Doctor 021

PHARMACOLOGY

Sheet no. 6

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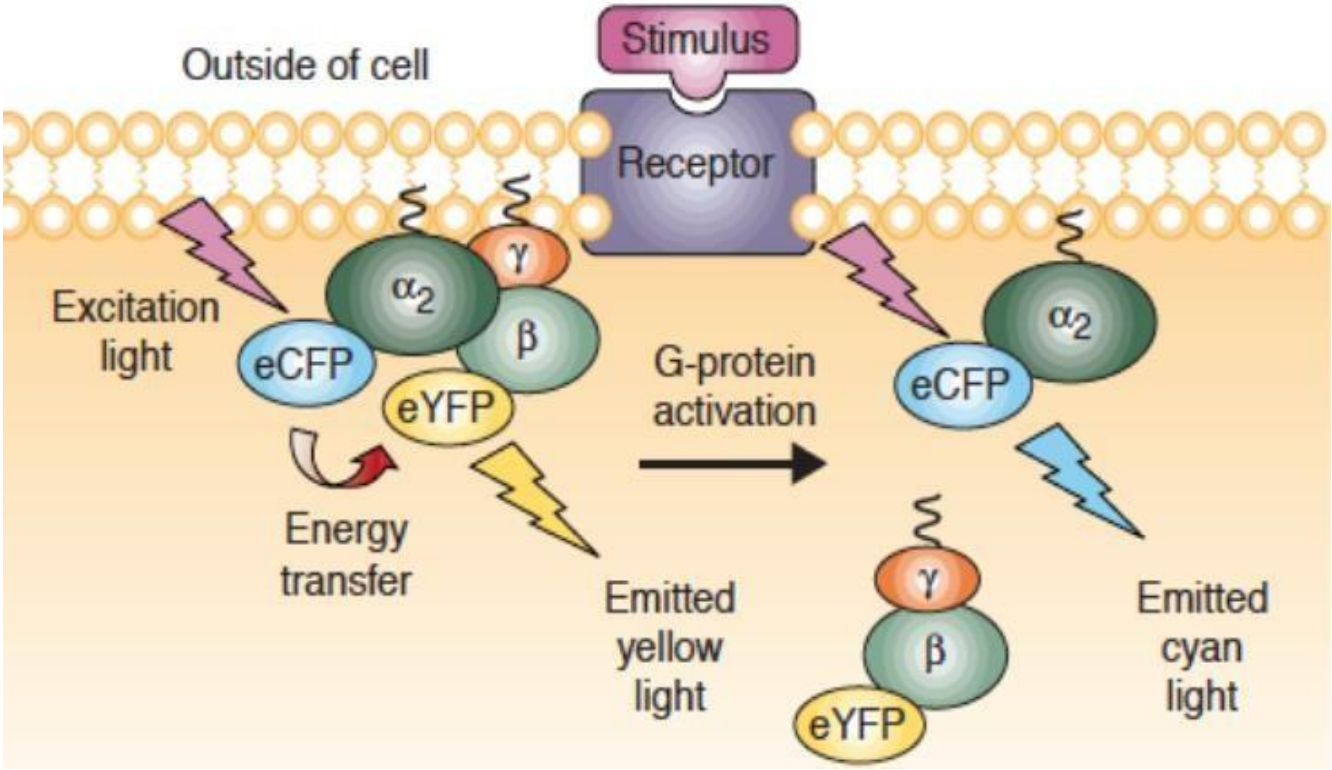
G PROTEIN-COUPLED RECEPTORS

• Receptors on the inner face of the plasma membrane regulate or facilitate effector proteins through a group of guanosine triphosphate (GTP) proteins known as G proteins.

• Some hormones peptide receptors and neurotransmitter receptors (e.g., adrenergic and muscarinic receptors depend on the G proteins) mediate their action on cells.

G protein-coupled receptors are a target for many drugs. Muscarinic receptors which exist in parasympathetic nervous system and adrenergic receptors which exist in sympathetic nervous system, are examples of G protein-coupled receptors, which indicates its existence all over the body because sympathetic and parasympathetic nervous systems control homeostasis by controlling multiple organs in the body. These receptors are present in the inner face of the plasma membrane, they regulate and facilitate the effector proteins through a group of proteins known as G proteins as you can see in the picture below : a receptor coupled to three subunits α, β and γ . α subunit is activated by the conversion of GDP into GTPwhich leads to dissociation of these units and after that each one of them leads an activation pathway on its own.



ENZYME-LINKED RECEPTORS

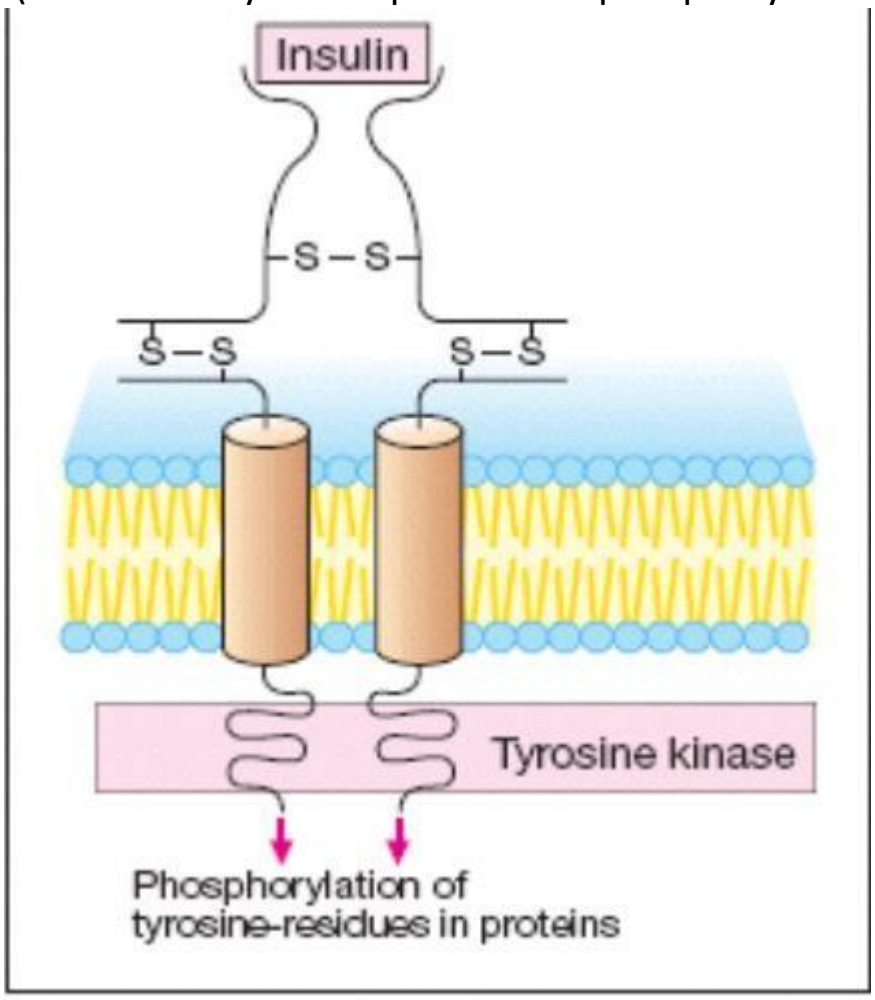
•Binding of the ligand to the extra cellular domain activates or inhibits the related cytosolic enzyme.

• The most common are the receptors that have a tyrosine kinase activity as part of their structure, in which the binding results in the phosphorylation of tyrosine residues of specific protein.

• The addition of phosphate group can modify the three dimensional structure of the target protein, and so resulting in molecular switch.

These receptors contain a catalytic domain, thus apart of the receptor is capable of enzymatic activity, ex: kinases in insulin receptors. Insulin receptors are coupled to a tyrosine kinase enzyme, which is going to phosphorylate another protein or even itself to auto activate further signaling mechanisms.

(kinases : enzymes responsible for phosphorylation).

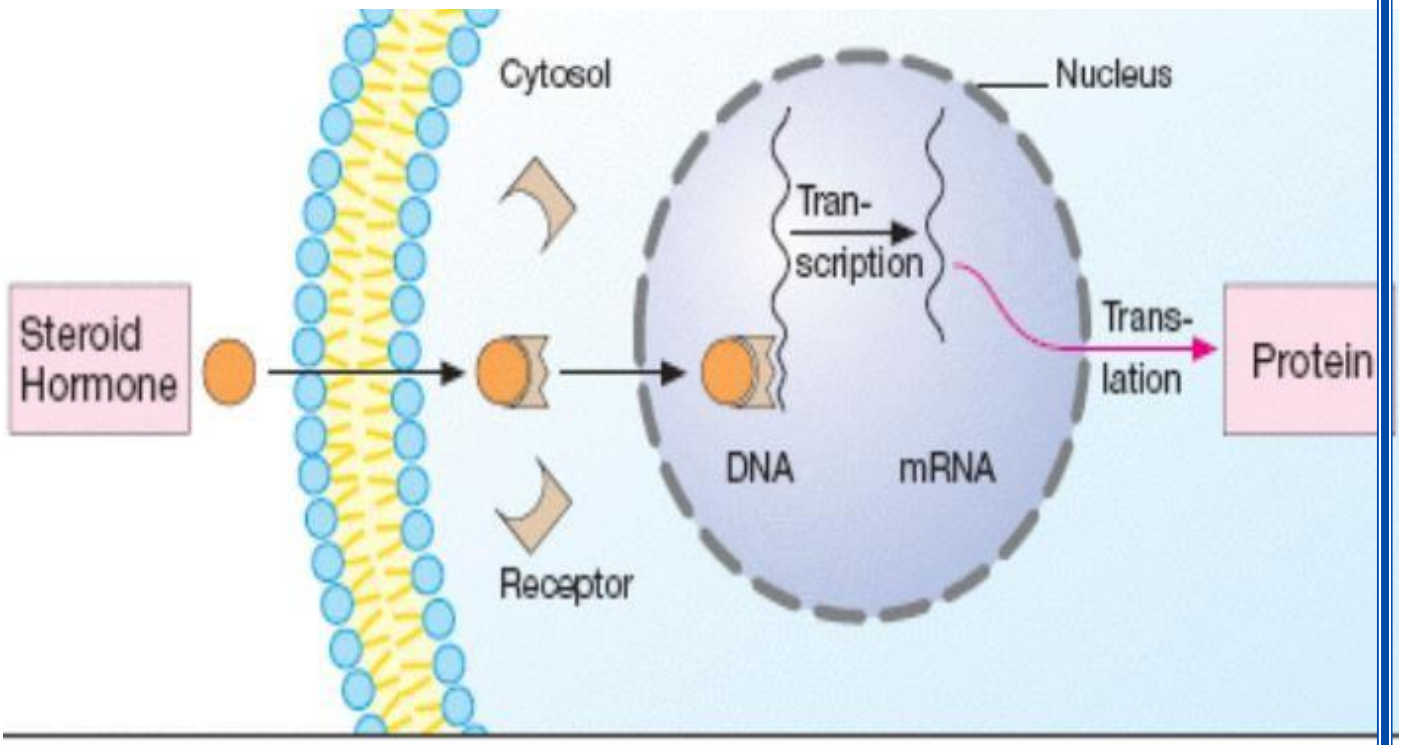


C. Ligand-regulated enzyme

INTRACELLULAR RECEPTORS

- **In this family the ligand must diffuse into the cell to interact with the receptors.**
- **Therefore, the ligand must have sufficient lipid solubilities to be able to move across the target cell membranes.**
- **The best example being the steroids hormones. In which the activated ligand-receptor complex migrates to the nucleus, where it binds to a specific DNA sequences, resulting in regulation of the gene expression.**

Cortisone is an example of steroid Hormones, It has lipid based structure so it crosses plasma membrane easily, and its receptor is located intracellular. Once the hormone connects to the receptor, it activates it leading to its translocation to the nucleus. That's why these receptors are considered to be part of the transcription factors family. Transcription factors bind to the DNA and they modulate the transcription of genes either by increasing or decreasing It.
For example, steroids are given to athletes to increase their muscle build up by increasing expression of certain proteins.



Protein synthesis-regulating receptor

HOW DO DRUGS WORK?

Most work by interacting with endogenous proteins:

- **Some antagonize, block or inhibit endogenous proteins**
- **Some activate endogenous proteins**
- **A few have unconventional mechanisms of action (very few).**

Our body proteins (receptors and enzymes) have ligands (neurotransmitters and hormones) , once theligand connects to the receptor it may activate it to give a response, one example is Adrenalin.

Adrenalin connects to beta2 adrenergic receptor, causing dilatation of bronchi so it's used to treat asthma. some drugs like "vintolin" mimic adrenalin's action and act as bronchodilators **(activation process).**

In case of fear (ex. Stage anxiety), Adrenalin will get secreted resulting in undesirable symptoms like sweating, high heart rate, high blood pressure. In this case we use a "beta blocker drug" that will connect to beta receptors in my body , kicking out Adrenalin and stopping it from connecting to its receptors, preventing their activation. And this is what antagonists do.

**beta blockers are serious medications given to patients with heart disease. They basically reduce stress or fear, however those are indications to use it.

Antagonist is a drug that binds to a receptor and prevents the agonist from binding. While Agonist is a drug or a ligand that will bind a receptor and activate it.

A lot of drugs are antagonists or blockers. Tubocurarine is a drug used to hunt animals earlier, now we use it as muscle relaxant, it binds to the nicotinic receptors, and blocks acetylcholine from binding, resulting in blocking the activation of the channels preventing the entry of Na+ into the cells.

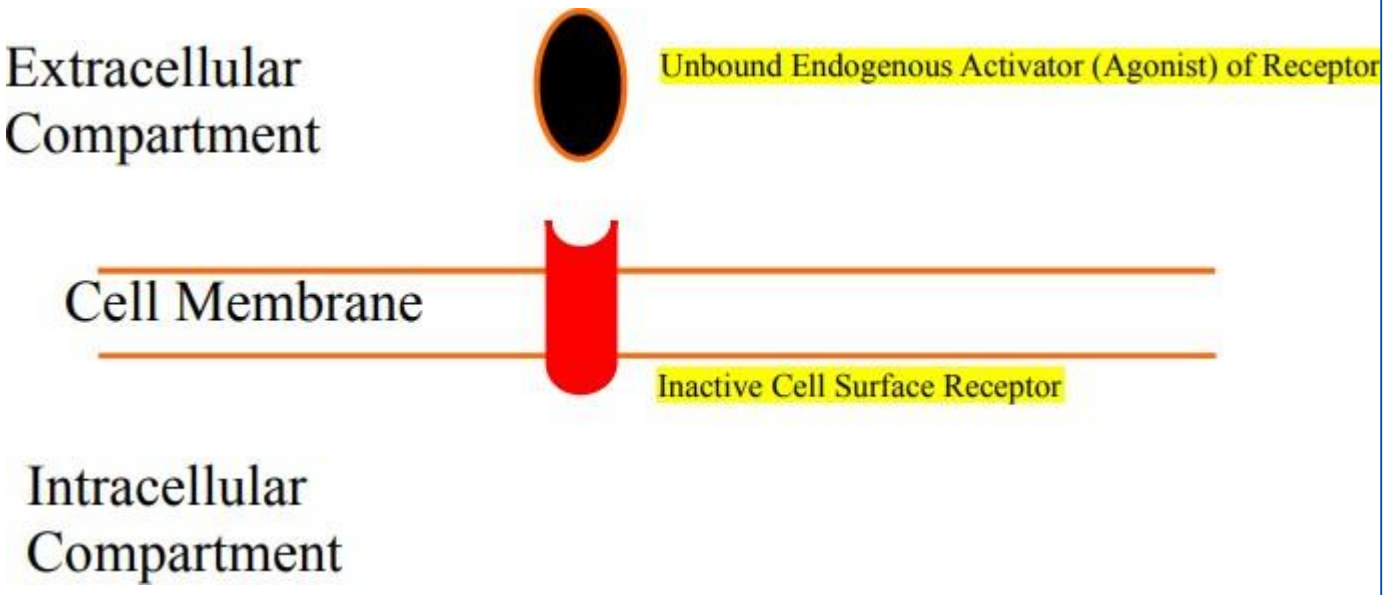
**Inhibitors: slows or stops the function of a specific enzyme, ex : cox inhibitors (aspirin).

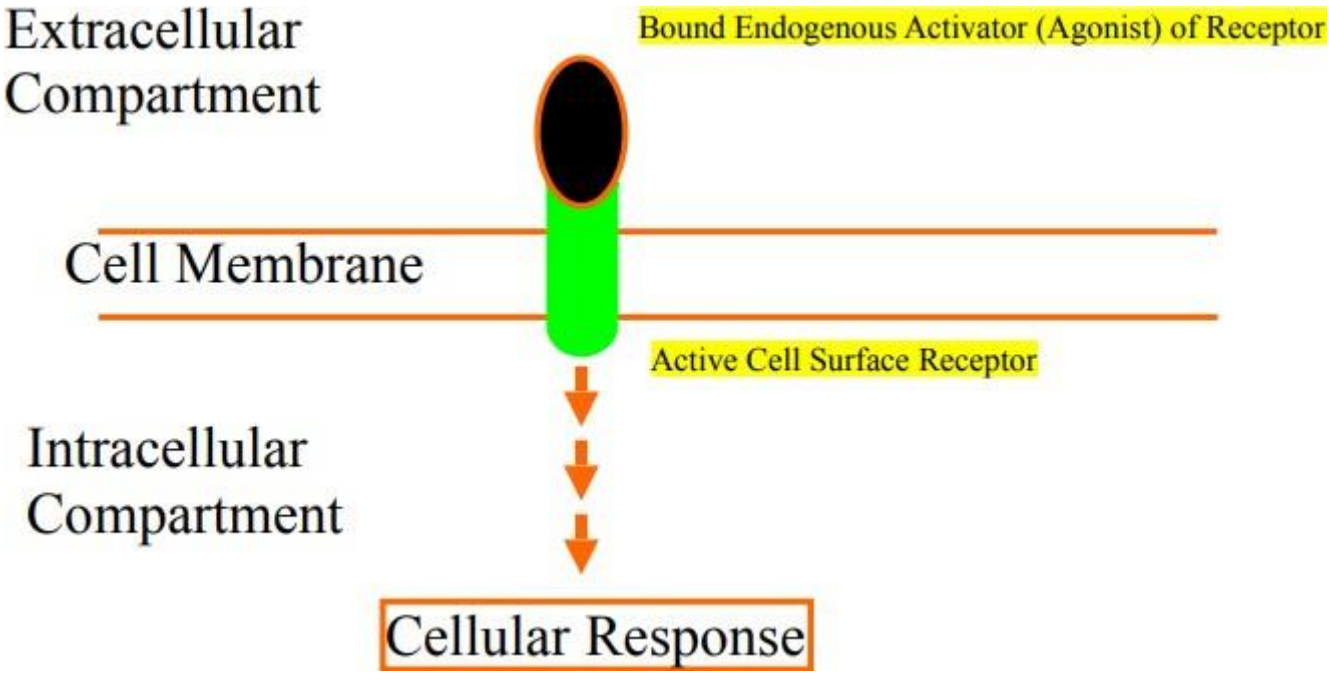
HOW DO DRUGS ANTAGONIZE, BLOCK OR INHIBIT ENDOGENOUS PROTEINS?

- Antagonists of Cell Surface Receptors
- Antagonists of Nuclear Receptors
- Enzyme Inhibitors
- Ion Channel Blockers
- Transport Inhibitors
- Inhibitors of Signal Transduction Proteins

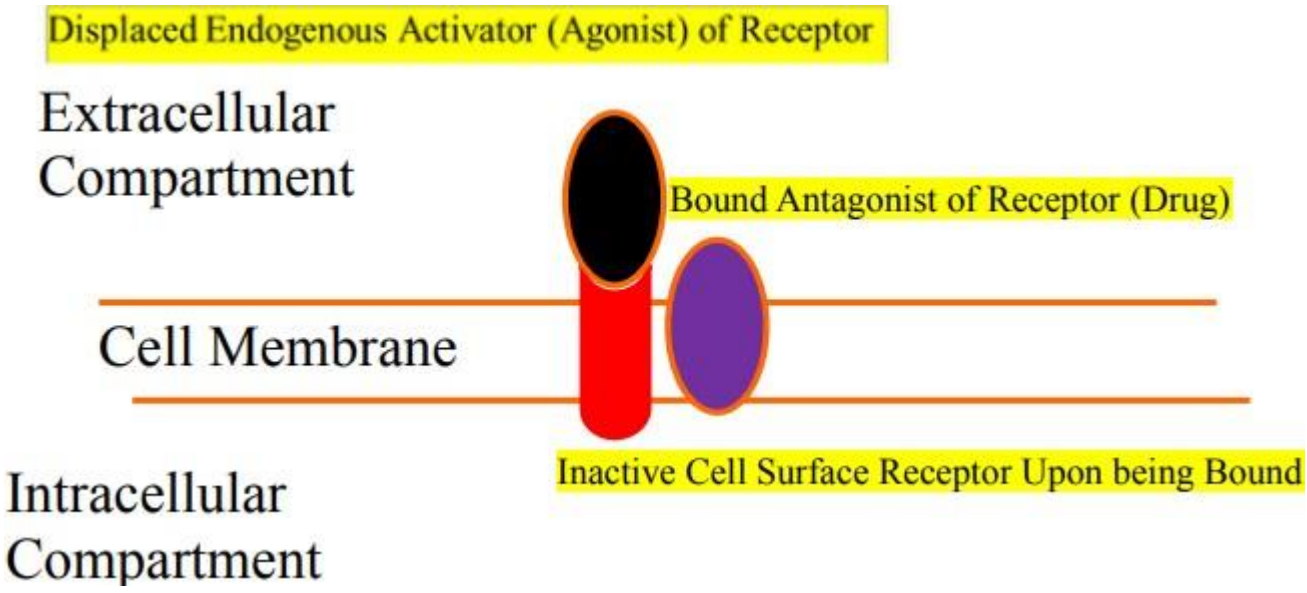
DEFINITION OF CELL SURFACE RECEPTOR

A receptor that is embedded in the cell membrane and functions to receive chemical information from the extracellular compartment and to transmit that information to the intracellular compartment.





In this diagram When adrenaline binds to its cell surface receptor, it activates it.



The purple circle is the antagonist

Footnote: Most antagonists attach to the agonist binding site on the receptor sterically (by occupying its space) prevent endogenous agonist from binding.

Lets get into the first conventional method of drug action :

FIRSTLY: to antagonize, block or inhibit endogenous proteins

Summery of such method : the antagonist drug binds to the receptor preventing endogenous agonist (effector hormone or neurotransmitter) from binding and stopping its activation process.

- "Endogenous proteins" indicate:
  Cell Surface Receptors +Nuclear Receptors +Enzymes+ Ion Channels +Transporter+ Signal Transduction Proteins.

*very important note is that they didn't decrease the heart rate (they don't exert an opposite mechanism), what they actually did is only preventing the increase of the heart rate. so the effect that we see is reduced heart rate.

We call these antagonists "neutral antagonists" because their net effect on the receptor is zero.

- We will come into the antagonization of each type of receptor in separate points of view:

HOW DO DRUGS WORK BY ANTAGONIZING CELL SURFACE RECEPTORS? KEY CONCEPTS:

• Cell surface receptors exist to transmit chemical signals from the outside to the inside of the cell.

• Some compounds bind to cell surface receptors, yet do not activate the receptors to trigger a response. (These are the antagonizing agent)

Possible patterns of binding: reversibly (noncovalently) OR irreversibly (covalently)

Resulting in to two modalities of inhibition:

a- Competitive inhibition: when the exogenous antagonist (drug molecule), sterically, reversibly, noncovalently binds the receptor at its active binding site , <u>for short period</u> (about millisecond) . in this case the receptor binding capability can be shared between both the endogenous ligand and the exogenous antagonist drug molecule .

- What actually determines the predominance in occupying the available cell-surface receptor are :
  1- Amount(concentration) 2- affinity towards the binding

- Therefore increasing the concentration of endogenous ligand gives it a stronger binding behavior to kick out the antagonist and overcome its interfering effect and so that it provides its physiological cascade of processes once again .
- On the other hand, a better therapeutic effect can be gained out of stronger binding competitive behavior of the antagonist drug , aided by agreater dose.
- Finally: this pattern of binding is preferrable, because it shortens the half-life of the drug, and prevent the permanent conformational damage of the cell-surface receptor.

  a- Noncompetitive inhibition: when the exogenous antagonist (drug molecule), sterically, irreversibly, covalently binds the receptor, occupying the available certain number of receptors, causing permanent conformational damage.
- In such a case, endogenous ligand have NO competitive behavior (can not kick out the noncompetitive antagonist), thus changing concentrations is useless. Instead to overcome that non-competitive antagonist ,an expression of new receptors take place.
- Such therapies are distinguished by a long half-life .

  c- Allosteric (suffix allo refers to other)
  In this case, the antagonizing agent binds at remote critical site. Therefore, similarity to the endogenous agonist and complementarity to the substrate binding site is NOT REQUIRED .
- Allosteric effects contain permanent conformational defect the prevents endogenous agonist from binding .
- It's a noncompetitive binding and you can only overcome such inhibition by expression of new receptors .

• When cell surface receptors bind the molecule, the endogenous chemical cannot bind to the receptor and cannot trigger a response.

• The compound is said to "antagonize" or "block" the receptor and is referred to as a receptor antagonist.

Binding sites among receptors: Substrate binding site, Allosteric activator binding site, allosteric inhibitor binding site.

a- Substrate binding site :

For both

1- endogenous agonist ligand (hormones , neurotransmitter).

2-exogenous agent, either activator drug molecule (exogenous agonist).

Or antagonizing inhibitor .

However, ALL the binding endogenous and exogenous sharestructural similarities.

- Endogenous and exogenous agonists both generate identical activatory pathways

An example to illustrate :

Beta adrenergic receptor substrate binding site receives both :endogenous adrenaline and exogenous Ventolin (salbutamol) and both activate the same sympathetic autonomic response of bronchial dilatation . Such as cases of treating Asthma .

- The more intensively the substrate site is occupied by agonists the more effective the generated pathway is .

b- Allosteric activator binding site

c- Allosteric inhibitor binding site

Most antagonists attach to binding site on receptor for endogenous agonist and sterically prevent endogenous agonist from binding. If binding is reversible - Competitive antagonists

If binding is irreversible - Noncompetitive antagonists

However, antagonists may bind to remote site on receptor and cause allosteric effects that displace endogenous agonist or prevent endogenous agonist from activating receptor. (Noncompetitive antagonists)

ARE DRUGS THAT ANTAGONIZE CELL SURFACE RECEPTORS CLINICALLY USEFUL?

Examples on cell-surface receptor targeting drugs: THERAPUETIC EFFECT OPPOSITE TO THE CELLULAR FXN

1-Angiotensin Receptor Blockers (ARBs) for treatment of high blood pressure, heart failure, chronic renal insufficiency (losartan [Cozaar®]; valsartan [Diovan®]) .

2-Beta-Adrenoceptor Blockers for treatment angina, myocardial infarction, heart failure, high blood pressure, performance anxiety (propranolol [Inderal®]; atenolol [Tenormin®]) :

- The original cellular response by the endogenous agonist-adrenaline. (that is inhibited by the antagonist) : To provide a sympathetic autonomic response (fight or flight), for example bound beta1 receptor provides an increased heart rate
- Therapeutic action :(NO more increased heart rate)

Note : names between brackets indicate the commercial nomenclature of the drug .

**Remember we are dealing with antagonizing drugs for various endogenous proteins, we here have covered the cell-surface receptors and here the remaining come.

SECONDLY, nuclear receptors :

In this case, the endogenous agonist must retain lipophilicity (steroids are an example) .

ARE DRUGS THAT ANTAGONIZE NUCLEAR RECEPTORS CLINICALLY USEFUL?

• Mineralocorticoid Receptor Antagonists for treatment of edema due to liver cirrhosis and for heart failure (spironolactone [Aldactone®]) Some important examples:

• Estrogen Receptor Antagonists for the prevention and treatment of breast cancer (tamoxifen [Nolvadex®])

THIRDLY : Enzyme Inhibitors

HOW DO DRUGS ANTAGONIZE, BLOCK OR INHIBIT ENDOGENOUS PROTEINS?

Enzymes catalyze the biosynthesis of products from substrates.

• Some drugs bind to enzymes and inhibit enzymatic activity.

• Loss of product due to enzyme inhibition mediates the effects of enzyme inhibitors.

ARE DRUGS THAT INHIBIT ENZYMES CLINICALLY USEFUL?

Here are important examples

• Cyclooxygenase Inhibitors for pain relief, particularly due to arthritis (aspirin; ibuprofen [Motrin®])

- The original cellular response by the active enzyme. (that is inhibited by the antagonist): Arachidonic acid metabolized into PGs which are critical inflammatory mediators responsible for pain and fever response .
- Therapeutic action :(stopping the production of PGs decreasing its inflammatory symptoms.

  COX inhibitor : analgesic-anti pyruvic-anti inflammatory.

• Angiotensin Converting Enzyme (ACE) Inhibitors for high blood pressure, heart failure, and chronic renal insufficiency (captopril [Capoten®]; ramipril [Altace®])

• HMG-CoA Reductase Inhibitors for hypercholesterolemia (atorvastatin [Lipitor®]; pravastatin [Pravachol®])

Fourthly: Ion Channel Blockers

ARE DRUGS THAT BLOCK ION CHANNELS CLINICALLY USEFUL?

Some important examples :

• Calcium Channel Blockers (CCBs) for angina and high blood pressure (amlodipine [Norvasc®]; diltiazem [Cardizem®]):

The original cellular response by the active enzyme. (that is inhibited by the antagonist): Na^+ ions influx causing depolarization of cellular membrane and propagation of electrical signals among neural pathway, which can finally release calcium ions out of their sarcoplasmic reticulum store and mediate vascular muscle contraction, blood vessel constriction and increased blood pressure .

• Sodium Channel Blockers to suppress cardiac arrhythmias (lidocaine [Xylocaine®]; amiodarone [Cordarone®])

Fifthly : Transport Inhibitors .

ARE DRUGS THAT INHIBIT TRANSPORTERS CLINICALLY USEFUL?

Some important examples

• Selective Serotonin Reuptake Inhibitors (SSRIs) for the treatment of depression (fluoxetine [Prozac®]; fluvoxamine [Luvox®]):

The original cellular response by the active transporter. (that is inhibited by the antagonist): serotonin neurotransmitter reuptake from the synaptic cleft- where it gets degraded, back to the pre-synaptic neuron causing termination of signaling.

- Therapeutic action: continuous neural stimulation and feeling of happiness.

• Inhibitors of Na-2Cl-K Symporter (Loop Diuretics) in renal epithelial cells to increase urine and sodium output for the

Sixthly: Signal Transduction Proteins.

ARE DRUGS THAT INHIBIT SIGNAL TRANSDUCTION PROTEINS CLINICALLY USEFUL?

Some important examples:

Tyrosine Kinase Inhibitors for chronic myelocytic leukemia (imatinib [Gleevec®]):

In cancer breakthrough, Such procedure is considered as critical pharmacological advance point to minimize non-targeted chemotherapy , and limit attacks to malignant cells only .

chronic myelocytic leukemia is characterized by an exaggerated activity of tyrosine kinase, and its corresponding activation signaling cascade

which finally results in the rapid proliferation of such cancerous leukemic cells.

you can notice the specificity in targeting one molecular signal , whereas conventional chemotherapy generalize inhibition into multiple cellular pathways in highly replicated tissues , causing severe wide range of adverse effects.

Type 5 Phosphodiesterase Inhibitors for erectile dysfunction (sildenafil [Viagra®])

AND NOW after visualizing antagonists, lets move on to the other conventional method of drug action: exogenous agonists.
which mimics the endogenous hormone or neurotransmitter and generates a similar but stronger signaling pathway.

HOW DO DRUGS WORK BY ACTIVATING ENDOGENOUS PROTEINS?

Important examples :

- **Agonists of Cell Surface Receptors (e.g. alpha-agonists, morphine agonists)**

Example, Morphine (a pain killer) mimics endogenous analgesic (enkephalins) andbinds to their brain receptor providing and activating identical pain relief response.

HOW DO CHEMICALS WORK BY ACTIVATING CELL SURFACE RECEPTORS?

KEY CONCEPTS:

- **Cell surface receptors exist to transmit chemical signals from the outside to the inside of the cell.**

Some chemicals bind to cell surface receptors and trigger a response.

Chemicals in this group are called receptor agonists.

Some agonists are actually the endogenous chemical signal, whereas other agonists mimic endogenous chemical signals

So, a patient can be supplied with an agonist that mimics endogenous chemical effector (hormone).
Or by the chemical effector hormone itself.

- **Agonists of Nuclear Receptors (e.g. HRT for menopause, steroids for inflammation)**
- **Enzyme Activators (e.g. nitroglycerine (guanylyl cyclase), pralidoxime)**
- **Ion Channel Openers (e.g. minoxidil (K) and alprazolam (Cl))**

Lets now move to the second minor field of drug action, nonconventional drugs, which don't behave as either antagonist or agonist.

HOW DO CHEMICALS WORK BY UNCONVENTIONAL MECHANISMS OF ACTION?

- **Disrupting of Structural Proteins e.g. vinca alkaloids for cancer, colchicine for gout**

- Tubulin cytoskeletal structures polymerize into a higher level of organization, known as microtubules, which are essential building blocks in multiple cellular organelles such as
  a- Mitotic spindles that separate chromatids of duplicated chromosomes during mitotic division.

Therefore , these structural proteins can be targeted to control the proliferation of cancerous cells .

b- Microtubules mediate chemotactic process of inflammatory phagocytic macrophages .

Therefore , these structural proteins can be targeted to control the chemotactic ,phagocytic inflammatory arthritic response in gout disease .

HOW DO CHEMICALS WORK BY UNCONVENTIONAL MECHANISMS OF ACTION?

- **Disrupting of Structural Proteins**

e.g. vinca alkaloids for cancer, colchicine for gout

you have to know how chemicals work by an unconventional mechanism of action

for example you don't have to know that the colchicine used for gout, but you should know that some drugs are involved in tubulins polymerization (structural proteins) and they prevents its function.

- **Being Enzymes**

e.g . streptokinase for thrombolysis

this drug (streptokinase) is found in microorganisms (bacteria) and it works on lysing blood clotting, so we utilize the presence of this enzyme and generate it by recombinant DNA technology, using in emergency situations of clotting disorders

- **Covalently Linking to Macromolecules**

e.g. cyclophosphamide for cancer

some drugs bind covalently to macromolecules like DNA. a lot of drugs that treat cancer work by interfering with DNA.

- **Reacting Chemically with Small Molecules**

e.g. antacids for increased acidity

if someone ate a heavy meal he will have Heartburn, as a result of increasing the acid secretion in the stomach, one way to control the high amount of acid is to give him a base(antiacid)like: Calcium carbonate

here we will get a chemical reaction between the calcium carbonate and the HCl2 producing salt and water , so this is a chemical reaction that resulted in treatment of the problem.

- **Binding Free Molecules or Atoms**

e.g. drugs for heavy metal poisoning, infliximab (anti-TNF)

we have a certain drugs that target a small molecules in our body, HOW?

By generating immunoglobulins antibodies against these proteins.

TNF: tumor necrosis factor, it has been associated with multiple diseases, so by targeting these small molecules that are present in our body by small immunoglobulins we can target these cases of immune diseases, cancers or other conditions.

OTHER EAMPLES OF UNCONVENTIONAL MECHANISMS OF ACTION

- **Being Nutrients**

e.g. vitamins, minerals

we take some drugs to supply us with something that we need or something we don't have like: calcium supplement (for deficiency in calcium), vitamin E or C .

- **Exerting Actions Due to Physical Properties e.g. mannitol (osmotic diuretic), laxatives**

Some drugs that are used to treat constipation, work by just being a bulk forming compounds. so we basically give the patient a fiber (substance that like to absorb water because of its physical property),so it will form a bulk in the intestine and stimulates the intestine to contract and evacuate the body from the stool.

So just by their physical property of being absorbent for water we have utilize these drugs.

- **Working Via an Antisense Action**

e.g. fomivirsen for CMV retinitis in AIDS

one of the antiviral drugs ex. Fomivirsen that can bind to the DNAor the RNA of the virus and prevent its transcription. So the target here is either a DNA or RNA.

- **Being Antigens**

e.g. vaccines

vaccines stimulate the immune response in our body to have protection from certain conditions such as: polio, viral illness.

- **Having Unknown Mechanisms of Action**

e.g. general anesthetics

general anesthetics have been used for long period of time, more than hundred years and they actually do their work, but we don't know their mechanism of action yet. For example: halogenates hydrocarbons (a group of chemicals structure drugs) have been used for general anesthetics, we know that They anesthetize the patient, but we don't know how exactly they do that. Why is that? Because in the past they discovered those drugs ether by trial, error or coincidence, like the discovery of penicillin

Alexander Fleming was working on discovering an anti infectant for the bacteria, so he was growing a bacteria in petri dishes contaning nutrients for bacteria. Fleming left his little experiment for 3-4 days and came back after when he noticed the growth of molds **(**if I were him, I will just through it**)** but he was smart enough to notice a hallow (empty ring)in the dish and he thought that probably the molds produce a substance that killed the bacteria. so he started studying penicillin molds more and isolated them away.

Someone who was invaded by a rose thorn that caused an infection which made him fatally ill, accepted to let them try the penicillin on him, after that he was recovered. Unfortunately, The penicillin was not enough, they tried to extract it from his urine to give it to him again, but after two months he died.

In conclusion, scientists used to try the drug to know its function, but nowadays we absolutely can't give a patient drug from molds immediately. But we try to discover what happen at signaling level of the cell for example, after that we try it on animal models or cell models , then we can know the dose by trail and error.

So we really don't know how some drugs work yet. For example the cyclooxygenase inhibitors, we know for sure that they inhibit cyclooxygenase but we can't tell how.

Important note

After the great development in technology and by sequencing of genes and sequencing the whole genome of humans ,we were able to identify a lot of receptors in our body ,but we don’t know the ligand of them yet so we call them the **orphanreceptors.**