DRUG-RECEPTOR INTERACTION

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- Various Protein Target for Drug action
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INTRODUCTION

- What is Drug ?
- Effects of Drug
  - Type of Response ~ Effect
  - How & where it is produced ~ Action.

So, Effect is measured while action is identified.

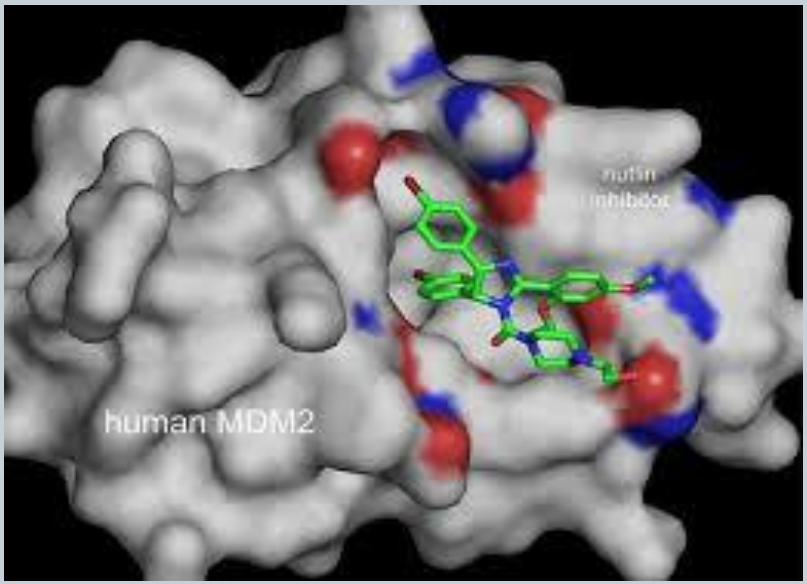
- How drug acts,
- Where it acts,
- How it produces the pharmacological response.

SITES OF DRUG ACTION

- Extra Cellular Sites
  - * Antacids - Neutralize Gastric Acidity
  - * Chelating Agents - Complexes with Heavy Metals.
  - * Osmotic Purgatives - Retaining Fluid inside Intestine.
- Intracellular Sites
  - * Sulpha Drugs - Interfering Synthesis of Folic acid
  - * 5-Flurouracil - Replacing Uracil into RNA
- Cellular Sites
  - * Acetyl Choline - Motor end plate of skeletal muscles
  - * Ranitidine - H_2-receptor of Parietal cell

TARGETS FOR DRUG ACTION

- Protein targets for drug action on mammalian cells -
    1. Receptor
    2. Ion Channels
    3. Enzymes
    4. Carrier Molecules



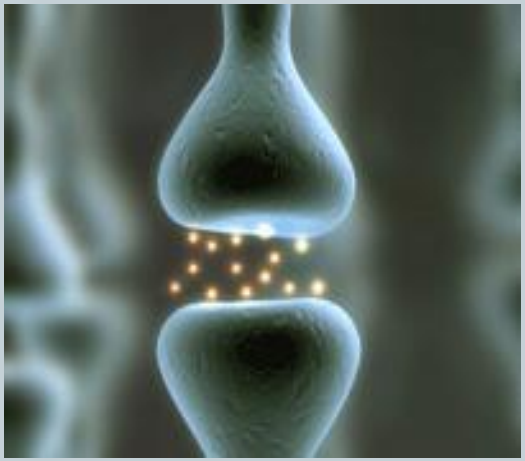
Type of target	Effectors	
Receptors	**Agonists**	**Antagonists**
Nicotinic ACh receptor	Acetylcholine	Tubocurarine
	Nicotine	α-bungarotoxin
β-adrenoceptor	Noradrenaline	Propranolol
	Isoprenaline	
Histamine (H_1 receptor)	Histamine	Mepyramine
Histamine (H_2 receptor)	Impromidine	Ranitidine
Opiate (μ-receptor)	Morphine	Naloxone
5-HT_2 receptor	5-HT	Ketanserin
Dopamine (D_2 receptor)	Dopamine	Chlorpromazine
	Bromocriptine	
Insulin receptor	Insulin	Not known
Oestrogen receptor	Ethinylestradiol	Tamoxifen
Progesterone receptor	Norethisterone	Danazol
Ion channels	**Blockers**	**Modulators**
Voltage-gated Na^+ channels	Local anaesthetics	Veratridine
	Tetrodotoxin	
Renal tubule Na^+ channels	Amiloride	Aldosterone
Voltage-gated Ca^{2+} channels	Divalent cations (e.g. Cd^{2+})	Dihydropyridines
		β-adrenoceptor agonists
Voltage-gated K^+ channels	4-aminopyridine	
ATP-sensitive K^+ channels	ATP	Cromokalim
		Sulphonylureas
GABA-gated Cl^- channels	Picrotoxin	Benzodiazepines
Glutamate-gated (NMDA) cation channels	Dizocilpine, Mg^{2+}	Glycine
	Ketamine	

Enzymes	Inhibitors	False substrates
Acetylcholinesterase	Neostigmine Organophosphates	
Choline acetyltransferase		Hemicholinium
Cyclo-oxygenase	Aspirin	
Xanthine oxidase	Allopurinol	
Angiotensin-converting enzyme	Captopril	
Carbonic anhydrase	Acetazolamide	
HMG-CoA reductase	Simvastatin	
Dopa decarboxylase		Methyldopa
Monoamine oxidase-A	Iproniazid	
Monoamine oxidase-B	Selegiline	
Dihydrofolate reductase	Trimethoprim Methotrexate	
DNA polymerase	Cytarabine	Cytarabine
Enzymes involved in DNA synthesis	Azathiaprine	
Enzymes of blood clotting cascade	Heparin	
Plasminogen*		
Thymidine kinase	Acyclovir	
HIV protease	Saquinavir	
Reverse transcriptase	Didanosine (ddI) Zidovudine	

Carriers	Inhibitors	False substrates
Choline carrier (nerve terminal)	Hemicholinium	
Noradrenaline uptake 1	Tricyclic antidepressants Cocaine	
		Amphetamine Methyldopa
Noradrenaline uptake (vesicular)	Reserpine	
Weak acid carrier (renal tubule)	Probenecid	
$Na^+/K^+/2Cl^-$ co-transporter (loop of Henle)	Loop diuretics	
Na^+/K^+ pump	Cardiac glycosides	
Proton pump (gastric mucosa)	Omeprazole	

RECEPTOR

- Macromolecular component of organism that binds drug and initiates its effect.
- Specific Macromolecular Protein.
- Membrane bound or Intracellular.



- Capable to bind with specific functional groups of drugs.
- 3-Dimensional Configuration.

DRUG-RECEPTER BINDING

- Most drugs act (bind) on *receptors*
  - In or on cells
  - Chemical Bond: Ionic, Hydrogen, Hydrophobic, Vander Waals & Covalent.
  - Exact requirements (Size, Shape, Stereo specificity)
  - Saturable
  - Agonists (Salbutamol), or Antagonists (Propranolol)
- ***Receptors have signal transduction mechanism***

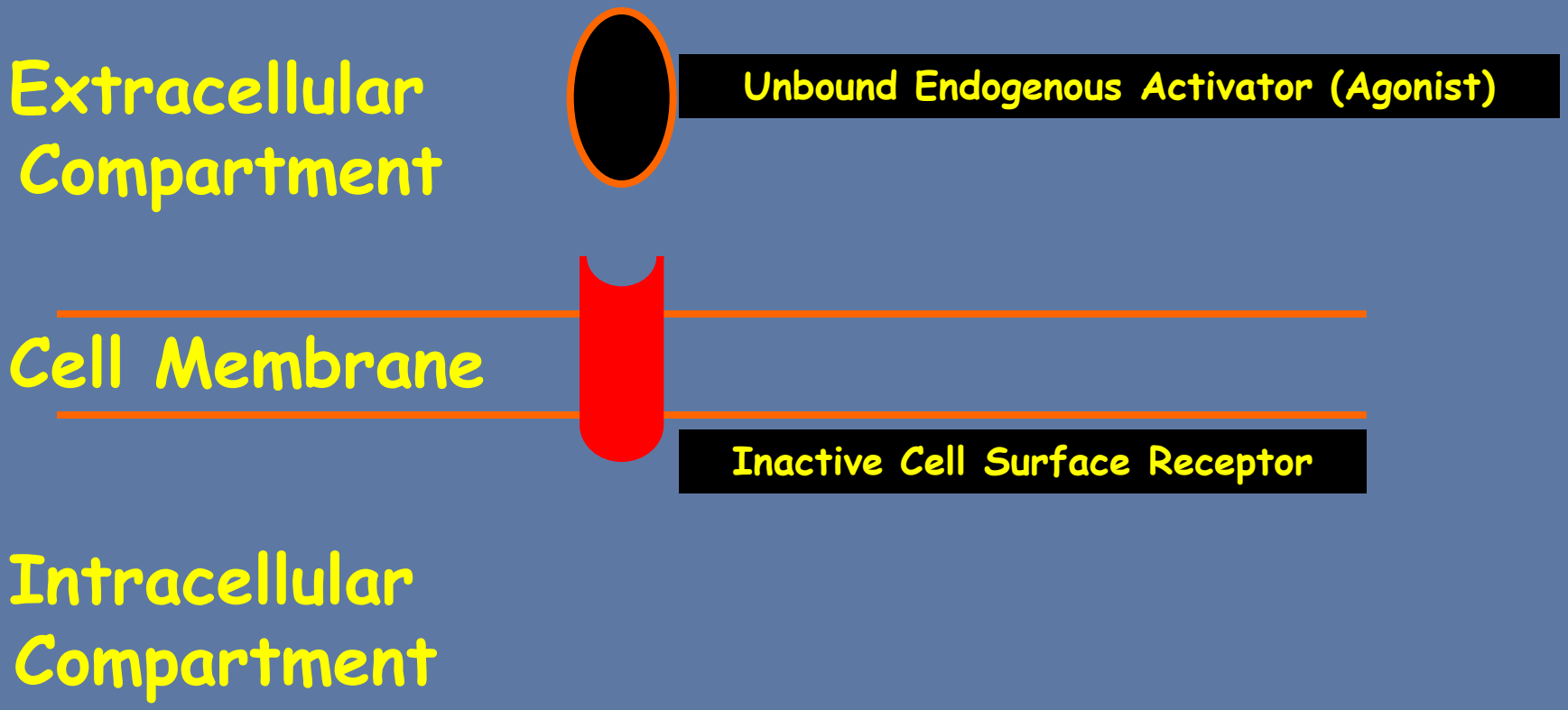
D + R  DR Complex

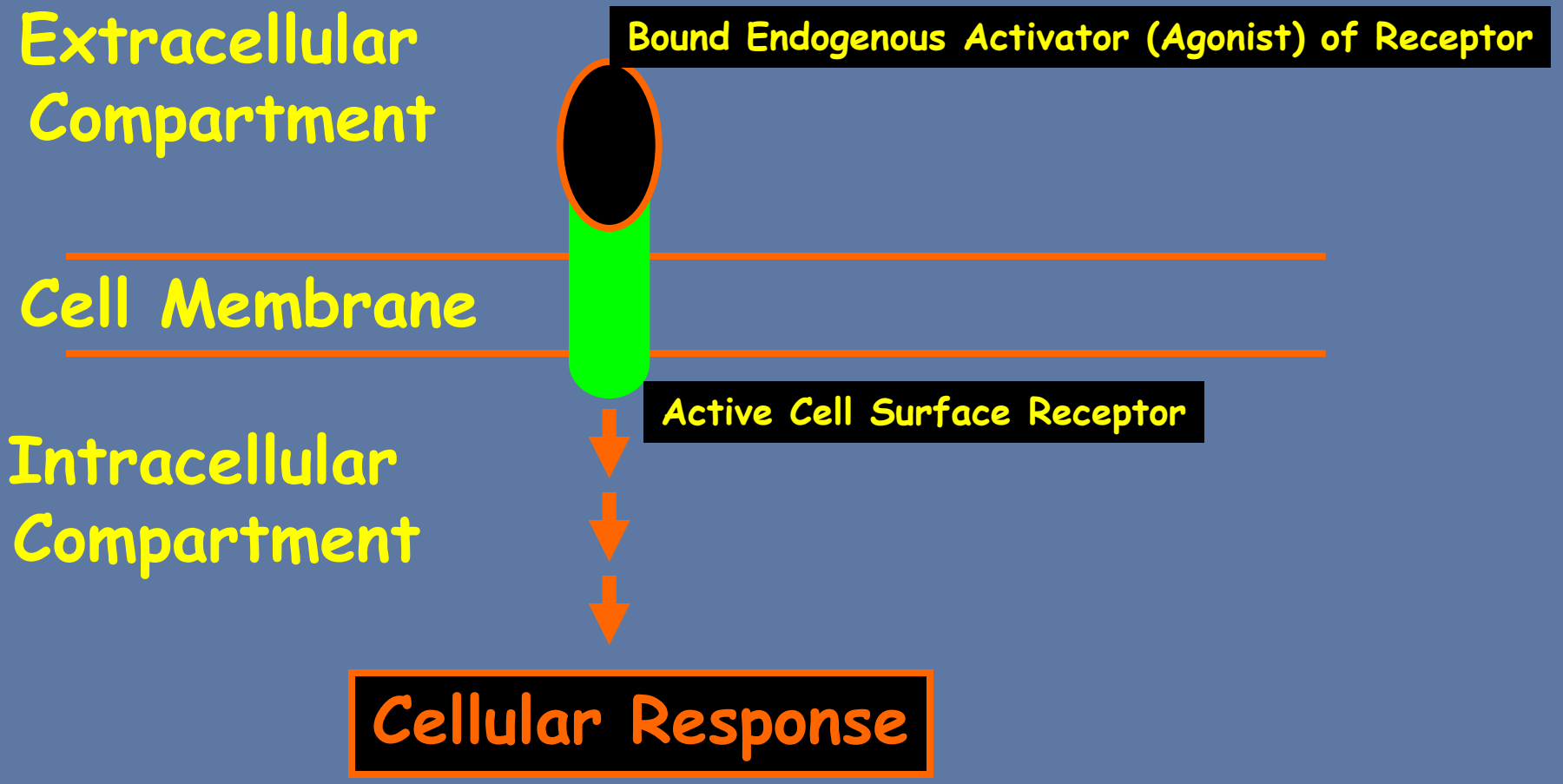
AFFINITY – Attractiveness B/W Drug & Receptor.

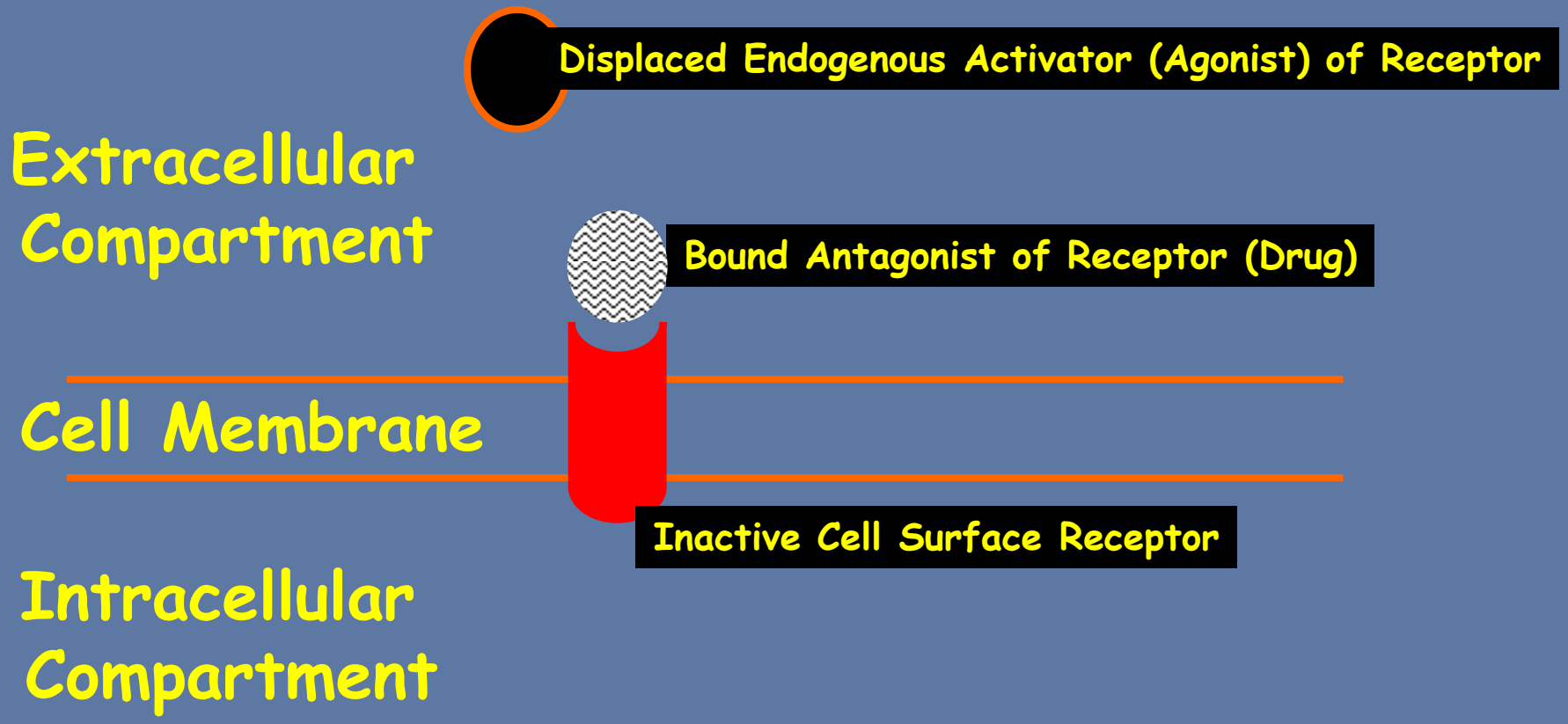
* Covalent bonds stable & essentially irreversible.
* Other bonds strong or weak, but usually reversible.

Efficacy – Ability of a bound drug to change receptor in a way that produces effect.

Some drugs possess affinity but NOT efficacy.


Extracellular Compartment
Unbound Endogenous Activator (Agonist)
Cell Membrane
Inactive Cell Surface Receptor
Intracellular Compartment


Extracellular Compartment
Bound Endogenous Activator (Agonist) of Receptor
Cell Membrane
Active Cell Surface Receptor
Intracellular Compartment
Cellular Response


Displaced Endogenous Activator (Agonist) of Receptor
Extracellular Compartment
Bound Antagonist of Receptor (Drug)
Cell Membrane
Inactive Cell Surface Receptor
Intracellular Compartment

Drug-receptor interaction

$$\text{Drug} + \text{Free Receptor} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Drug-receptor Complex}$$

D (100 - DR) DR

Where: D = drug concentration
DR = concentration of drug-receptor complex
100 - DR = free receptor concentration

- Drug binding obey the law of mass action.

At equilibrium,

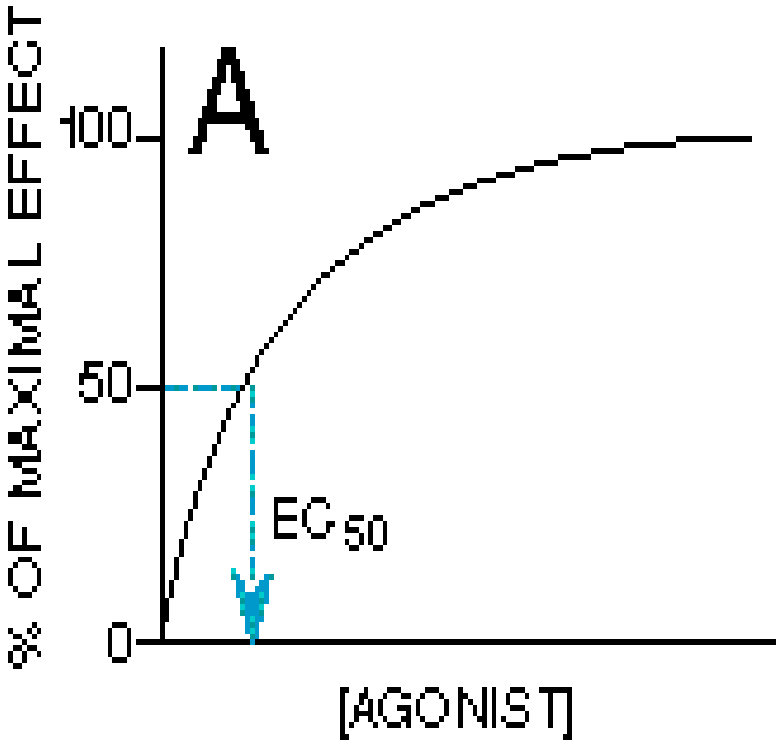
$$[D] \times [R] \times k1 = [DR] \times k\text{-}1$$

so that: $\frac{[DR]}{[D][R]} = \frac{k1}{k\text{-}1}$

k1/k-1 = dissociation constant (kd)

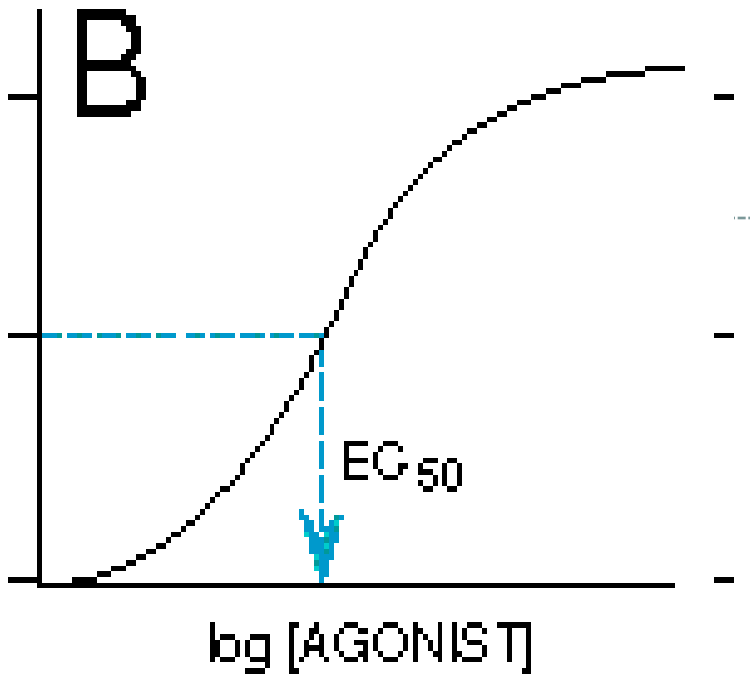
Lower kd = more potent drug

Arithmetic Dose Scale



- Rate of change rapid at first and becomes progressively smaller as dose increased.
- Eventually, increments in dose produce no further change in effect i.e., maximal effect for that drug is obtained
- Difficult to analyze mathematically

Log Dose Scale



- Transforms hyperbolic curve to sigmoid (almost straight line)
- Compresses dose scale
- proportionate doses occur at equal intervals
- Straightens line
- Easier to analyze mathematically

EFFECTIVENESS, TOXICITY, LETHALITY

- **ED50 (Median Effective Dose 50)** –

  * 50% Population manifests a given effect.

- **TD50 (Median Toxic Dose 50) -**

  * 50 percent Population manifests a given toxic effect.

- **LD50 (Median Lethal Dose 50) -**

  * Dose which kills 50 percent of the subjects.

$$\text{Therapeutic Index (TI)} = \frac{\text{TD50 or LD50}}{\text{ED50}}$$

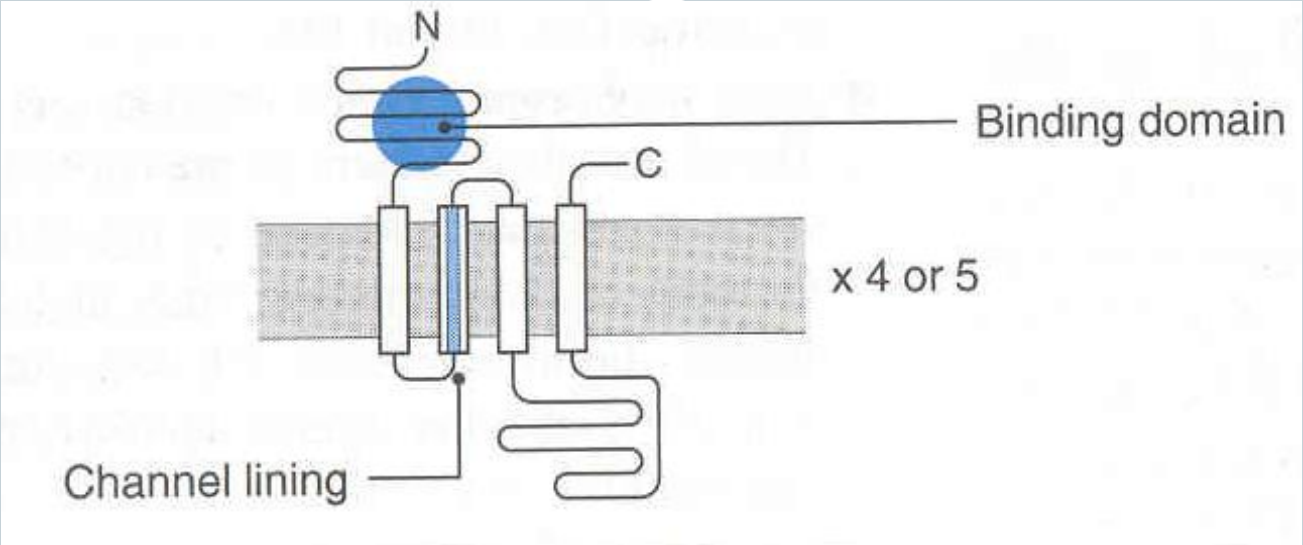
- Provides a very crude measure of safety of drug.
- Higher the TI = Safer the drug.
- TI vary from: 1.0 (some cancer drugs) to >1000 (penicillin).

Receptor Families: Signal Transduction Mechanism

- Ion-channel linked receptor
- G-protein coupled receptor
- Enzyme linked receptor
- Nuclear receptor

Ion- channel linked receptor

- Located on cell membrane.
- Directly coupled with Effectors (channels).
- Takes millisecond to produce action.
- Mainly involved in fast Synaptic transmission.
- Examples are—
  - η-Ach receptor
  - $GABA_A$ receptor
  - Glutamine receptor
  - Glycine receptor
  - $5\text{-}HT_3$ receptor



Structure:-

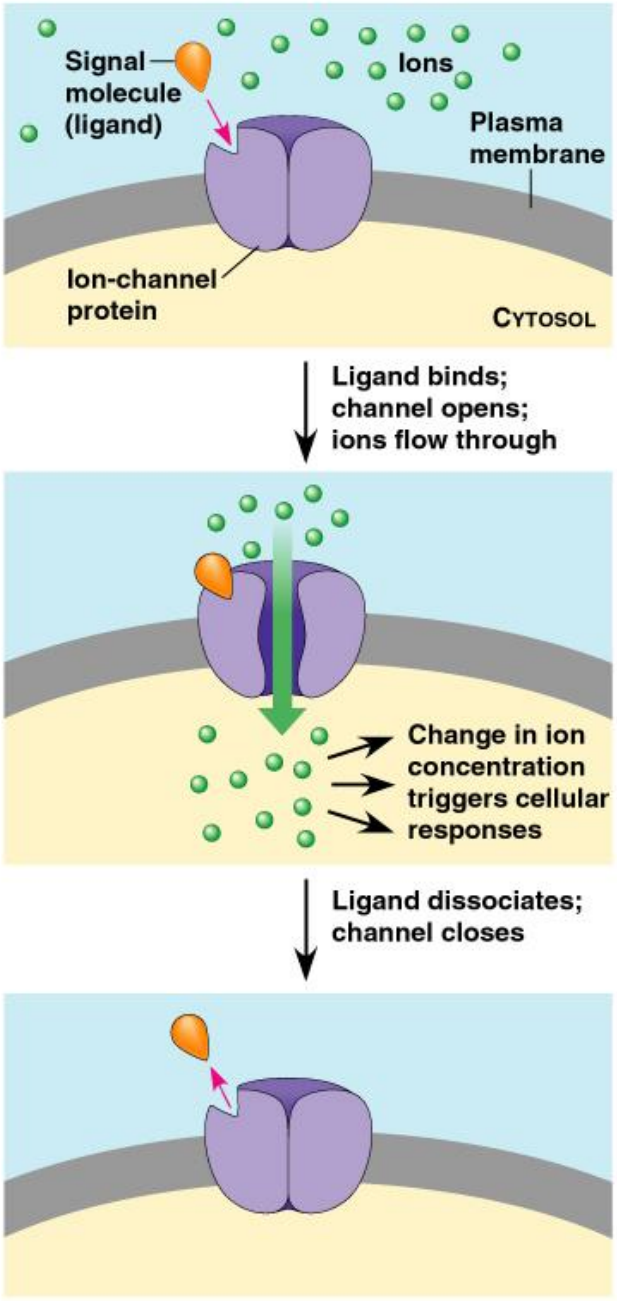
* Made of Oligo protein containing four subunits which enclosing a cylindrical Ion channel.
* Each subunit have 4-5 Transmembrane Segments. Which crosses the lipid bi layer 4-times.

Ion channel receptors

Signal molecule binds as a ligand at a specific site on the receptor

Conformational changes open the channel allowing ions to flow into the cell

The change in ion concentration within the cell triggers cellular responses

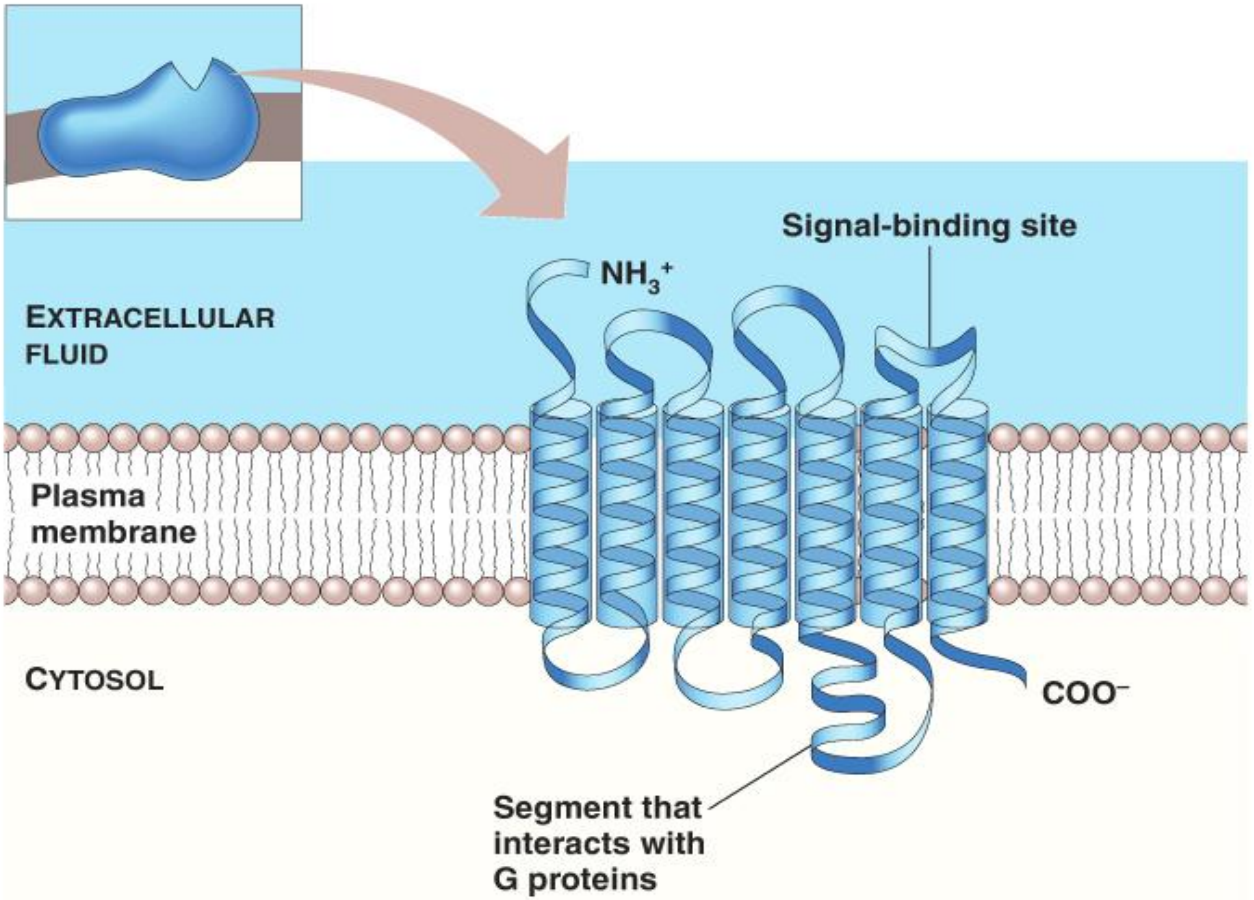


G-protein coupled receptor

- Also called metabotropic receptor.
- Located on cell membrane.
- Coupled through G-protein with Effectors.
- Effector may be channels or enzymes.
- Take seconds to produce action.
- Mainly involved in Hormones & slow transmission.
- Examples -

  * m-Ach receptor
  * Dopamine receptor
  * Adrenergic receptor
  * Opiate receptor

G protein-linked receptors



Structure:

- Single polypeptide chain that crosses the lipid bilayer 7 times, resulting in 7 transmembrane helices
- There's a G protein attached to the cytoplasmic side of the membrane (functions as a switch).

Chemical signal binds as a ligand to a G protein-linked receptor

G protein linked receptor changes shape and interacts with a G protein

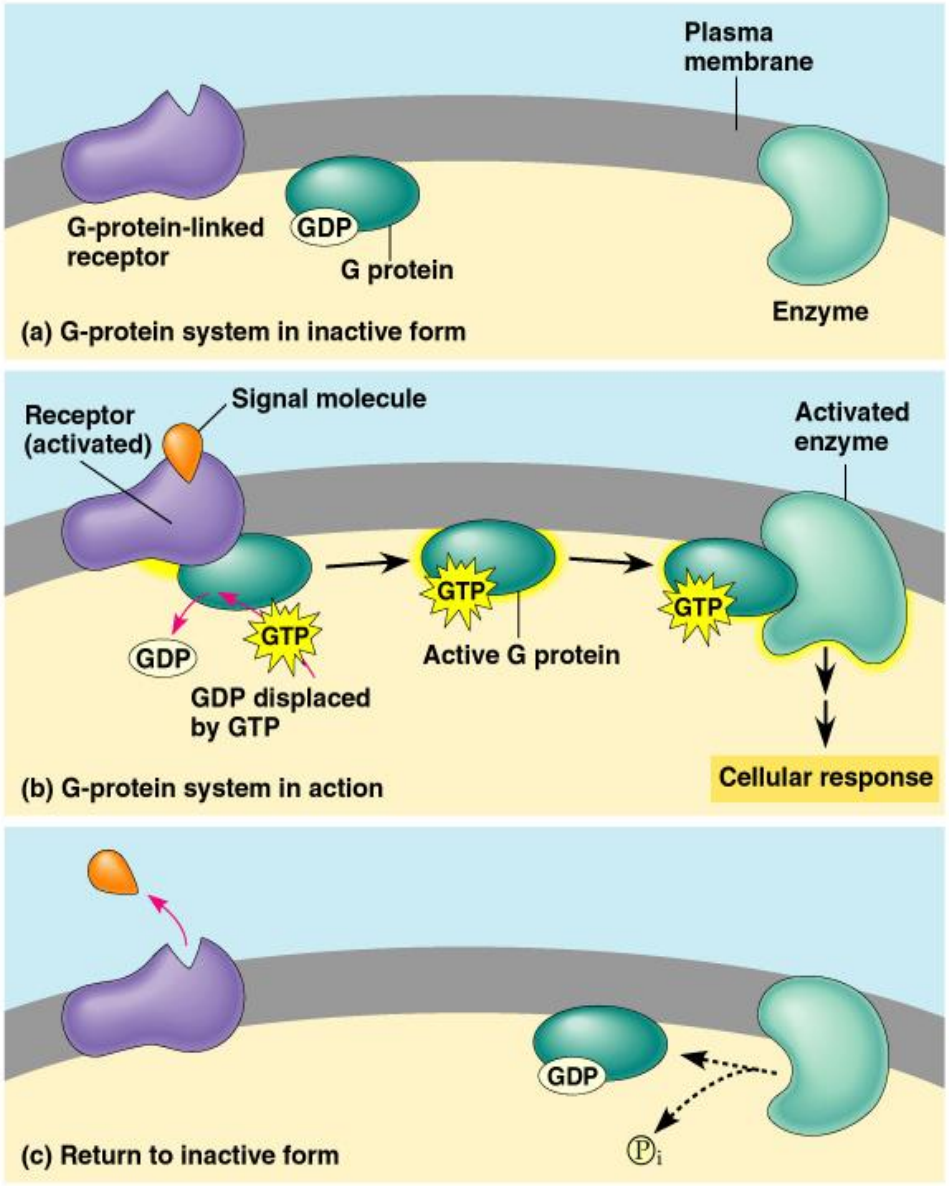
Interaction causes GDP to be displaced and GTP to be bound to the G protein

The active G protein binds to another protein, usually an enzyme

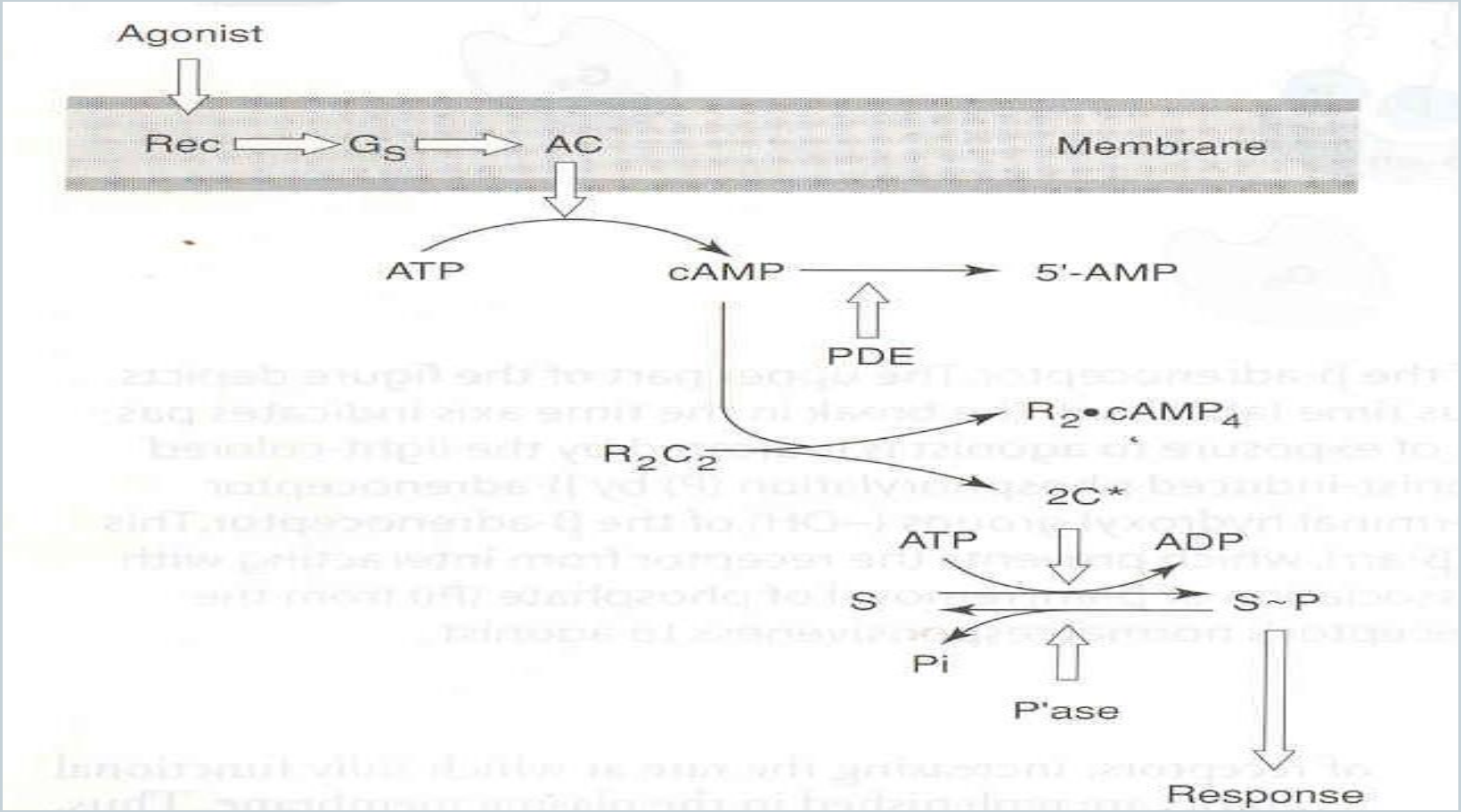
The enzyme is activated

G protein hydrolyzes GTP back to GDP

G protein releases from the enzyme and the reaction stops



C-AMP Activation



G protein

- "G" refers that protein binds Guanine nucleotides (GDP, GTP)
- G proteins integral membrane protein, i.e. hetertrimers ($\alpha\beta\gamma$);
- G proteins have similar β and γ subunits, but differ in type of α-subunits;
- When G-protein activated, α subunit dissociates to interact with an enzymes that generate second messengers (e.g. cAMP).

The Nobel Prize in Physiology and Medicine 1994

"for their discovery of G-proteins and the role of these proteins in signal transduction in cells"

Alfred G. Gilman
USA
1941-

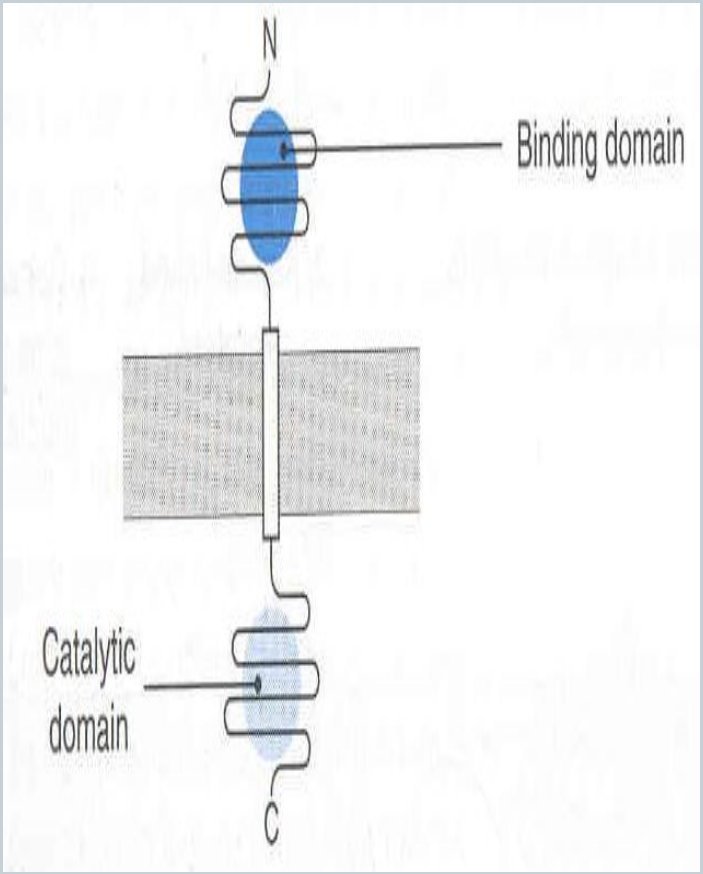
Martin Rodbell
USA
1925-1998

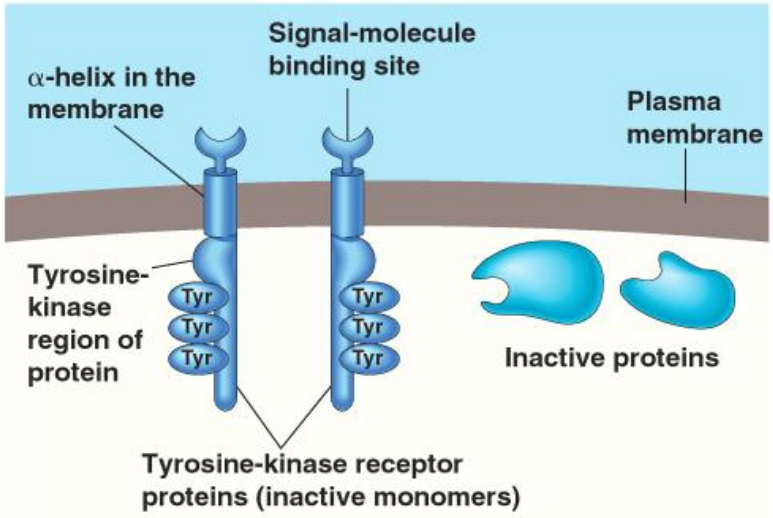
Enzyme linked receptor

- Located on Cell membrane.
- Also known as kinase linked receptor.
- Coupled with intracellular Tyrosine kinase.
- Take minutes to produce Action.
- Mainly involved in Growth factor and certain hormones.
- Examples –
  - Insulin receptor
  - Cytokinase receptor

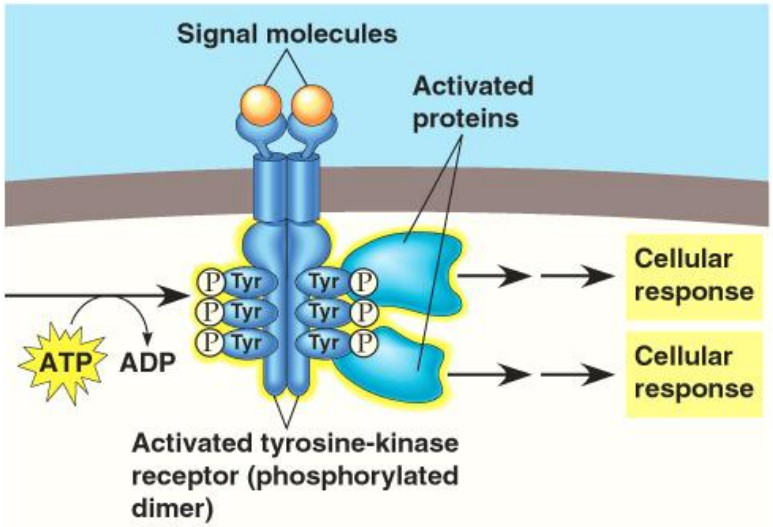
Structure:-

- Exist as individual polypeptides
- Very large binding domain, present in extra cellular and
- large effector domain present in intracellular.
- Directly linked tyrosine kinase which binds to specific Protein.





(a) Inactive tyrosine-kinase receptor system



(b) Activated system

Ligands bind to both receptors

The two receptor polypeptides aggregate forming a dimer

Activates the tyrosine-kinase parts of the dimer

Each phosphorylates (using ATP) the tyrosines on the tail of the other polypeptide

Receptor proteins are now recognized by relay proteins inside the cell

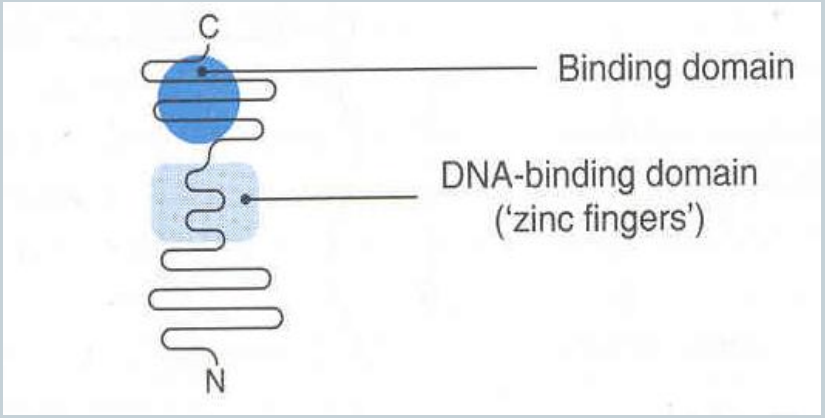
Relay proteins bind to the phosphorylated tyrosines (may activate 10 or more different transduction pathways)

Nuclear receptors

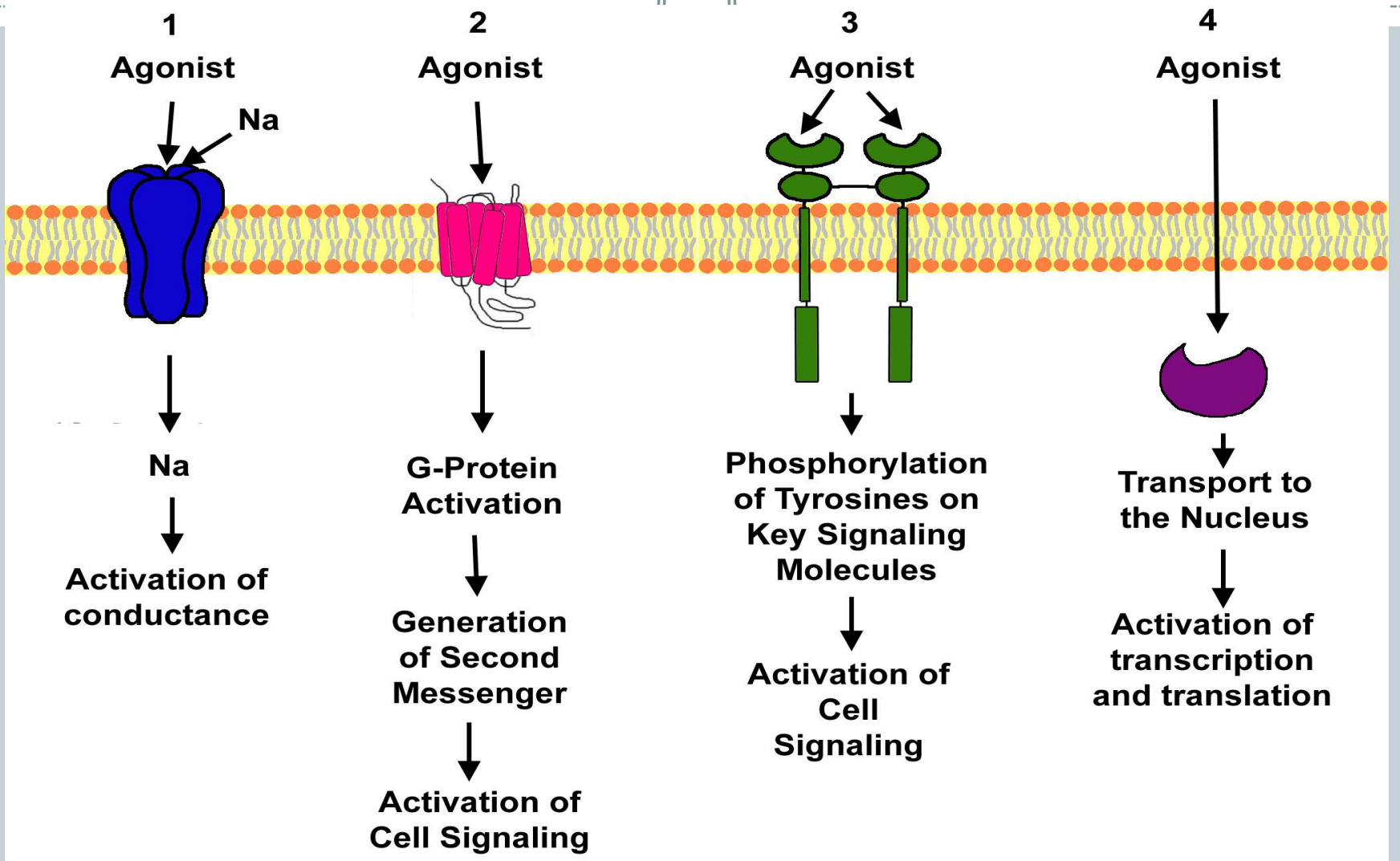
- Also known as Gene-Transcription receptor.
- Present in intracellular either Cytoplasm or Nucleus.
- Intracellular protein so agonist must first inter cells.
- Coupled via DNA.
- Take hours to produce action.
- Examples –
  - * Steroid receptor
  - * Thyroid receptor
  - * Vit-D receptor

Structure:-

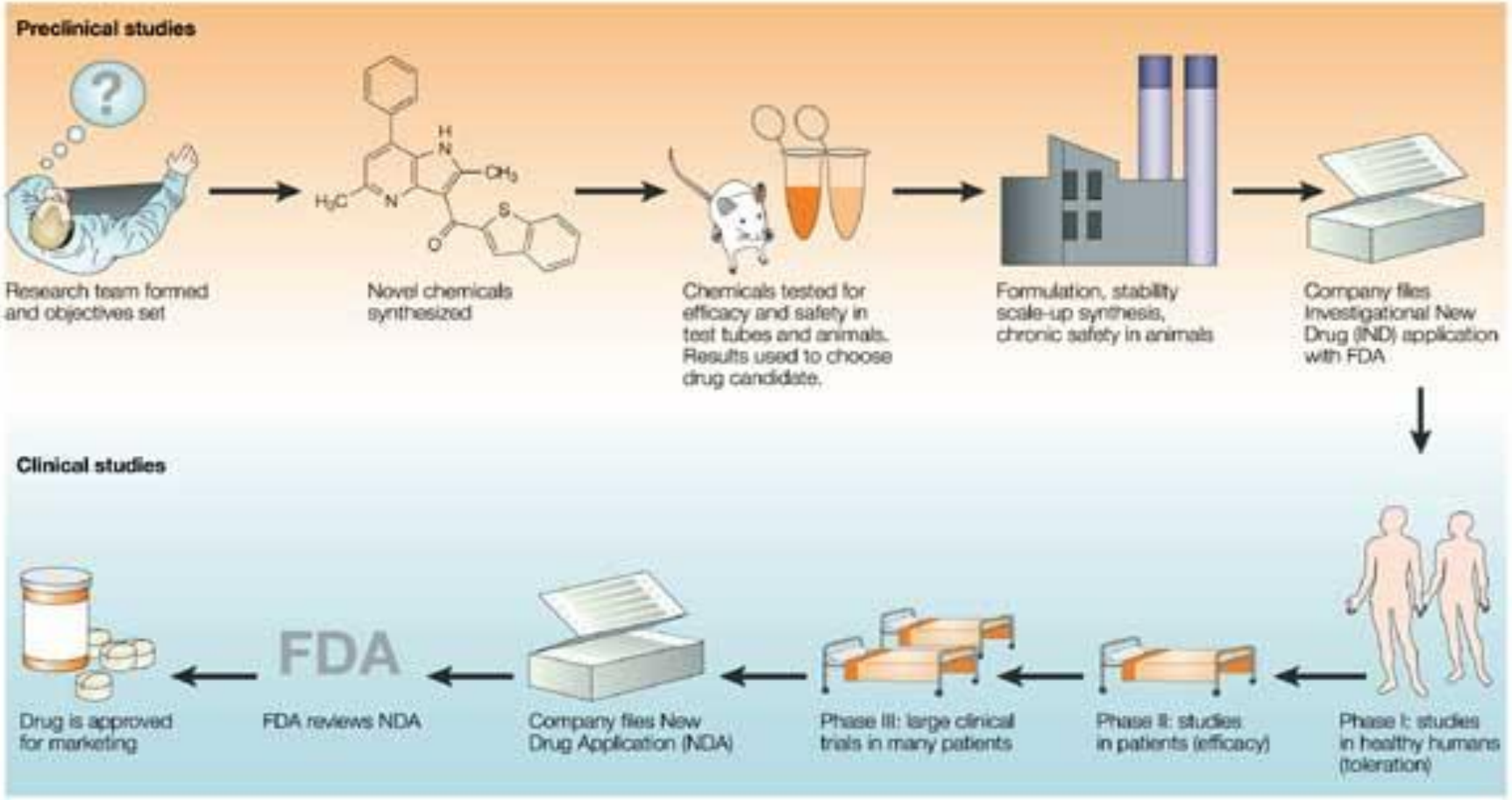
- Heat Sensitive Protein(HSP-90),
- DNA binding domain (zinc finger),
- Transcriptional control domain and
- Ligand binding domain.



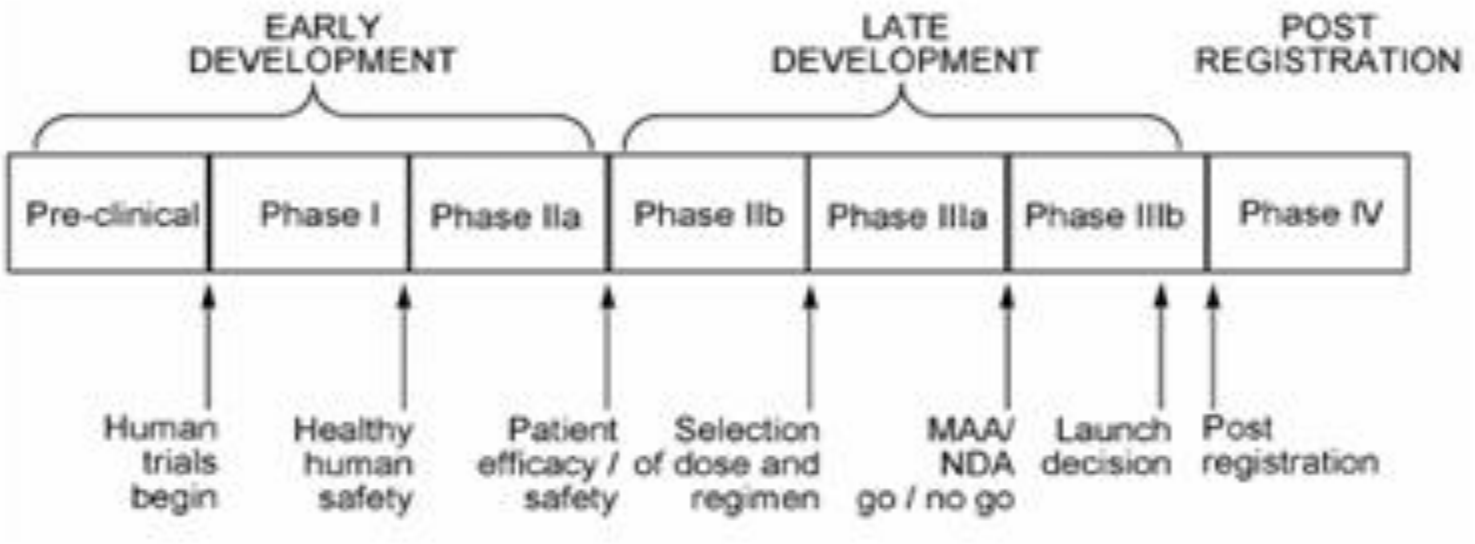
- Agonist binding release HSP-90, then exposes DNA binding domain.
- Exposed DNA binding domain associate with DNA of specific gene; stimulate RNA polymerase activity; Specified m-RNA synthesized.
- m-RNA regulate production of specific proteins that gives specific physiological functions.


1
Agonist
Na
Na
Activation of conductance
2
Agonist
G-Protein Activation
Generation of Second Messenger
Activation of Cell Signaling
3
Agonist
Phosphorylation of Tyrosines on Key Signaling Molecules
Activation of Cell Signaling
4
Agonist
Transport to the Nucleus
Activation of transcription and translation

Drug Discovery and Development



Checkpoints



Safety & toxicity in animals

- Acute toxicity profile
- Chronic toxicity profile

-- 14 day toxicity test in one rodent and one non-rodent species before use in man.

-- 3 month study read out at 28 days

-- longer studies (12 & 24 month)

Three dose levels (below, about, well above human dose).

It is insufficient to use doses which are not toxic; the doses producing toxic effects and the nature of these effects MUST be established.

Clinical Trials

- Research studies involving people
- Try to answer scientific questions and find better ways to prevent, diagnose, or treat disease
- Clinical trials translate results of basic scientific research into better ways to prevent, diagnose, or treat disease
- The more people take part, the faster we can:
  - Answer critical research questions
  - Find better treatments and ways to prevent disease

Clinical testing

- {Phase 0 (non-clinical)}
- Phase 1 (volunteers)
- Phase 2 (patients)
- Phase 3 (large scale multi-centre)
- Phase 4 (post registration monitoring)

phases can also be defined by the information you are trying to get out of the testing

Clinical trials

(The Way We Make Progress Against Disease)

Drug action depends on:

- Pharmacodynamics
- Pharmacokinetics and dose regimen
- Drug interactions
- Receptor sensitivity of patient
- Mood/personality of patient & doctor
- Patients expectations and past experience
- Social environment of patient
- Clinical state of patient

Clinical trial controls these variables and examines action of drug in defined set of circumstances

CONCLUSION

- Most drugs act through receptors.
- Interaction b/w drug & receptor can be described mathematically and graphically.
- Higher therapeutic index, Safer the drug.
- There are 4 common signal transduction mechnism.
- Second messengers carry signal inside cell; often use protein phosphorylation as a signaling device.

Thank you

Various types of G-protein families α-subunits

G protein	Signal	Effected enzyme	Effect
G_s	epinephrine glucagon	adenylyl cyclase	stimulatory
G_i	catecholamines	adenylyl cyclase	inhibitory
G_q	acetylcholine catecholamines	phospholipase C	stimulatory
G_t	photons	cGMP phosphodiesterase	stimulatory