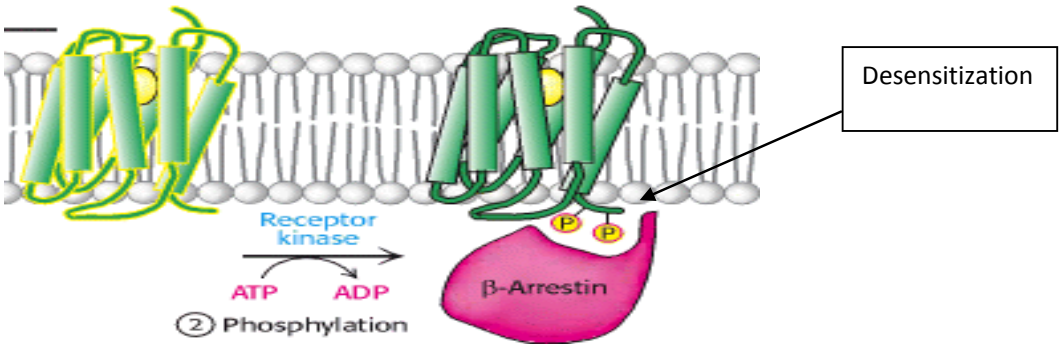
  The ***termination of the signal*** should act on all levels:

  Hormone *dissociation* from the receptor. The mode of hormone-receptor binding is non-covalent interactions, so that at the end, dissociation occurs. (If the binding was covalent, then the ligand is a ***toxin*).

  **Active alpha subunit becoming inactive again through the slow *GTPase activity*, which hydrolyses the GTP with the active alphasubunit to GDP. Now it is inactive and it re-associates with the beta-gamma dimer.

  **Breaking cAMP by *Phosphodiesterase* enzyme.

  The receptor itself contains many Ser/Thr residues in the Cytoplasmic part, which constitute a site for phosphorylation. After the hormone has done its work (by conveying the signal inside the cell and changing the cell's metabolism), these Ser/Thr residues get phosphorylated ''*by receptor kinase''*, which leads to a conformational change in the receptor, which makes the receptor have high affinity to a protein called **β-Arrestin. This protein binds to the intracellular side of the receptor (the coupling domain), and now that this domain is masked/covered by β-Arrestin, even if the hormone is binding the receptor, the G protein will NOT get activated (the conformational change in the receptor due to hormone binding cannot affect the G protein because β-Arrestin lies between them, preventing direct contact between the receptor and the G protein).
  → This iswhat we call Desensitization of the receptor, meaning that even when the hormone is bound to the receptor, there is NO signal being transduced/propagated/conveyed in the cell.



  ****Note: Another way is by the action of phosphatases, to remove the effect of PKA, but since PKA phosphorylates many proteins, this effect is not of significance.

- ***Cholera toxin:***
  It is a toxin produced by *Vibrio cholerae,* which is transmitted by contaminated water. If one ingests it and it reaches the intestines, it binds to a 7-transmembrane receptor, activates G protein, activates Adenylate cyclase, and produces many cAMP molecules. Due to extreme binding affinity between the receptor and the toxin, ***huge*** amount of cAMP is produced, which affects membrane channels; it causes Cl- release, and pumping of Na+ out, which causes increased osmolarity and water getting out of cells as well. All of this leads to excessive and uncontrolled diarrhoea which may be fatal.

  **I asked the doctor whether cholera toxin produces its action via inhibition of GTPase activity in the active alpha subunit (which will also lead to huge production of cAMP) and he said that this activity wouldn't be of such importance without the presence of extreme binding affinity between the toxin and the receptor, meaning that even if it really inhibits it but the toxin can dissociate, the effect would be temporary, but because of the high affinity, there is constant and persistent cAMP production.

****

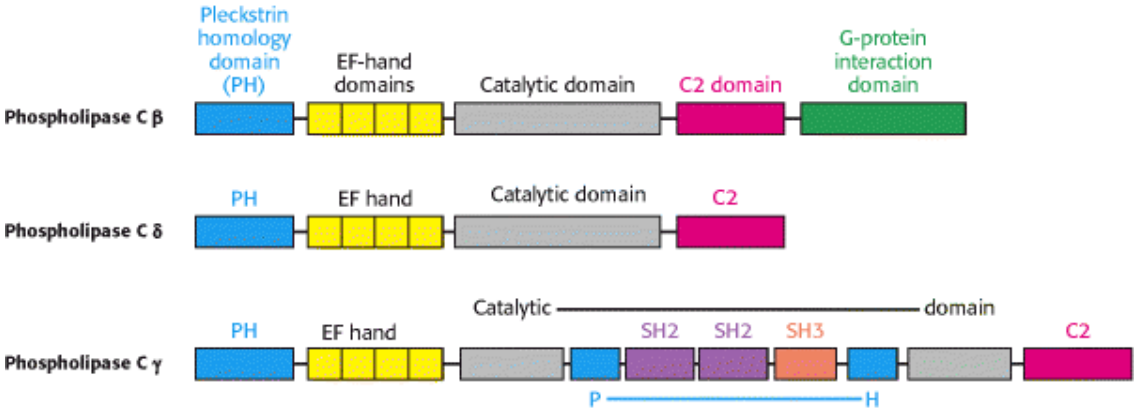
The phosphoinositide Cascade

- ''*This pathway is used by many hormones, like ADH*'' .The active alpha subunit can target another enzyme, called *Phospholipase C.* It is an enzyme that is attached to the cell membrane. Since it's a protein, it contains domains. The major ones include:

  1- Catalytic domain (does the catalysis of the reaction).
  2-Domain to bind the cell membrane
  3-Domain to receive/bind the active G protein alpha subunit.

- This enzyme has isozymes (multiple forms of the enzyme, each with certain tissue localization). There are Phospholipases beta, gamma, and delta. Only Phospholipase beta contains the G protein binding domain, so it is the only one involved in this pathway.

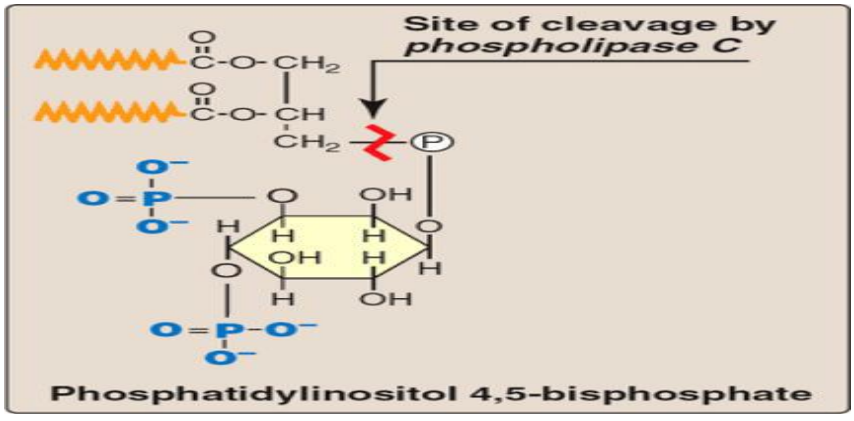
- The PH''*bind lipid head group*''and C2 domains *''bind phospholipid head group''*→membrane attachment.



- This enzyme breaks down phospholipids, particularly PIP2 (phosphatidyl-inositol 4,5bisphosphate). It consists of glycerol (3 carbons), two of which are connected to fatty acyl chains; the 3rd is connected to a phosphate group (so far this is the structure of phosphatidic acid). This phosphate is connected to inositol (sugar that forms a hexagonal ring, each carbon has an OH group→hydrophilic). On carbons number 4 and 5 of the inositol ring, there exists two phosphate groups, so the result is PIP2.

- This structure (PIP2) is present in the cell membrane and the enzyme as well. When a signal comes (hormone), the G protein gets activated, activate phospholipase C, breaks down PIP2bond between the phosphate and the carbon of the glycerol, producing inositol 1,4,5trisphosphate (IP3) and diacylglycerol (DAG). IP3 is totally hydrophilic, so as soon as it is formed, it leaves the membrane directly towards the cytoplasm, while DAG contains two fatty acids, so it can still hang in the membrane. It is an amphipathic structure (having both h.philic and h.phobic parts).

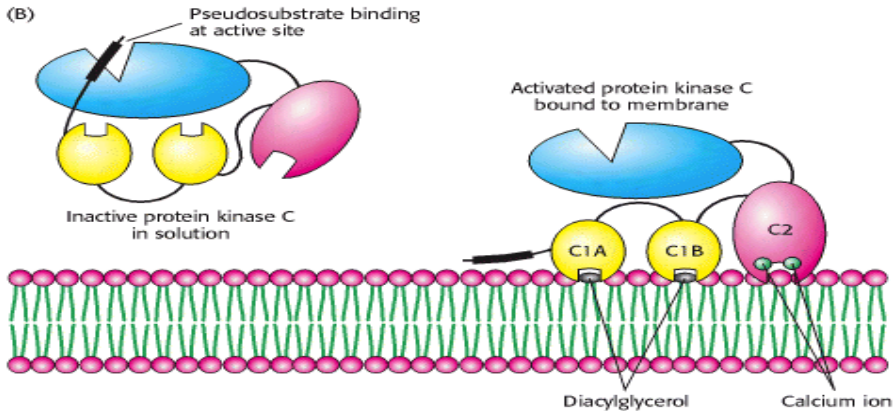
  The ***main*''*actual*''second messenger in this system is the IP3. DAG also works as a 2nd messenger.



- IP3 destination is the sarcoplasmic reticulum (smooth ER), which is a reservoir of Ca. IP3 binds to Ca protein channels on sER to cause Ca release. Each channel binds four IP3 molecules to fully open, and if (at least) three IP3 molecules bind they cause considerable opening of the channel (but not full). So, three IP3→ can do the job, four→ better. Note that the IP3 binding to the channel is cooperative, meaning that binding of the first IP3 makes it easier for the second IP3 to bind, which makes the 3rd binding easier, which makes the 4th binding easier (*recall:* haemoglobin and oxygen).

- Ca release into the cytoplasm occurs. Since Ca is positively charged, it binds with negatively charged proteins (not one, they are a group), which are called *Calcium binding proteins.* Once bound to calcium, they get activated. Another target of Ca is protein kinase C (maybe considered as a Ca binding protein, and the reason for calling it Protein kinase **C** is that it gets activated by **C**alcium). This PKC is a membrane enzyme, and gets partially activated when bound to Ca, now it is able to bind to what is left of PIP2 in the membrane,DAG, and get fully activated.

  **PKC which is a protein attached to the membrane, contains these domains:
  1-Catalytic domain ''*protein kinase domain''.*
  2-Membrane binding domain ''*C2''*, which must consist of hydrophobic amino acids and it may also contain fatty acids, in order to attach to the membrane.
  3-DAG binding domain*''C1A-C1B''*
  4-Ca binding domain.
  5-Pseudosubstrate domain: PKC is an enzyme that phosphorylates proteins; it contains this domain, which resembles/looks like the substrates of this enzyme. This domain looks like the sequence that enters the active site to be phosphorylated, but instead of having Ser/Thr, it contains hydrophobic amino acids, such as Alanine. This domain (since it resembles the substrate) can fit in the active site, but because there doesn't exist Ser/Thr, no phosphorylation occurs. Since the active site is occupied/full, the enzyme is inactive. Before Ca binding, it is not closely related to the membrane (only C2 domain faces the membrane and attach phospholipids), but when Ca binds→ conformational change→ DAG domains flip to face the membrane and be able to interact with DAG present within the membrane. In addition, when they flip, they draw/pull the pseudosubstrate domain with them, exposing the active site. Now, the enzyme is active and can act on other proteins.



''*The pseudosubstrate domain acts as a competitive inhibitor''.*

- ***Termination of the signal;*** by two ways, both of them end the activity of the IP3 molecule (inositol 1,4,5trisphosphate), which is a short lived messenger: **either** we remove a phosphate via cellular phosphatases **or** we add a phosphate producing IP4 (inositol 1,3,4,5 tetrakisphosphate), and this is a faster way as an initial solution, then when the cell has the time, it starts removing the phosphates out of the IP4 molecule. And when you remove the phosphates, do not remove the last phosphate added first, meaning that if you remove the last phosphate added –which is on carbon number 3- the molecule will return to active inositol 1,4,5trisphosphate, but if you remove any other phosphate you will have the bonds (1,3,4 or 1,3,5 or 3,4,5) which are all inactive and the signal is terminated.

******Clinical hint:*** Lithium based drugs (psychiatric medicine, used for depression). Lithium is a heavy metal, which inhibits enzymes in the CNS, such as phosphatases. Now, IP3 cannot be broken ''*inhibit IP3 recycling''*→IP3 is active→treatment of depression.

- All this pathway is based on Ca release, so why Ca?? What characteristics Ca has that make it suitable for this pathway's function?

  1- Ca***is positively charged*** (+2), it has the ability to bind negatively charged structures (including proteins with negative charges).

  2-Concentration: there is a very ***hugedifference in the concentration*** of Ca between the cytoplasm and sER (around 10000 times) and between the cytoplasm and outside the cell is also 10000. This difference is not present for other molecules/ions. This difference produces huge impact when Ca channels open. Note that when Ca channels open, if we just want to wait until Ca is released and then pump it back, large amounts of Ca will be released due to the high difference which acts as a driving force and after that the driving force is over, so this must not be allowed. What happens is that as soon as Ca is being released (just small amount), Ca pumps

start pumping it back to the sER, in order to maintain this large difference between the cytoplasm and the sER, and thus maintain the driving force. So small release→ do the desired function→ maintain large difference.

3-It can make up to 8 bonds (called ligations), so it can ligate up to 6-8 bonds with polar charges on oxygen on amino acids/water/ negative amino acids…etc. These bonds ensure ***tight binding,*** thus change in its target.

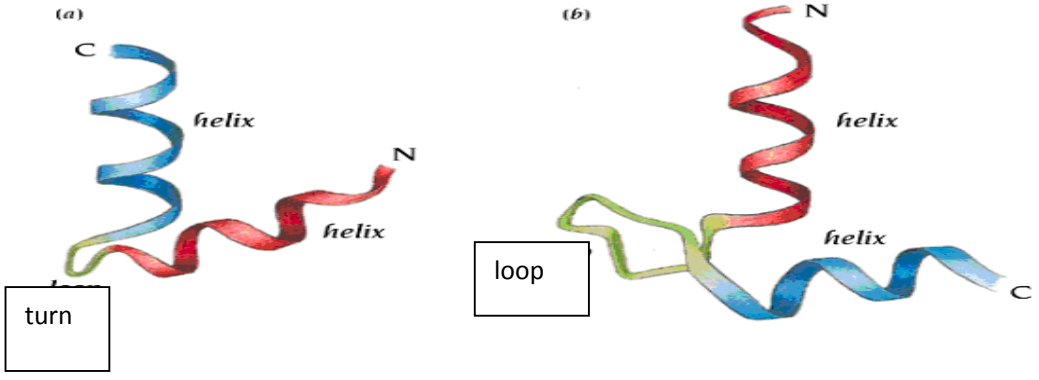
4- Ca is ***bulky***, so when it binds to the protein it produces the desired effect, which is the conformational change in that protein.

- Ca target is Ca binding proteins, which are a group of proteins that get activated when bound to Ca, thus changing cell metabolism. Examples include:
  A-Calmodulin
  B-Troponin C
  C-Parvalbumin (the first discovered, it has 6 alpha helices, called: A-B-C-D-E-F. This proteins binds Ca, the site of Ca binding is a loop between helix E and helix F. If you look at the structure: helix E-Ca binding loop-helix F (Helix-loop-Helix), this is a domain, and wherever you see this domain (in other proteins), most probably it is a Ca binding domain). This domain is called an **EF hand.**

So, EF hand is a Ca binding domain that consists of helix-loop-helix, first discovered in parvalbumin protein but is present in other proteins.

Q/ Why a loop, not a turn? What is the difference between a loop and a turn?

Ans: A turn is very small; it consists of 4 amino acids only, and it makes a sharp edge, so it is hard for Ca to fit there. A loop is not a regular structure, more amino acids so they can move to accommodate Ca binding.



**Helix-turn-helix…….DNA binding proteins
Helix-loop-Helix…….Ca binding proteins.

- Ca binding proteins characteristics:

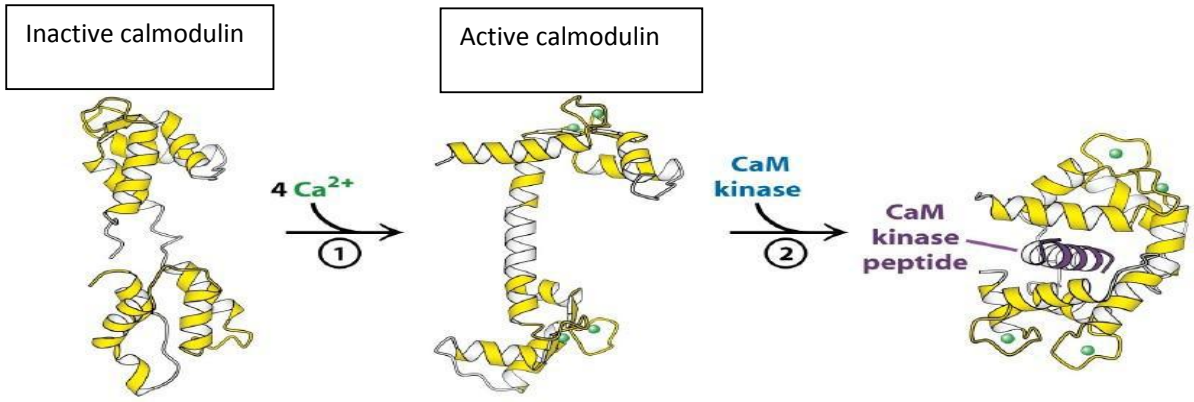
  1- Contain EF hand.

  2-Contain negatively charged amino acids, to bind the Ca.

- ''*Ca binding proteins have similar structures:*
  →Rich in Asp and Glu
  ▪Gln, Asn, Ser
  →Several α helical segments

  →Binding site is formed by
  ▪Helix Loop Helix, which is a Super-secondary structure''

- A common example is ***Calmodulin*** (17kD), consists of two globular domains, each one has two EF hands. Since each EF hand binds one Ca, in total, calmodulin molecule can bind four Ca molecules (each globular domain binds 2 Caions). Following Ca binding, it becomes active, and activates other proteins, including Calmodulin-dependent protein kinase. Also, it activates Ca ATPase pump, in order to return Ca inside and terminate the signal.



- When Ca is bound to calmodulin, calmodulin underwent a tremendous conformational change. Also some hydrophobic amino acids were exposed, which means that they will interact with other hydrophobic regions of other proteins, causing activation of these proteins.

  ''*Calmodulin structure: 17kD, consists of 149 amino acids, comprised of 2 globular regions connected via a flexible region, contains 2 EF hands, 4 Ca binding sites*''

- ***Ca ATPase pump***: activated directly after Ca release (fast activation). It will pump Ca against its large concentration gradient (from the cytoplasm to the sER), so it is energy expensive: for each 2 Ca ions pumped, 1 ATP is hydrolysed. This pump is present in large amounts on the surface of the sER; it constitutes around 80% of all proteins present on the surface. ''*It consists of 10 membrane spanning helices. This pump is highly ATP expensive, and depletion of ATP leads to tetany, Rigor mortis*''

Good luck

At the end of this sheet, there is a brief summary for the whole lecture. If you don't have time, just go through it.

Review

There are two types of receptors:

1- 7TM; which are always bound to a G protein "GPCR". This pathway may lead to activation of adenylyl cyclase or phospholipase C
2- Receptor Tyrosine kinase

Receptor Tyrosine Kinases Cascade

This receptor is a kinase enzyme, and the pathway involves Tyrosine amino acid phosphorylation. This pathway is used by most growth-related hormones (Insulin, GH, growth factors…). There are two classes of this receptor:

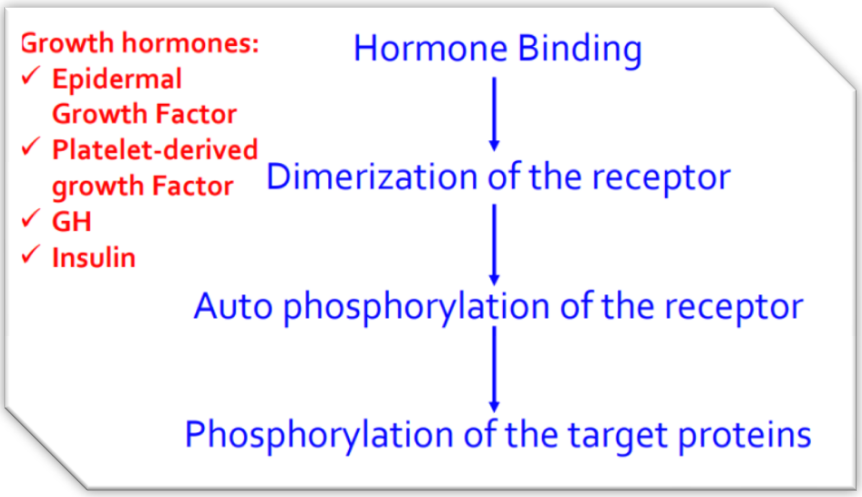
- Monomer, which dimerizes after ligand binding. All receptors of this family are monomers except for insulin receptor.
- Dimer, and the subunits are bound by disulfide bridges; such as insulin receptor.

The receptor spans the membrane, and has several subclasses (class II, Insulin R).

Receptor Domains

The receptor is a hormone receptor has a Tyrosine kinase portion.The coupling domain of this receptor has Tyrosine residues. Tyrosine is common target for phosphorylation.

The Pathway



- Binding of the ligand leads to conformational change, which results with monomers dimerization. Dimerization is a hallmark of this pathway that can be noted in many levels of the pathway. Binding has two forms:
  1- One ligand binds to a monomer and another binds to another monomer. Conformational changes of the monomers leads to their dimerization. So 2 ligands bind here.
  2- One ligand binds to a dimer receptor (ex. Insulin receptor).
- Dimerization is not enough for activation. Dimerization induces a conformational change that leads to auto- and cross-phosphorylation of the Tyrosine residues in the coupling domain, and thus fully activating the receptor. Notice that the monomers phosphorylate themselves and each other. Remember that the receptor is itself an enzyme and so perform a kinase activity when activated.
- This receptor is distinct in that it has its second receptor (tyrosine kinase) within itself. So, it does not need a second messenger system.
- Target activities may be alterations in membrane transport of ions & amino acids & the transcription of certain genes; Phospholipase C is one of the targets. Insulin-sensitive protein kinase: activates protein phosphatase 1.

  activation of an originally dimerized receptor (ex. Insulin receptor) is similar to the activation of the monomer receptor, and involves:

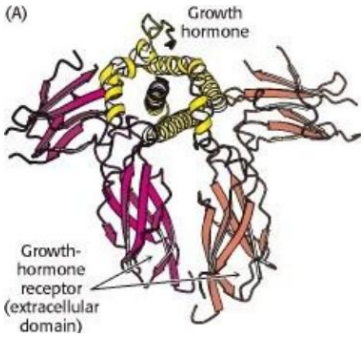
  Binding – conformational change – activation – tyrosine residues phosphorylation – kinase activity

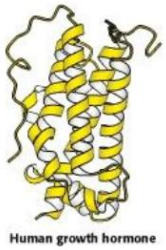
NOTE: although subtle, conformational changes allow the functionality of the proteins to take place.

NOTE: the monomers before dimerization are not so moving, they rather stand facing each other, and when they bind a ligand, the resultant conformational change allows them to get dimerized.

Growth Hormone & GH receptor

- GH:
  - Monomeric Protein
  - 217 Amino Acids
  - Compact Four-helix Bundle
- GH receptor (cooperative binding)
  - 638 A.Acid
  - Extracellular Domain (≈250 A.A) & Intracellular Domain (≈350 A.A)
  - Single Membrane-Spanning Helix
  - Monomeric (free) vs. Dimeric (bound)

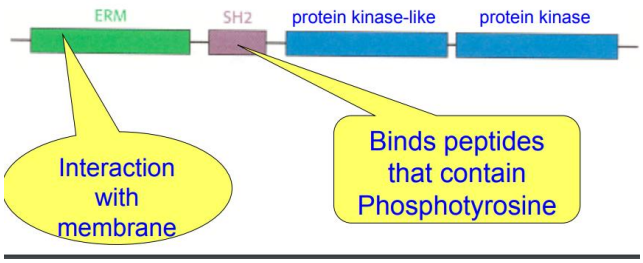



Human growth hormone

Janus Kinase

With each monomer, a Janus kinase, or JAK, is bound. Janus is a Greek god that has two identical faces, and this is how JAK is bound to the monomers that get dimerized. JAK also has Tyrosine residues.

JAK kinase domains



JAK kinase domains include:

- Membrane binding domain (to be close to the receptor)
- Kinase domain
- SH2 domain (discussed below)

Dimerization of the receptor monomers allows JAK kinases of each monomer to get closer. This will lead to a conformational change. After that, auto- and cross-phosphorylation occurs between the two JAK kinases, resulting with their activation. Activated JAK kinases phosphorylate target molecules, and STAT, or *SignalTransducer&Activators ofTranscription*, is the most common one. STAT leads to transcription activation, having the DNA as its final target.

STAT phosphorylation leads to dimerization of STAT molecules. *How is that?*

STAT is phosphorylated on a Tyrosine residue nearthe carboxyl terminus. Phosphorylated Tyrosine binds to SH2 domain of anotherSTAT 5 molecule. *But what is SH2 domain already?*

Src Homology 2; SH2

SH2 domain is a phosphorylated Tyrosine binding domain. It is present in JAK kinase as well as STAT molecules.

After that, the resultant STAT dimer heads towards its final target, the DNA, to activate transcription.

Note that JAK/STAT pathway is an example of the pathways tgat follow the binding of a ligand to a receptor tyrosine kinase.

Examples on Receptor Tyrosine Kinases

Epidermal Growth Factor Receptor :♣Monomeric (inactive) ♣ EGF binding

⎩Dimerization ⎩Cross Phosphorylation ⎩Activation

Insulin Receptor:♣ Tetramer (2 α ; 2 β), dimer (2 αβ pairs) ♣ Disulfide bridges

Insulin Binding →Activation of the Kinase

RAS Protein

RAS protein is a monomeric G protein. It works in a similar manner to α-subunit of G proteins. So, its activation includes:

Ligand binding leads to receptor activation – RAS conformational change – GDP for GTP replacement – activation – activates another effector protein

Like G protein α-subunit, RAS protein also has a slow GTPase activity that leads to GTP for GDP replacement and signal termination.

RAS includes several groups or subfamilies.

RAS has a major role in growth, differentiation, cellular transport, motility etc…

Mammalian cells contain 3 Ras proteins.

Mutation of RAS GTPase domain: Loss of ability to hydrolyze GTPRas is locked in "ON" position. Continuous stimulation of growth.

- In mammals, three known types of RAS proteins are known, which are known to be mutated. If RAS (regardless of type) is mutated, this might affect its GTPase activity, so RAS is locked in GTP-bound form and it remains active. This might lead to cancer.

Eicosanoids

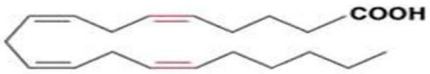
- ✓ They are examples of paracrine and autocrine hormones. They are small molecules which are derived of fatty acids in their nature. They do not go through the blood to far destinations (act locally). They are very potent, have short half lives and are not stored.

- ✓ Mainly include: Prostaglandins (PGs), Thromboxanes (TXs), Leukotrienes (LTs). All these are derived from one parent molecule called Arachidonic acid (fatty acid).

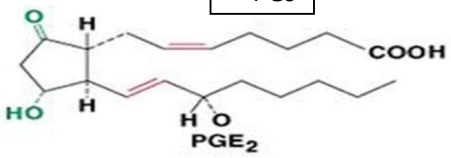
- ✓ This fatty acid is present in the plasma membrane's phospholipids. It is always bound to carbon number 2 of glycerol, and is released by the action of Phospholipase A2, which breaks it from glycerol. Then, it undergoes modifications in several reactions to produce either one of the above classes.

- ✓ Eicosanoids (Eicosa=20): group of molecules, each consists of 20 carbon unit. How to differentiate between them?

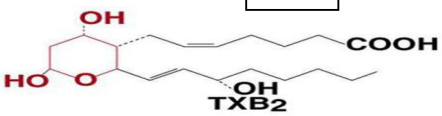
Arachidonic acid



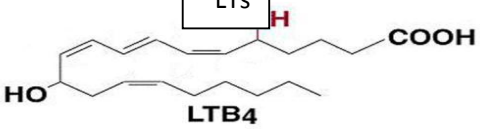
PGs



TXs



LTs



→Arachidonic acid is a 20 carbon unit molecule, doesn't contain rings, has 4 double bonds.

→Prostaglandins, 20 carbon unit molecules, all have five-membered ring

→Thromboxanes, 20 carbon unit molecules, all have six-membered ring.

→Leukotrienes, 20 carbon unit molecules, have at least 3 conjugated double bonds, meaning they alternate: double—single—double—single—double—single, don't have ring.

- ✓ There are many Prostaglandins, Thromboxanes, and Leukotrienes. They have diverse functions and may be opposite to one another. Some of them promote platelet aggregation; some inhibit platelet aggregation, some cause vasodilation while others cause vasoconstriction, some cause bronchodilation while others promote bronchoconstriction but all act locally. The dominating function is determined by the signal coming to that area and according to what is being secreted, the effect appears.

- ✓ Some of their functions (mainly physiology):

PGI2, PGE2, PGD2 :
Increase Vasodilation, cAMP
Decrease
- **▪Platelet Agregation**
- **▪Lymphocyte Migration**
- **▪Leucocyte Aggregation**

PGF2α Increases
***Vasoconstriction**
***Bronchoconstriction**
***Smooth Muscle Contraction**

Thromboxane Increases
***Vasoconstriction**
*** Platelet Aggregation**
***Lymphocyte Proliferation**
***Bronchoconstriction**

- ✓ The rate limiting step in Eicosanoid synthesis is the release of Arachidonic acid from glycerophospholipids by Phospholipase A2. Now, cyclooxygenases convert it to PGs and TXs, while lipoxygenases convert it to LTs.

Synthesis and degradation of Hormones

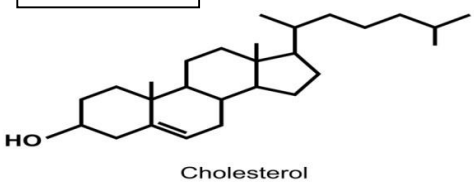
- Hormones are classified according to mechanism of action into 2 groups: some bind on receptors outside the cell (membrane), some bind intracellular receptors.

  **Hormones binding intracellular receptors:

- **Steroids**: all steroids are synthesized from cholesterol, which contains 27 carbons. All steroids have the four sterane rings, accounting for 17 carbons. These 17-carbon rings are not metabolized/cleaved in the human body. Rather, the rings are conjugated to something else for excretion (mainly with bile products, small amount in the urine). What we can actually metabolize is what is attached to the ring (we can increase or decrease the number of carbons to produce different steroids). We have 18-carbon unit steroids, 19-carbon unit steroids, 21-carbon unit steroids….until we reach the parent (cholesterol) with 27-carbons.

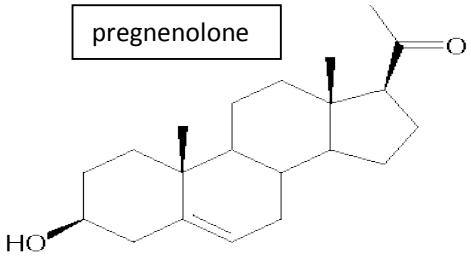
  **You must know these 18,19,21 carbon steroids. To count quickly, it is a given that the rings have 17 carbons, just count what is extra.

Cholesterol



Cholesterol

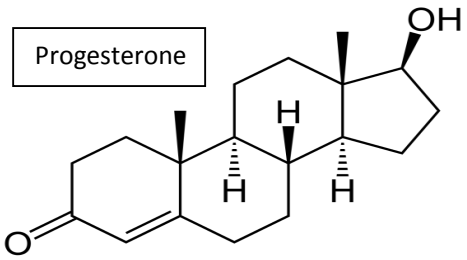
pregnenolone



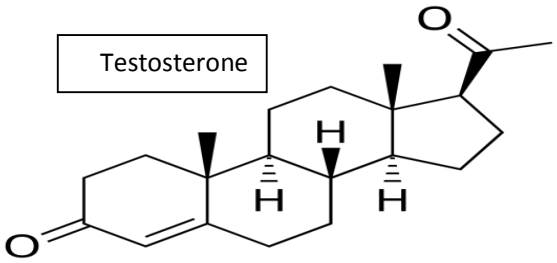
→**Pregnenolone**, 21-carbon steroid, is a parent molecule of sex hormones.

By further modification on it, like desaturation, we get the 21-carbon steroid of **Progesterone** (parent for others like Aldosterone and cortisol, both have 21Carbons)

Progesterone

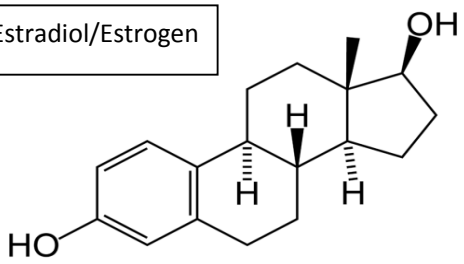


Testosterone



Removal of 2 carbons from progesterone (the acetyl group) produces the 19-carbon steroid **Testosterone.** If testosterone loses one carbon, Estrogen is produced (18 carbons). This step (Testosterone→Estrogen) is catalyzed by Aromatase.

Estradiol/Estrogen



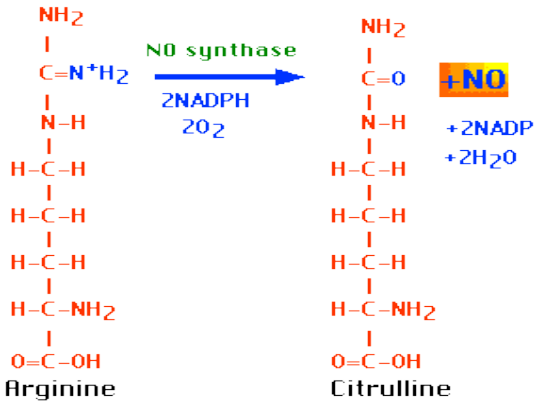
Side Note: Aromatase enzyme is affected by some pesticides. This may pose a problem for farmers by affecting male/female characteristics, and may also affect the ability to have children

- **Small molecules – Nitric oxide (NO):** it works locally (paracrine hormone). NO is made by Nitric Oxide Synthase (NOS), which has different isozymes in different tissues: it is present in neurons (neurotransmission), macrophages (kills bacteria), and most importantly in smooth muscles of blood vessels. NO is a local vasodilator.

  →Clinical application: Nitrates, due to their vasodilatory action, are used to treat conditions resulting from vasoconstriction (leading to decreased blood flow to organs and damage). A famous example is the use of nitroglycerin pills (sublingual pills) to treat Angina Pectoris (Chest-pain causing disease, due to decreased blood flow to the heart, major cause is obstruction/ constriction of coronary vessels).

  →Septic shock: due to presence of bacterial toxins in the circulation. These toxins interfere with this pathway, causing huge NO synthesis, and extreme vasodilation→ severe hypotension, might cause coma.

*NOS:



- **Thyroid hormones:** tyrosine molecule attached to a phenol (benzene ring and OH). Depending on how many iodines are added, we get T3 or T4.

Hormones with receptors outside the cell:

- **Catecholamines:** group of molecules, all contain a catechol ring present on Tyrosine, they also have an amino group in their backbone. They include epinephrine, norepinephrine and dopamine.

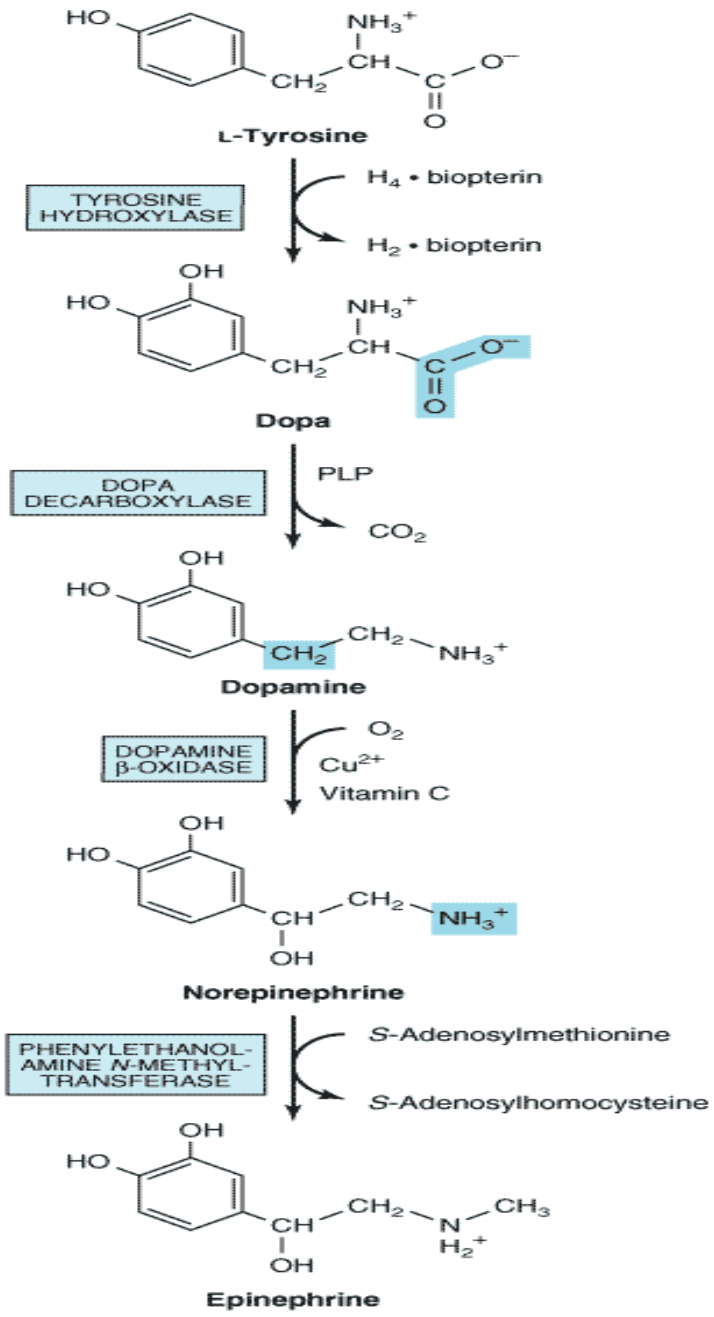
  *Their synthesis is important and required:

  1. First we have Phenylalanine (essential amino acid which is the base for synthesis of all catecholamines). This amino acid is hydroxylated by Phenyalanine hydroxylase, the deficiency of which causes Phenylketonuria disease (PKU), and this step yields Tyrosine.

  2. Tyrosine is also hydroxylated by Tyrosine hydroxylase (also called Tyrosinase) to yield DihydroxyPhenylAlanine (DOPA). This enzyme deficiency results in variable degrees of Albinism, because it is involved in Melanin biosynthesis.

  3. DOPA is decarboxylated by removing the –COOH from the amino acid backbone to yield Dopamine.

  4. Hydroxylation of dopamine to yield Norepinephrine. Lastly, methylation of norepinephrine to produce epinephrine.



****_The degradation of Catecholamines_**: It is done through 2 pathways: either we start with the ring, or we start with the backbone. If we start with the backbone, we remove the amino group from the backbone through an oxidation process, and the enzymes which remove the amino group oxidatively are called monoamine oxidases (MAO). MAO inhibitors are used in psychiatric medicine as Anti-depressent, by preventing degradation of Catecholamines. The other pathway (start with the catechol ring) is by transferring a methyl group to one of the OHs present on the ring. This step is done by Catechol-O-methyl tranferases (COMT). Thus, catecholamines lose their activity. COMT inhibitors maybe used therapeutically.

- **Proteins and peptide hormones:** we previously discussed their synthesis. We have more than one way:

  1- synthesis of one major very long polypeptide chain, then fractionate it to more than one hormone (POMC→ACTH,MSH,Endorphines).

  2-Synthesis of one big immature protein, then cleave to get mature hormone (preproinsulin→proinsulin→insulin).

  3-Parent gene like neurophysin present in posterior pituitary gland, to which certain codons are attached, which make Oxytocin in one place and Vasopressin in another.

  **Degradation of protein hormones: Proteins generally are degraded as follows:

  I. If the protein is outside the cell ***(this applies to protein hormones)***, it undergoes endocytosis→vesicle→fuse with lysosomes→degradation.
  This is the energy-independent pathway (no need for energy)

  II. If the protein is inside the cell, it's degraded by the energy dependent Ubiquitin-proteasomal pathway. But this ***does not apply to protein hormones***.
  **Some protein hormones are excreted in the urine or broken down in blood.

Good luck