PHARMACODYNAMICS

by

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PHARMACODYNAMICS

Greek : dynamis – power

- 'What the drug does to the body'

- **Definition:** Pharmacodynamics is the branch of pharmacology which deals with the physiological and biochemical effects of drugs and their mechanism of action at macromolecular/subcellular/organ system levels.

Principle of drug action:

- Alter the rate of body function.
- They do not create new effects.
- They modulate intrinsic physiological functions.

Types of drug action

- **Stimulation**– Epinephrine (heart) pilocarpine (salivary glands)
- **Depression**––barbiturates (CNS), quinidine (heart)
- **Irritation**––irritant purgatives
- **Replacement**––levodopa (parkinsonism), insulin (DM)
- **Cytotoxic**––chemotherapeutic agents

Mechanisms Of Drug Action

Drugs may act by following mechanisms:

1. Drug receptor interaction: The most common MOA

A. Drug interaction may be with:

- Regulatory proteins i.e. Physiological receptors for:
  - i. Neurotransmitter (Adrenergic drugs)
  - ii. Autacoids (Antihistamines)
  - iii. Hormones (Corticosteroids)
- Transport proteins i .e. Na^+-K^+-ATPase (Digoxin)
- Structural proteins i.e. Tubulin (Colchicine)

B. Drug-enzyme interaction

Enzymes (functional proteins) are very important target of drug actions.

The drug molecule is a substrate analogue that acts as competitive inhibitor of the enzyme, either reversibly or irreversibly.

Carbonic anhydrase (inhibited by Acetazolamide)

Xanthine oxidase (inhibited by Allopurinol)

Cyclooxygenase (inhibited by aspirin irreversibly)

C. Drug-channel interaction

- i. e Calcium channels are blocked by Verapamil.

  Sodium channels are blocked by Local Anesthetics.

D. Interaction with nucleic acid

- i.e.5 F U (Anticancer drug)

2. Biophysical interaction

i.e. Alcohol , Volatile

E Alteration of specific processes unique to micro-organisms / Cancer cells:

Antibacterial i.e. Streptomycin ,Penicillins.

Antiviral i.e. Ribavirin

Anti fungal i.e. Clotrimazole.

Antiparasitic i.e. Chloroquine.

Alkylating agents i.e. Cyclophosphamide

Antimetabolites i.e. Methotrexate

Spindle poisons i.e. Vincristine

Hormone / Hormone antagonists i.e. Prednisolone / Tamoxifen

F. **Chemical interaction:** i.e. Chelating agents & heavy metals

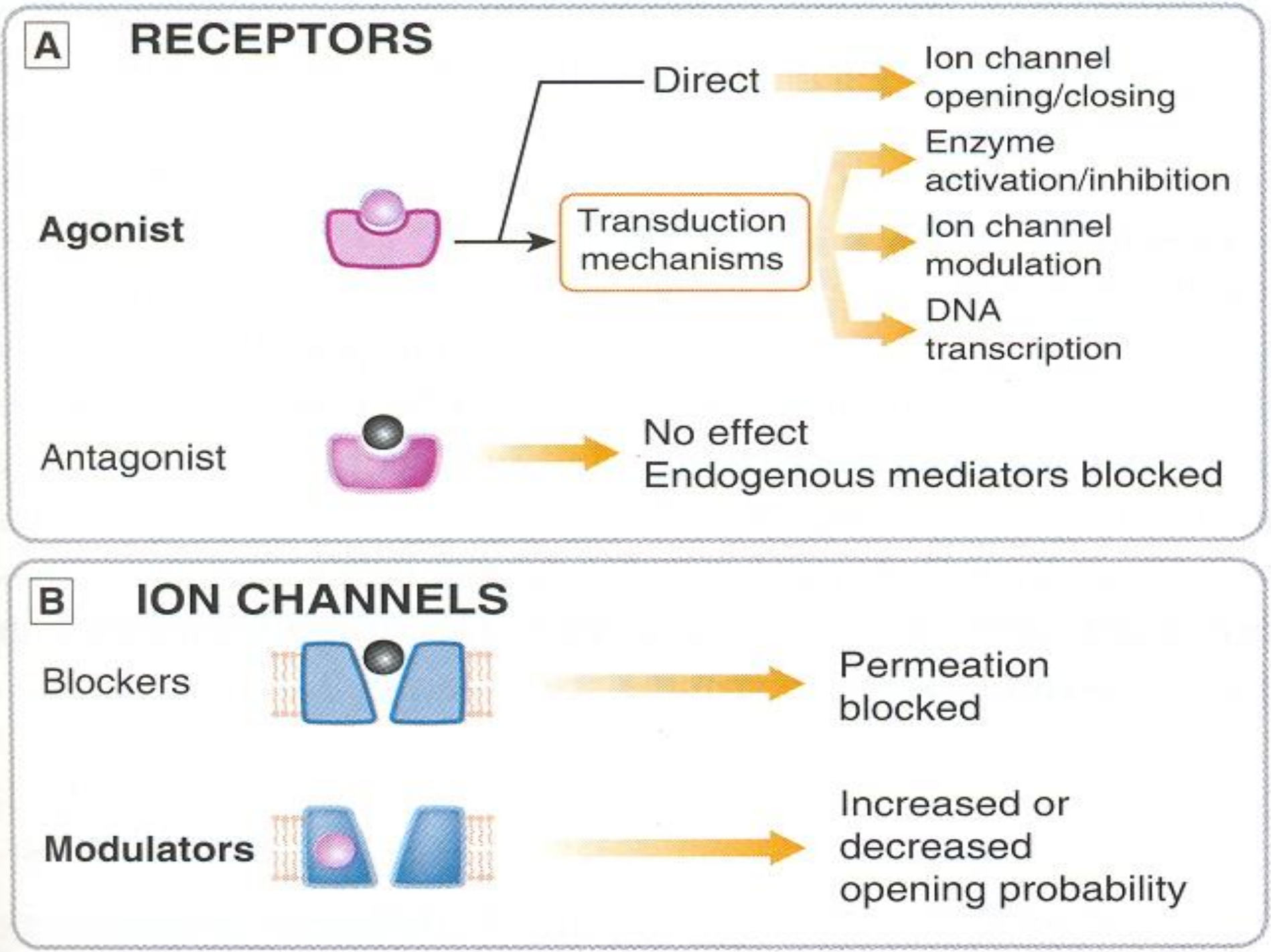
Heparin & Protamine Sulphate

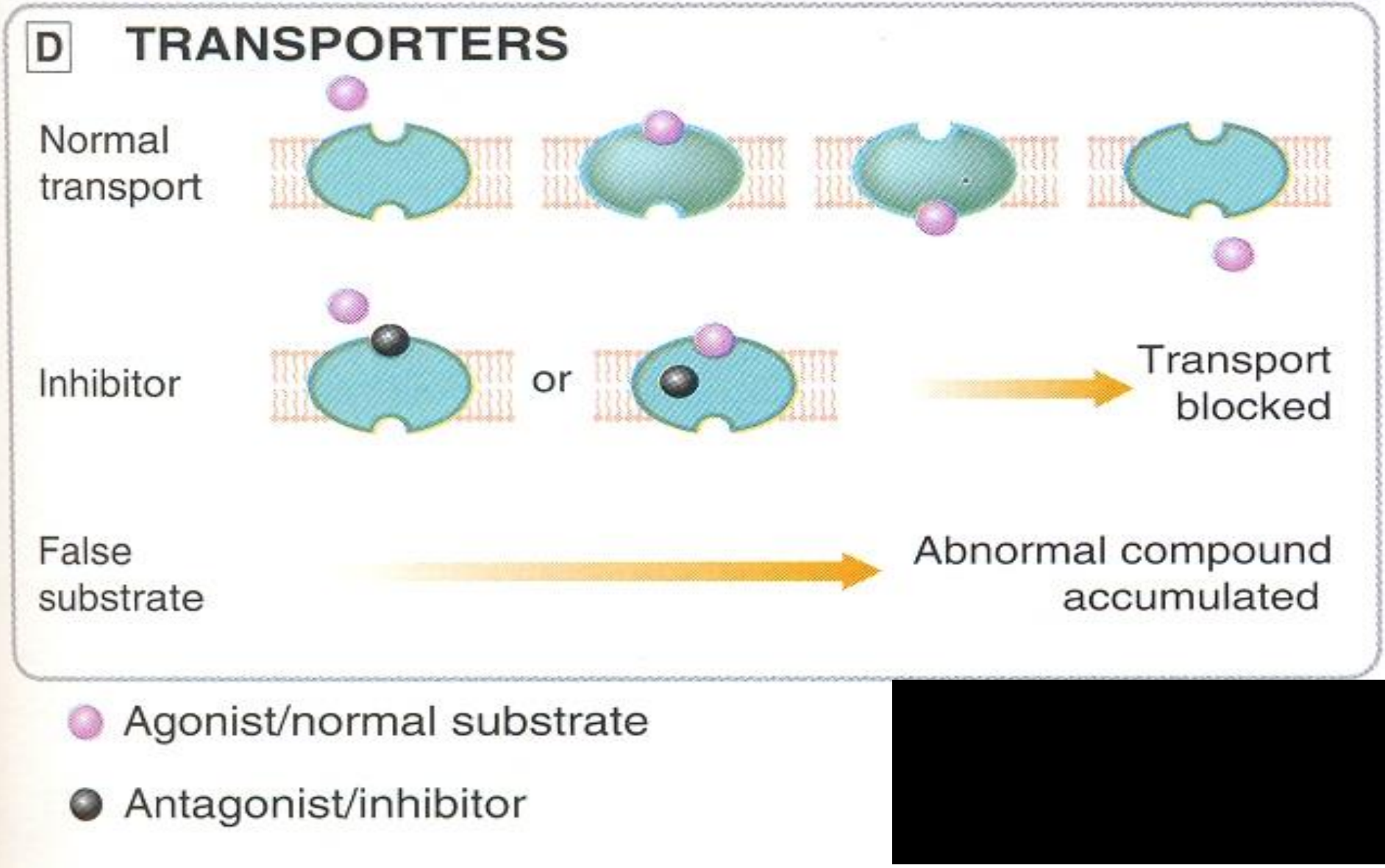
G. **Physical Action**

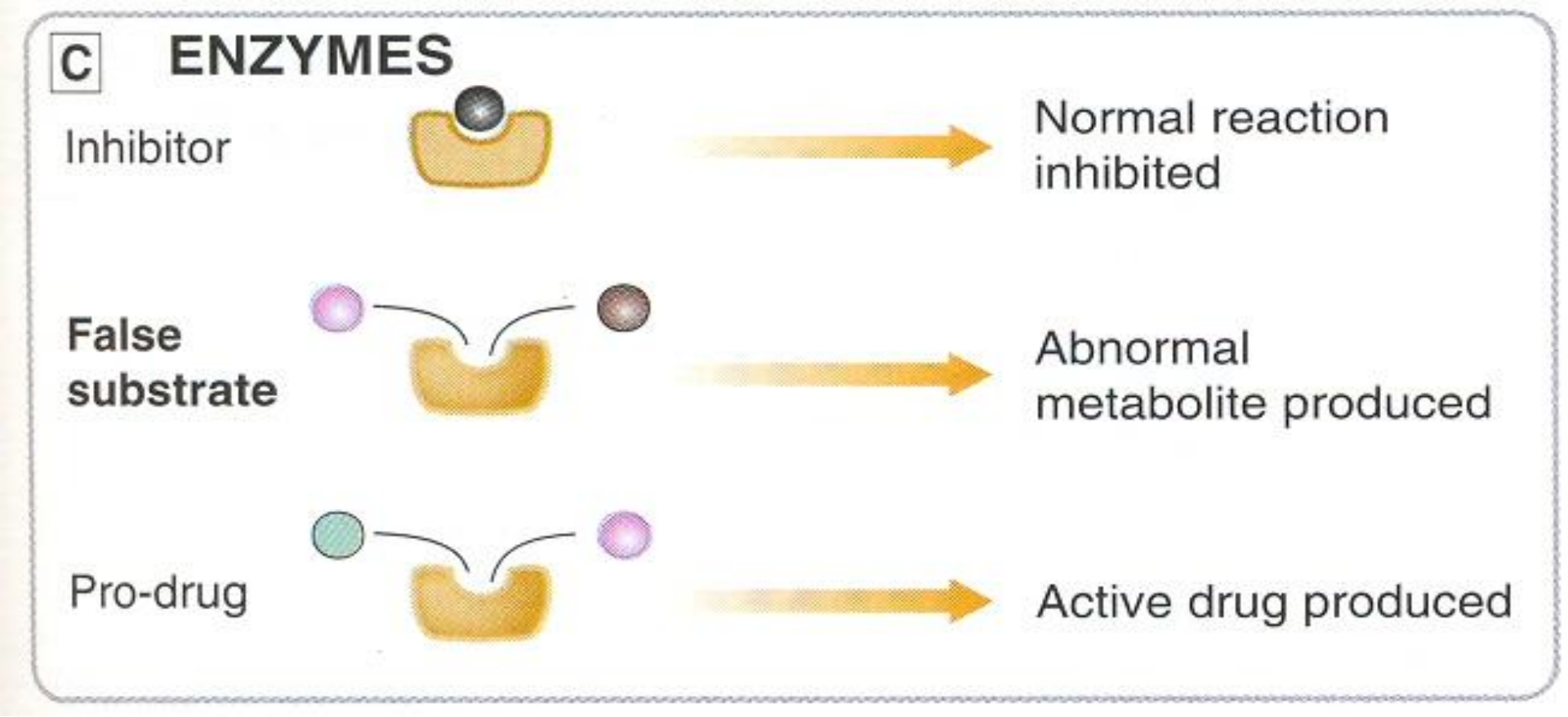
- Mass of the drug ---- Bulk laxatives
- Adsorptive property ---- Charcol, Kaolin ,
- Osmosis i.e. Diuretic (Manitol)
- Radioactivity ----- ^{131}I
- Radioopacity ---- contrast media , Urografin

6. **By interfering with synthesis storage release uptake or metabolism of neurotransmitters.**

e.g Anti-adrenergic drug.


A
RECEPTORS
Direct
Ion channel opening/closing
Agonist
Transduction mechanisms
Enzyme activation/inhibition
Ion channel modulation
DNA transcription
Antagonist
No effect
Endogenous mediators blocked
B
ION CHANNELS
Blockers
Permeation blocked
Modulators
Increased or decreased opening probability


D TRANSPORTERS
Normal transport
Inhibitor
or
Transport blocked
False substrate
Abnormal compound accumulated
Agonist/normal substrate
Antagonist/inhibitor


C ENZYMES
Inhibitor
Normal reaction inhibited
False substrate
Abnormal metabolite produced
Pro-drug
Active drug produced

Drug receptor interaction

Receptors:

A receptor may be defined as any target molecule to which a drug binds in order to elicit its specific effects in a biological system. It is usually a protein.

OR

Receptors are the specific cellular macromolecule to which the drug binds to produce its biological effect/response by bringing a conformational change in the macro molecule & initiating a chain of reaction.

Biological effect may be :

- Increased secretion of a gland
- Contraction of a muscle.
- Decrease in heart rate
- Change in permeability.

Characteristics of receptors

- Most of the receptors are proteins, a few are other molecules such as DNA.
- They have a specific region to which drug molecule binds with high selectivity & specificity -- Receptor site (Recognition site)
- Receptors determine the quantitative relationship between the dose and pharmacologic effects of a drug.
- Are responsible for selectivity / specificity of drug action.
- They mediate the actions of different ligands---- pharmacologic agonists & antagonists, partial agonists and inverse agonists.

Types of drug receptor binding / occupancy interaction:

- Reversible: Drug molecule binds to receptor by weak bonds--- hydrogen bonds
- Irreversible: Drug molecule binds to receptor by strong bonds--- covalent bonds

Coupling:

The transduction process between the occupancy of receptors & drug response is called coupling.

The NT can be thought as a signal & receptor as signal detector & transducer

So receptors serve two essential functions:

1. Recognition of the specific ligand molecule.
2. Transduction of the signal into a response.

Transducer Mechanisms:

These are highly complex multistep processes that provide for amplification & integration of concurrently received extra-and intra-cellular signals at each step.

Effectors:

These are molecules that translate the drug-receptor interaction into a change in cellular activity.

Examples:

- Enzyme Adenylyl cyclase.
- Some receptors are also effectors:

Tyrosine kinase is part of Insulin receptor.

Molecular / Signaling mechanisms and drug action

Five basic mechanisms of transmembrane Signaling:

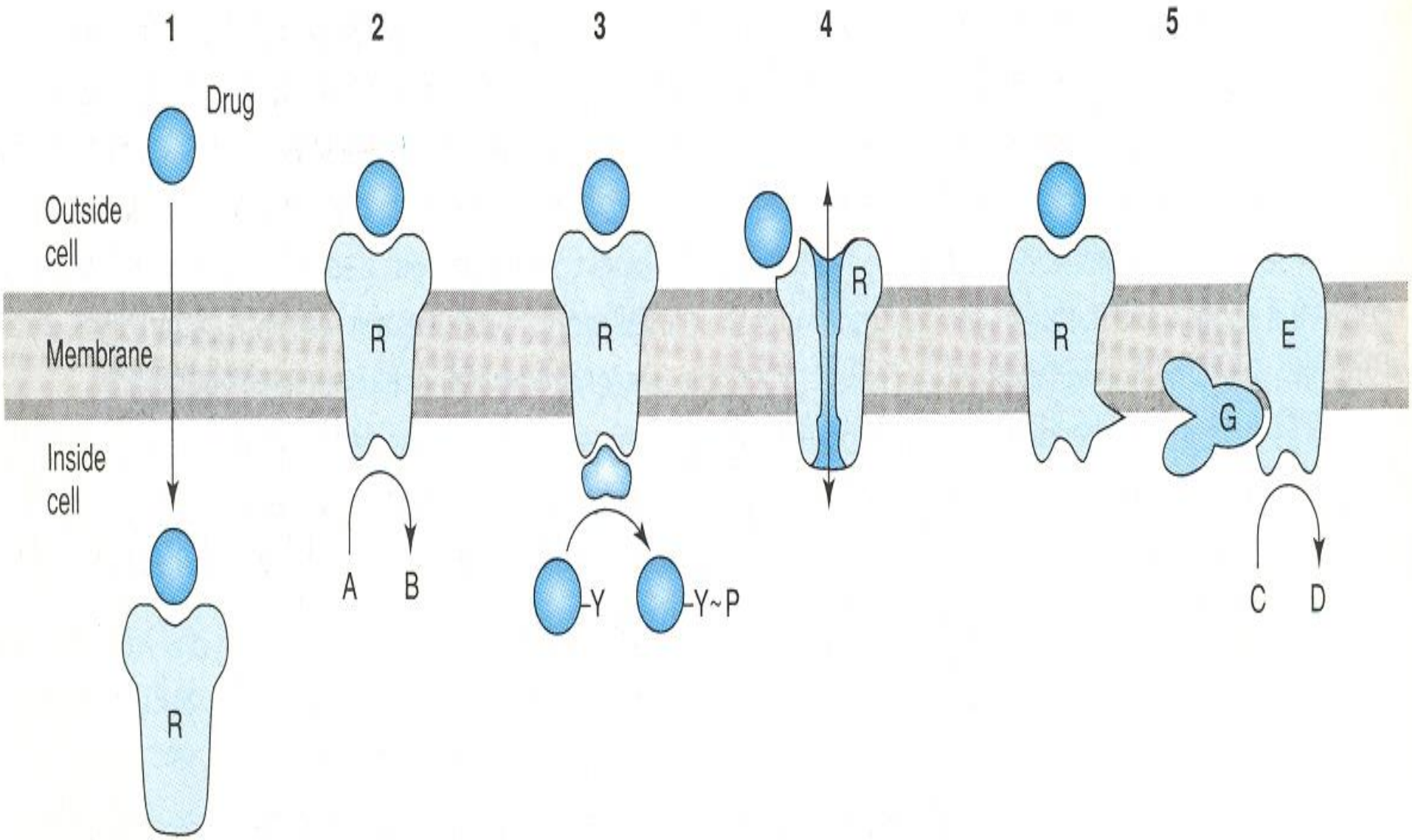
a. Lipid soluble ligand that crosses the membrane and acts on intracellular receptors

b Transmembrane receptor protein whose intracellular enzymatic activity is allosterically regulated by a ligand that binds to a site on the protein's extra cellular domain

c. Binding to extra cellular domain of trans membrane receptor bound to separate intracellular protein tyrosine kinase molecule

d. Binding to and directly regulating the opening and closing of an ion channel.

e. Binding to a cell surface receptor linked to an effector enzyme by a G protein (GTP binding signal protein) which in turn changes the concentration of intracellular second messenger.


1
2
3
4
5
Drug
Outside cell
Membrane
Inside cell
R
R
R
R
R
A
B
Y
Y~P
G
E
C
D

Receptor & Signaling Mechanisms for drug effects:

1. Trans membrane diffusion and action on intracellular receptors:

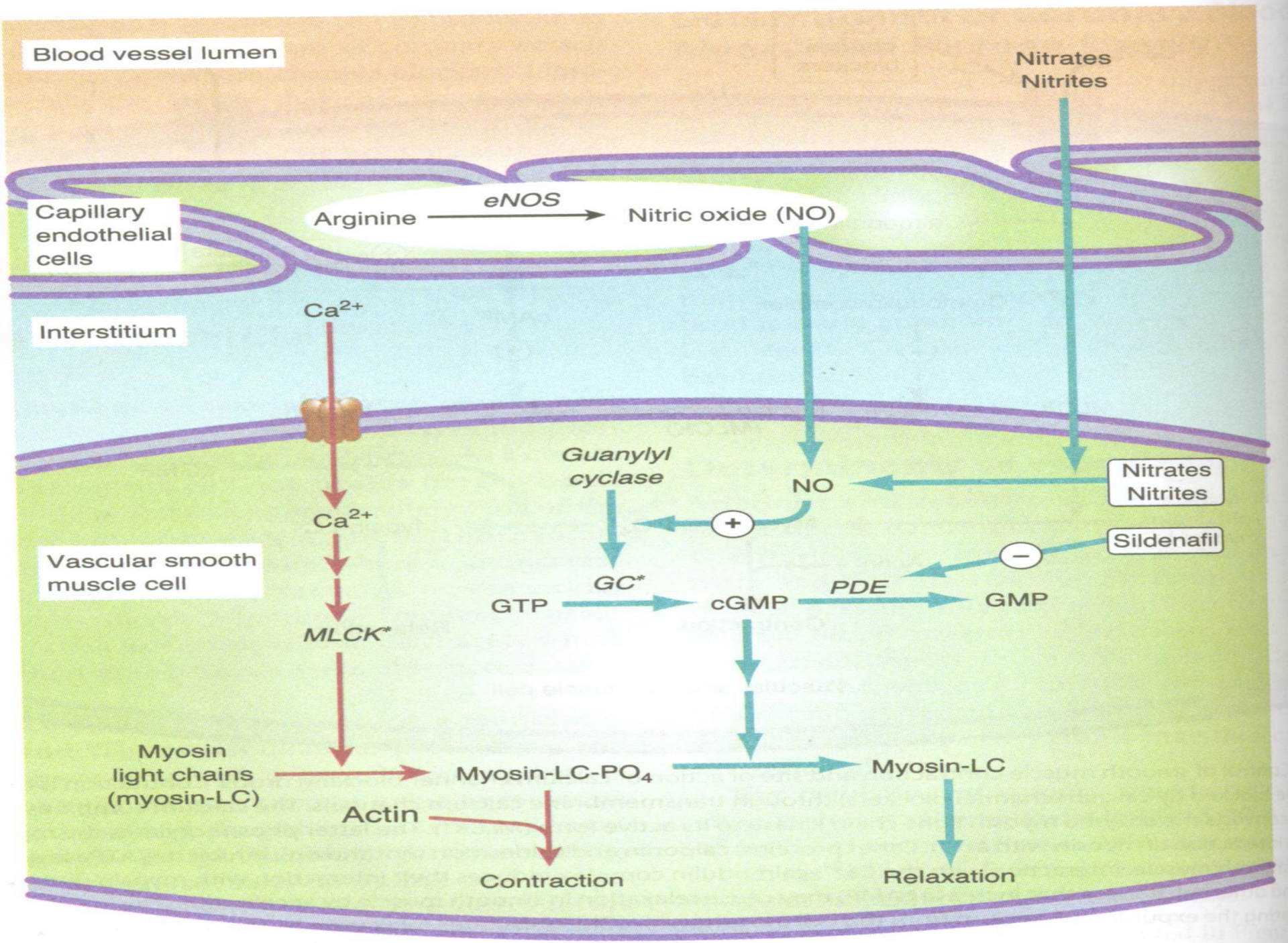
Several biological ligands & related drugs molecules are sufficiently lipid soluble to cross the plasma membrane by lipid diffusion & act on intracellular receptor by:

a: Stimulation of cytoplasmic enzyme

b: Stimulation of gene transcription.

a: Stimulation of cytoplasmic enzyme : Nitric oxide diffuses into the vascular smooth muscles cell by lipid diffusion.

- Stimulates Guanylyl cyclase enzyme (effector)
  - Accumulation of cGMP (Second messenger)
  - Relaxation of smooth muscles



Mechanism of action of nitrates, nitrites, and other substances that increase the concentration of nitric ox

Stimulation of gene transcription by:

- Corticosteroids
- Thyroid hormone
- Vitamin D

These receptors control gene transcription (gene active receptors).

- Receptors are intracellular proteins, so ligands first enters the cells; by lipid diffusion.
- Receptors consist of three domains:
  i. DNA-binding domain.
  ii. Ligand-binding domain.
  iii. Transcription activating domain.

- Without ligand receptor(R)is stablized in inactive state by hsp90.

- When steroid binds to receptor site hsp90 is dissociated, receptor is converted into active configuration.

- Activated R can initiate transcription of genes by binding to Response Elements (specific DNA sequences near the genes).

Important therapeutic consequences:

- Effects are slow in onset (30 min /several hrs) as they are due to altered protein synthesis.

- Effects can persists for hours / days after the agonist conc. has been reduced to zero ----- due to slow turn over of enzymes & proteins.

- One type of nuclear receptor is responsible for the increased expression of drug-metabolising enzymes induced by many therapeutic agents.

2. Receptor Located on Transmembrane Enzymes :

- Trans-membrane proteins
- Large extra cellular domain
- Intracellular domain having effector enzymatic activity:
  - tyrosine kinase
  - guanylate cyclase
  - serine / Threoninekinase activity.

Examples:

Epidermal growth factor, Insulin , Atrial natriuretic peptide

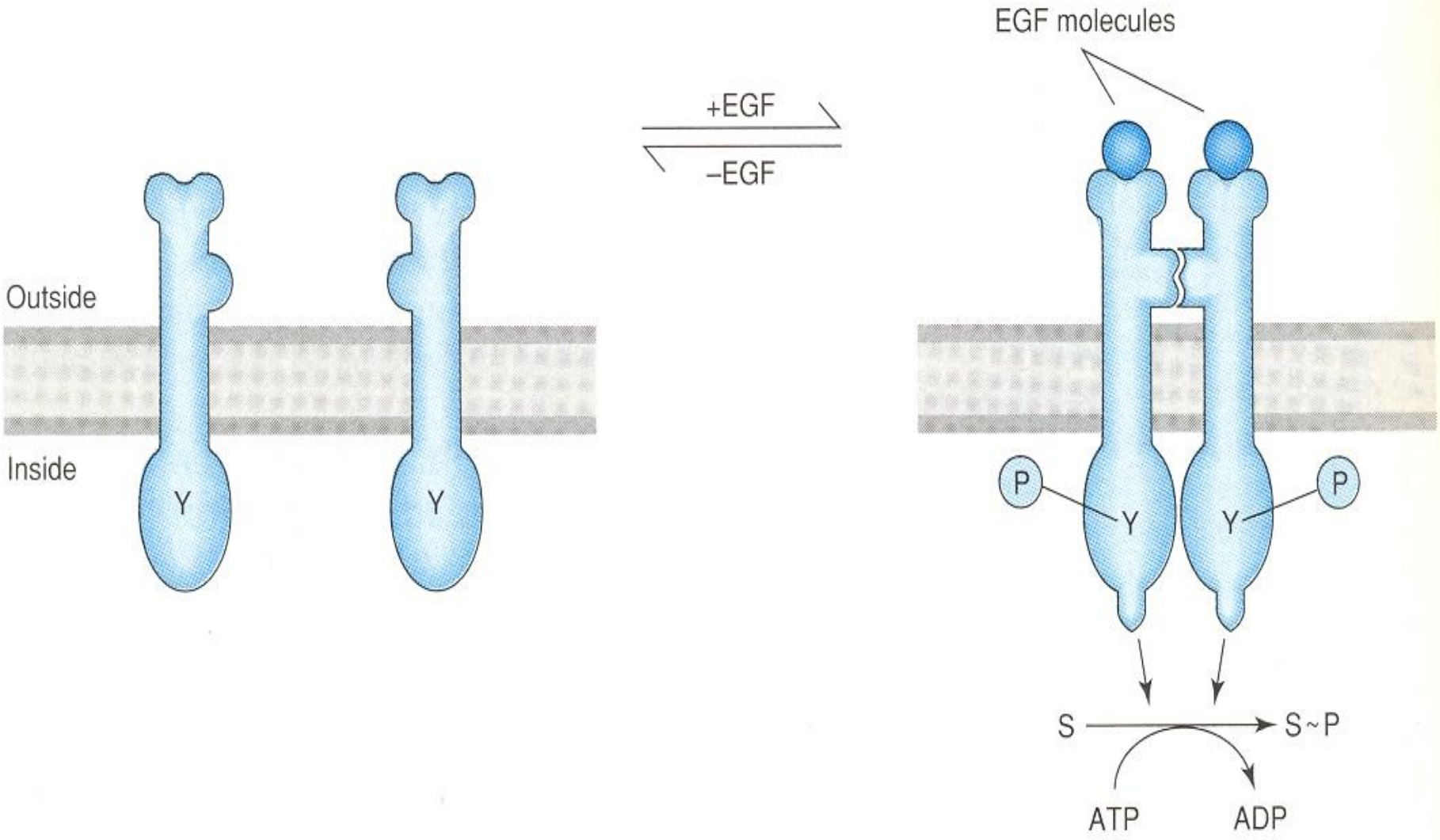
Platelet derived growth factor.

Transforming growth factor –β (TGF- β)

- Binding of drug to receptor site on extra cellular domain
- Conformational change.
- Activates enzymatic activity of **effector** enzyme---- Tyrosine kinase.
- Dimerisation & enzymatic activation of tyrosine residues ; they phosphorylate one another.
- Activated receptors catalyze phosphorylation of tyrosine residues on different target signaling proteins.
- So single type of activated receptor can modulate a number of biochemical processes

i.e Insulin increases glucose & amino acids uptake & to regulate the metabolism of glycogen & triglycerides in the cells.

- Each growth factor initiates in its target cell a complex program of cellular events ranging from altered membrane transport of ions & metabolites to expression of many genes.
- Inhibitors of Tyrosine kinase are useful in those cancers where excessive signaling by growth factors is involved.
- Ligand binding to Tyrosine kinase receptors also produces endocytosis- & degradation ---- down regulation of receptors ----decreased response; if denovo synthesis occurs at slower rate.
- Genetic mutation s which decrease endocytosis---increased risk of certain cancers.


EGF molecules
+EGF
–EGF
Outside
Inside
Y
Y
P
Y
Y
P
S
S~P
ATP
ADP

3. Cytokine receptor :

Trans membrane receptor having:

a) Extra cellular domain
b) Intracellular domain that binds non-covalently to a separate Tyrosine kinase from Janus-kinase family(JAK)

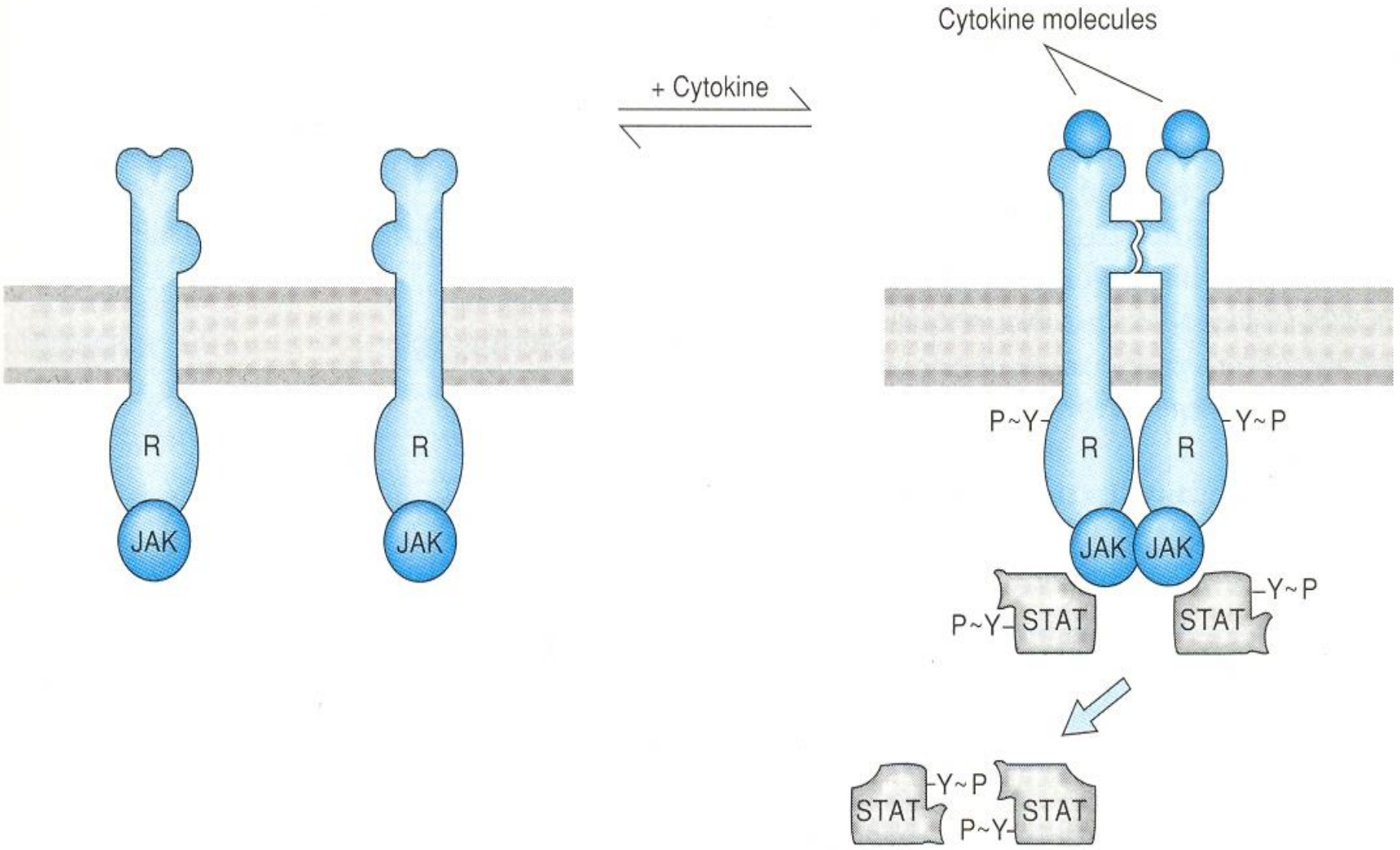
Examples:

- Growth hormone
- Erythropoietin
- Interferon

Signaling mech.

Like Tyrosine kinase (TK)receptors; the TK activity is not intrinsic to receptor but a separate Tk from Janus kinase (JAK)family binds non-cavalently to the receptor

- When the receptor is occupied , dimerization and activation of JAK – which phosphorylates tyrosine residues.
- The phosphorylates tyrosine residues set in a chain of reactions by binding to STATs (Signal transducers and activators of transcription).
- The bound STATs are themselves are phosphorylated by the JAKs
- Finally the STAS-STAT dimers travel to the nucleus and regulate transcription of specific genes.


Cytokine molecules
+ Cytokine
R
R
JAK
JAK
P~Y
Y~P
R
R
JAK
JAK
P~Y
STAT
STAT
Y~P
STAT
Y~P
P~Y
STAT

4. Ligand & voltage gated ion channel (ionotropic receptors):

Many useful drugs act by mimicking /blocking the actions of endogenous ligands that regulate the flow of ions through channels in plasma membrane.

Examples

Synaptic transmitters:

- Acetylcholine at nicotinic receptor
- Gammaaminobutyric acid at $GABA_A$ receptors
- Glycine
- 5hydroxytryptamine at $5\text{-}HT_3$ receptors

Ligand & voltage gated ion channels (ionotropic receptors)

Ligand gated ion channels :

- Several structural families, the commonest 4-5 trans-membrane sub units, arranged around a central aqueous channel.
- The drug binds to receptor site & directly opens the channel **within milliseconds.**
- Involved mainly in fast synaptic transmission– important for moment to moment transfer of information across synapses.

Ligand gated ion channels can be regulated by several mechanisms– phosphorylation & endocytosis--- this contributes to synaptic plasticity involved in learning & memory.

Examples _Synaptic transmitters:

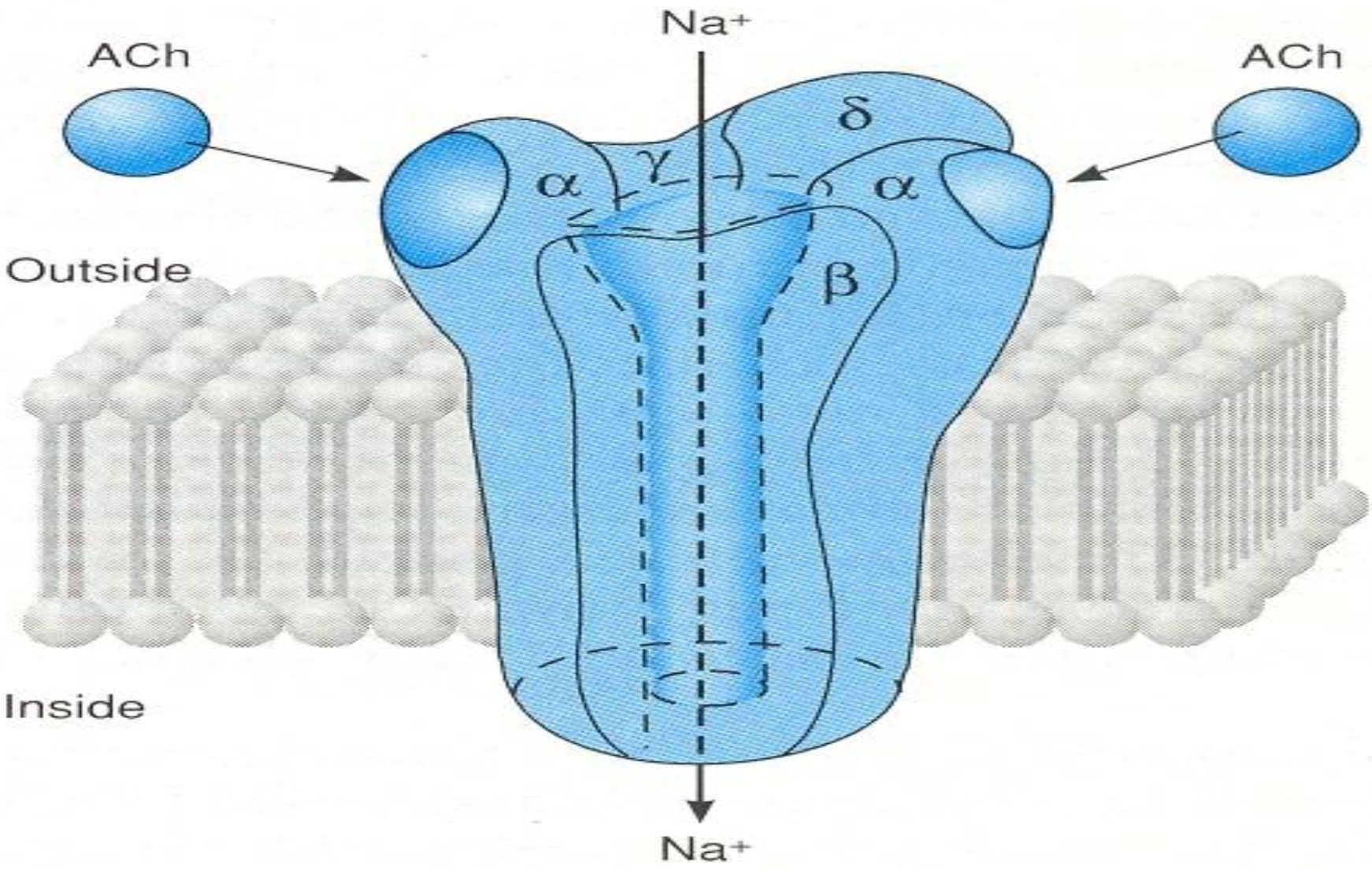
Acetylcholine at nicotinic receptor

Gammaaminobutyric acid at $GABA_A$ receptor

5hydroxytryptamine at $5\text{-}HT_3$ receptors

The best example is **Acetylcholine nicotinic receptor (N_M).**

- It is a pentapeptide **composed of** 2 α & one β, δ , γ subunits
- **Location:** Skeletal Muscles:Neuromuscular junction
- **Binding of Agonist:** Ach/ Nicotine at binding sites on 2 α subunits--there is conformational change – opening of the sodium channel--- Influx of Na+ ions from ECF into the cell.
- **Response**: End-plate depolarization, skeletal muscle contraction.


Na+
ACh
ACh
δ
γ
α
α
Outside
β
Inside
Na+

Voltage gated ion channels:

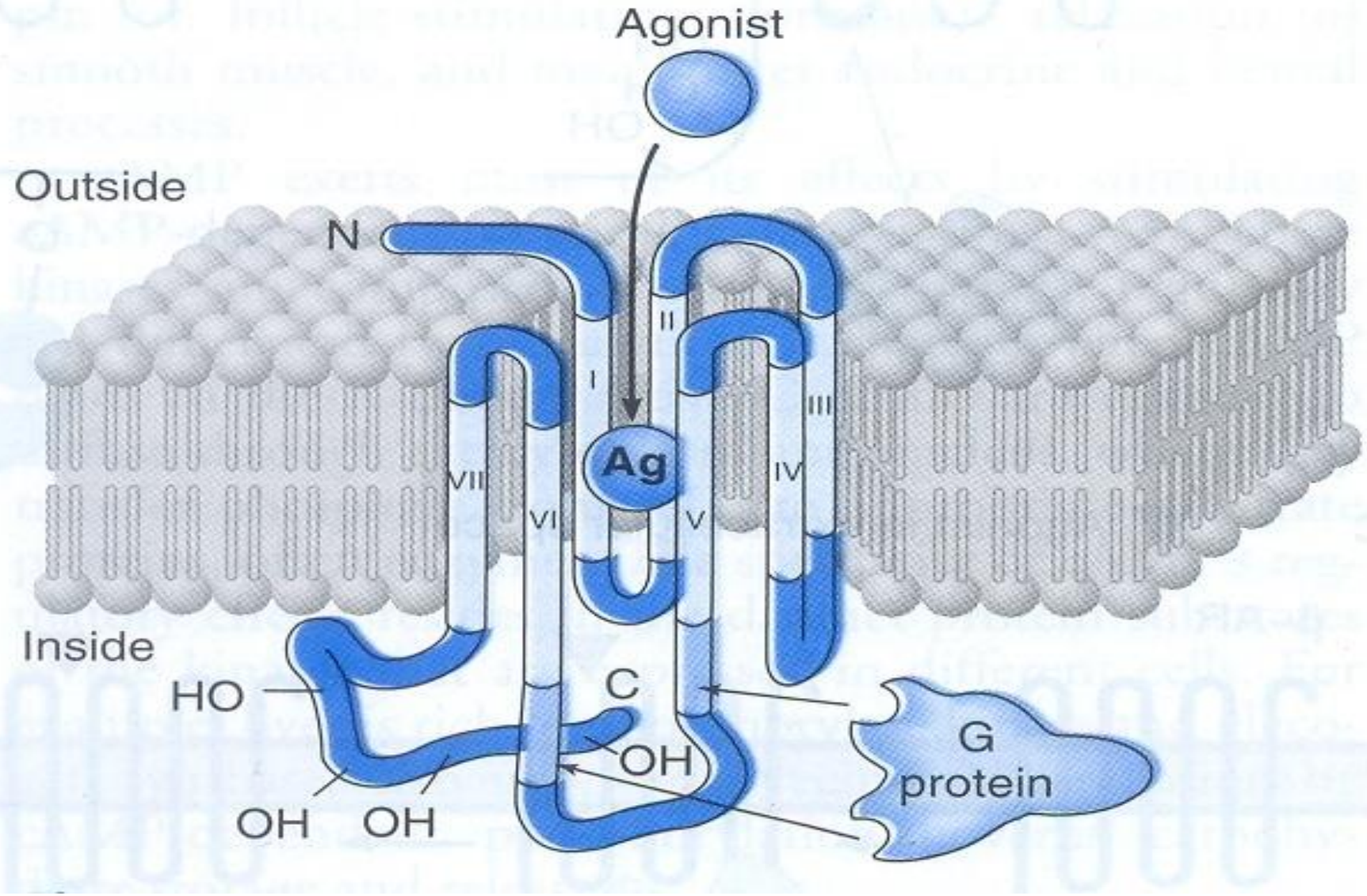
- They do not bind neurotransmitters directly but are controlled by membrane potential.
- They are also important drug targets
- Example:--Verapamil blocks calcium channels present in heart & BV---- useful in lowering high blood pressure or controlling arrhythmias

5. G protein-coupled receptors (GPCR) Or Metabotropic receptors

- Family of 7 trans membrane or serpentine receptors.
- One of the intracellular loops is larger than the others and interacts with the G-protein.

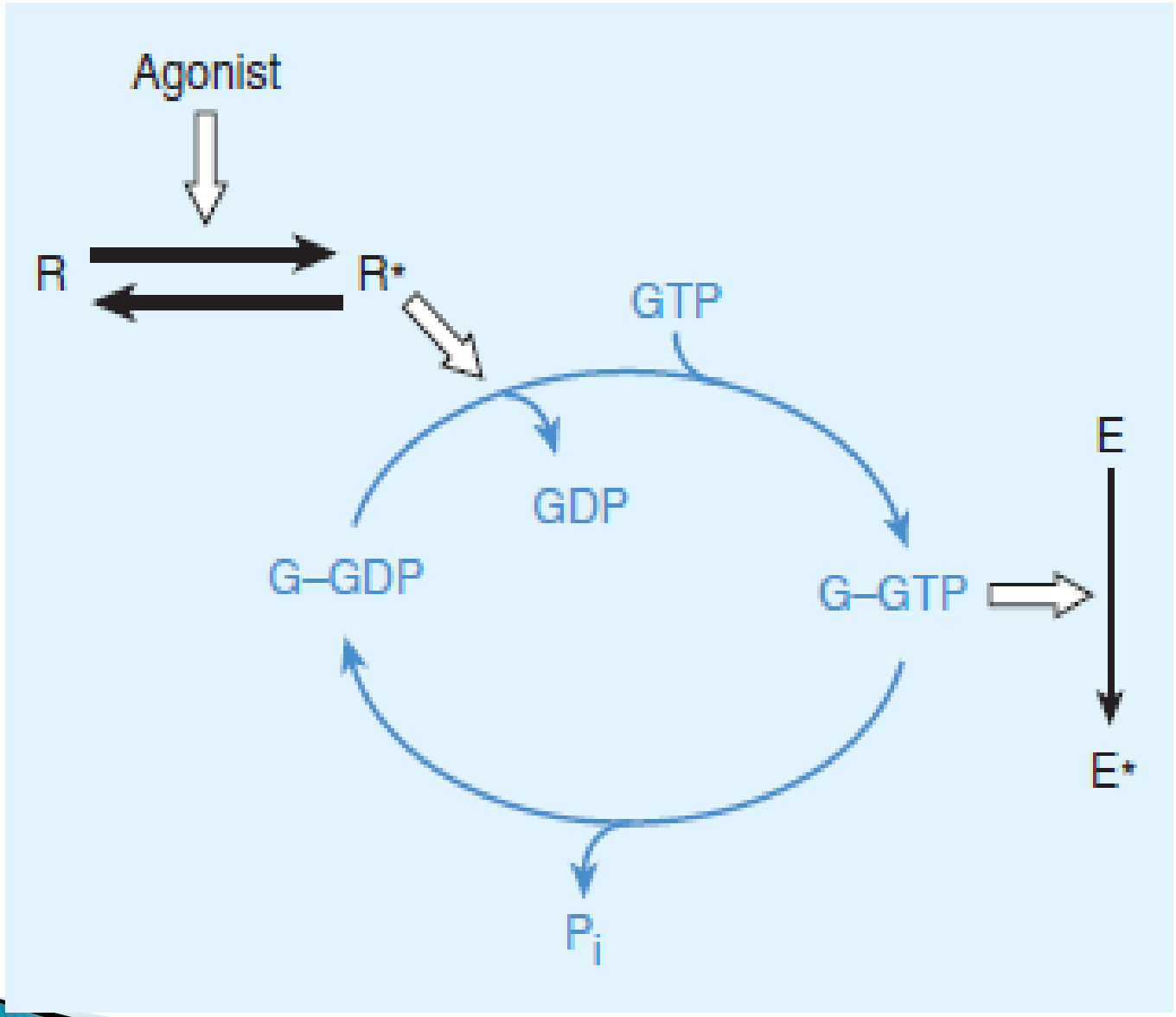
The G-protein : it is a membrane protein.

- Several types of G-proteins: Gq, Gs, Gi
- It comprises of:
  - 3 subunits **αβγ**
  - α-subunit possesses GTPase activity .


Agonist
Outside
N
I
II
III
IV
V
VI
VII
Ag
Inside
HO
C
OH
OH
OH
G
protein

- **When drug binds to a cell surface receptor.**
  - Conformational change.
  - Transmitted to C loops
  - The α-subunit dissociates.
  - Release of GDP from G pr. & entry of GTP.
  - Then it can activate an **effector:**
    - A membrane enzyme i.e. Adenylyl cyclase
    - Ion channel

(In some cases the βγ-subunit may be the activator species as at M_2 receptors in heart)


Agonist
R
R*
GTP
GDP
G–GDP
G–GTP
E
E*
P_i

- This changes the concentration of intracellular second messenger i.e.
  - Cyclic Adenosine Monophosphate (cAMP)
  - Calcium and Phosphoinositides (IP3 and diacyl glycerol)
  - Cyclic Guanosine Monophosphate (cGMP)
- Activation of the effector is terminated when the bound GTP molecule is hydrolyzed,
- The αsubunit recombines with βγ.

Examples

- **Muscarinic acetylcholine receptor**
- **Alpha & Beta Adrenoceptors**

Second messengers:

These are chemical substances, produced in response to NT binding to a receptor, their intracellular concentration increases/ decreases in response to receptor activation by agonist through "effectors".

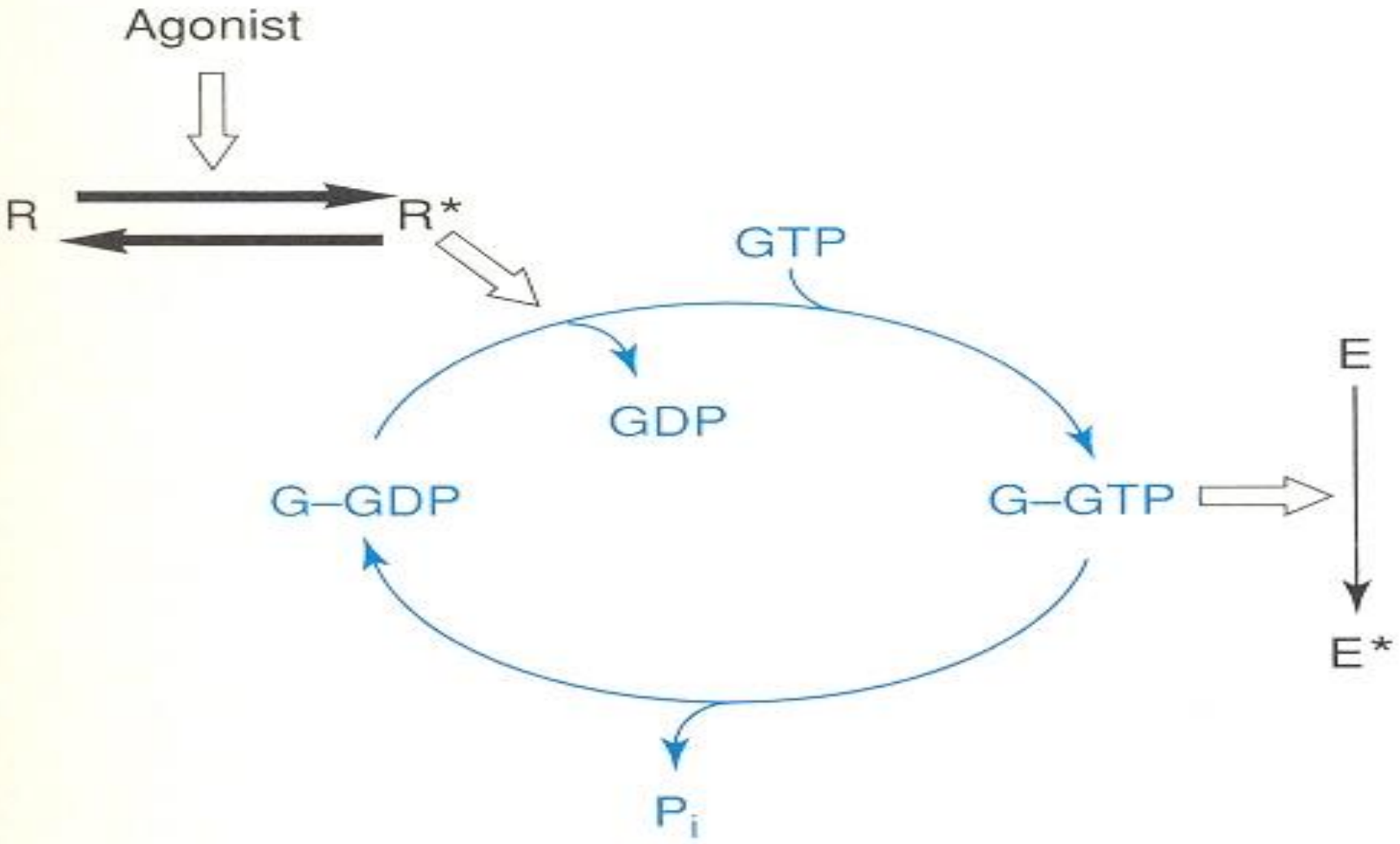
called second messengers because they intervene between the original message (NT/Hormone) and the ultimate effect on the cell.

They amplify & further transmit the signal to trigger processes that eventually produce the response---they translate the extracellular signal into a response that may be further propagated or amplified within the cell.

e.g. Cyclic Adenosine mono phosphate(cAMP)

Diacylglycerol (DAG)

Second messengers are part of the cascade at G-protein coupled receptors.


Agonist
R
R*
GTP
GDP
G–GDP
G–GTP
E
E*
P_i

Receptor Types	Coupling protein	Effector	Effector substrate	Second messenger response	Result
M1,M3, Alpha	Gq	Phospholipase C	Membrane lipids	↑IP3 ↑ DAG	↑Ca^{2+} ↑ Protein kinase
Beta , D1	Gs	Adenyl cyclase	ATP	↑cAMP	↑Ca^{2+} influx ↑Enz. activity
$Alpha_2$, M2	Gi	Adenyl cyclase	ATP	↓cAMP	↓Ca^{2+} influx & Enz activity

G Protein	Ligand for receptors	Effectors	Second Messenger
Gs	Beta adrenergic amines	↑ Adenylyl cyclase	↑ cAMP
G_{i1}, G_{i2}, G_{i3}	α_2 adrenergic amine Acetylcholine Opioids	↓ Adenylyl cyclase	↓ cAMP Open cardiac K+ channels
G_{olf}	Odorants	↑ Adenylyl cyclase	↑ cAMP
G_q	Acetylcholine, serotonin & others	↑ Phospholipase C	↑IP3 ↑ DAG & Cytoplasmic Ca++
G_{t1} G_{t2}	Photons	↑ cGMP - Phosphodiesterase	↓ cGMP

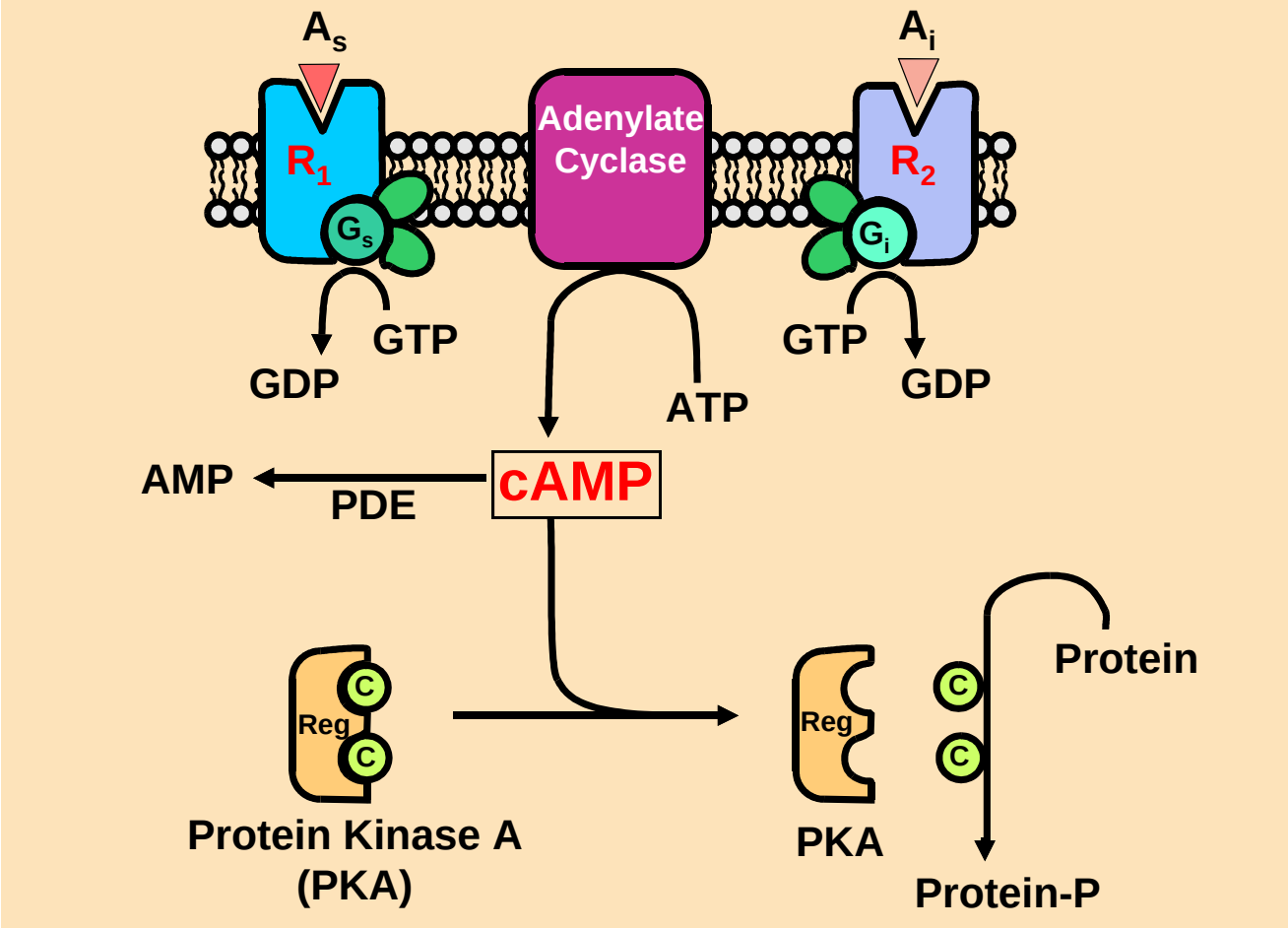
Effectors activated or inhibited by G-proteins

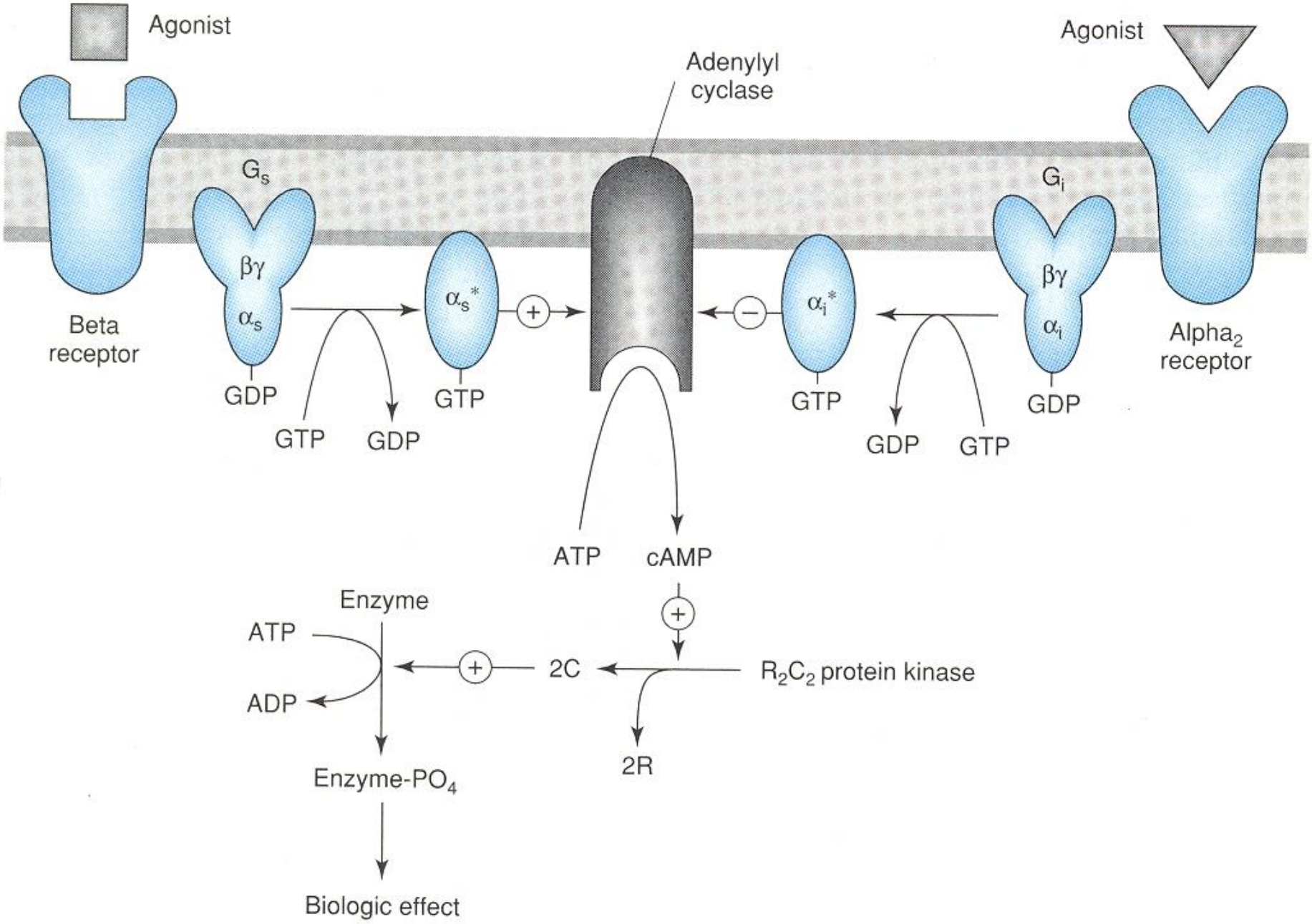
- Adenylate cyclase (AC):
  - AC catalyses formation of the intracellular messenger cAMP.
  - cAMP activates various protein kinases.
  - PK control cell function in many different ways by causing phosphorylation of various

    Enzymes

    Carriers

    Other proteins.


A_s
A_i
R_1
R_2
Adenylate Cyclase
G_s
G_i
GTP
GDP
GTP
GDP
ATP
AMP
PDE
cAMP
Reg
C
C
Protein Kinase A (PKA)
Reg
C
C
PKA
Protein
Protein-P


Agonist
Agonist
Adenylyl cyclase
Beta receptor
G_s
$\beta\gamma$
α_s
GDP
GTP
GDP
α_s^*
GTP
+
−
α_i^*
GTP
G_i
$\beta\gamma$
α_i
GDP
GDP
GTP
$Alpha_2$ receptor
ATP
cAMP
Enzyme
ATP
ADP
+
2C
+
R_2C_2 protein kinase
2R
Enzyme-PO_4
Biologic effect

Phospholipase C

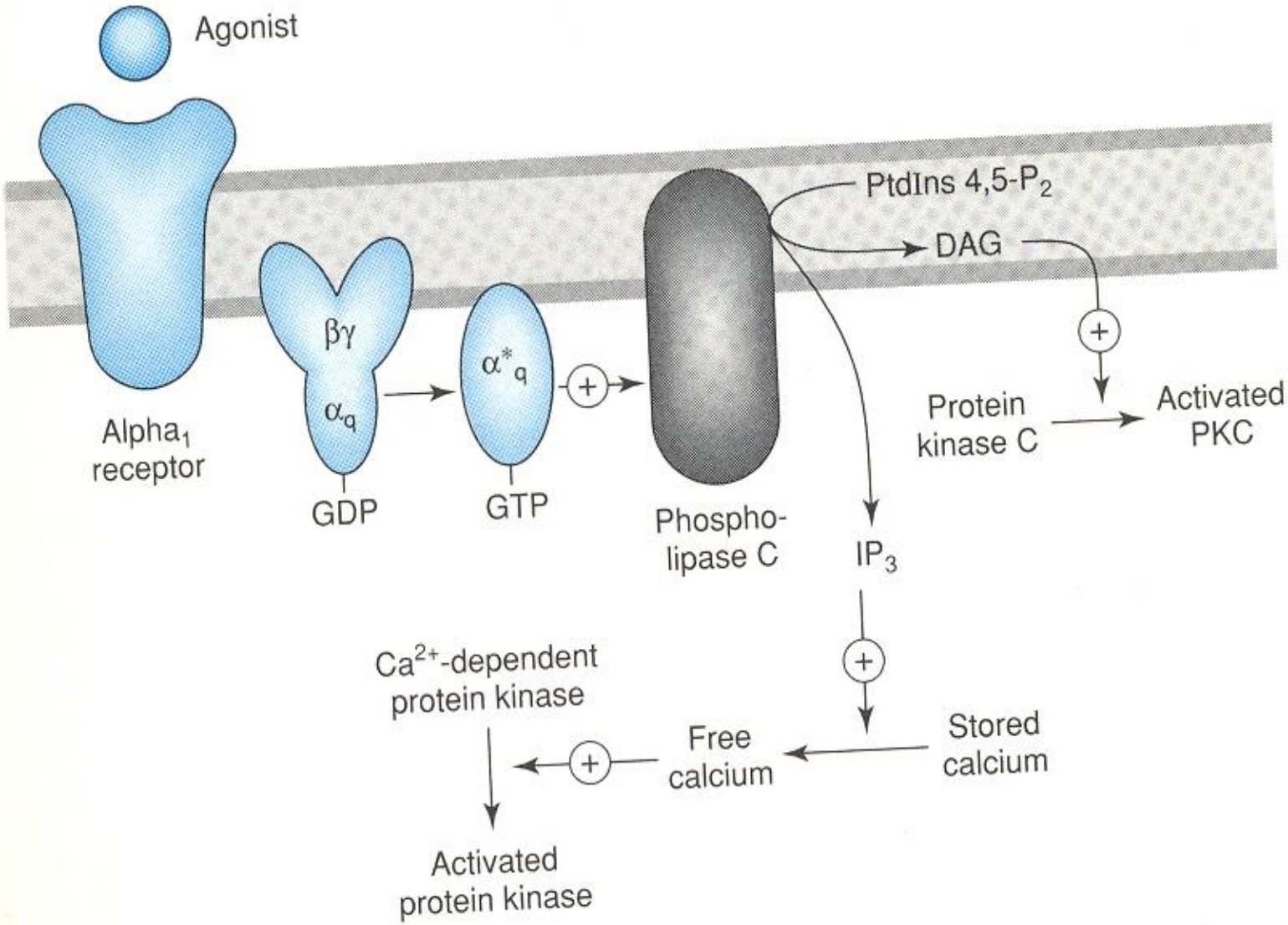
- Catalyses the formation of two intracellular messengers from membrane phospholipids:
  i) Inositol trisphosphate (IP_3)
  ii) Diacylglycerol (DAG)

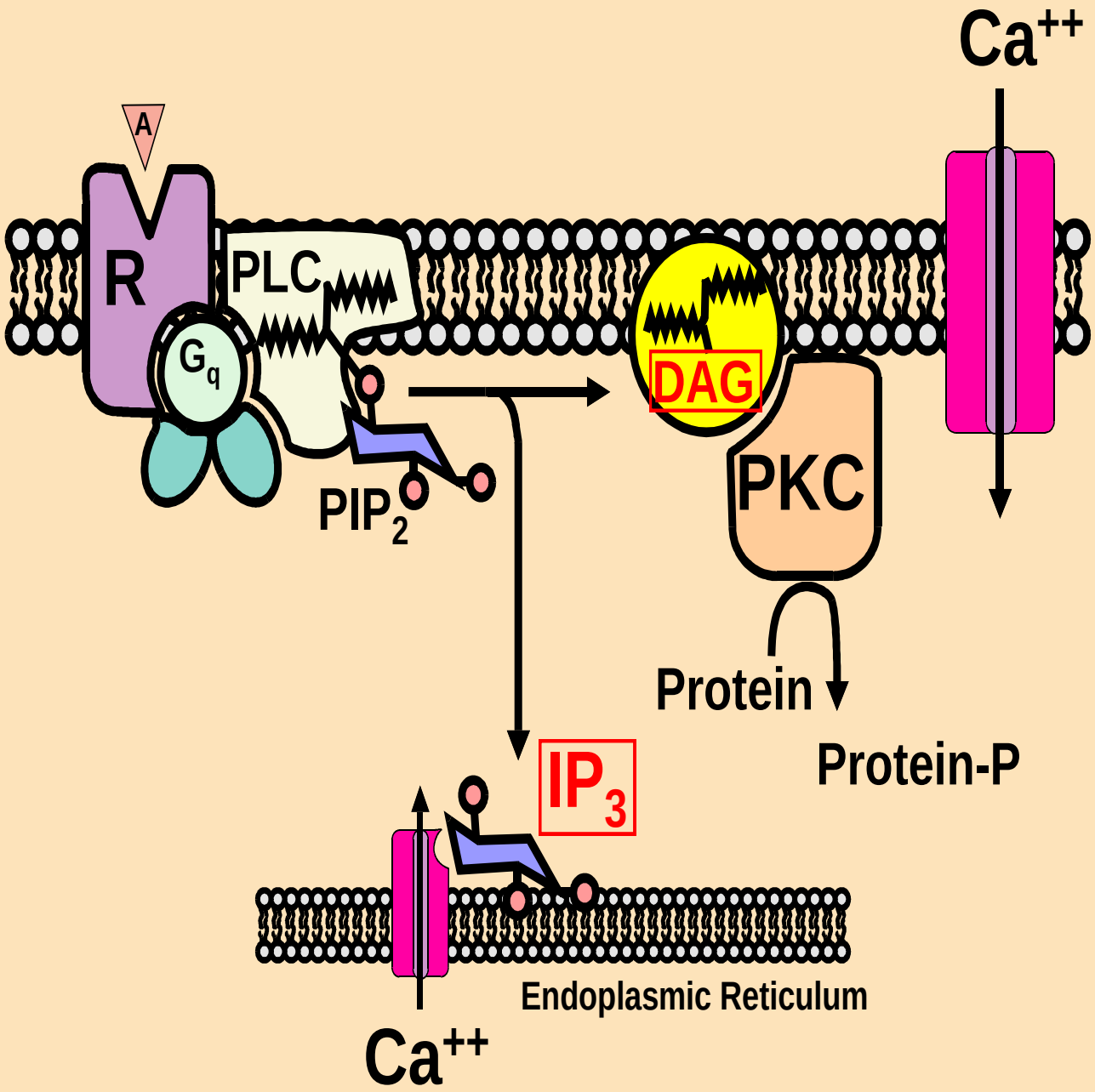
- IP_3 releases Ca^{2+} from intracellular compartments

So ↑ free cytosolic Ca^{2+} .

- **↑ free Ca^{2+} initiates many events:**
  Contraction
  Secretion
  Enzyme activation
  Membrane hyperpolarisation.

- DAG activates protein kinase C
- Protein kinase C controls many cellular functions by phosphorylating a variety of proteins

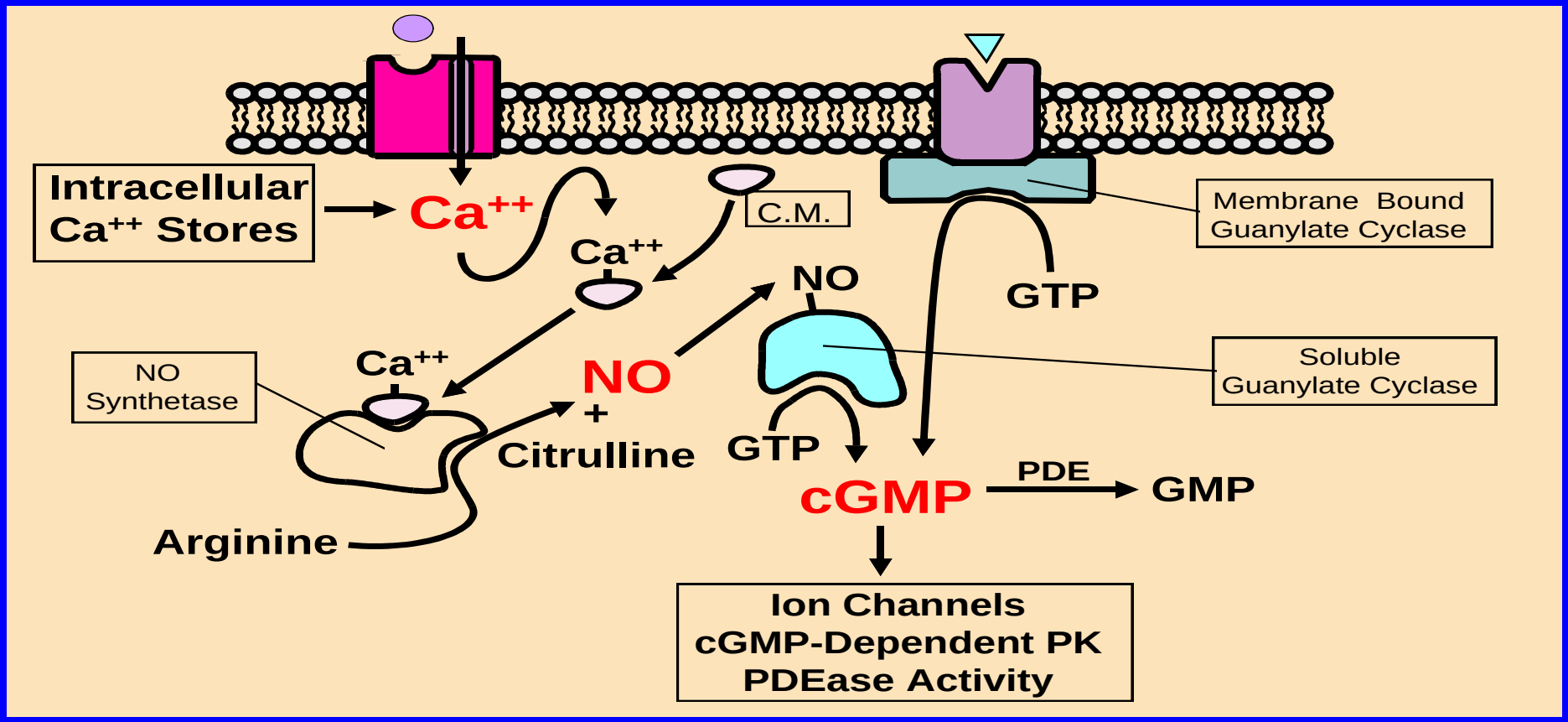

Agonist
Alpha1 receptor
βγ
αq
GDP
α*q
GTP
Phospho-lipase C
PtdIns 4,5-P2
DAG
Protein kinase C
Activated PKC
IP3
Ca2+-dependent protein kinase
Free calcium
Stored calcium
Activated protein kinase


Ca++
A
R
PLC
Gq
DAG
PIP2
PKC
Protein
Protein-P
IP3
Endoplasmic Reticulum
Ca++

Second Messenger	Examples of Hormones Which Utilize This System
Cyclic AMP	Epinephrine and norepinephrine, glucagon, luteinizing hormone, follicle stimulating hormone, thyroid-stimulating hormone, calcitonin, parathyroid hormone, antidiuretic hormone
Protein kinase activity	Insulin, growth hormone, prolactin, oxytocin, erythropoietin, several growth factors
Calcium and/or phosphoinositides	Epinephrine and norepinephrine, angiotensin II, antidiuretic hormone, gonadotropin-releasing hormone, thyroid-releasing hormone.
Cyclic GMP	Atrial naturetic hormone, nitric oxide

GUANYLATE CYCLASE: (cGMP) & NITRIC OXIDE

(AS SECOND MESSENGERS)



Three second messangers: Ca^{++}, Nitric oxide (NO), and cGMP.

Increased intracelluar Ca^{++} can occur through receptor operated channels or by release of intracellular calcium stores. Calcium binds with a calcium binding protein such as calmodulin (C.M.), and this complex in turn activates Nitric Oxide Synthatase (NOS). NOS produces nitric oxide (NO) from the amino acid arginine. The NO that is prduced acivates a soluble form of guanylate cyclase to make cGMP. cGMP levels can also be increaed by receptor activation of a membrane bound form of the guanylate cyclase enzyme. cGMP has a variety of tissue-specific effects

Spare & Orphan receptors

- Orphan receptors : Their ligand is not known
- Spare receptors: A fraction of receptors may need to be occupied to elicit a maximum response from a cell by a drug & the receptors that do not bind drug are spare receptors .

This is variable for different ligands .

Spare receptors allow maximal response *without* total receptor occupancy – increase sensitivity of the system

Spare receptors can bind , and *internalize* extra ligand preventing an exaggerated response if too much ligand is present

Examples: of Spare receptors

a. Insulin receptors.;99% are spare- so there is functional reserve that ensures the adequate amounts of glucose to enter the cells.

b. In the heart , only 5-10% Beta receptors are spare, when catecholamines bind & produce their effects--- so little reserve exists in failing heart.

- Phenomenon of spare receptors may occur at GPCRs due to amplification of the initial signal , because :

  a. A single drug- receptor complex may interact with many G protiens..

  b. Activated G proteins may persist for longer than the drug -receptor complex .

Regulation of receptors:

Regulation of receptors:

Receptors are dynamic structure i.e. there may be increase,/decrease in synthesis or function of receptors; leading to:

Up regulation / Super sensitivity

Increase in number of receptors on long term use of some antagonists e.g. Beta blockers (propranolol) sudden with drawl after prolonged use may lead to an attack of angina.

Down regulation / Desensitization of receptors

Decrease in number of receptors on prolonged use of some agonist drugs leading to refractoriness e.g. Adrenergic $Beta_2$ agonists(salbutamol) may become ineffective as bronchodilators in asthmatic patients on chronic use.

Receptor regulation

1. Homeostasis
2. Up and down regulation
3. Desensitization
4. Tolerance

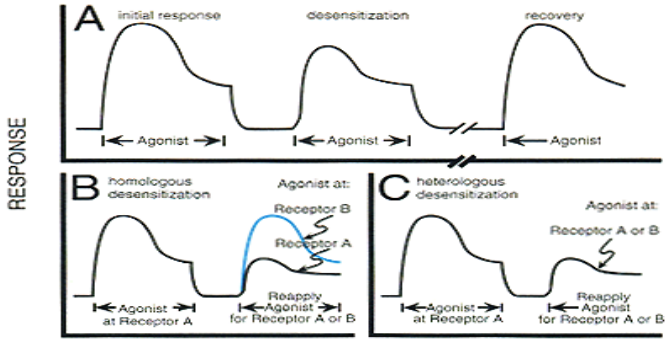


Figure 2–4. Desensitization in response to an agonist.

A. Upon exposure to an agonist, the *initial response* usually peaks and then decreases to approach some tonic level, elevated but below the maximum. If the drug is removed for a brief period, the state of *desensitization* is maintained such that a second addition of agonist also provokes a diminished response. Removal of the drug for a more extended period allows the cell to "reset" its capacity to respond, and *recovery* of response usually is complete. (***B*** and ***C***) Desensitization may be *homologous* (***B***), affecting responses elicited only by the stimulated receptor, or *heterologous* (***C***), acting on several receptors or on a pathway that is common to many receptors.

agonists desensitize receptors

- **homologous**

  (decreased receptor number)
- **heterologous**

  (decreased signal transduction

Antagonists tend to up regulate receptors

- Desensitization of G-protein-coupled receptors may occur as a result of phosphorylation by specific receptor kinases, causing the receptor to become non-functional and to be internalized.
- There is a large family of phosphatases that act to reverse the effects of kinases.
- Receptor-linked G-proteins also control:
  - **Phospholipase A** → Formation of arachidonic acid and eicosanoids
  - **Potassium and calcium channels** → affecting:
    Membrane excitability
    Transmitter release
    Contractility

Parameters of drug action:

- **Affinity:** The ability to occupy / to bind to the receptors.
- **Intrinsic activity / Efficacy:** The ability to activate the receptor or initiate a chain of reactions leading to a response.

Receptor Ligands:

Latin Ligare ---- to bind

Ligand is a molecule of drug which attaches selectively to a particular receptor

Agonist: A drug which has affinity as well as intrinsic activity-- it binds to & activates the receptor as it initiates a chain of reactions leading to response.

e.g. Epinephrine, Isoprenaline , Acetylecholine

Antagonist: A drug which has affinity for the receptor, but no intrinsic activity . It binds to receptor but can not activate it .

Moreover it blocks the effect of Agonist.

e.g. Propranolol , Atropine

Partial agonist: **A drug which has affinity & low degree of intrinsic activity at full receptor occupancy, & it blocks the effect of full Agonist.**

e.g. Oxpranolol, Pentazocine

Inverse agonist: **A drug which has affinity as well as intrinsic activity, it activates the receptors but the effects produced are opposite to those produced by Agonist**

e.g. Betacarbolines at Benzodiazepines receptor