Heterotrimeric G-proteins

Proteins binding **GDP or GTP**

mostly freely membrane-bound (they can move along the inner surface of the plasma membrane).

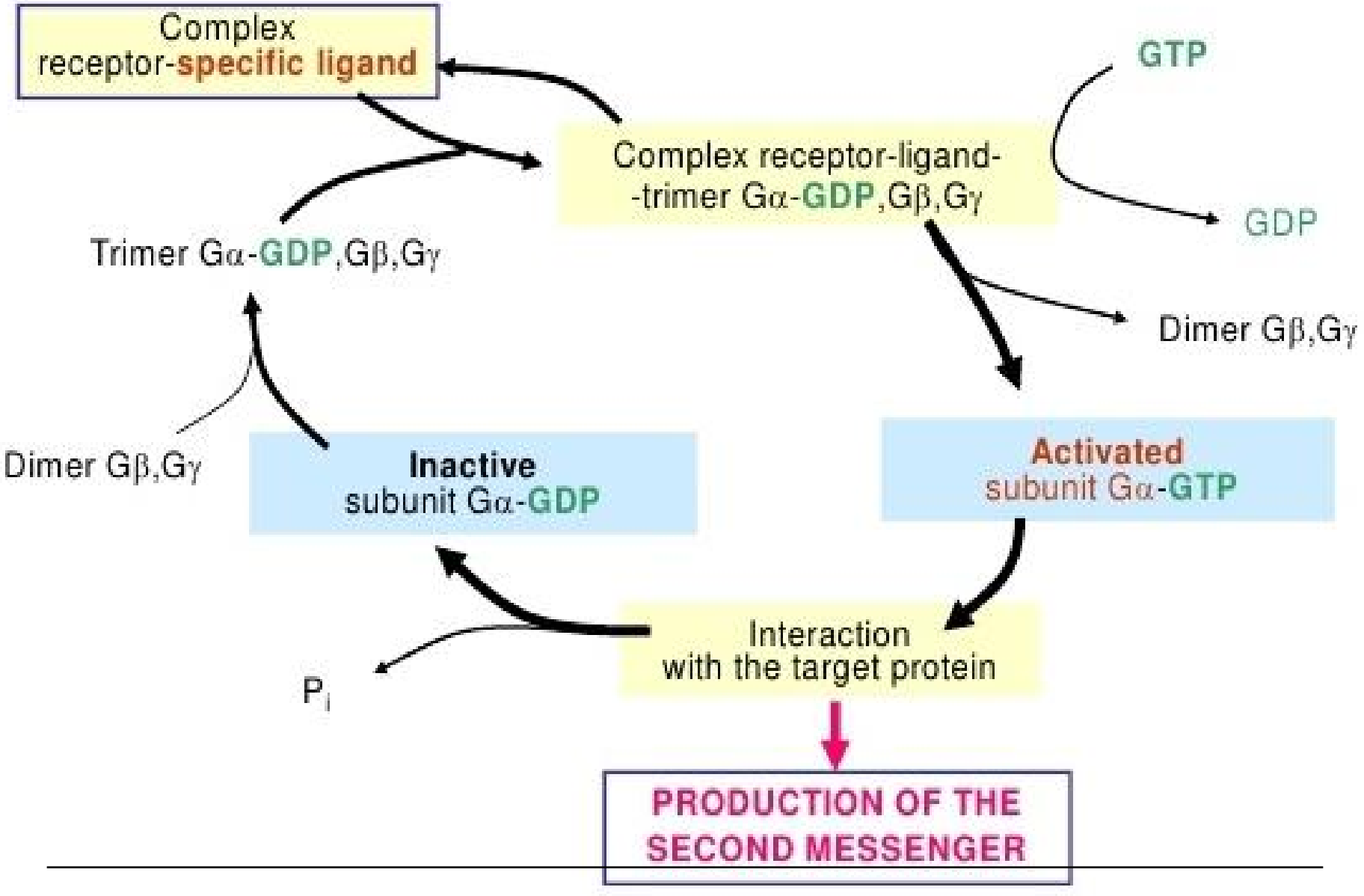
Subunits α, β a γ.

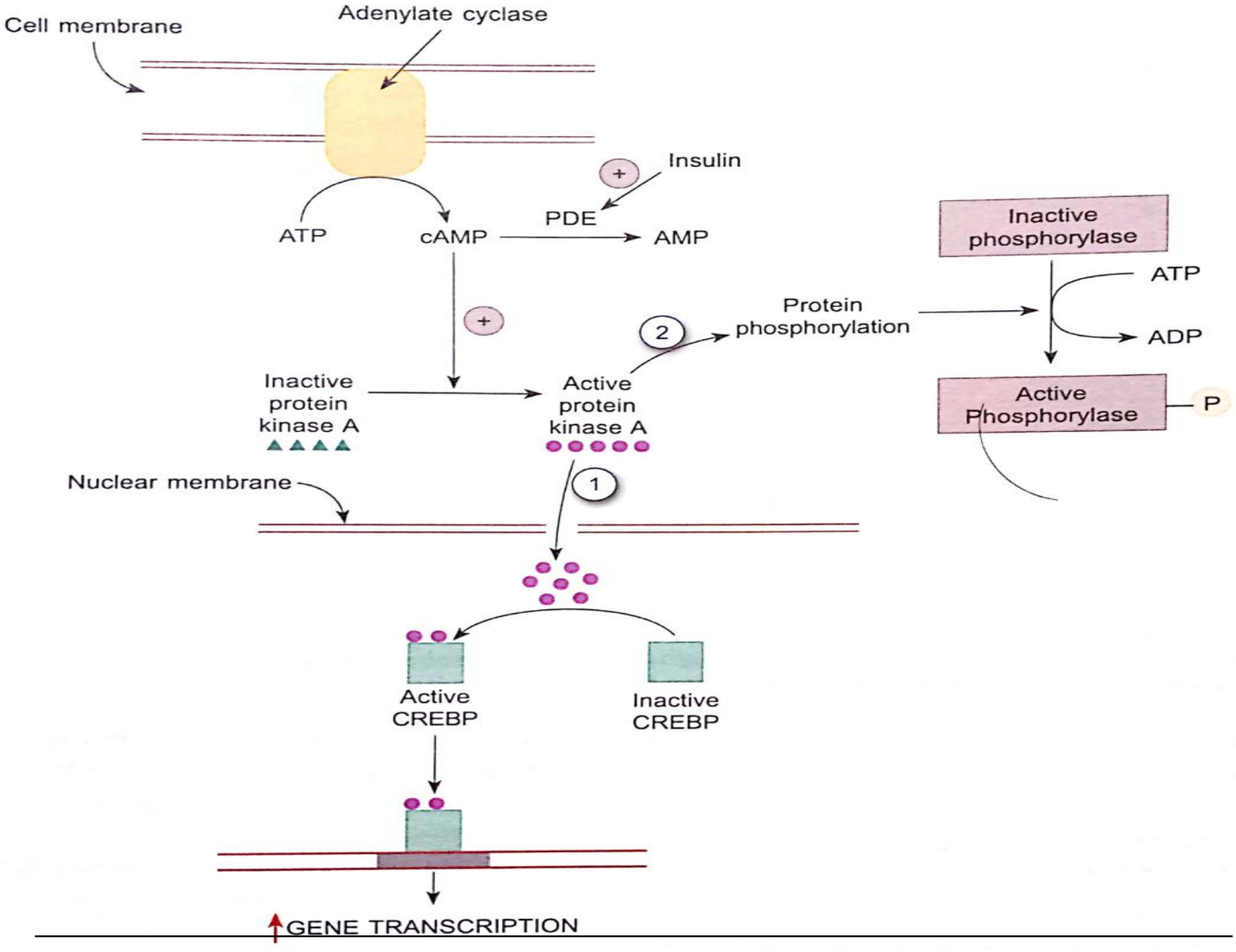
Subunits Gβ and Gγ **are** hydrophobic and non specific

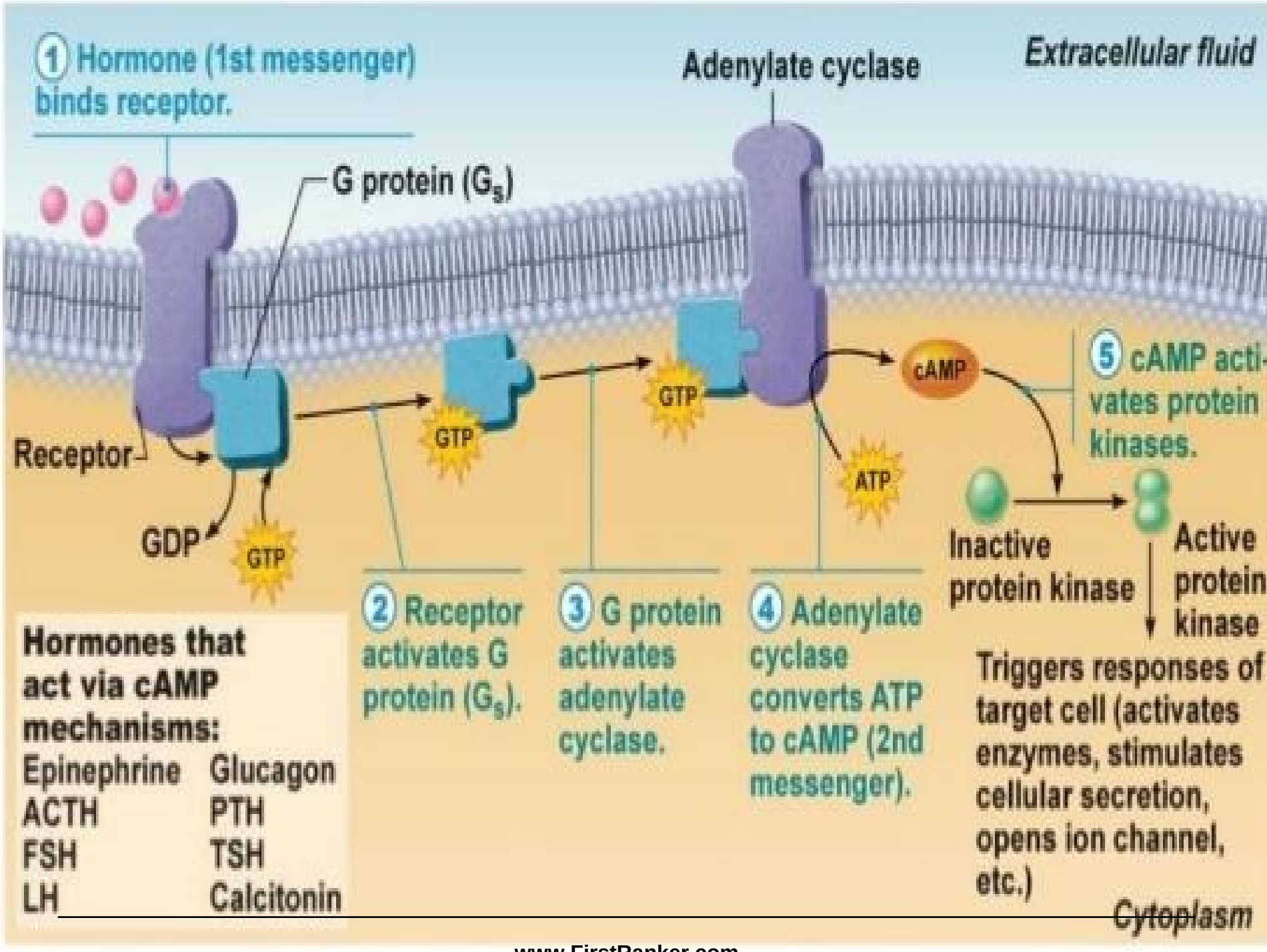
Gα subunit is the largest, hydrophilic, it binds GTP or GDP, and
It is **specific** for particular mechanism of second messenger production.

More than 20 different α subunits have been identified.

The cycle of G-proteins activation

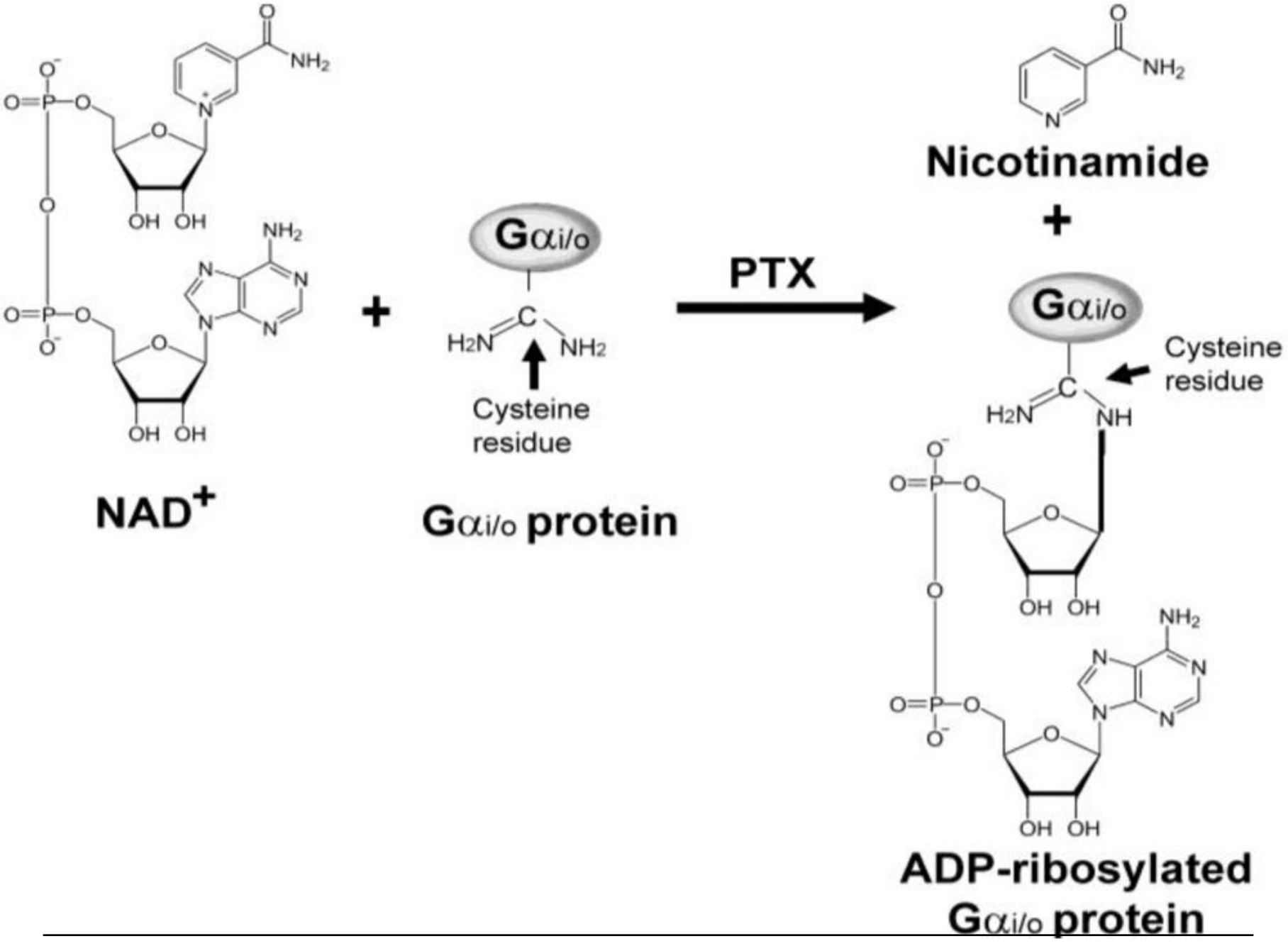




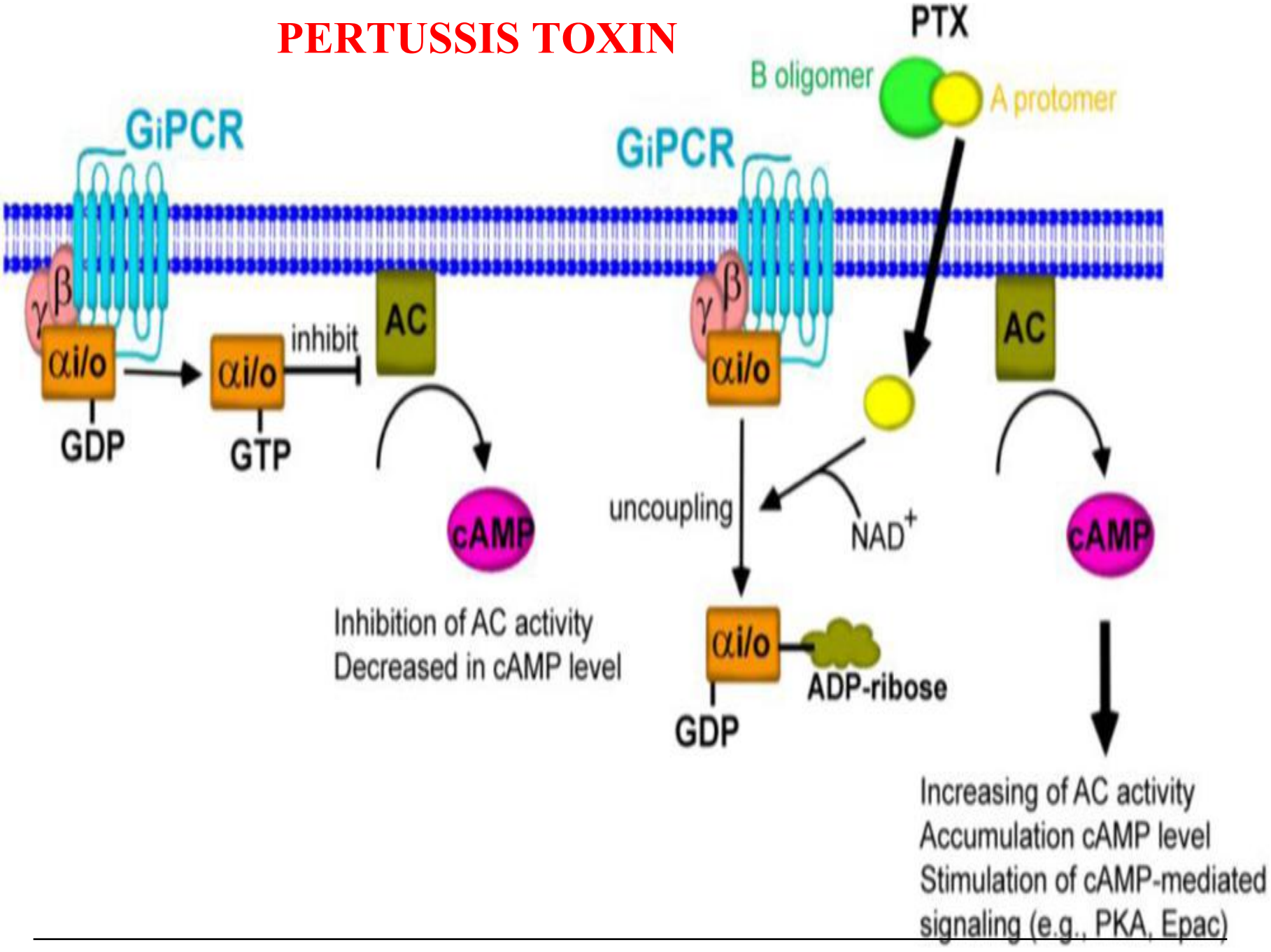


Hormones that act via cAMP mechanisms:

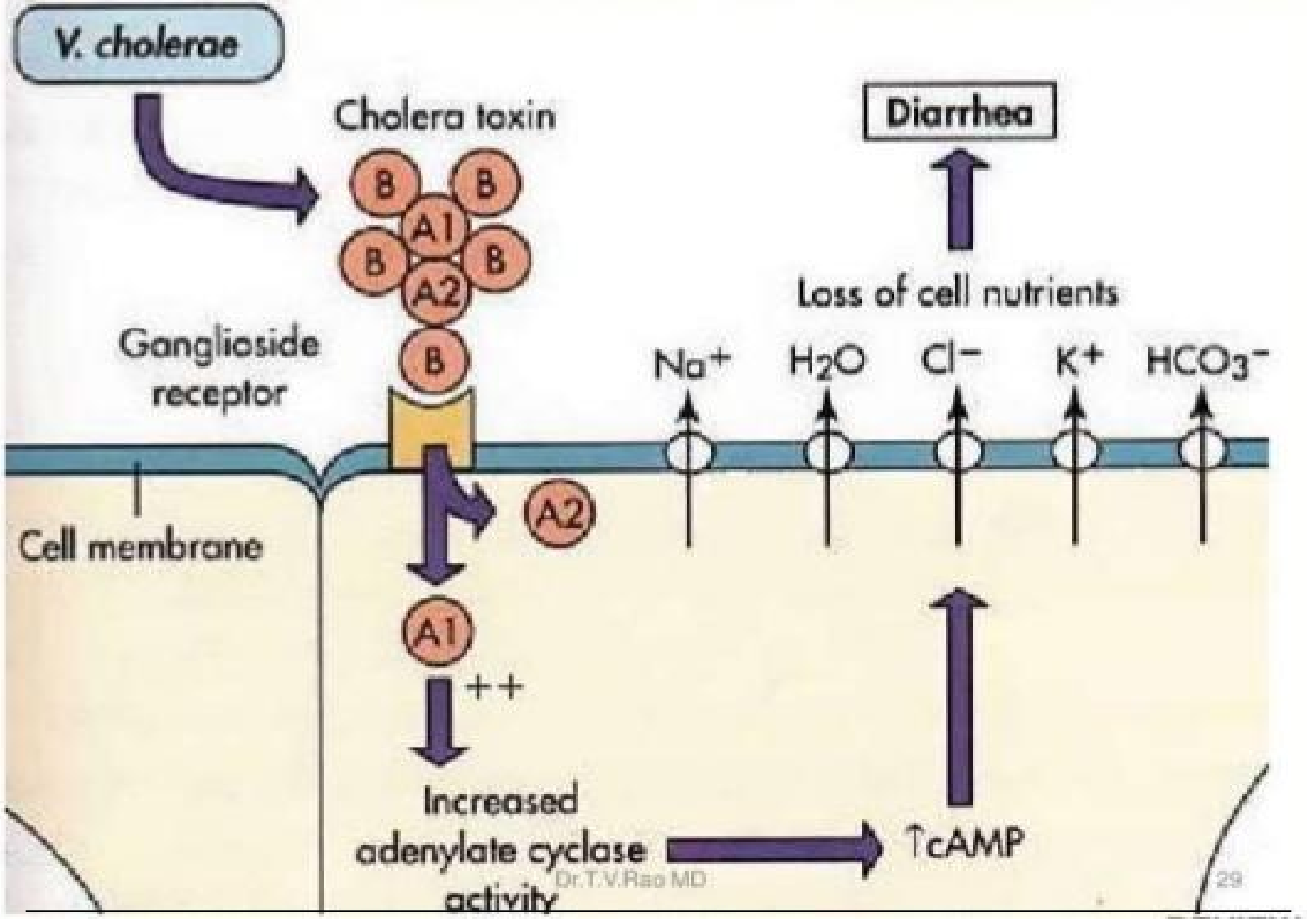
Epinephrine	Glucagon
ACTH	PTH
FSH	TSH
LH	Calcitonin



PERTUSSIS TOXIN



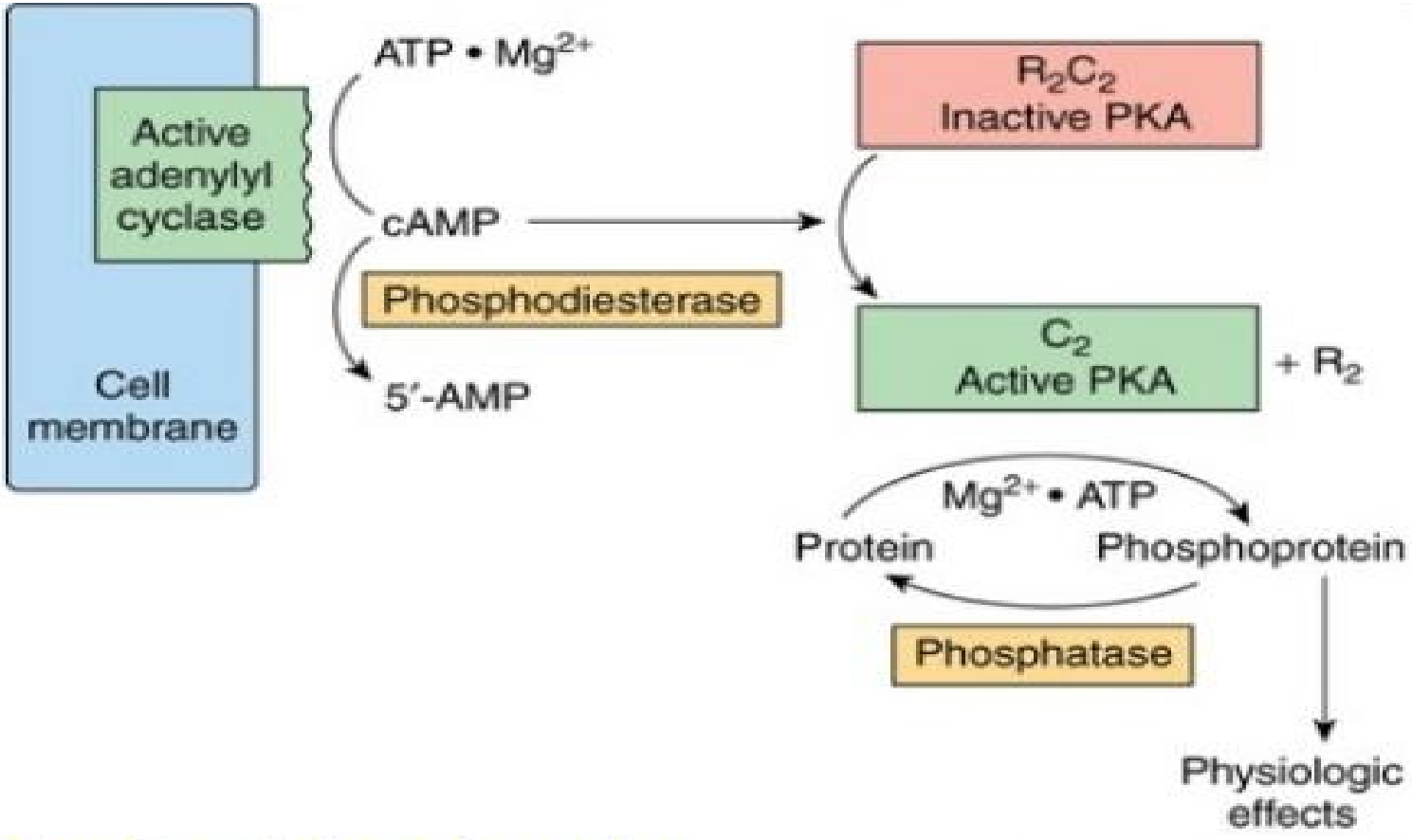
Mechanism of Action of Cholera Toxin



Protein Kinase

- In prokaryotic cells, cAMP binds to a specific protein called catabolite regulatory protein (CRP) that binds directly to DNA and influences gene expression.
- By contrast, in eukaryotic cells, cAMP binds to a protein kinase called protein kinase A (PKA), a heterotetrameric molecule consisting of two regulatory subunits (R) that inhibit the activity of the two catalytic subunits (C) when bound as a tetrameric complex.
- cAMP binding to the R2 C2 tetramer results in the following reaction:

$$4cAMP + R_2C_2 \rightleftarrows R_2 \cdot 4cAMP + 2c$$



Phosphorylation of proteins.

In cytoplasma - mostly metabolic enzymes (rapid response)

In the nucleus – phosphorylation of gene specific transcription factor CREB (cAMP response element-binding protein) (slower response)

- The R2 C2 complex has no enzymatic activity, but the binding of cAMP by R induces dissociation of the R–C complex, thereby activating the latter.

- The active C subunit catalyzes the transfer of the γ phosphate of ATP to a serine or threonine residue in a variety of proteins.

- Protein phosphorylation is now recognized as being a major and ubiquitous regulatory mechanism.

- The effects of cAMP in eukaryotic cells are all thought to be mediated by protein phosphorylation-dephosphorylation, principally on serine and threonine residues.

- The control of any of the effects of cAMP, including such diverse processes as
  - steroidogenesis,
  - secretion,
  - ion transport
  - carbohydrate and fat metabolism,
  - enzyme induction
  - Gene regulation,
  - synaptic transmission, and
  - cell growth and replication,

  could be conferred by a specific protein kinase, by a specific phosphatase, or by specific substrates for phosphorylation

- The array of specific substrates define a target tissue, and are involved in defining the extent of a particular response within a given cell.
- For example, the effects of cAMP on gene transcription are mediated by CREB, the cyclic AMP response element binding protein.
- CREB binds to a cAMP responsive DNA enhancer element (CRE) in its nonphosphorylated state and is a weak activator of transcription.
- When phosphorylated by PKA, CREB binds the coactivator CREB-binding protein CBP/p300 and as a result is a much more potent transcription activator.

- CBP and the related p300 contain histone acetyltransferase activities, and hence serve as chromatin-active transcriptional coregulators.
- Interestingly, CBP/p300 can also acetylate certain transcription factors thereby stimulating their ability to bind DNA and modulate transcription.

Phosphodiesterases

- Actions caused by hormones that increase cAMP concentration can be terminated in a number of ways, including the hydrolysis of cAMP to 5′-AMP by phosphodiesterases
- Phosphodiesterases are subject to regulation by their substrates, cAMP and cGMP; by hormones; and by intracellular messengers such as calcium, probably acting through calmodulin.
- Inhibitors of phosphodiesterase, most notably methylated xanthine derivatives such as caffeine, increase intracellular cAMP and mimic or prolong the actions of hormones through this signal

cGMP :an Intracellular Signal

- Cyclic GMP is made from GTP by the enzyme guanylyl cyclase, which exists in soluble and membrane-bound forms.
- Each of these enzyme forms has unique physiologic properties
- The atriopeptins, a family of peptides produced in cardiac atrial tissues, cause natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion.
- These peptides (eg, atrial natriuretic factor) bind to and activate the membrane-bound form of guanylyl cyclase

- This results in an increase of cGMP by as much as 50-fold in some cases, and this is thought to mediate the effects mentioned above.
- A series of compounds, including nitroprusside, nitroglycerin, nitric oxide, sodium nitrite, and sodium azide, all cause smooth muscle relaxation and are potent vasodilators.
- These agents increase cGMP by activating the soluble form of guanylyl cyclase, and inhibitors of cGMP phosphodiesterase (the drug sildenafil [Viagra], for example) enhance and prolong these responses.
- The increased cGMP activates cGMP-dependent protein kinase (PKG), which in turn phosphorylates a number of smooth muscle proteins leading to relaxation of smooth muscle and vasodilation.

Receptors with guanylate cyclase activity

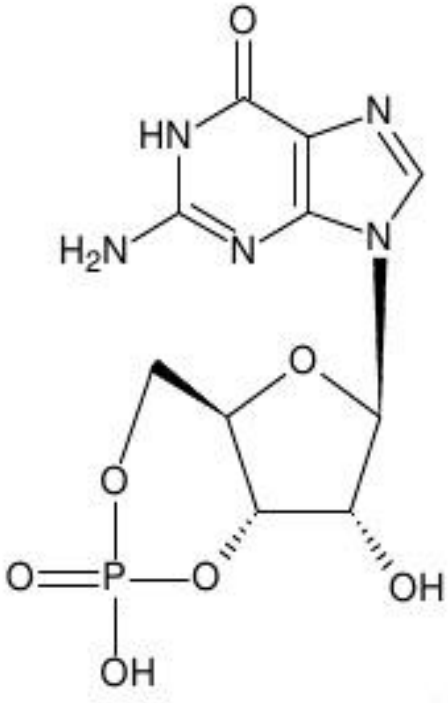
After binding of ligand they convert GTP to cGMP

cGMP is the second messenger

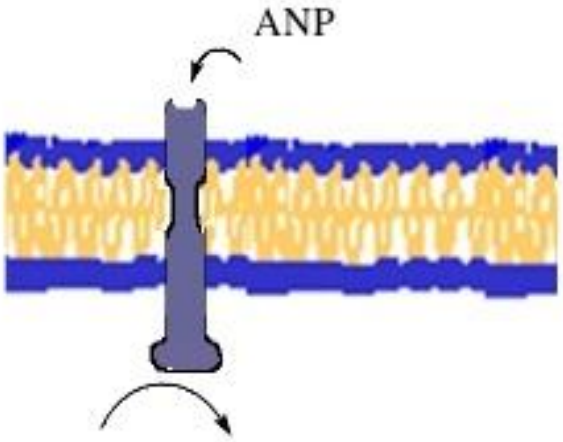
It activates proteinkinase G

Two types of receptors:

- membrane-associated
- soluble (cytoplasmic)



Membrane receptors with guanylate cyclase activity



Receptors for ANP (atrial natriuretic factor)

Mainly smooth muscle of vessels and in kidneys

$GTP \rightarrow cGMP + PP_i$

phosphodiesterase

H_2O

GMP

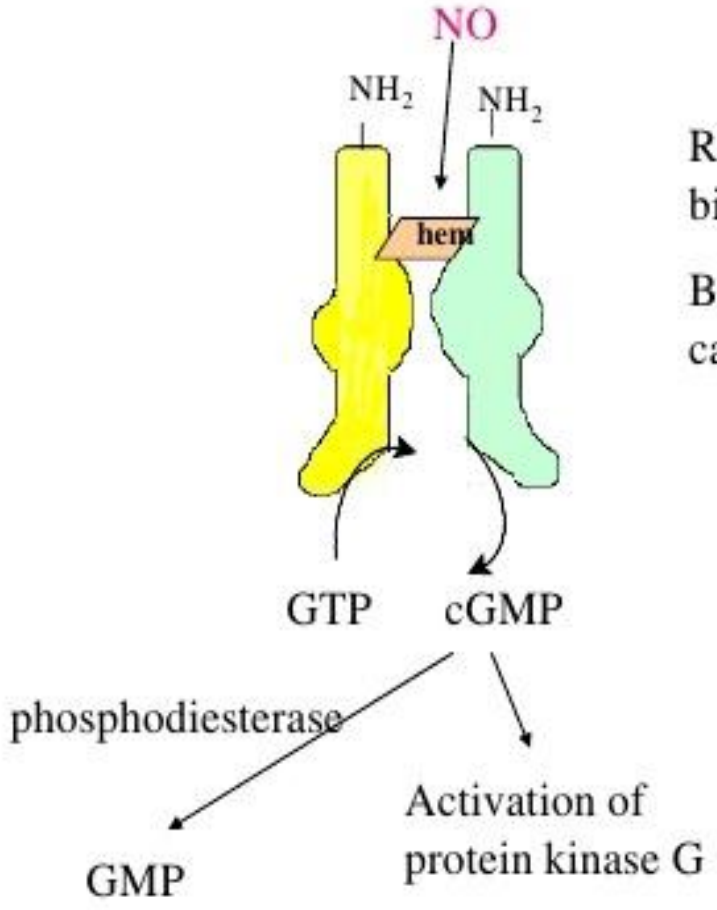
Protein kinase G
inactive

active protein kinase G (PKG)

Phosphorylation of proteins

ANP is produced by cardac atrial tissue in response to increase of blood volume or pressure

Soluble receptors with guanylate cyclase activity



Receptor je dimeric complex and binds hem

Binding NO to the hem increases catalytic acitivity guanylate cyclase

NO is generated by the action of nitroxid synthase (NOS)

NO readily permeates membranes, it can be produced by one type of the cell and rapidly diffuse into neighboring cell types

Proteinkinase G

cGMP sensitive proteinkinase G

Widely expressed in many cells

It phosphorylates various proteins (enzymes, transportion proteins ect.)

Effect of PKG in smooth muscle

Phosphorylation of proteins:

- inactivation of proteins attenuating Ca^{2+} release from ER $\Rightarrow \downarrow Ca^{2+}$
- activation of MLC phosphatase $\Rightarrow$ repression of actin-myosin interaction
- decrease of K^+-channnels activity $\Rightarrow$ decrease of hyperpolarization $\Rightarrow$ increased influx of Ca^{2+} into the cell

Relaxation of smooth muscle