Mechanism of action of Hormones

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HORMONES

- **Cellular communication** is mediated by signalling molecules - eg. hormones, neurotransmitters, prostaglandins, growth factors
- A hormone is synthesized in an endocrine (ductless) gland eg. thyroid, pituitary and secreted into blood which takes it to target tissue(s) on which it acts.

Hormone classification

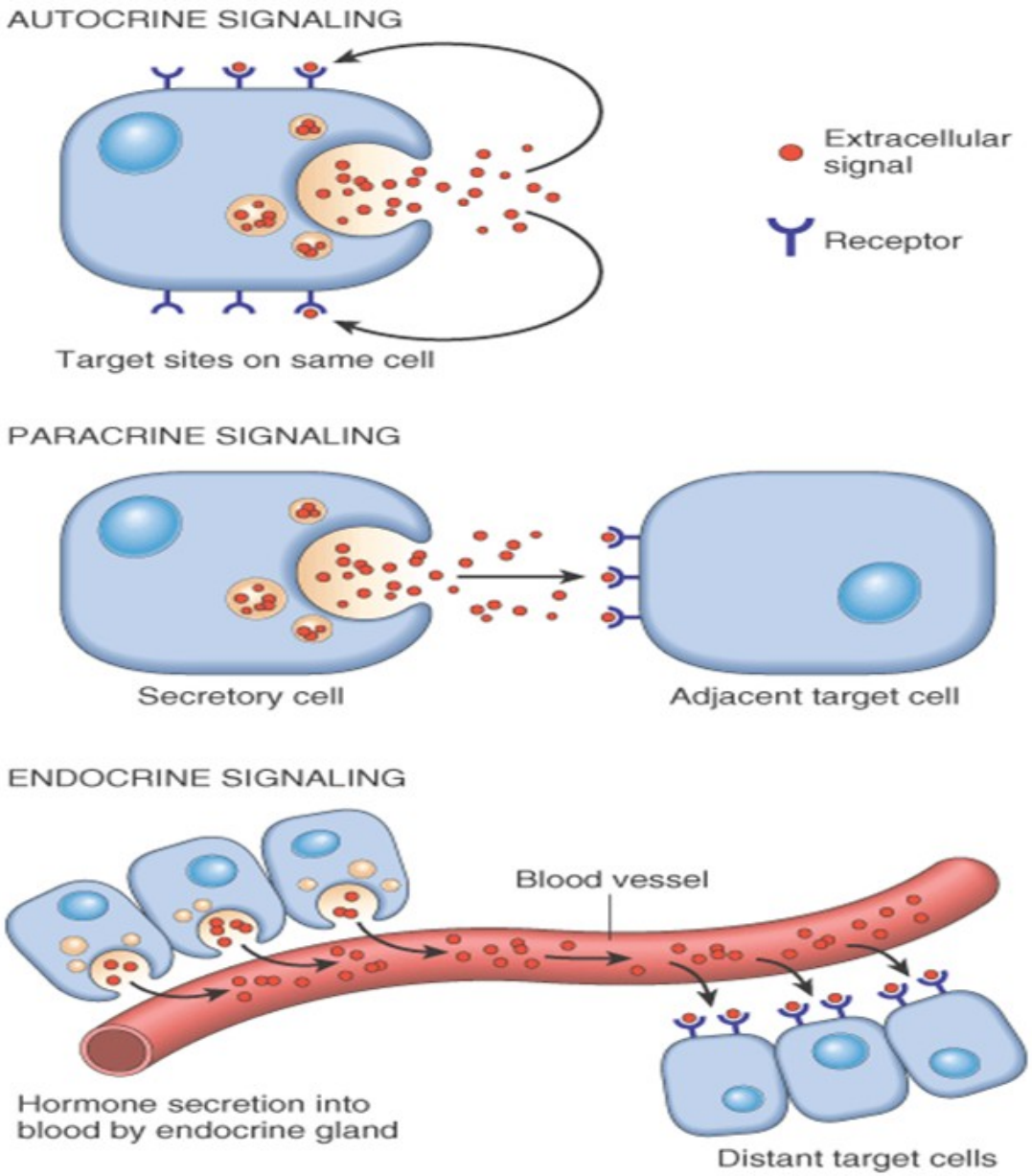
(1) Composition

- AA & their derivatives –thyroid hormones
- Proteins – hormones of GIT, pancreatic hormones
- Steroid compounds – glucocorticoids, sex hormones
- Fatty acid derivatives - prostaglandins

(2) Type of signal

- Autocrine -cells respond to substances they themselves secrete eg.growth factors secreted by tumor cells
- Paracrine – acts on neighbouring cells(separated by extracellular space)
- Endocrine – acts on cells at distant sites (carried by blood or lymph)

Three types of signaling – autocrine, paracrine, endocrine



(3) Receptor location

- Group I – Hormones which bind with intracellular receptors (Lipophillic hormones)

  eg: steroid and thyroid hormones, calcitriol

- Group II – Hormones which bind with cell membrane receptors (Water soluble hormones)

  eg. insulin, glucagon, epinephrine

Mechanisms of hormone action

- Hormones mediate their actions by binding with cellular recognition molecules called **receptors** found in target cells.
- Some cells have more than one receptor type for a hormone.

  eg. Epinephrine can bind to **β-adrenergic** receptor and to **α-adrenergic** receptor in the skeletal muscle each inducing a different reaction.

- All receptors have at least two domains –
  a) hormone binding domain
  b) functional domain- couples hormone recognition to an intracellular function
- Receptors are proteins in nature.
- Location –
  intracellular – bind with Gp.I hormones
  cell membrane – bind with Gp. II hormones

Mechanism of action of Gp.I hormones-

eg. steroid hormones, thyroid hormones

- Hormones diffuse through plasma membrane and bind with **intracellular receptors** present in target cells.

  nuclear receptors: thyroid & sex hormones

  cytosolic receptors: glucocorticoids, aldosterone

- **Cytosolic receptors**: Associated with a dimer of heat shock proteins (HSP) located in the DNA binding region of the receptor.
- Hormones bind with the unactivated receptor forming a hormone-receptor complex (HR complex).

- The HR complex next undergoes activation leading to the release of HSP dimer exposing the DNA binding region.
- The activated HR complex translocates to the nucleus via a nucleophore and bind with DNA at specific sites (hormone receptor element- HRE).
- Binding of HR complex to DNA leads to activation or inactivation of specific genes, selectively affecting gene transcription and protein synthesis.

- **Nuclear receptors**: Hormones diffuse through the perinuclear membrane and bind with receptors and becomes activated.
- The activated complex binds with DNA and affects transcription and protein synthesis.
- Nuclear receptors are not associated with HSP`s and are able to associate with DNA in the absence of the hormones.
- In absence of hormone transcription is repressed. Binding of hormone leads to a conformational change in the receptor activating transcription.

- DNA binding region of the receptors of GP I hormones consist of 2 zinc fingers which-

  -determines the specificity of binding of receptor to HRE

  -stabilizes the receptor to HRE

Mechanism of action of Gp.II hormones-

- Bind with **cell membrane receptors** which are associated with a group of proteins known as **G-proteins** (GTP binding proteins).

G-proteins:

-linked with cell membrane receptors

-heterotrimeric; contain α,β and γ subunits

-activated by binding of GTP

-inactivated by exchange of GTP for GDP (hydrolysis of GTP to GDP by GTPase)

G-protein-coupled receptors (GPCRs, 7-TMRs, Serpentine)

Integral membrane proteins that contain **seven membrane-spanning helices**

Coupled to heterotrimeric G proteins on the intracellular side of the membrane

On ligand binding GPCR undergoes a conformational causing G-protein activation.

Hormone + Receptor → HR complex

↓

Activation of G-protein By binding GTP

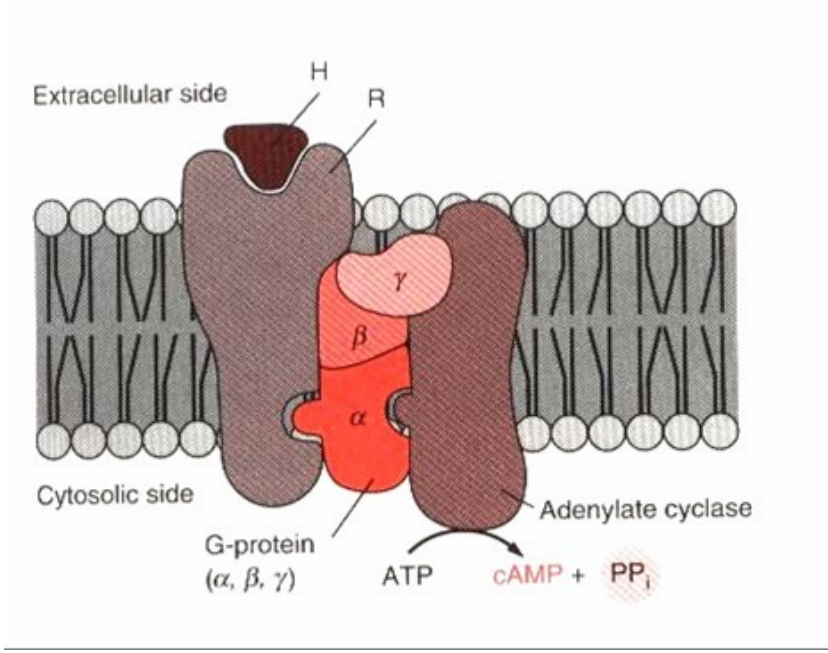
↓

Dissociation of GTP bound α-subunit (catalytic subunit)

↓

Alter the concentration of a intracellular signalling molecule- **second messenger**

eg. cAMP, cGMP, DAG, IP3



- Second messengers activate or inactivate enzymes mediating a cellular response.

  eg. cAMP- activate protein kinase A

  cGMP- activate protein kinase G

  DAG- activate protein kinase C

  IP3- release Ca^{+2} from ER and

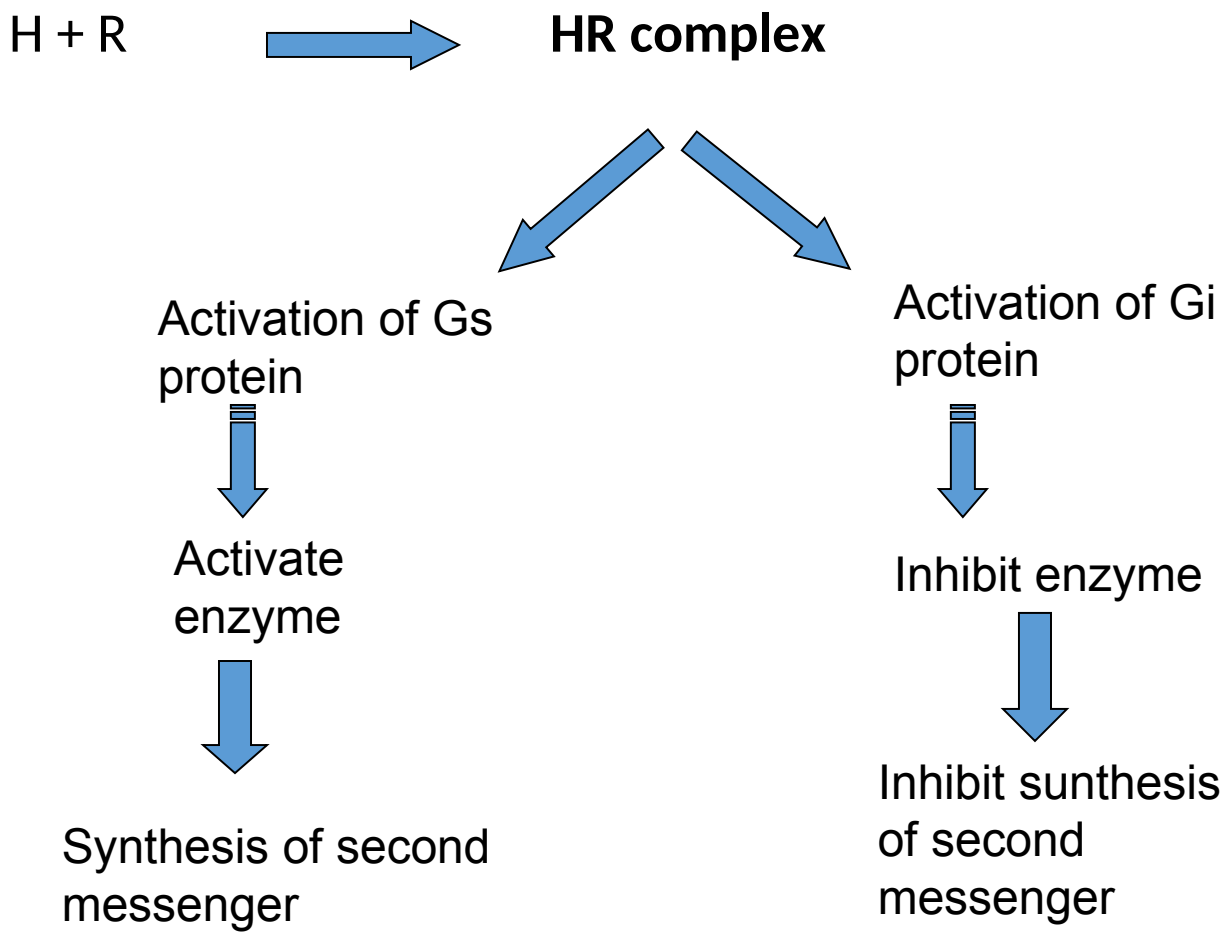
  activate Ca+2 binding enzymes

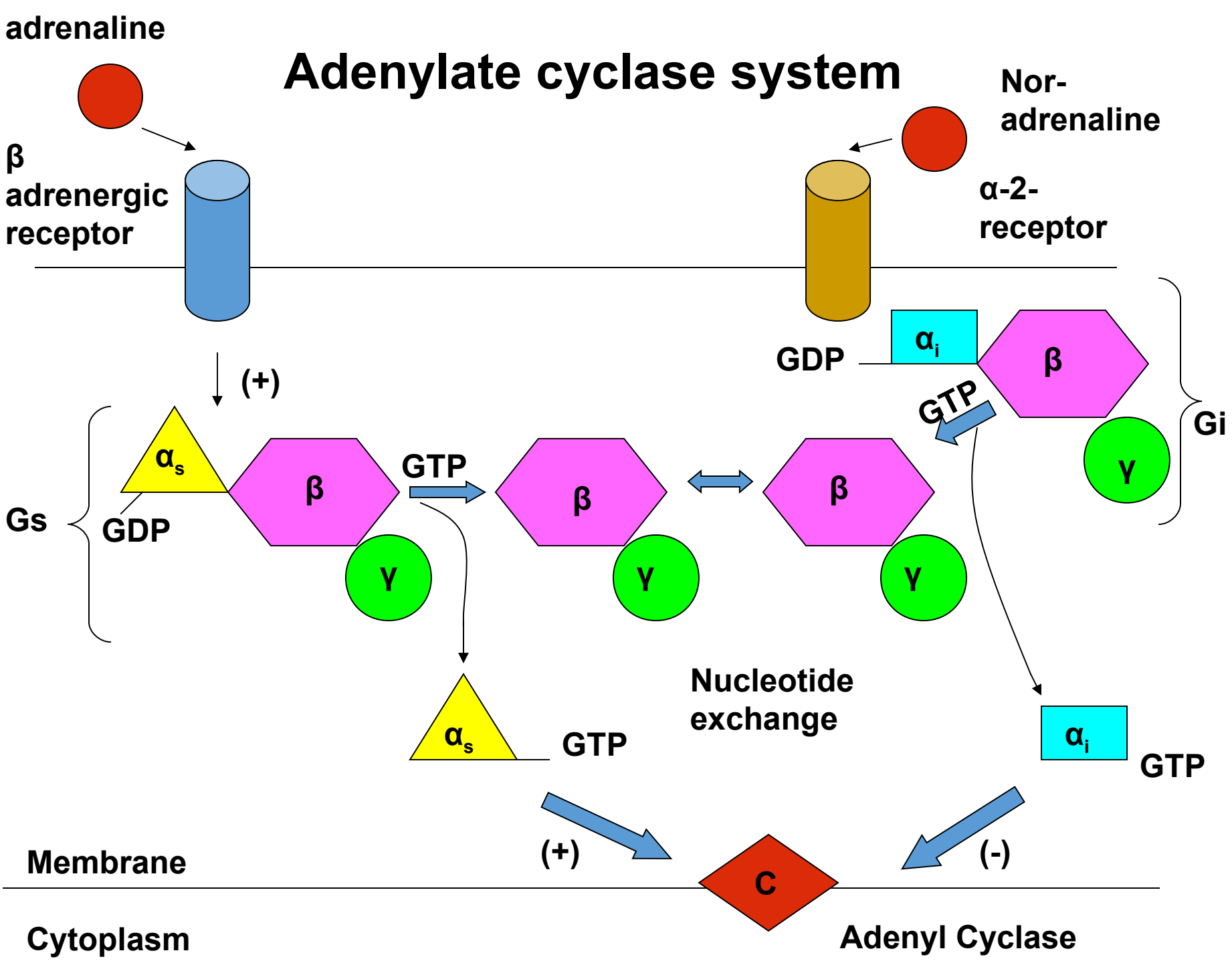
Protein kinases- activate or inactivate enzymes through **phosphorylation**

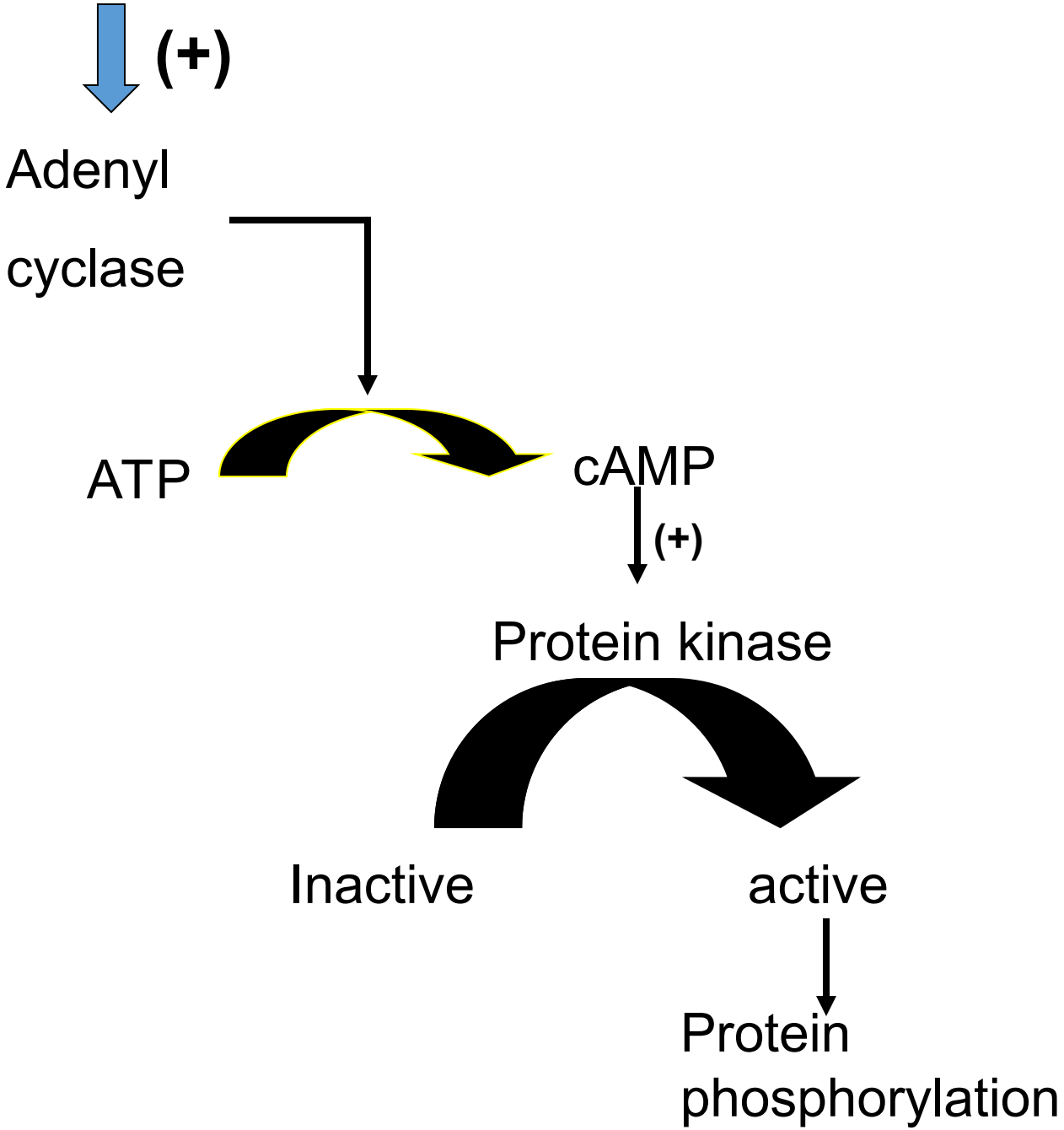
G-proteins are of two types-

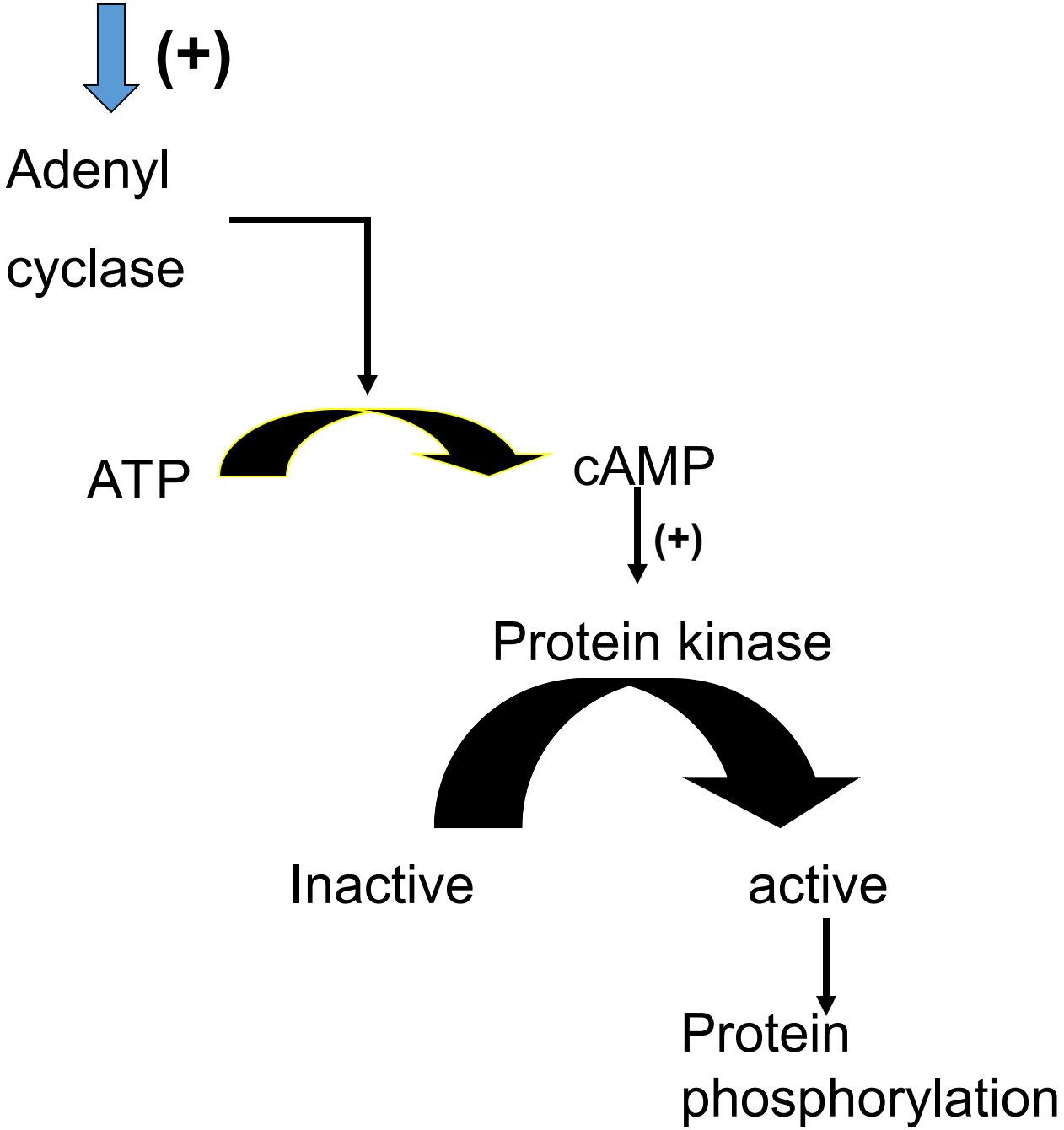
-stimulatory G-proteins (Gs proteins)

-inhibitory G-proteins (Gi proteins)

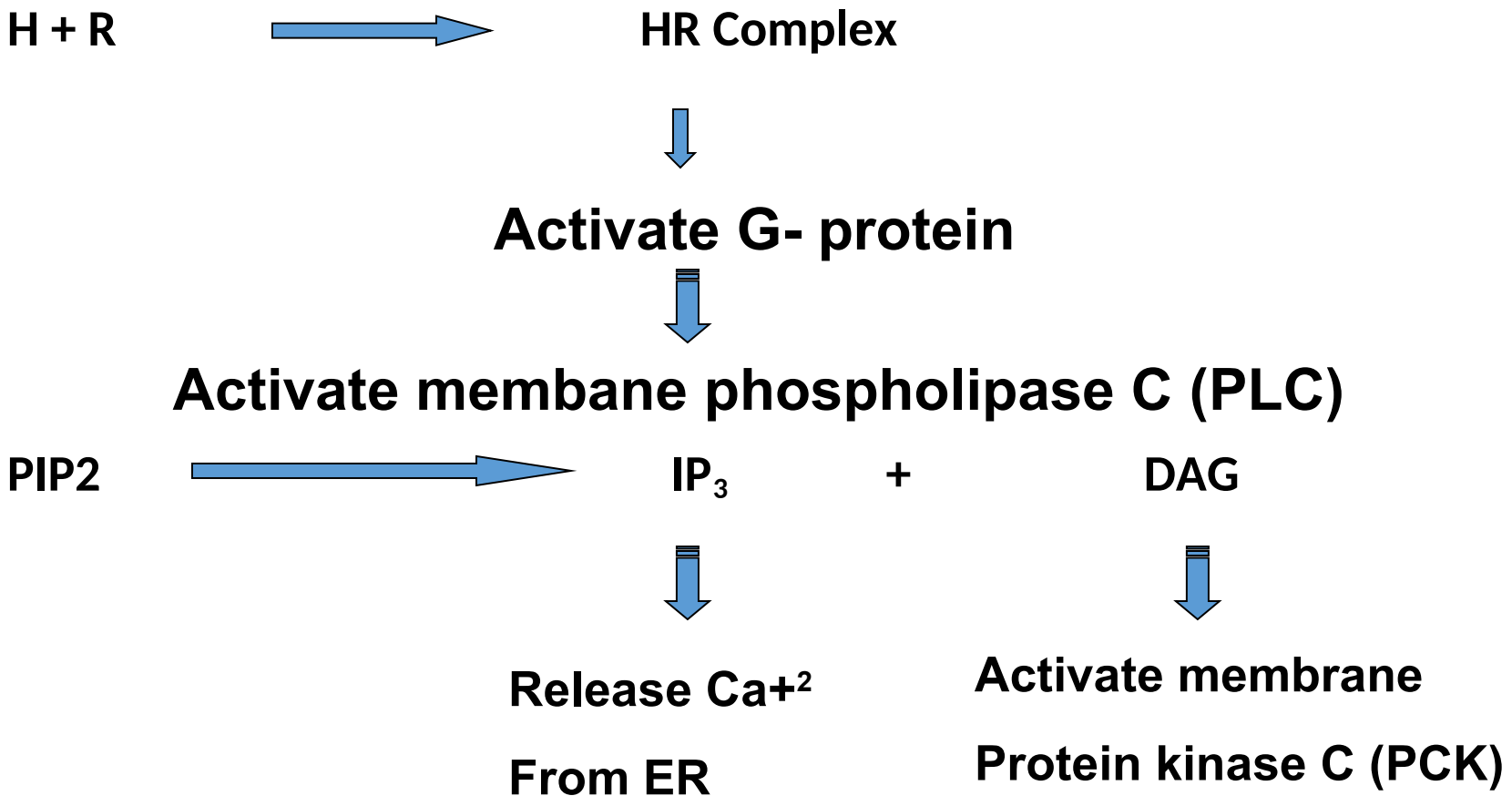



adrenaline
Adenylate cyclase system
Nor-
adrenaline
β
adrenergic
receptor
α-2-
receptor
(+)
GDP
α_i
β
GTP
Gi
γ
α_s
β
GTP
β
β
Gs
GDP
γ
γ
γ
Nucleotide
exchange
α_s
GTP
α_i
GTP
(+)
(-)
Membrane
C
Cytoplasm
Adenyl Cyclase


(+)
Adenyl
cyclase
ATP
cAMP
(+)
Protein kinase
Inactive
active
Protein
phosphorylation


(+)
Adenyl
cyclase
ATP
cAMP
(+)
Protein kinase
Inactive
active
Protein
phosphorylation

Inositol triphosphate(IP_3) system



Eg : Adrenalin on α-1 receptor. (liver cell and muscle)

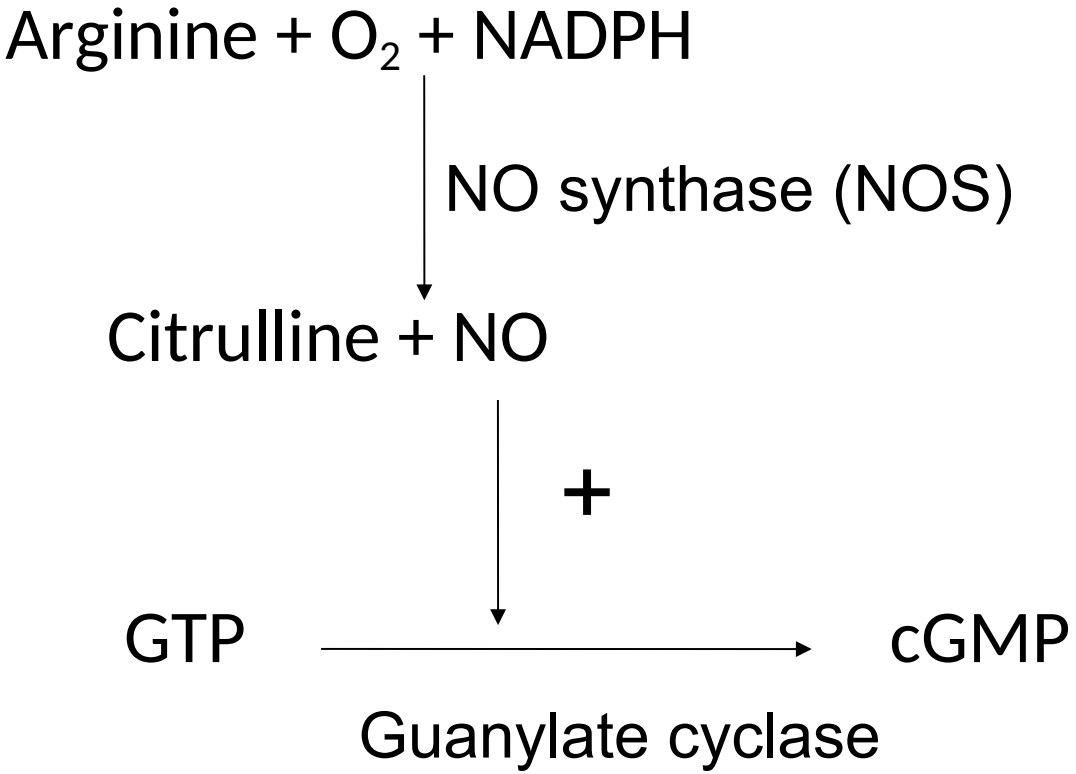
Acetylecholine on muscarinic cholinergic receptors. (muscle)

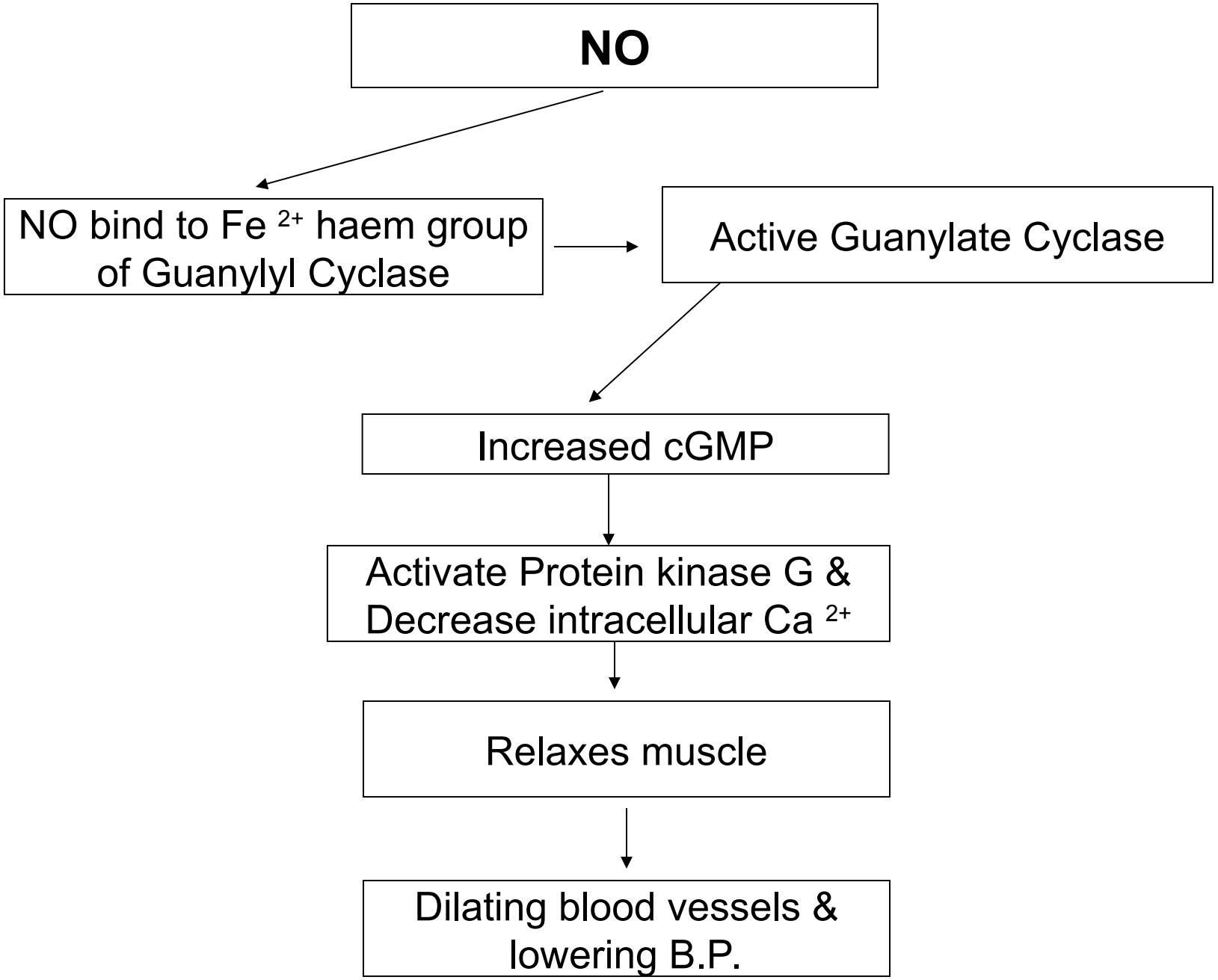
cGMP as second messenger –

Nitric oxide (NO) -

- Used by the body as a signaling molecule.
- Serves different functions depending on body system. i.e. neurotransmitter, vasodilator (relaxes smooth muscles), involved in the cytotoxic activity of macrophages.
- First gas known to act as a biological messenger

The relaxing effect of NO on smooth muscles is associated with increased production of cGMP.



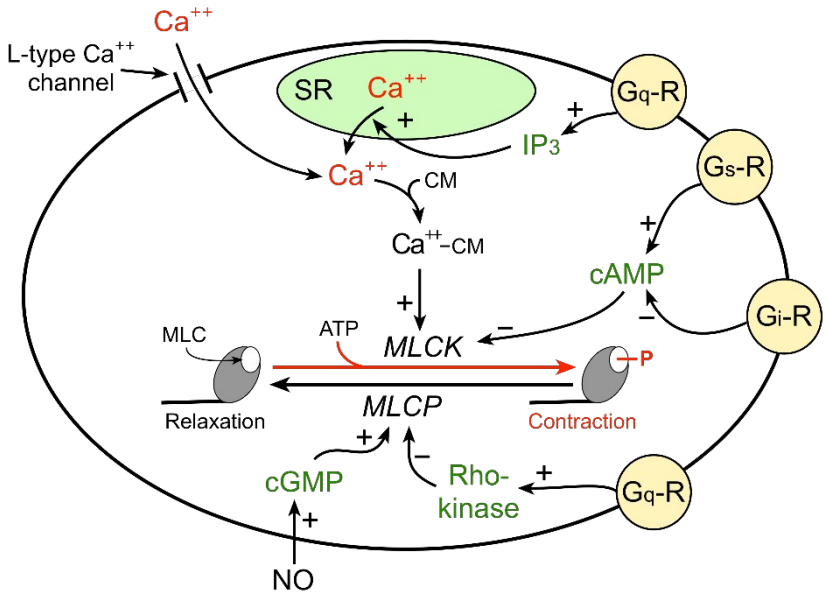

NO
NO bind to Fe 2+ haem group of Guanylyl Cyclase
Active Guanylate Cyclase
Increased cGMP
Activate Protein kinase G & Decrease intracellular Ca 2+
Relaxes muscle
Dilating blood vessels & lowering B.P.

The mechanism by which an increase in intracellular calcium stimulates VSM contraction is illustrated in the figure to the right.

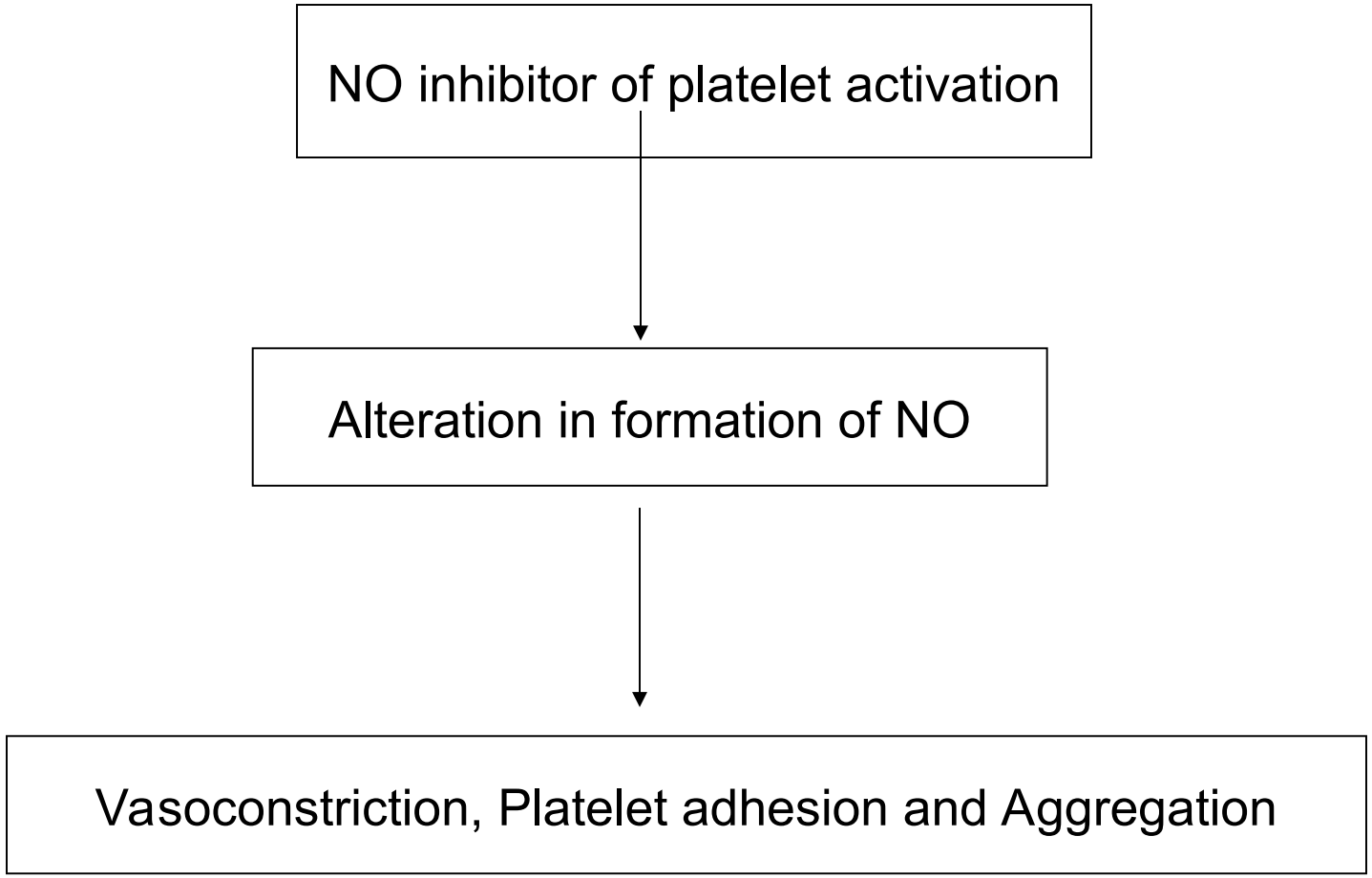
An increase in free intracellular calcium can result from either increased flux of calcium into the cell through calcium channels or by release of calcium from internal stores (e.g., sarcoplasmic reticulum; SR).

The free calcium binds to a special calcium binding protein called **calmodulin**.

Calcium-calmodulin activates **myosin light chain kinase** (MLCK), an enzyme that is capable of phosphorylating myosin light chains (MLC) in the presence of ATP. Myosin light chains are 20-kD regulatory subunits found on the myosin heads. MLC phosphorylation leads to cross-bridge formation between the myosin heads and the actin filaments, and hence, smooth muscle contraction.

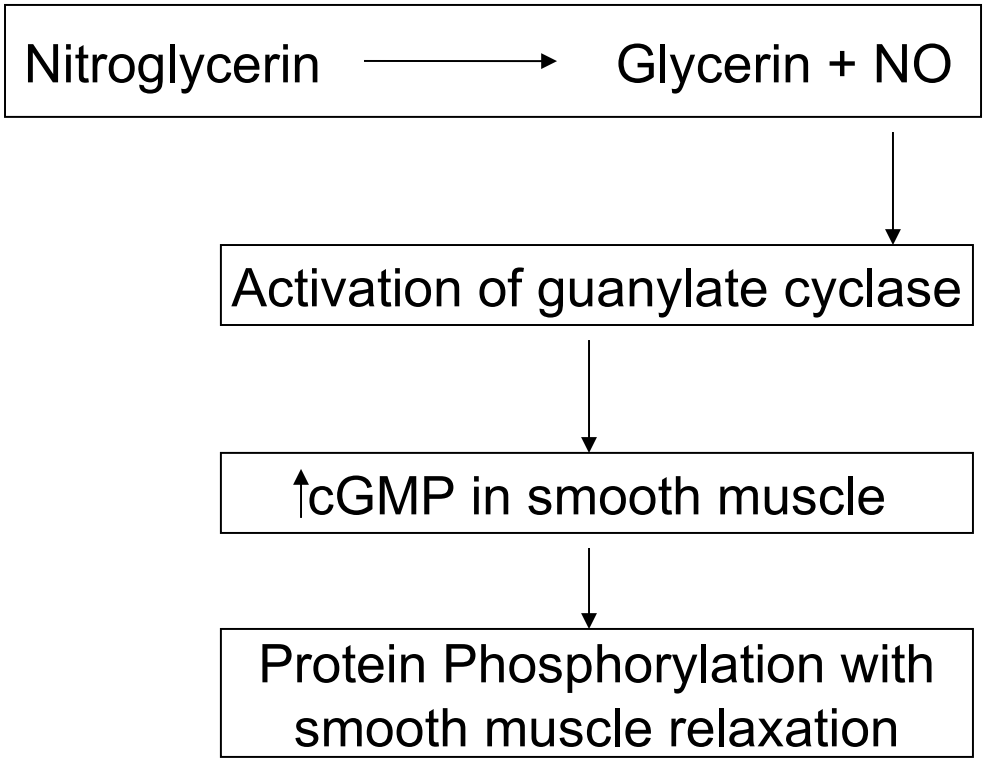


In coronary artery disease, basal level of NO as well as stimulated release of NO reduced



Pharmacological Role of NO

GTN (glyceryl trinitrate, nitroglycerin) – used in the treatment of angina (chest pain) due to reduced blood flow.



Sildenafil (Viagra):

- in vascular smooth muscle blocks degradation of cGMP to GMP by inhibiting

$$NO \longrightarrow cGMP \xrightarrow{\text{phosphodiesterase-5}} GMP$$

- Prolong the effect of cGMP on vasodilation.

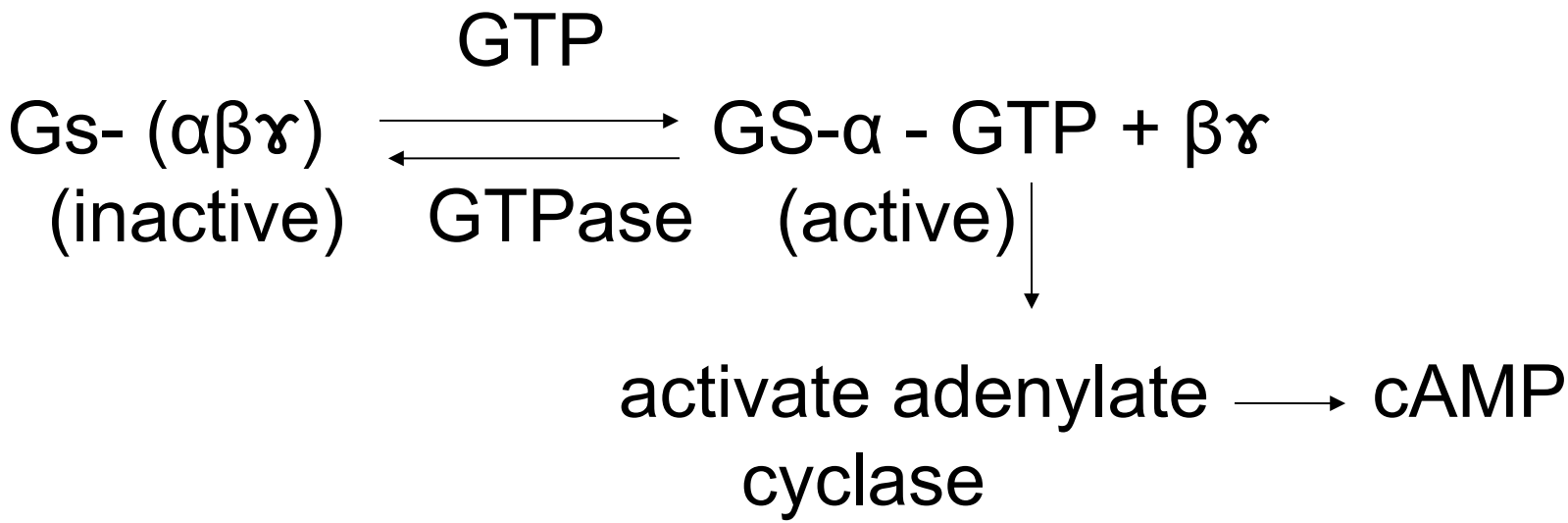
Disorders associated with G-proteins –

Cholera-

- caused by bacterium *Vibrio cholera*. The cholera toxin (CTX) is responsible for the massive, watery diarrhoea characteristic of the cholera infection.
- CTX enters the enterocytes through a membrane ganglioside and activates adenylate cyclase to synthesize cAMP.
- Mechanism –
  - CTX mediates the **ADP-Ribosylation** (ADPR) of the alpha-subunit of a stimulatory G-protein forming Gs-ADPR. This binds with a GTP which activates adenylate cyclase to synthesize cAMP.
  - CTX inhibits GTPase and Gs is continuously activated.

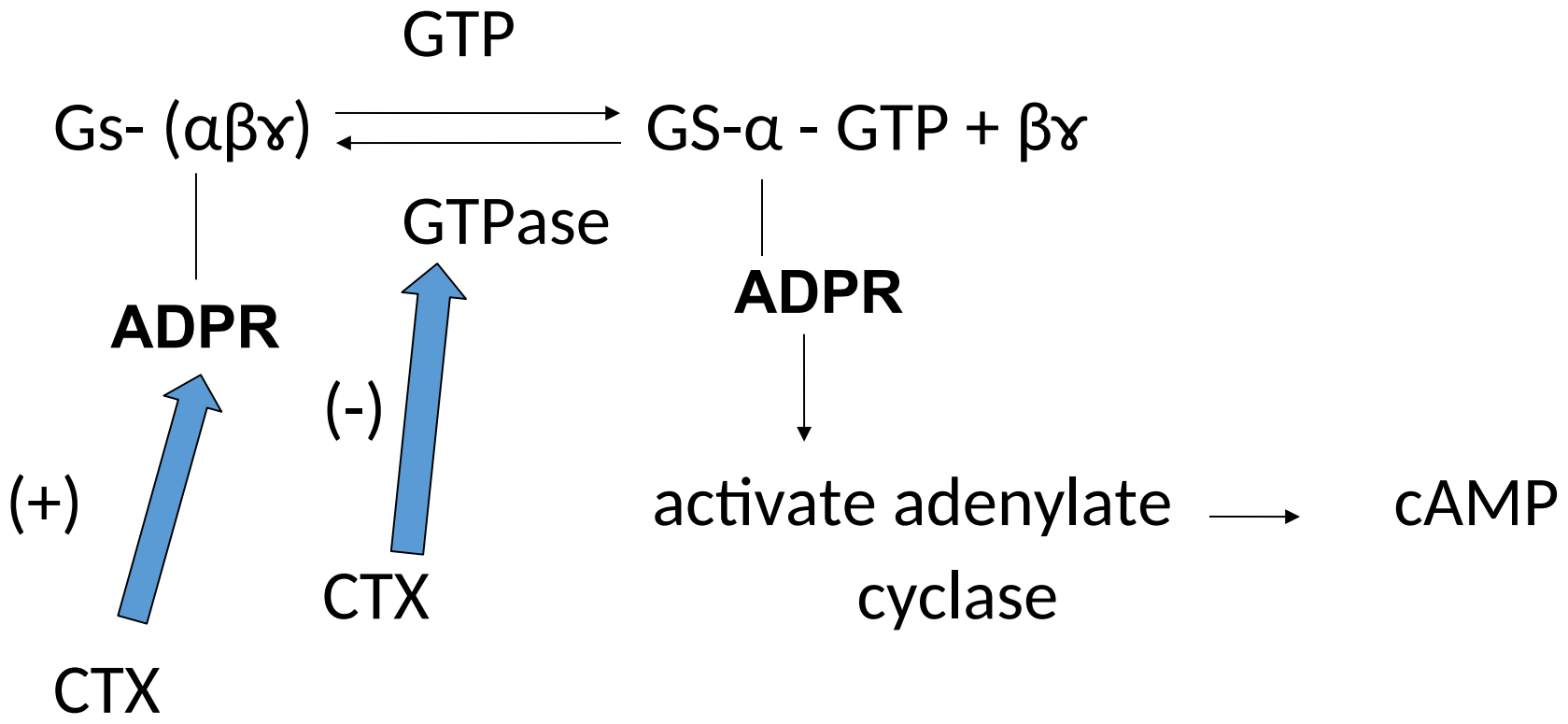
Normal conditions-

H + R interaction activates Gs protein.

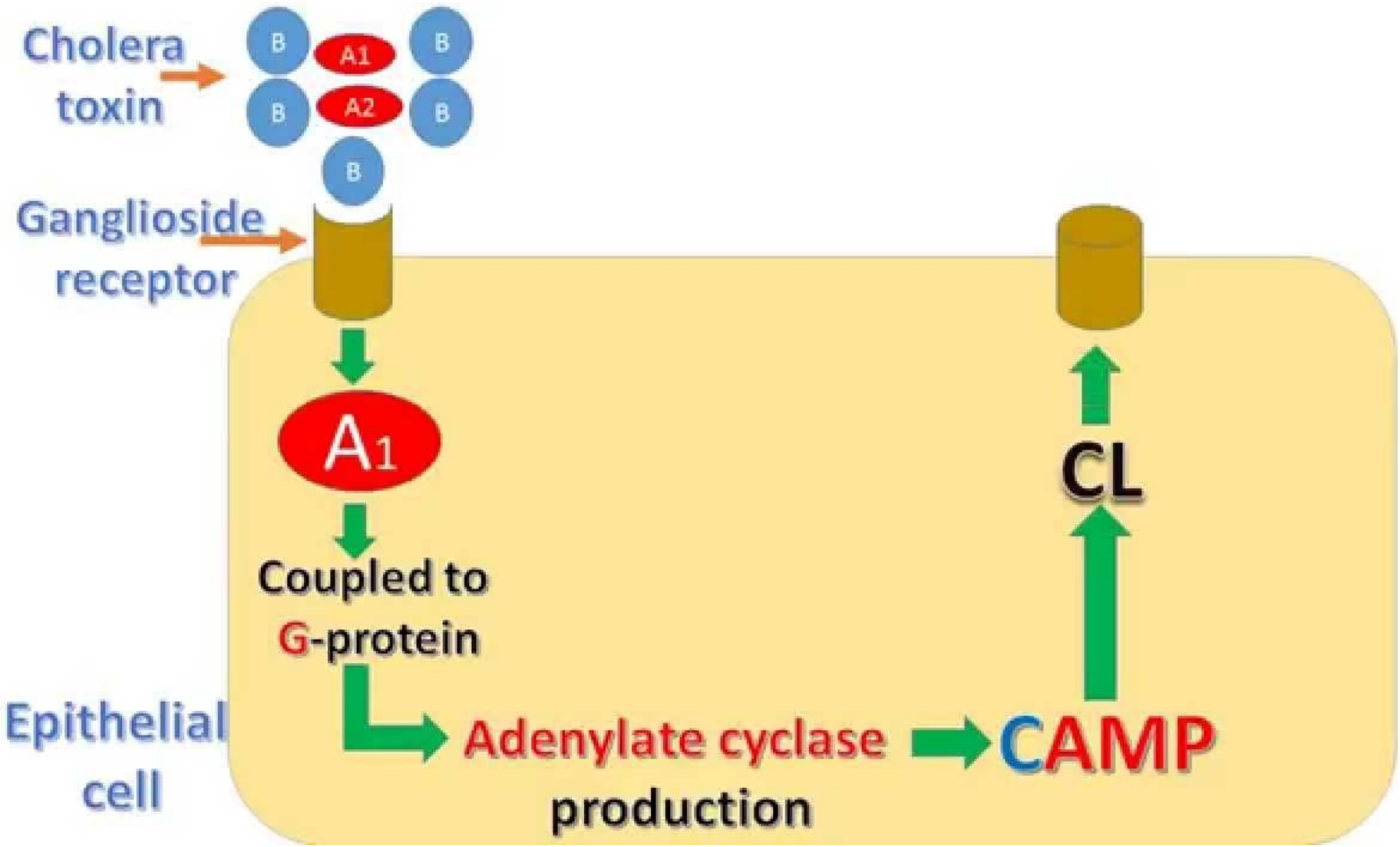


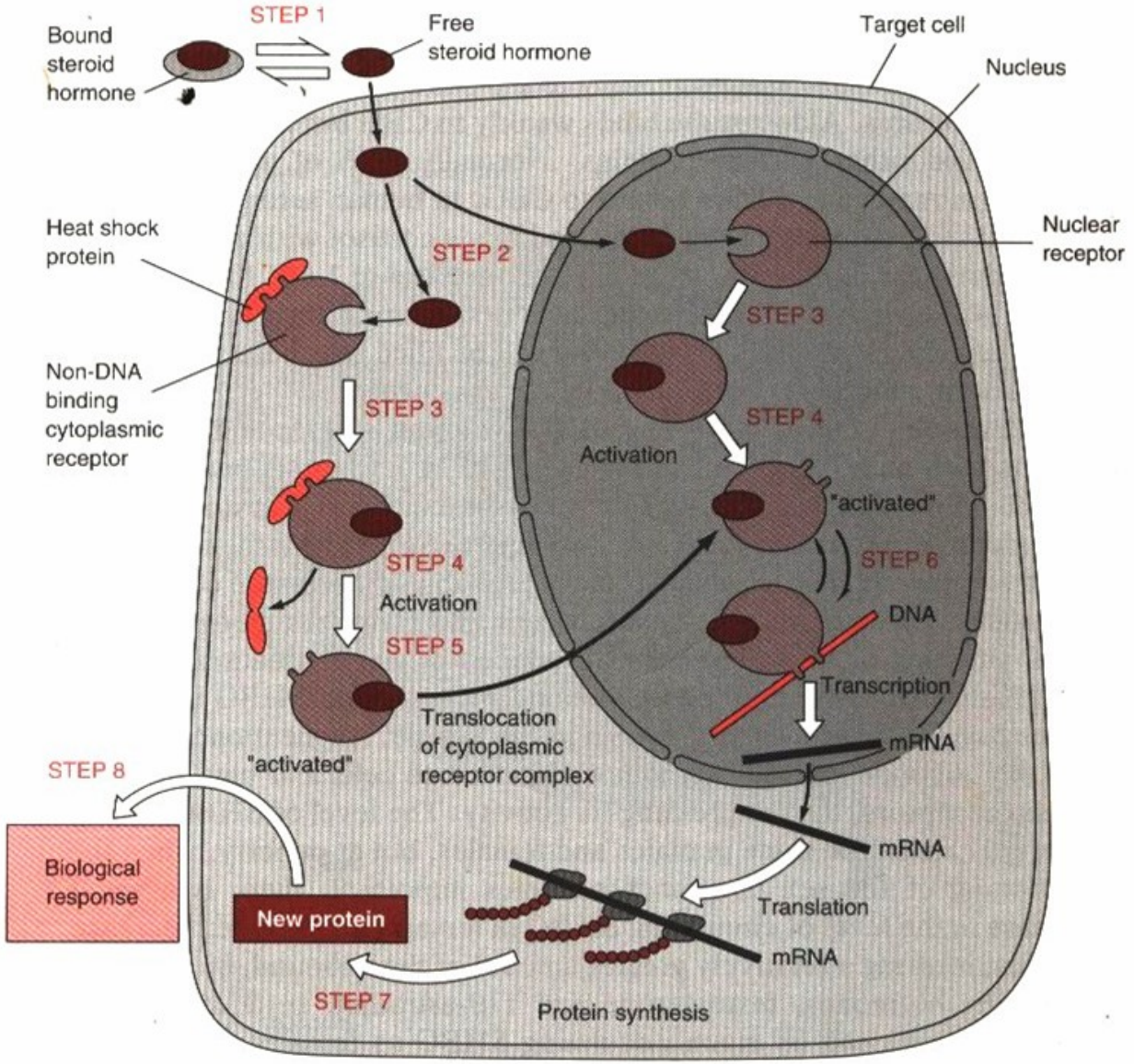
In cholera-

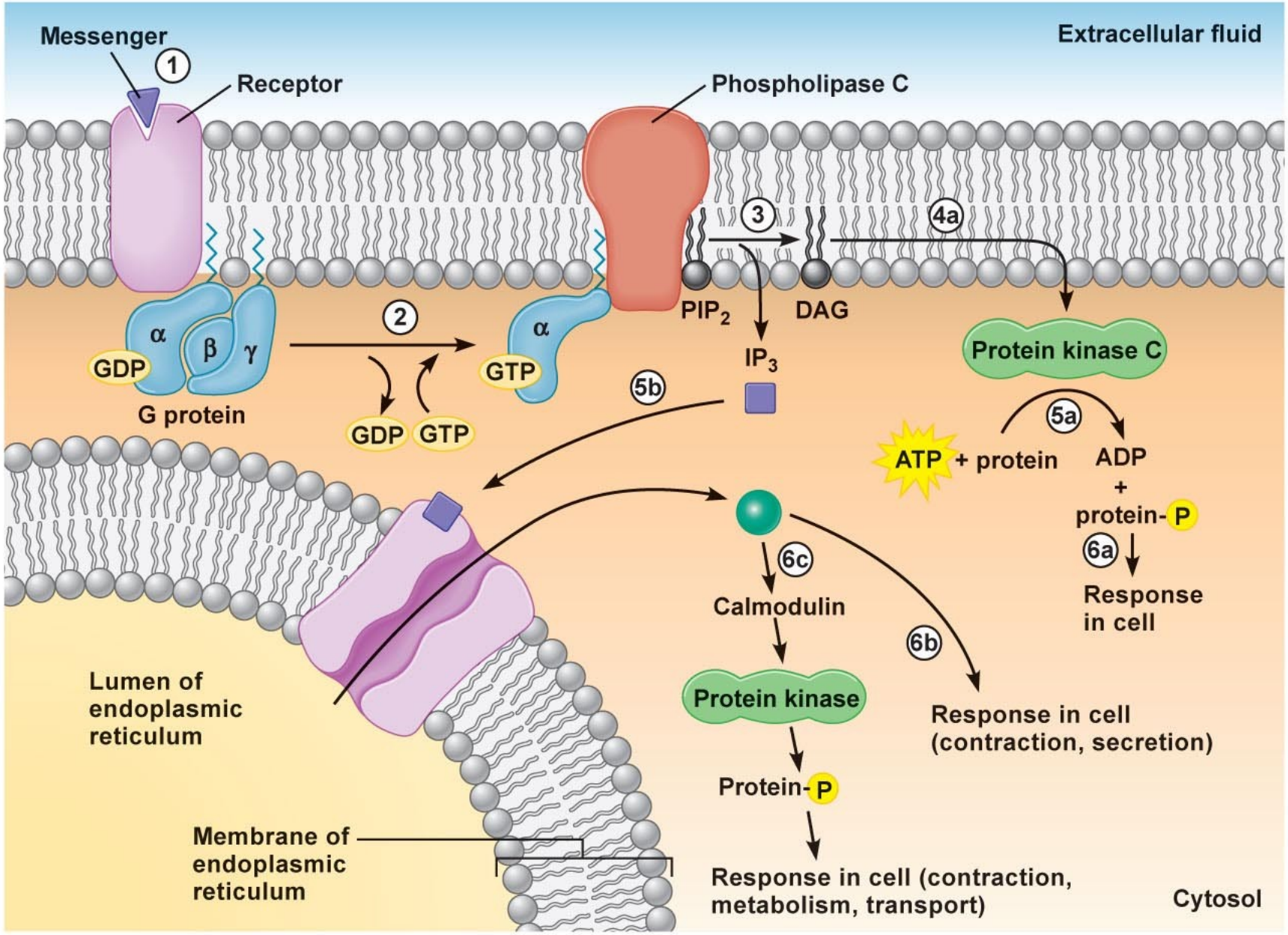
CTX interacts with Gs protein and ADP-ribosylate the alpha subunit.

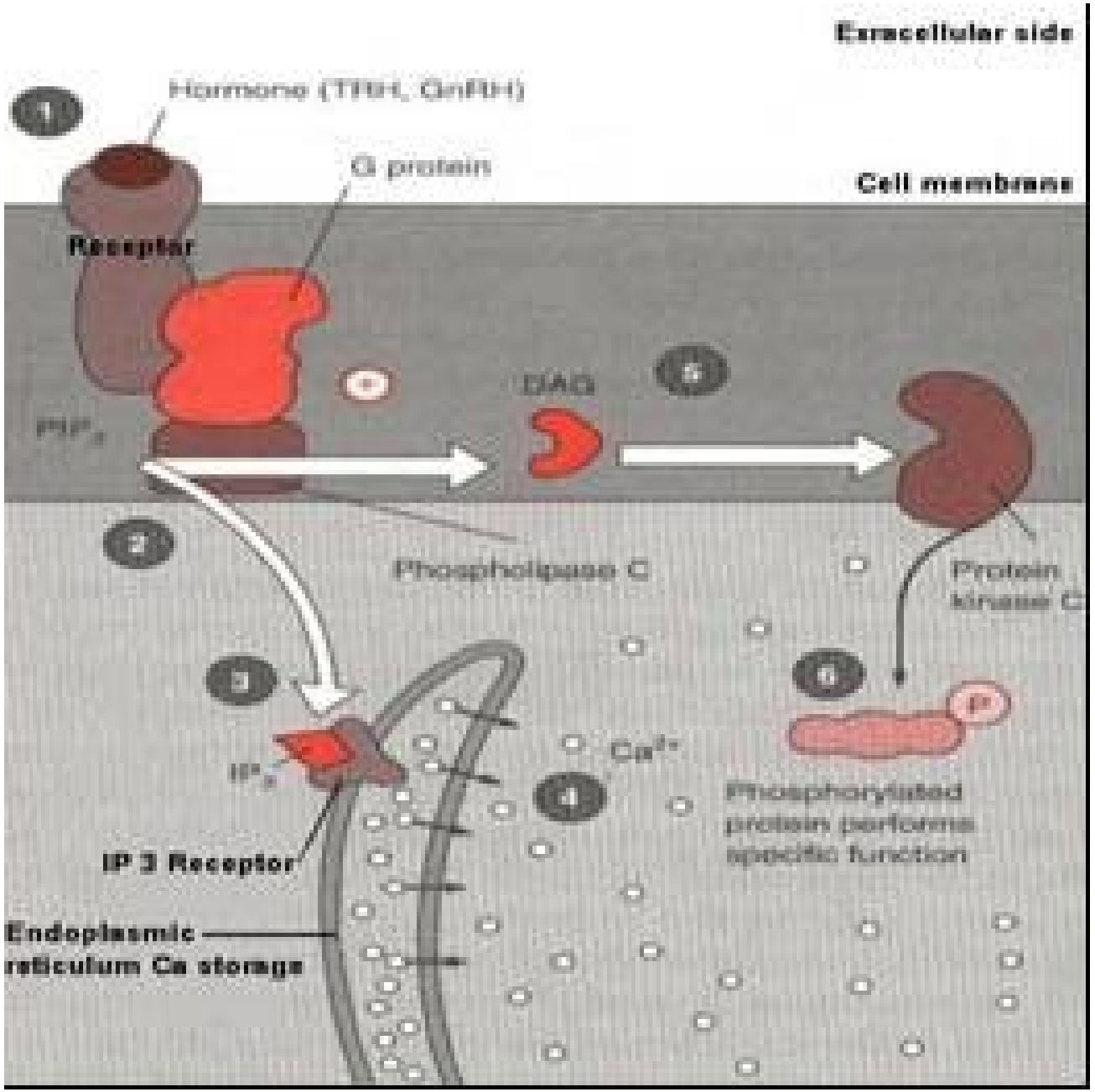


- The GTP bound Gs-ADPR cannot be inactivated by hydrolysis of the GTP to GDP by GTPase.
- Adenylate cyclase remains continually activated.
- The net effect of the toxin is to cause cAMP to be produced at an abnormally high rate which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal lumen.
- H_2O, Na+ and other electrolytes follow due to the osmotic and electrical gradients caused by the loss of Cl^-.
- The lost H_2O and electrolytes in mucosal cells are replaced from the blood.
- Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhea, loss of electrolytes, and dehydration that are characteristic of cholera.


Cholera toxin
B
A1
B
B
A2
B
B
Ganglioside receptor
A1
Coupled to G-protein
Epithelial cell
Adenylate cyclase production
CAMP
CL


STEP 1
Bound steroid hormone
Free steroid hormone
Target cell
Nucleus
Heat shock protein
STEP 2
Nuclear receptor
STEP 3
Non-DNA binding cytoplasmic receptor
STEP 3
STEP 4
Activation
"activated"
STEP 6
DNA
STEP 4
Activation
STEP 5
Transcription
Translocation of cytoplasmic receptor complex
mRNA
"activated"
STEP 8
mRNA
Biological response
New protein
Translation
mRNA
STEP 7
Protein synthesis


Messenger
1
Receptor
Extracellular fluid
Phospholipase C
3
4a
α
β
γ
GDP
G protein
2
α
GTP
GDP
GTP
PIP2
DAG
IP3
5b
Protein kinase C
5a
ATP + protein
ADP
+
protein- P
6a
Response
in cell
6c
Calmodulin
6b
Protein kinase
Response in cell
(contraction, secretion)
Lumen of
endoplasmic
reticulum
Protein- P
Membrane of
endoplasmic
reticulum
Response in cell (contraction,
metabolism, transport)
Cytosol


Extracellular side
Hormone (TRH, GnRH)
G protein
Cell membrane
Receptor
DAG
Phospholipase C
Protein kinase C
Ca^{2+}
Phosphorylated protein performs specific function
IP 3 Receptor
Endoplasmic reticulum Ca storage

THANK YOU