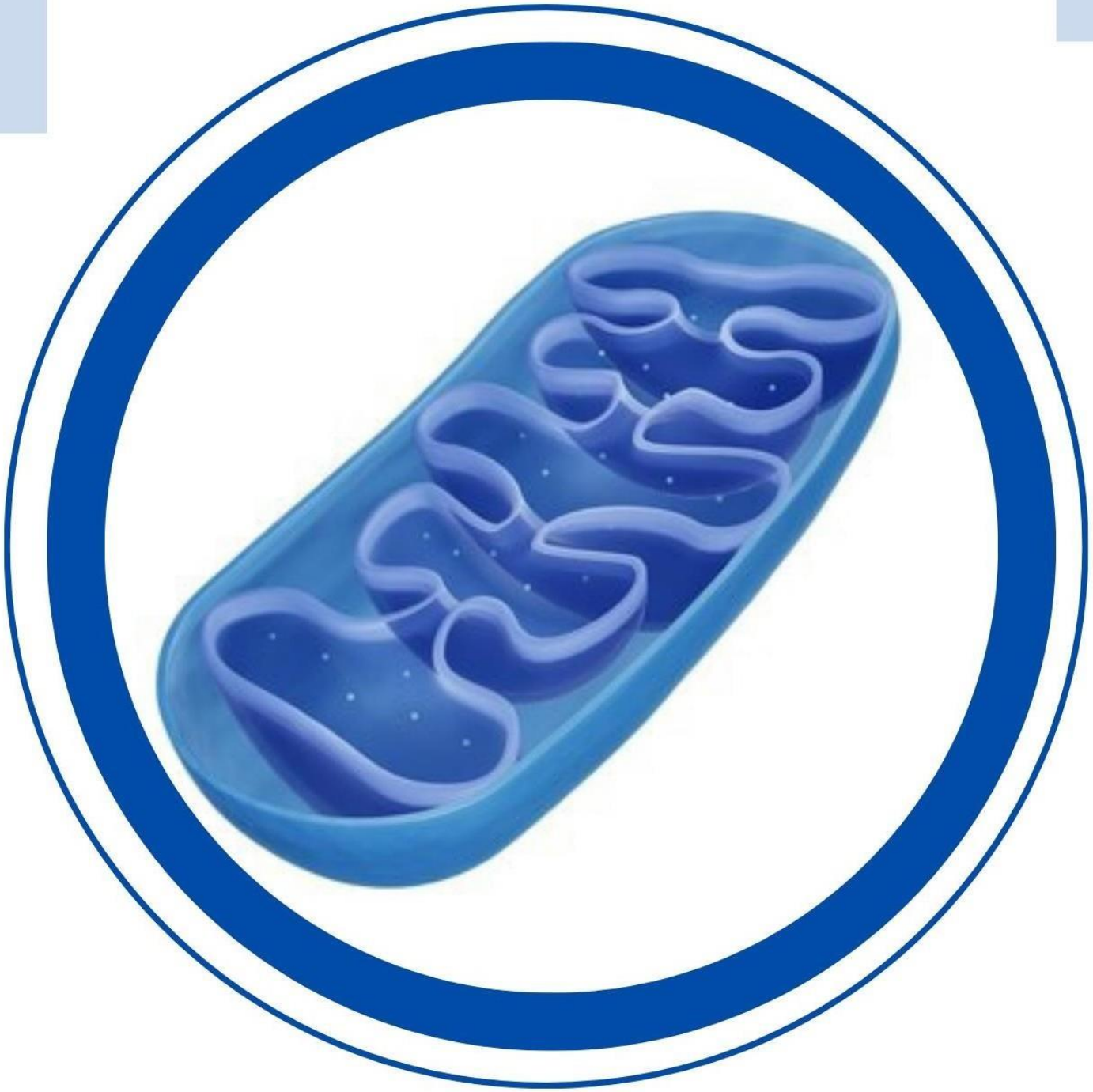
ENDOCRINE
METABOLISM

 # 2



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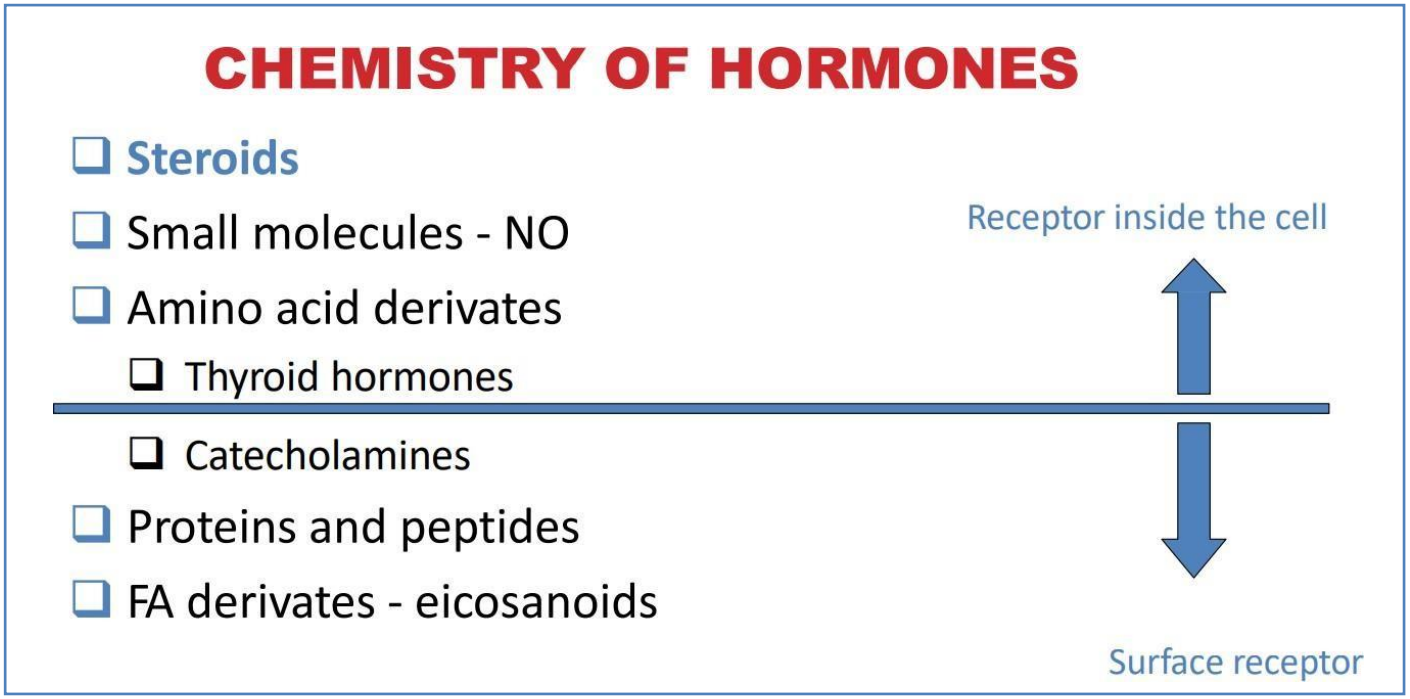
7.SYNTHESIS AND DEGRADATION OF HORMONES ACCORDING TO THEIR CHEMISTRY

- Hormones can have their receptors either outside or inside the cell and what determines the location of a hormone's receptor is its chemistry.



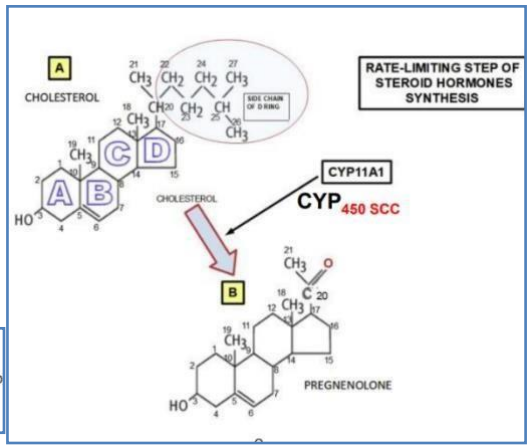
7.1 STEROIDS

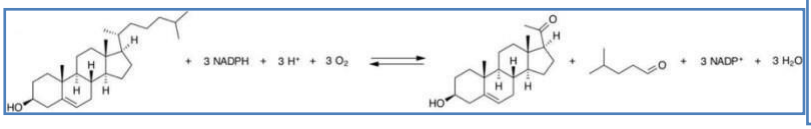
- All steroidal hormones are derived from cholesterol; regardless of their action and their nature.
- Cholesterol is a 27 carbon unit molecule that is synthesized inside the body; mainly in the liver.
- General structure of cholesterol: a **sterane ring** (composed of 4 small rings that have 17 carbons in total) then this ring is modified by adding two methyl groups (C 18 and 19) and then we add an isoprenoid chain to make it a 27-carbon molecule.
- Steroidal hormones are formed by the modification of the attributes of this sterane ring.
- **Synthesized in the mitochondria and SER of respective tissues.**
- **Specific enzymes involved (CYP450 SCC).**
- **Sterane core cannot be cleaved.**
- Once the sterane ring is formed, it cannot be degraded inside the body.
- So, how do we get rid of these synthesized hormones?

- **In the liver: hydroxylation and conjugation with glucuronides or sulphates** (to increase their solubility)**.**
- **Urinary excretion:**
  - **Of unchanged hormones.**
  - **Of metabolites.**

The rate limiting step in the conversion of cholesterol into the parent molecule that steroidal hormones are derived from is the action of the enzyme **CYP450 SCC** (*SCC: side chain cleavage).*

- This enzyme moves out 6 carbons from the side chain and leaves us with a 21-carbon molecule which is the **pregnenolone** (The parent molecule of steroidal hormones).
- This enzyme is given to pregnant woman to mediate the production of progesterone.

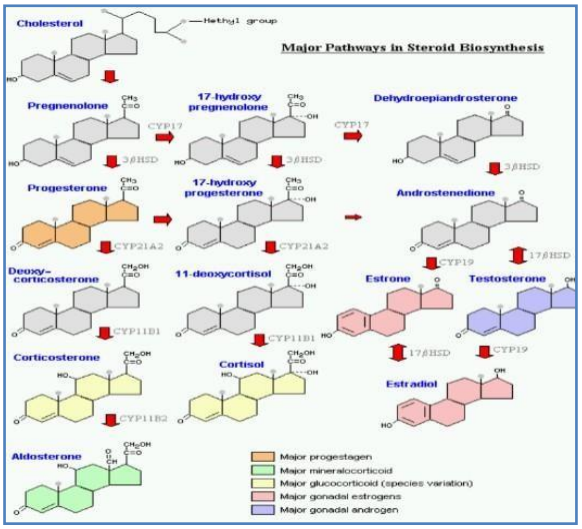




- We classify steroidal hormones according to their carbons' number.
- **C21:**
  - **Progesterone: directly from pregnenolone.**
  - **Cortisol & Aldosterone: from progesterone.**
- **C19:**
  - **Testosterone: From progesterone** (modified pregnenolone) **or pregnenolone; 2 carbons shortage.**
  - From Testosterone we can derive the main estrogen which is estradiol (18 C molecule).
- **C18 (estrogen):**
  - **Aromatase** (catalyzes the conversion of testosterone into estradiol)**, Cleaves C18, Reduction reaction** (converts the keto group into an alcoholic group)**.**
  - Again, it is only one carbon unit that makes a female "female", and a male "male" by the effects of estrogen and testosterone, which tells you more about the high specificity of hormones.

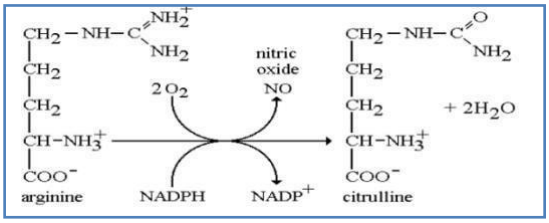
- Glyphosate is the most widely used insecticide to control weed growth, but it also can kill the plants we actually need, so in order to resist the insecticides; we modify plants genetically.
- Another problem we face when we use glyphosate is that it inhibits the aromatase enzyme (inhibits the conversion of testosterone to estrogen) and this might lead to sexual problems in farmers (infertility or congenital problems in their offspring). However, this topic is still under research.

-All what you need to know is the first step (rate-limiting), classification of hormones (C21, C19, C18) and details mentioned in the sheet only.



7.2 SMALL MOLECULES – NO

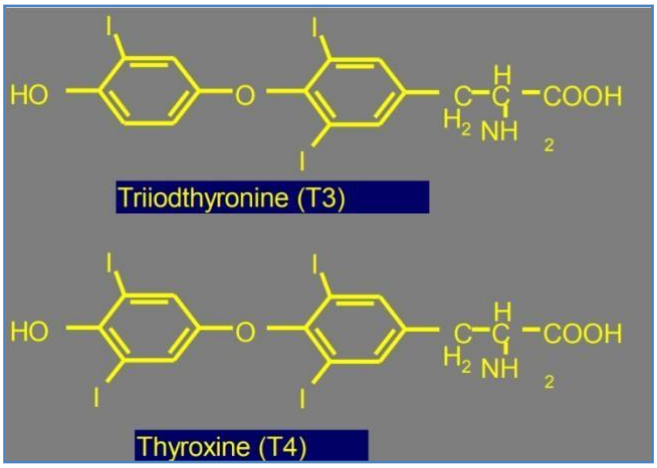
- It's a small molecule that has an intracellular receptor and it uses cGMP as a second messenger.
- **NO-synthase (NOS) isozymes**-the enzyme that catalyzes the reaction-**:**
- **In neurons (NOS-I): neurotransmission**
- **In macrophages (NOS-II): kills bacteria**
- **Endothelial (NOS-III): smooth muscle → cGMP→ vasodilation**-our interest-
- This reaction is NADPH-dependent, its substrate is arginine and its product is citrulline (basic amino acid).
- **Clinical correlation:**
  - **Nitrates in the treatment of angina.**
  - Nitrates cause the relaxation of smooth muscles, which accordingly leads to the widening of blood vessels.
  - **Refractory hypotension during septic shock** (NO is secreted in high amounts during septic shock causing refractory hypotension)**.**



- Trinitroglycerin is given to angina patients as a sublingual pill; it induces the widening of blood vessels and relieves angina momentarily until the patient seeks medical help.

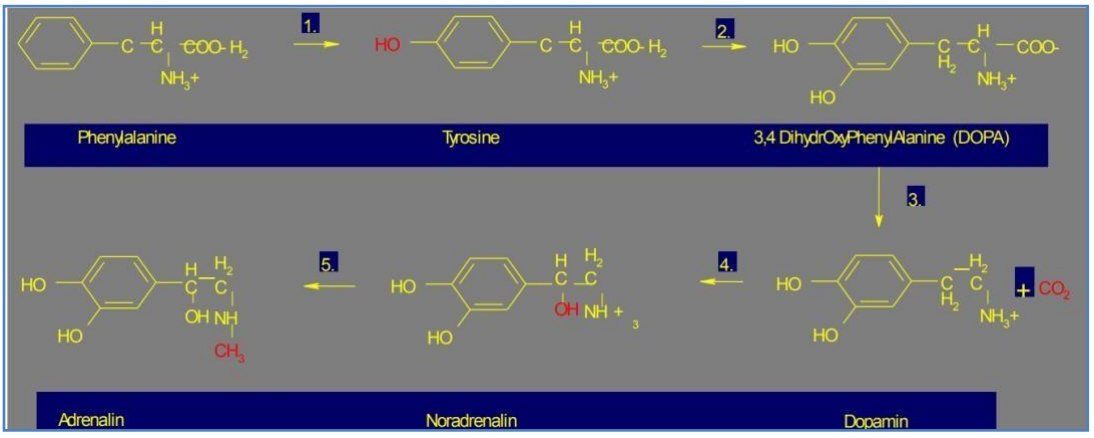
7.3 THYROID HORMONES

- Amino acid derived hormones.
- They are tyrosine based.
- We add a phenol group to the tyrosine molecule, and depending on the number of iodines attached to this ring, we either form T3 or T4.



7.4 CATECHOLAMINE

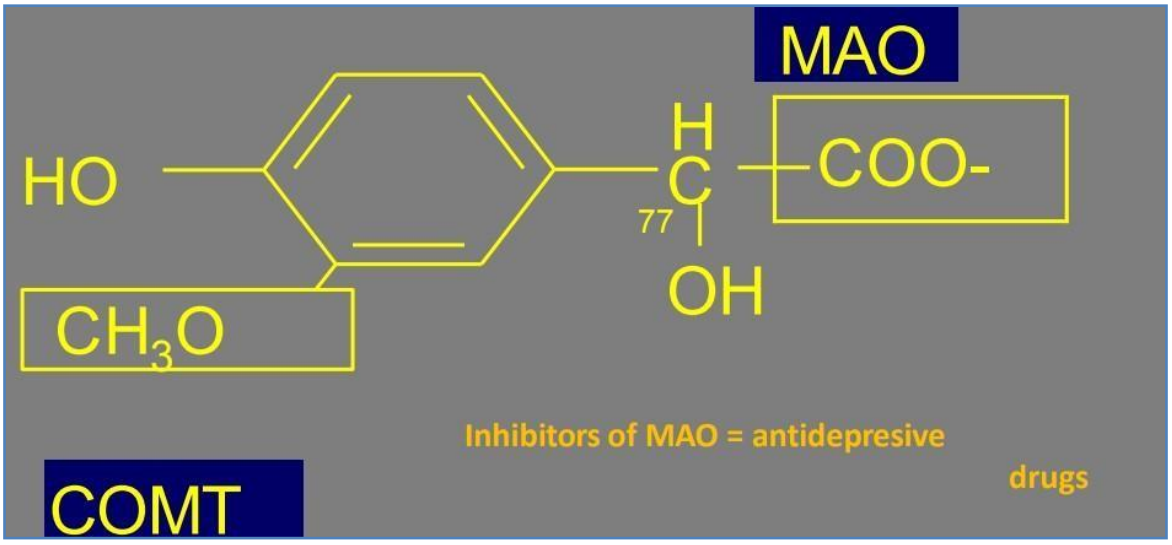
- They are Phenyalanine or tyrosine based **(Substrate = Phe or Tyr).**
- **Synthesis located in: adrenal medulla, nerve tissue.**
- Steps of synthesis:
  1. Phenylalanine, which is a hydrophobic amino acid, gets converted to tyrosine by a hydroxylation reaction.
  2. Then, tyrosine undergoes another hydroxylation reaction forming DOPA (Dihydroxyphenylalanine).
  3. Afterwards, the decarboxylation of DOPA gives Dopamine.
  4. The hydroxylation of Dopamine gives noradrenalin.
  5. Methylation of noradrenalin gives off adrenalin.



- Their receptors are extracellular.

- **Products:**
  - **Dopamine, adrenaline (hormones)**
  - **Noradrenaline (neurotransmitter)**

- We have two enzymes that help in the inactivation of monoamines:



1. MAO (monoamine oxidase): oxidative deamination reaction that removes the amino group, making the monoamines inactive.
2. COMT (Catechol O-methyltransferase): As the name implies, the action of the enzyme is transferring a methyl group to the oxygen on the catechol ring, making the molecule inactive.

- Inactivation occurs by the action of one enzyme or both.

7.5 PROTEIN AND PEPTIDE HORMONES

- They are synthesized inside cells and then secreted outside.
- The process of synthesis differs between hormones, but generally, the cells either form a large mRNA, then, by alternative splicing we get different hormones in different cells **or** the cell synthesizes a polypeptide then by cutting it off we produce different hormones in different cells **or** sometimes different genes give closely related hormones that perform different functions **or** the cell might synthesize one polypeptide and modify it differently.

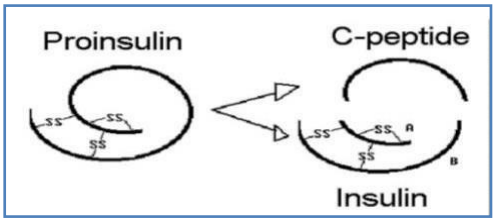
- **Examples on peptide hormones:**
  - **CNS mediators: neuropeptides, opioids.**
  - **Hypothalamic releasing hormones and pituitary peptides.**
  - **Insulin and glucagon.**

- **Growth factors: IGF, CSF, EPO.**

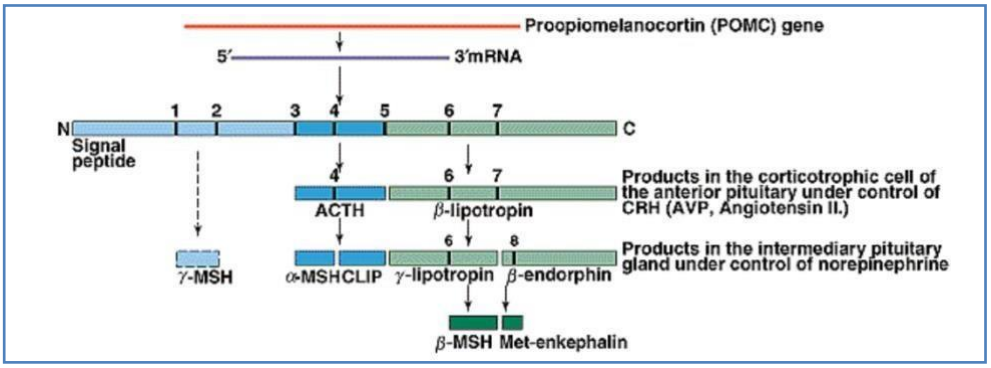
➢ GENERAL STEPS OF PEPTIDE SYNTHESIS:

1. PRECURSOR POLYPEPTIDES.

- **Expression of "pre-pro" protein.**
- **Transport to ER.**
- **Splitting the signaling sequence.**
- **Cleavage to definite peptide(s) and final modification in Golgi.**
- **Proinsulin to insulin.**
- **Proopiomelanocortin (POMC) to MSH and ACTH** (POMC splits to either one of these hormones).

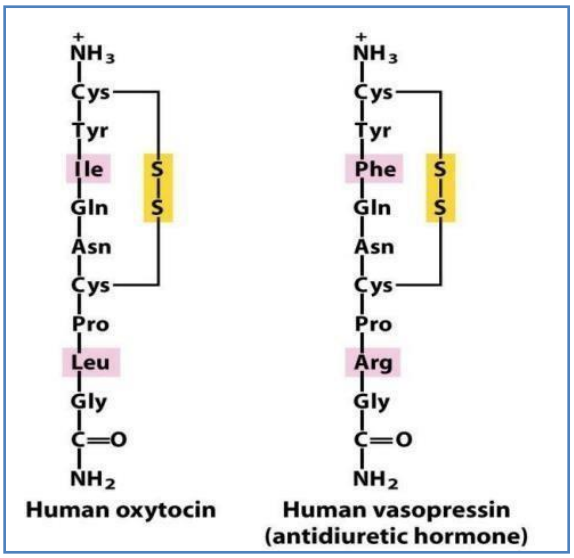


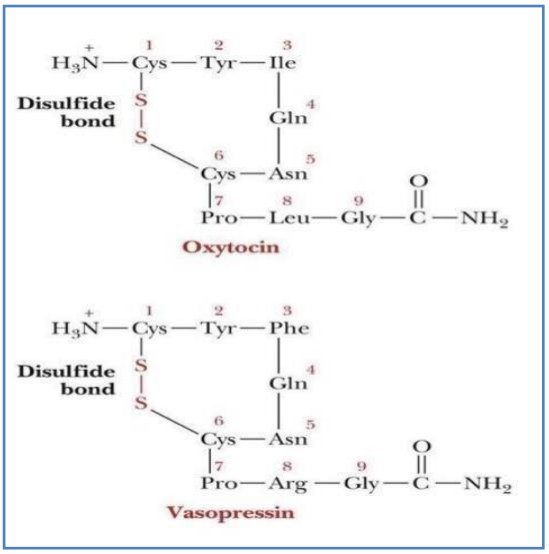
- **One gene may code more than one hormone (POMC).**
- **The cleavage depends on specific enzymes.**
- Over-activation of POMC gene causes hyperpigmentation (as an effect of MSH) and pain relief (enkephalins).



2. From precursor genes

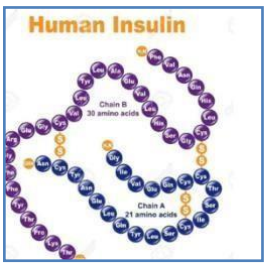
- **Vasopressin and oxytocin.**
- **Synthesis in separate cell bodies of hypothalamic neurons.**
- Very closely related genes and areas of secretion, meanwhile, they have different functions.
- Nonapeptides and they differ at position number 3 and 8 from each other while at position 1 and 6 they both have Cys that bridge themselves forming disulfide bonds creating the ring structure.

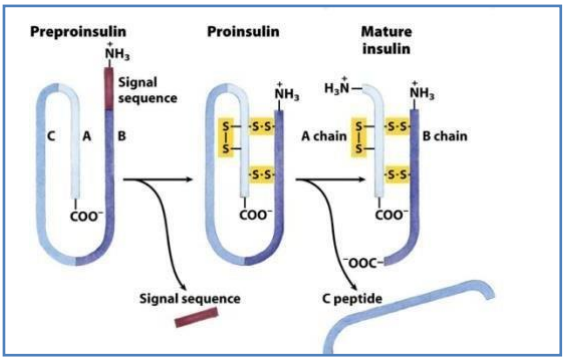




3. From Pre-pro-hormones.

- **A larger precursor pre-pro-insulin.**
  - **23 aa signal sequence**
  - **3 disulfide bonds;** 2 connecting α and β chains and the other one betweenα chain Cys.





- **Proinsulin**
  - **Remove the C peptide.**
- **Mature insulin**
  - **A and B chains**
- We can rely on the C peptide to assist the levels of insulin because every insulin molecule gives us one C peptide.

- **DEGRADATION of protein and peptide hormones:**
  1. **Lysosomal: After endocytosis of hormone-receptor complex.**
  2. **Chemical modification (liver): Rearrangement of S-S bridges, cleavage.**
  3. **Renal excretion of small peptides.**

8. SIGNAL TRANSDUCTION, CASCADES, AND RECEPTORS

- There are two types of receptors:
  - Intracellular: inside the cell, many types, hormones, domains, DNA binding, transcription related events, etc. We've already talked about it last lecture (the doctor said that he is not interested in further details).
  - Extracellular: on the cell surface, general mechanism of action that is applied to more than one hormone (unlike intracellular receptors) are our interest for this lecture.

8.1 SIGNAL TRANSDUCTION

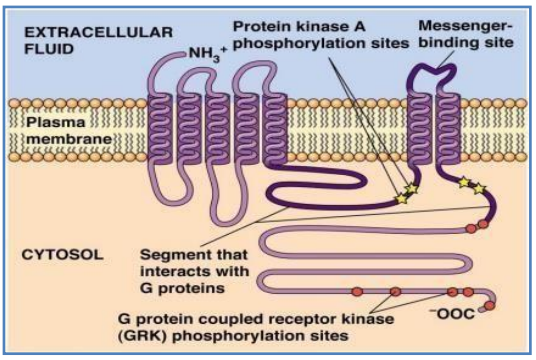
- **Transduction: conversion of one form of a signal to another so as cells can produce many kinds of responses in different ways.**
  Transmission of the signal to the inside of the cell.
- **Amplification is a MUST** for cell surface receptors, why? because:

- Hormones are available in small amounts.
- Receptors are few in number.
- If we had enough receptors and hormones, we obviously wouldn't need amplification, but why doesn't it work that way?
  - For energy conservation! There is no need to synthesize hundreds of receptors if only two receptors are enough to perform the same function.
  - However, doesn't making 2nd messengers also consume energy? Yes, but just a little; we need 2nd messengers to be small in size in order to be able to move to different targets. They should also be hydrophilic.
- Receptors' movement in the cell membrane is somewhat restricted, so molecules that can transduce the signals to anywhere inside the cell would be helpful.

- **Signal (polar, large) should bind receptors:**
  - **Intrinsic in** the membrane -or integral- but **not peripheral!**
  - **Transmembrane** so that the hormone binds from outside and the receptor convey its message inside the cell.
  - **Intra- & extracellular domains**
  - That have affinity for hormones
  - That are proteins
  - Aren't too rigid and able to change conformations easily

8.1 RECEPTOR DOMAINS

- **All receptors have at least two functional domains:**
  - **Recognition domain:** that recognize the structure of the hormone -hormone or ligand binding domain-.
  - **Coupling or signal transduction domain:** it couples or transduce the messages inside the cell.



- **Coupling occurs in two general ways:**
  - **Changing the activity of an enzyme (Polypeptide & catecholamines, plasma membrane),** or by the production of second messengers.
  - **Direct (steroids, retinoids, and thyroid hormones, intracellular)**
- **Steroid, thyroid, and retinoid hormone receptors:** intracellular receptors
- **Hormone binding site; DNA binding site; co-regulator proteins binding site** -repressors and activators-, **cellular trafficking proteins binding site**, dimerization domain, HSP binding domain, etc... So, they are higher in number compared to extracellular receptors which mainly involve 1) membrane domain, 2) extracellular/ligand binding/hormone binding/recognition and 3) intracellular/signal transduction/coupling domain. There are also G protein binding domains and catalytic domains.
- **Receptor–effector coupling provides the first step in amplification**

8.1 THE NEED FOR 2ND MESSENGER

- **Is that enough?**
  - **Few in number**
  - **Restricted movement**

8.1 2ND MESSENGERS

- **Ability to diffuse to other cellular compartments,** so they have to be hydrophilic.

- **Amplification of the signal**
    - **Enzyme activation**
    - **Membrane channels**
- **Some second messengers are common in multiple signaling pathways (≈ 30 hormones uses cAMP!!!)**
    - **Permits fine tuning but can pose problems**
- **Types of 2nd messengers:**
    - **Small molecules: cAMP, cGMP, Ca+2**
    - **Phosphorylation through kinases**

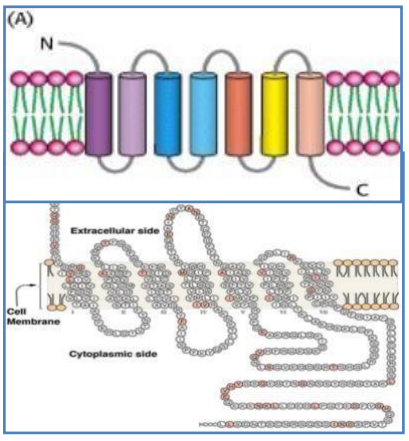
8.1 SIGNAL TERMINATION

- **Is it important?** Yes, it is actually a must; because the signaling cascade is very huge and it has to be terminated very fast
    - **Keeps cells responsive to new signals**
    - **Failure of termination may cause problem e.g GH & cancer**- caused by very high proliferation and activity especially in DNA binding structures whether they are intracellular receptors or extracellular ones that can affect transcription factors-.
- **How is it achieved?**
    - **Degradation of the second messenger**
    - **Dephosphorylation by hydrolysis**

8.1 TYPES OF MEMBRANE ASSOCIATED RECEPTORS

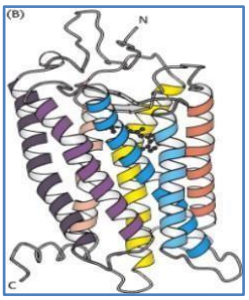
- **7-transmembrane helix receptor**
    - **G-protein coupled (cAMP, Phospholipase C, Ion channels)**
- **Tyrosine kinase receptor**

8.1 7-TRANSMEMBRANE HELIX RECEPTOR



- **7 α-helices: H-bonding, rigid, hydrophobic** so it can be inserted in the membrane, and fit there well. However, the extracellular and the intracellular domains must be hydrophilic.
- **Signal induces conformational changes**
- **Is it enough?**

- **Many Ser & Thr residues** in the intracellular domain, which are sites for phosphorylation. This fact can be used as a base for a mechanism of signal termination.
- They found that these receptors are all bound to G proteins intracellularly, so they called them GPCRs.



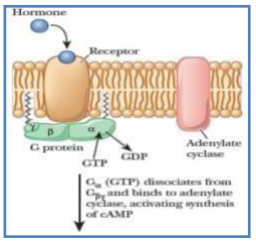
Rhodopsin receptor

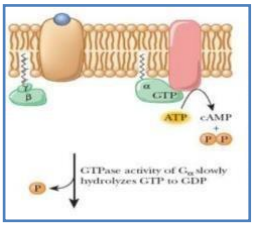
8.2 BIOLOGICAL FUNCTIONS MEDIATED

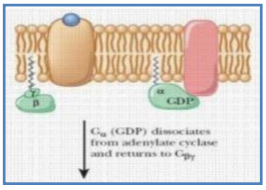
- **Smell, Taste, Vision**
- **Neurotransmission**
- **Hormone Secretion**
- **Chemotaxis**
- **Exocytosis**
- **Cell Growth, Development**
- **Viral Infection**
- **All these receptors share the same basic structure; however, they differ in their specificity and effects**
- Bottom line: GPCR are found in most cell types and have various actions.

8.2. A. G-PROTEIN COUPLED

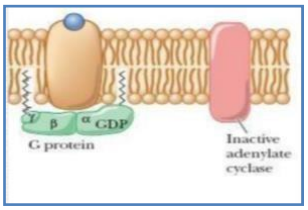
- It is a heterotrimeric protein, composed of alpha α (alone), and beta β and gamma γ subunits (as dimers).
- The α subunit is attached to the membrane by fatty acids.
- The β and γ subunits form a dimer to which the α subunit will be attached.
- The γ subunit - only from the dimer- is attached to the membrane by fatty acids.
- All the subunits are attached to the 7-transmembrane helix receptor.
- Binding of the hormone causes conformational changes; these changes will affect the G-proteins subunits by reducing the affinity for GDP and exchange it with a GTP molecule that has high affinity.



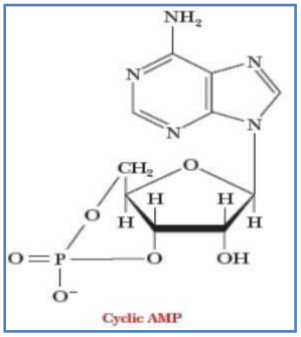




- This exchange will cause another conformational change causing the dissociation of the α subunit from the β and γ dimer due to decreased affinity between them, accordingly, the α subunit can move.



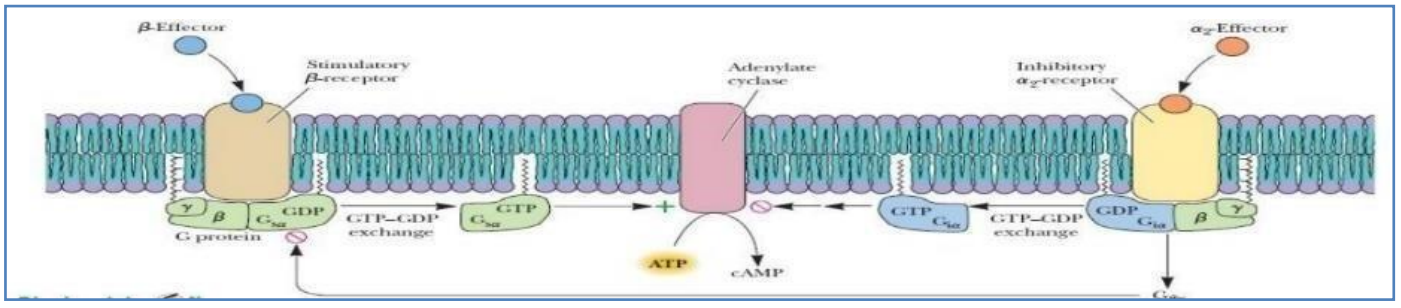
- Note that the α subunit can move freely in the membrane -since it is attached to fatty acids that can move within the membrane- and bind to other close respective structures with high affinity.
- Once the α subunit binds to the β and γ dimer, it will become inactive by hydrolyzing the third phosphate of the GTP molecule, restoring the original affinities.
- The β and γ dimer remains attached to the receptor.
- **cAMP: small & heat stable** compared to the regular molecules which are aliphatic in their nature.



- **Plasma membrane**
- **Hormone → Specific receptor (β1- or β2-adrenergic receptor) → G protein → Adenylate cyclase → cAMP → protein kinase A → phosphorylation**

8.2. A. G-PROTEIN COUPLED

- **Stimulatory or inhibitory?** It depends on:
  - The type of the receptor since the receptor is what is going to cause the conformational changes. Some receptors are inhibitory by their nature while others are excitatory.
  - The nature of the α subunit, which might be stimulatory (Gαs), inhibitory (Gαi), specific for the phospholipase (Gαq), etc...
- **Hormone → receptor (α2-receptor) → G protein → inhibits adenylate cyclase**



8.2. B. G-PROTEINS

- **More than 100 known G protein–coupled receptors and more than 20 known G proteins** and the combination between them is what causes the variations in their response within cells.
- **Can be activated by combinations of hormones**
  - **Epinephrine & glucagon act via a stimulatory G protein in liver cells**
- **Other than cAMP:**
  - **Stimulating phospholipase C**
  - **Opening or closing membrane ion channels**

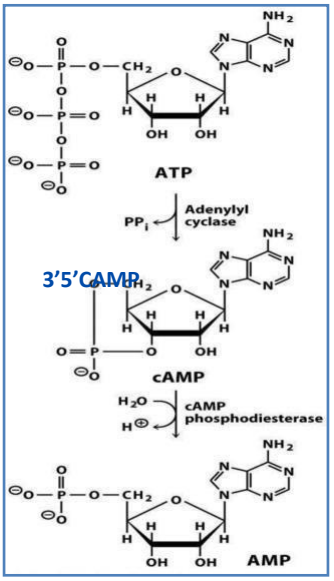
G-PROTEINS

G_α class	Initiating signal	Downstream signal
$G_{\alpha s}$	β-Adrenergic: amines, glucagon, parathyroid hormone, many others	Stimulates adenylate cyclase
$G_{\alpha i}$	Acetylcholine, α-adrenergic: amines, many neurotransmitters	Inhibits adenylate cyclase
$G_{\alpha q}$	Acetylcholine, α-adrenergic: amines, many neurotransmitters	Increases IP_3 and intracellular calcium
$G_{\alpha t}$	Photons	Stimulates cGMP phosphodiesterase
$G_{\alpha 13}$	Thrombin, other agonists	Stimulates Na^+ and H^+ exchange

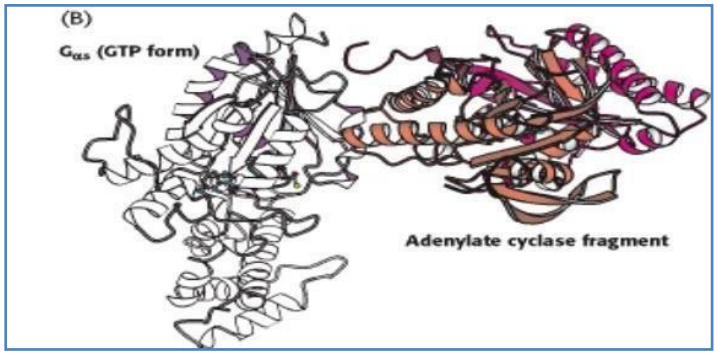
8.2. B. G-PROTEINS

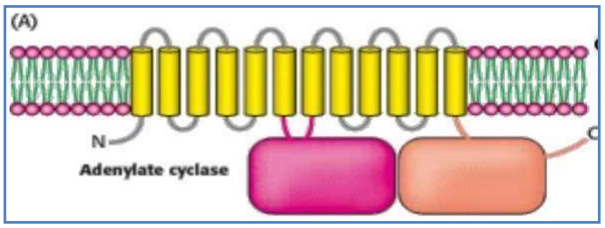
sorry we couldn't find the link of the original video, but this one is close enough.

8.2.C. ADENYLATE CYCLASE



- **Membrane protein.**
- **12 α helices.**
- **Two large intracellular** catalytic **domains.**
- **Activated by G protein**- by a signal from the α subunit-.
- It converts the ATP into cAMP. Why AMP in its cyclic form though?
  - Again, it's smaller and more stable chemically and heat wise.





8.2.C. CAMP CAN AFFECT WIDE RANGE OF PROCESSES

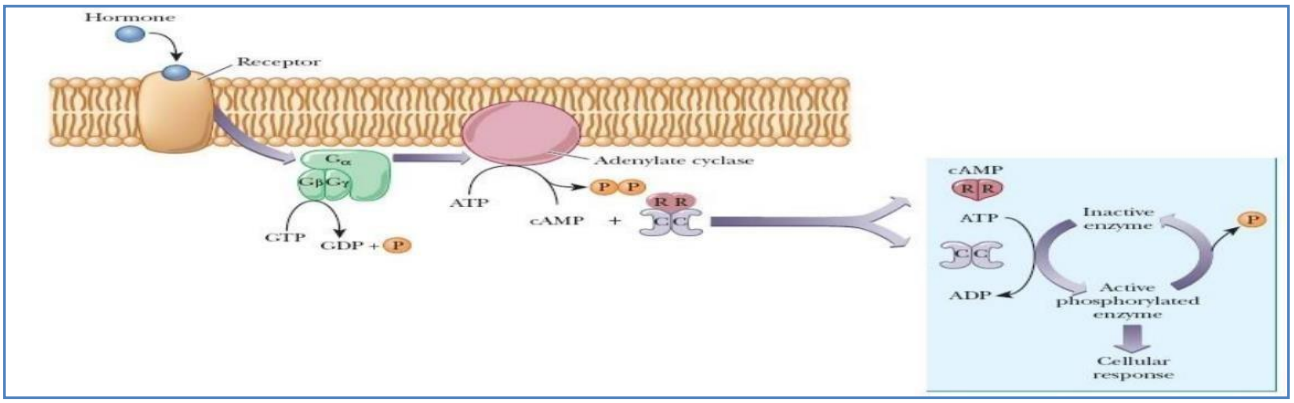
- **↑ degradation of storage fuels**
- **↑ secretion of acid by gastric mucosa from** the parietal cells **(caffeine-** which is found in coffee, Pepsi…-**:** blocks **phosphodiesterase & adenosine** receptors)
- Adenosine receptors are responsible for sleeping, that's why caffeine causes wakefulness.
- Phosphodiesterase is the enzyme that is responsible for the breakdown of cAMP; no Phosphodiesterase→ more cAMP → more acid secretion→ ulcers.
- **Dispersion of melanin pigment granules**
- **↓ aggregation of blood platelets**
- **Opening of chloride channels**

8.2.C. THEN WHAT? AMPLIFICATION

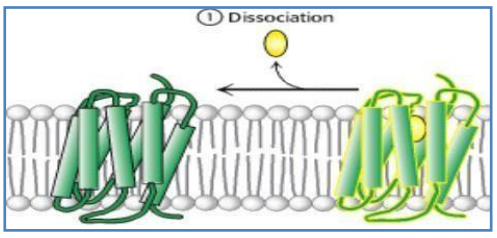
- Once cAMP is produced from adenylate cyclase, it is going to affect another enzyme, the protein kinase A.
- The PKA actually derived its name from AMP since cAMP activates it.

- PKA is a tetrameric protein with two catalytic subunits and two regulatory subunits.
- 4 cAMP molecules bind to regulatory subunits and cause dissociation of the catalytic from the regulatory subunits.
- The catalytic subunits are now active; it is a kinase, so it is going to phosphorylate a huge number of proteins and enzymes, causing their activation or inactivation and therefore changing the cellular metabolism.
- These subunits can also phosphorylate transcription factors present within the cytoplasm, activating them, then the phosphorylated transcription factorsare translocated into the nucleus to initiate the process of transcription. Therefore, not only intracellular hormones and receptors affect DNA transcription, but also extracellular receptors through activation of transcription factors in the cytoplasm.
- **Glycogen Synthase!!** (An example of a protein that is inactivated by phosphorylation in contradiction to what happens in glycogen breakdown).
- **Usually: Ser or Thr** are phosphorylated.

-For further clarification check this video.

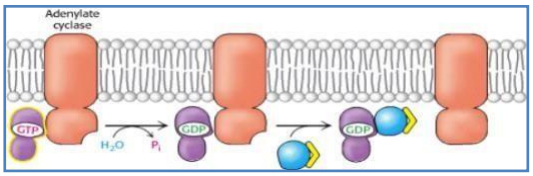


8.2.C. THEN WHAT? SWITCHING OFF

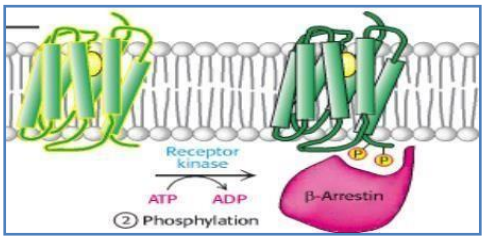


- **Dissociation of the hormone:** aided by the pulsatile secretion of the hormone - to reduce its concentration -, renal excretion.
  - Once the hormone dissociates from the receptor, conformational changes will affect the G-protein, accordingly, the cascade will cease.
- **GTPase activity of Gα subunit.**

- It is going to split the GTP molecule by hydrolyzing it, causing the subunit to become inactive and therefore it has a narrow window of activity.



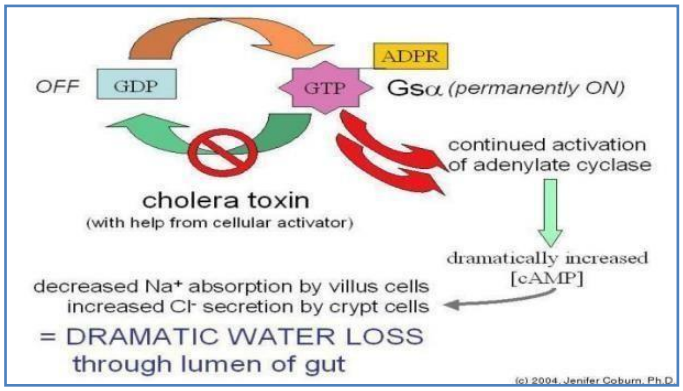
- **Hydrolysis of cAMP (phosphodiesterase** which was activated by PKA**)**
  - So PKA causes its inhibition by the hydrolysis of its activator.

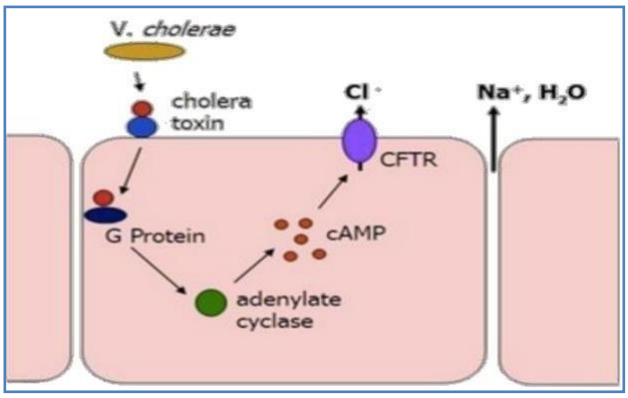


- **Phosphorylation of the hormone bound receptor** - on the Ser & Thr residues in the intracellular domain- **and followed by binding to β-Arrestin** with high affinity due to the phosphorylation.
  - β-Arrestin covers the signal transduction domain, so it won't transmit the signal to any other protein including the G-proteins.
  - Even if the hormone is bound, the signal will not be transduced.
- Desensitization (no transduction of the signal) can be caused by any ofthe formerly mentioned mechanisms.

8.2.C. CHOLERA

- It is active nowadays in many regions.
- It is associated with non-clean water.
- Vibrio cholera secrete **Cholera toxin →**binds to a 7-transmembrane helix → initiating signaling for G-proteins → activates Cl^- channels to increase the excretion of the ion, at the same time, **unregulated activity of adenylate cyclase in epithelial cells → Excessive cAMP in epithelial cells stimulates active transport of Na^+** outside the intestinal cells **→ large flow of Na+ and water from the mucosa →** Uncontrolled **diarrhea** followed by dehydration and death.
- Dehydration causes death due to electrolytes imbalance that can affect the nervous system as well as the heart resulting in arrhythmia.





PAST PAPERS:

1. **Which of the following is NOT true about cholera toxin:**
   a. Increases cAMP inside the cell
   b. Causes flow of NACL outside the cell and can lead to dehydration
   c. Associated with Tyrosine Kinase

Answer: C

2. **Which one of the following is correct about the 7TM receptors:**
   a. Dimer
   b. Can be phosphorylated on the intracellular domain
   c. Linked to tyrosine kinase activity
   d. Arrestin catalyzes the phosphorylation of the intracellular domain

Answer: B

3. **which statement of the following is incorrect regarding steroid hormone synthesis:**
   a. Oxidation of the 18-methyl group of corticosterone produces aldosterone
   b. Hydroxylation of progesterone occurs to synthesize androgens
   c. Testosterone can be produced by estrogen methylation

Answer: C

4. **One of the following isn't produced from a specific large precursor:**
   a. ACTH
   b. TSH
   c. MSH
   d. B-Endorphin
   e. Enkephalin

Answer: B

GOOD LUCK :)

V2

- **Page 4: Refractory hypotension during septic shock** (NO is secreted in high amounts during septic shock causing refractory hypotension)**.**
- **Page 5: Trinitroglycerin sublingual pills are given to angina patients (not the ones with septic shock).**