

Principles of Pharmacology - Study Guide

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Edited by Dr. Esam El-Fakahany and Becky Merkey, MEd



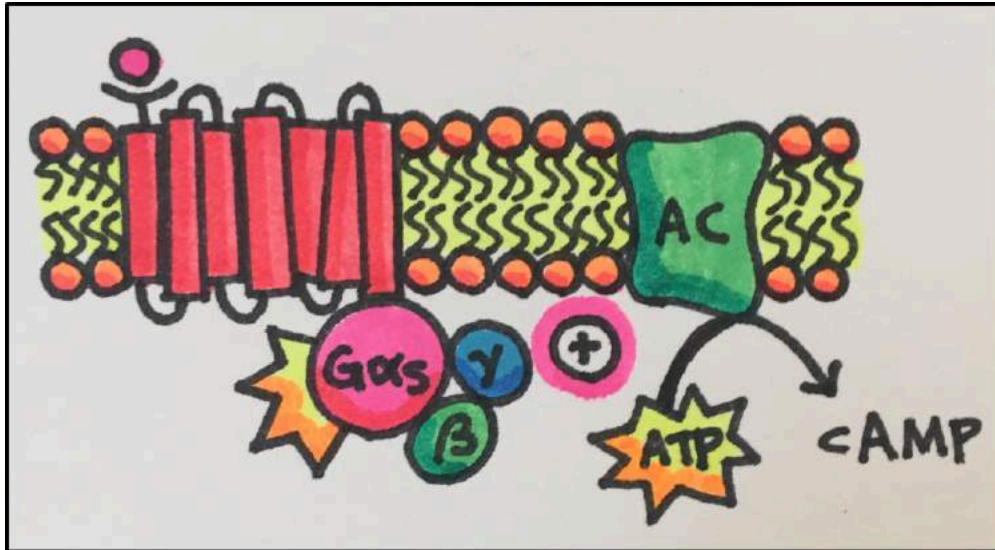
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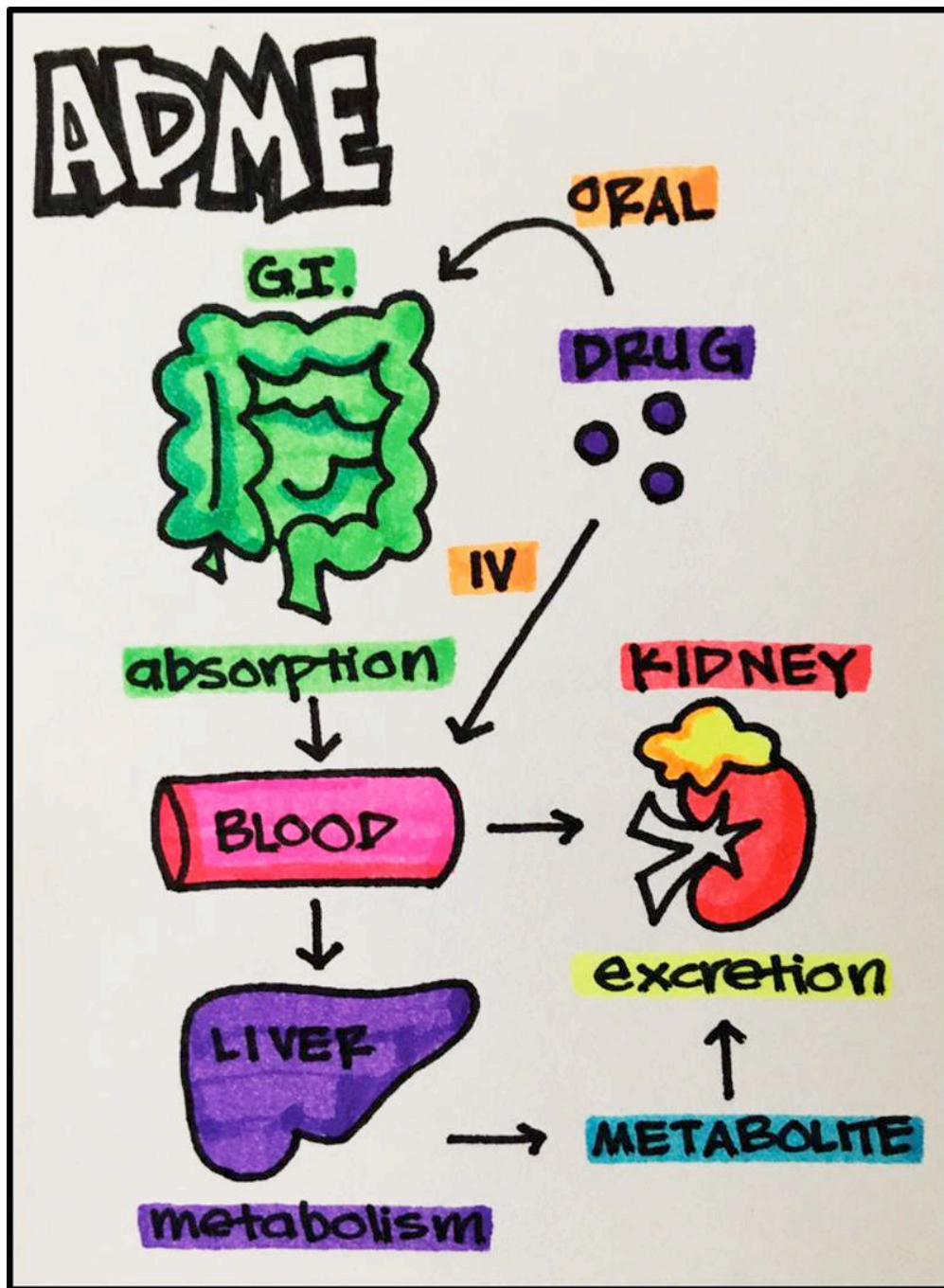
1. Introduction to Pharmacology

Pharmacology: the study of interaction of drugs with living systems.



Sub-Disciplines of Pharmacology

- **Pharmacodynamics:** effects and mechanisms of drug action
 - Drug-Receptor Interactions
 - Dose-Response Relationships
 - Signal Transduction
- **Pharmacokinetics:** movement of drug throughout the body including:
 - Absorption
 - Distribution
 - Metabolism
 - Excretion
- **Pharmacogenetics:** genetic factors play a role in the following:
 - Rate of Drug Metabolism
 - Drug-Induced Toxicity
 - Drug-Induced Allergies



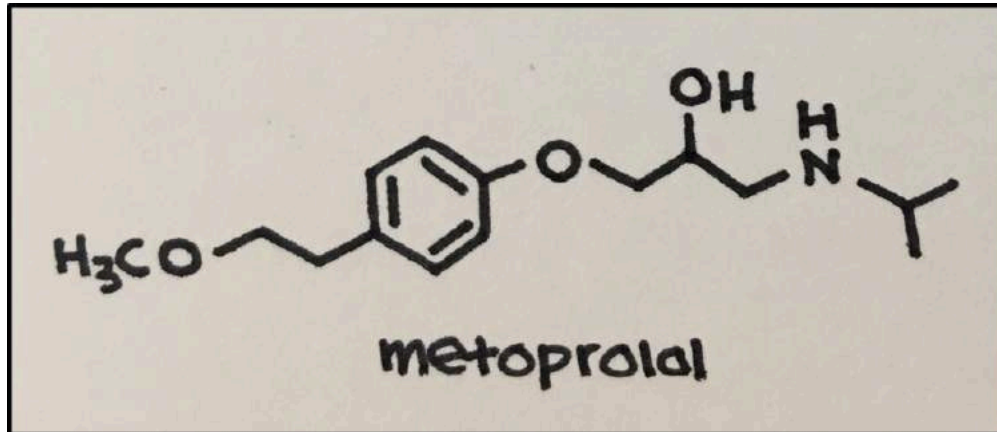
Pharmacology and the Pharmacist

Key Questions you should be asking as a Pharmacist:

- Where is the molecular **site of action**?
- What are the body function **changes** caused by a drug (pharmacodynamics)?
- What is the relationship between the **Dose vs. Effect**?
- How does a drug produce its **effect**?

- What is the **fate of the drug** once it enters the body (pharmacokinetics)?
- What is the interplay between **genetic makeup** and **drug response**?

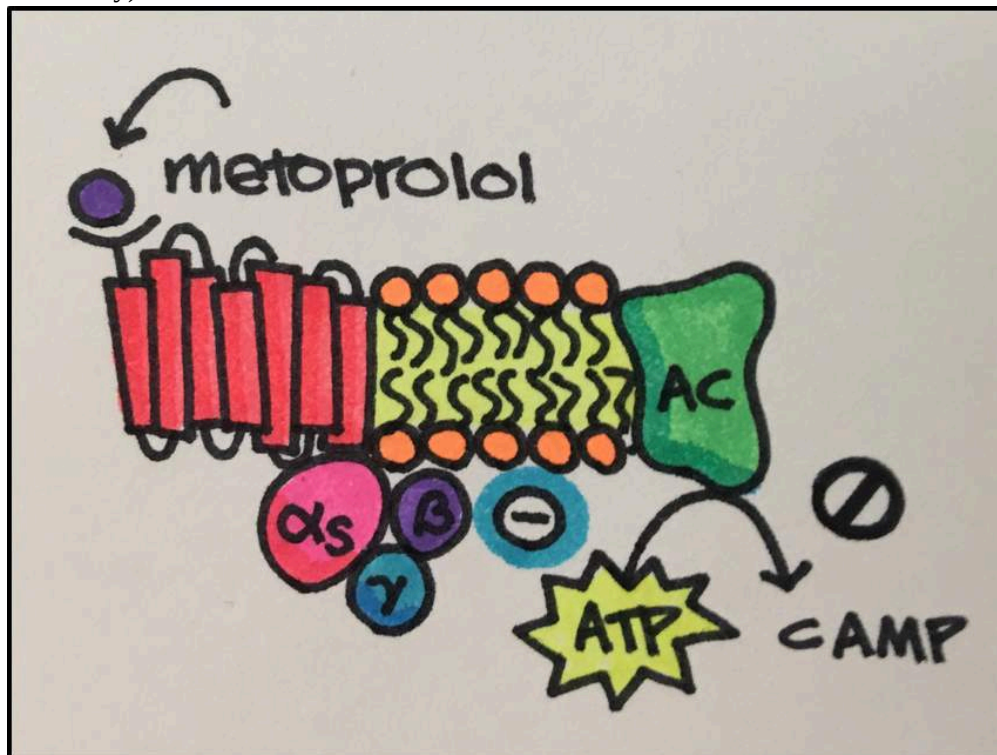
Example: Beta 1 Blocker: Metoprolol Succinate (oral)



Drug Action: selective binding to cardiac muscle beta 1 adrenergic receptors that respond to norepinephrine (at higher doses, also inhibits bronchial and vascular smooth muscle by acting on beta 2 adrenergic receptors) to inhibit the binding of norepinephrine.

Drug Effect: reduced inotropic effect (contractility) and chronotropic effect (heart rate)

Fate of the Drug (pharmacokinetics): 12% protein binding and distribution 5.6 L/kg; hepatic metabolism (CYP2D6 mainly): <5% renal excretion: $t_{1/2}$ 3-7 hours



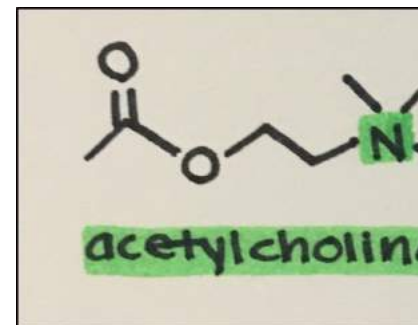
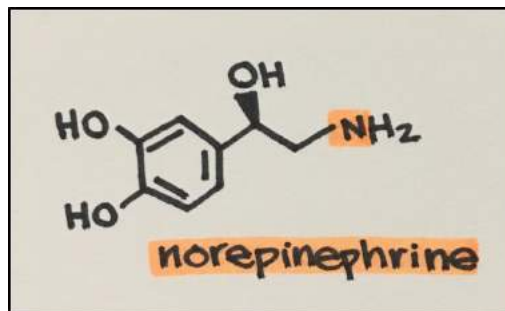
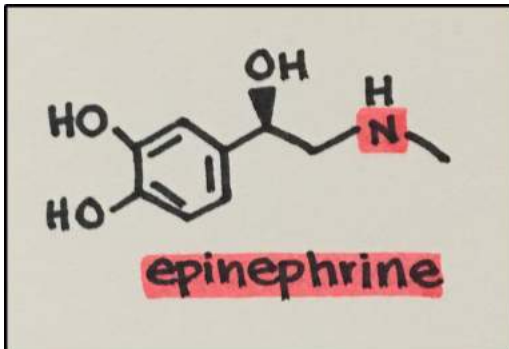
2. Introduction to Drug-Receptor Interactions and Pharmacodynamics

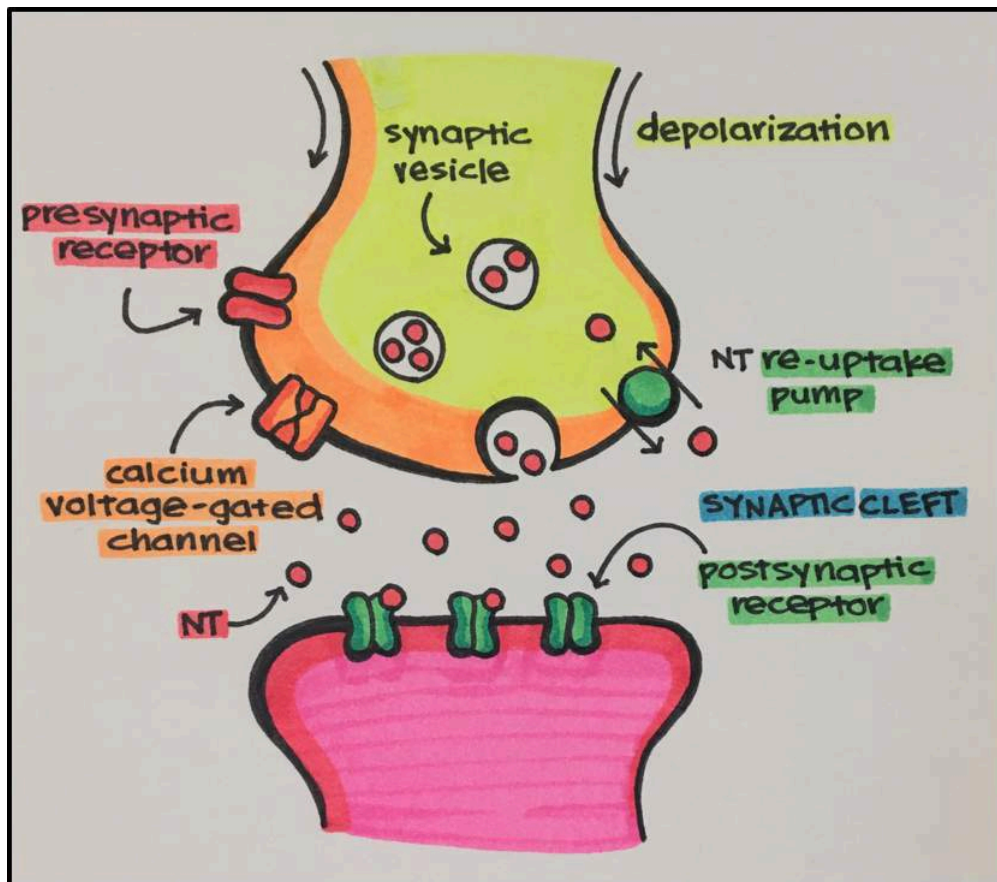
Receptors: protein molecules including enzymes, transporters and ion channels where a ligand (specific **endogenous** neurotransmitter/hormone or an external pharmacological agent (drug)) binds to, resulting in a cellular response.

- **Unique Exception: Orphan Receptors** are receptors for which the ligand remains unknown.
- **Reminder: Ligand** is an ion or molecule that forms a complex with a biomolecule to serve a specific biological purpose

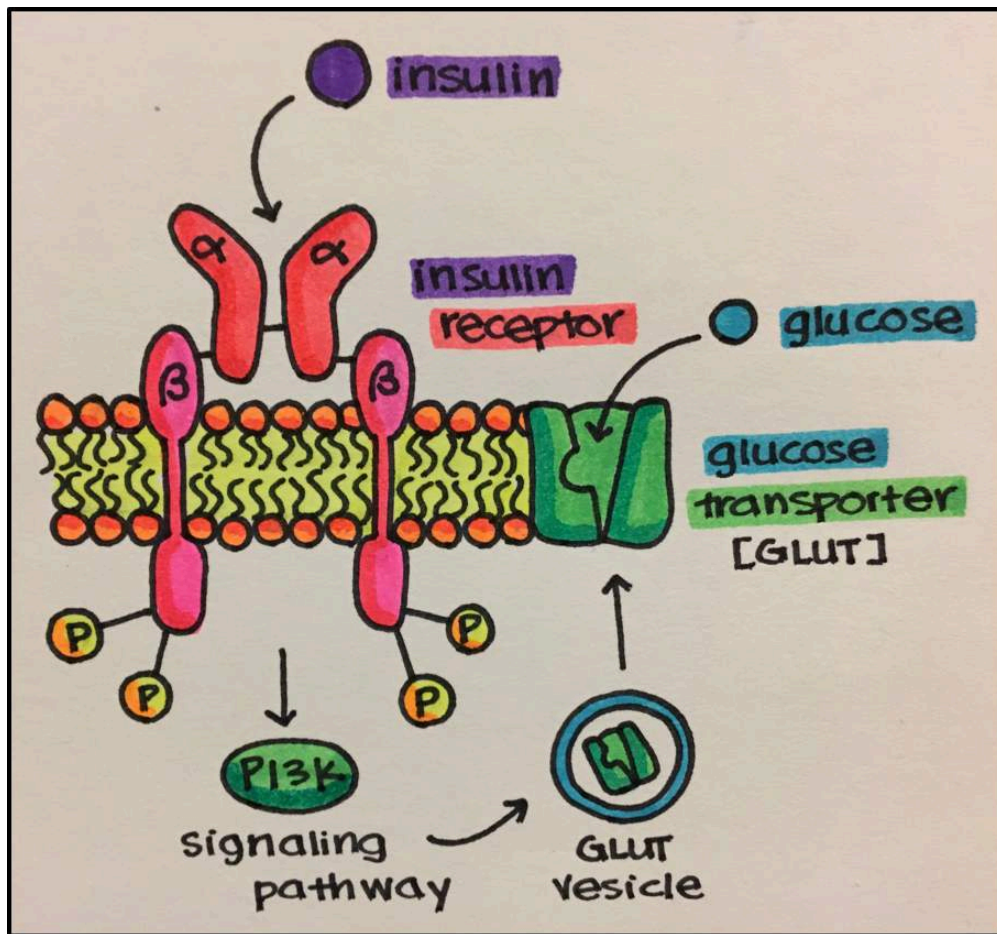
Examples of **Endogenous Ligands:**

- **Neurotransmitters[NT]:** chemical messengers signaling across a synaptic cleft
 - Acetylcholine [Ach]
 - Epinephrine [EPI]
 - Norepinephrine [NE]





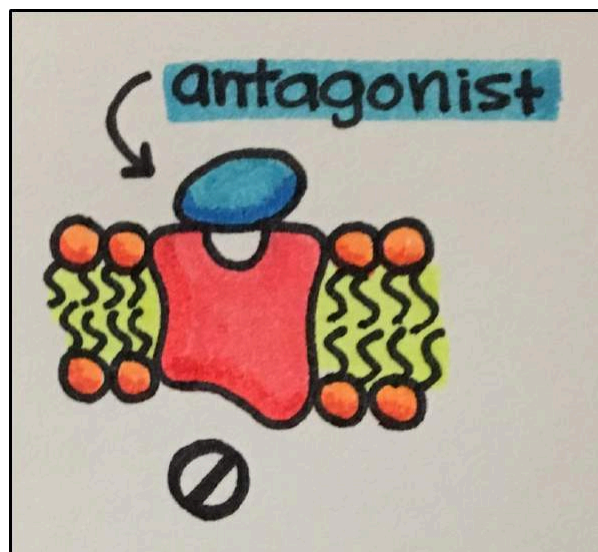
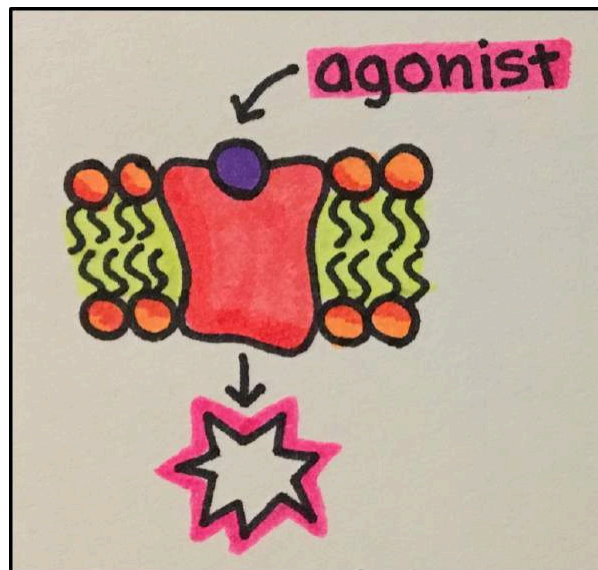
- **Hormones** (peptide): secreted from neuroendocrine cells into the blood to signal at distant cells and tissues.
 - Aldosterone
 - **Insulin**
 - Nerve growth factor [NGF]
 - Thyroid hormone [TH]



- **Insulin** is synthesized and released by pancreatic beta cells. It is transported through the blood to a variety of cells to stimulate those cells to express glucose transporters allowing those cells to bring glucose into the cell for energy utilization.

DRUG: A chemical agent that selectively interacts with specific target molecules (i.e. receptors) to alter their specific physiological functions.

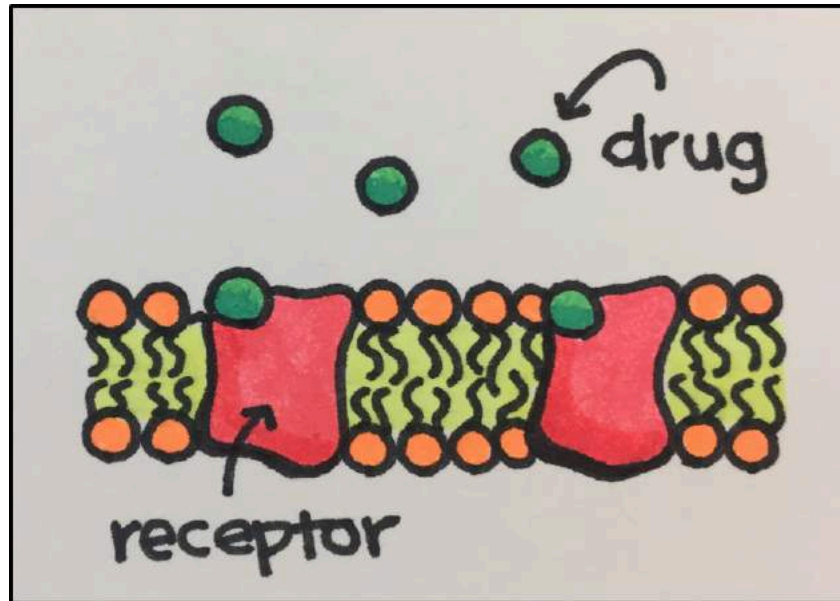
- **Agonist:** drug that activates receptors to result in either stimulation or inhibition of the function of various types of cells and organs.
- **Antagonist:** drug that prevents receptor activation by agonists.



Drug-Receptor Binding: drugs bind to their respective receptor in a variety of ways depending on their characteristics.

- **Ionic interaction:** cation & anion
- **Hydrogen bonding**
- **Lipophilic interaction**
- **Covalent bond:** irreversible

3. Factors Contributing to Drug Effect

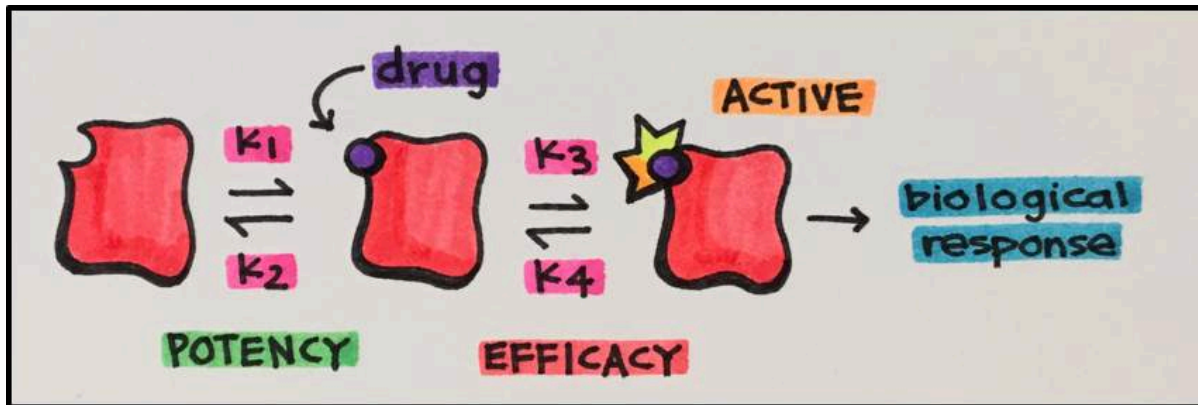


The Effect a Drug may have is Dependent on a Variety of Factors:

1. **Drug dosage:** effect of drug increases with increased amount of drug up to the **POINT OF RECEPTOR SATURATION**
2. **Number of Receptors:** receptors may be differentially expressed in one tissue to the next and, therefore, mediate different levels of biological responses.
 - **Example:** Beta-1 receptors are most concentrated in the heart; therefore, beta-1 agonists produce the greatest effects in the heart. Beta-2 receptors are most expressed in the bronchioles of the lungs and the arteries of skeletal muscle; therefore, beta-2 agonists produce the greatest effects in these tissues and organs.
3. **Disease states:** disease states can effect drug pharmacology
 - **Example:** pharmacokinetics of a drug change dramatically in a patient with chronic kidney disease
 - **Digoxin's** volume of distribution decreases when one has chronic kidney disease [CKD]. Clearance also decreases causing digoxin's half-life to increase, meaning that patient with CKD actually needs a lower dose than a patient with normal functioning kidney to achieve safe and effective digoxin levels.
4. **Drug Efficacy/Intrinsic activity:** ability to activate or block a receptor: maximum effect a drug can produce regardless of the dose

5. **Drug Potency/Affinity:** rate of drug-receptor binding and drug-receptor release: amount of drug needed to produce a particular effect: drug affinity for receptor:

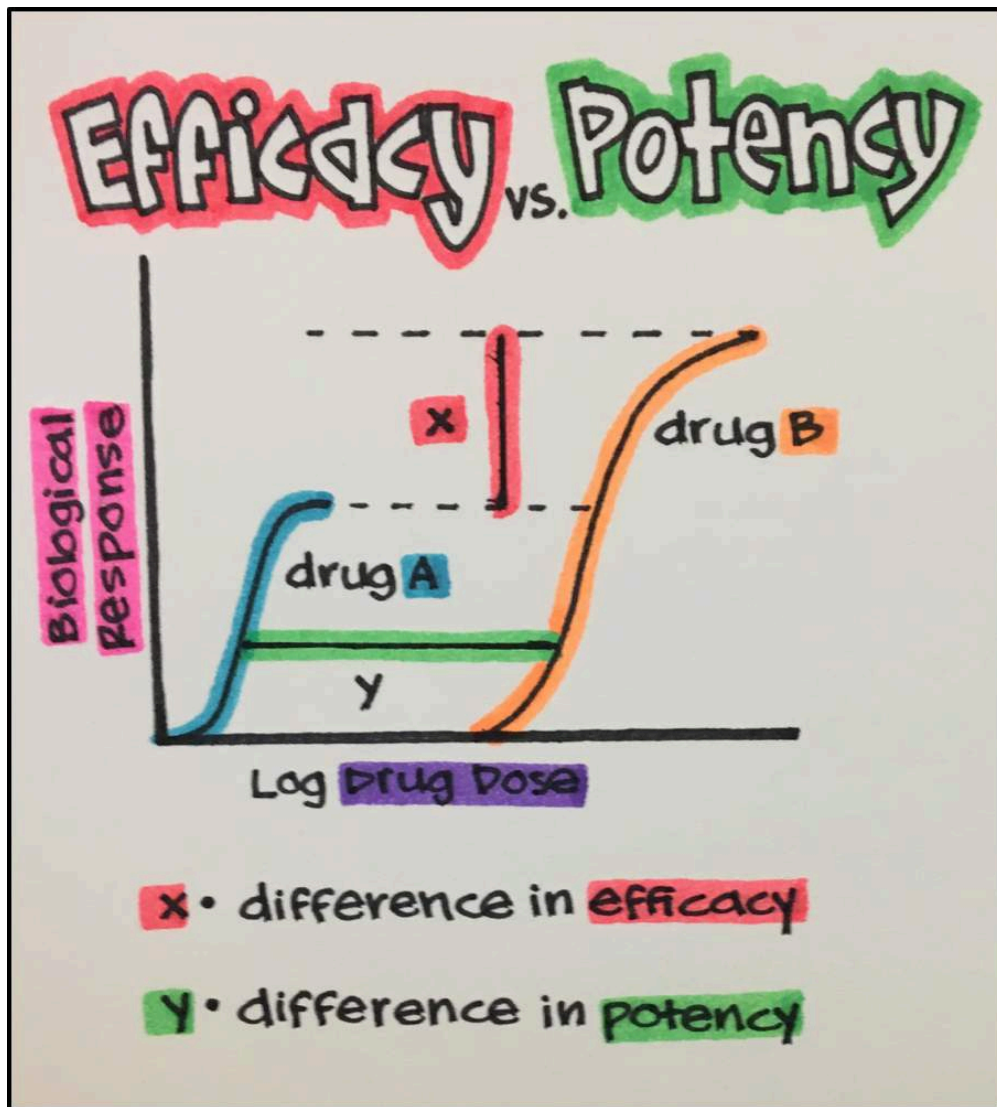
See below: the more **potent** the drug the quicker it binds its receptor (forward rate) and the slower it releases from its receptor (reverse rate).



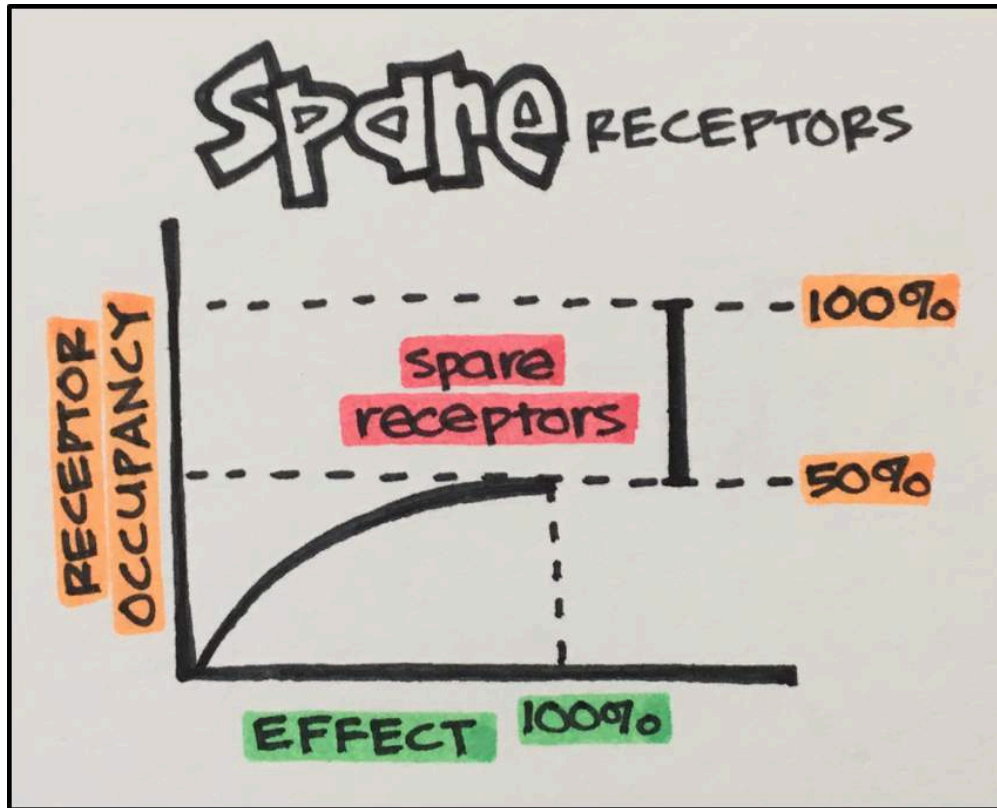
Efficacy vs Potency: As drug efficacy increases, so does the maximal biological response it can produce. Efficacy cannot be changed by increasing the dose beyond that which elicits a maximal response, since it is an inherent characteristic of the drug. While potency is also an intrinsic property of a given drug, effects of the drug may be increased by using higher doses because potency refers to the rate of drug-receptor binding and dissociation whereas efficacy refers to the resultant biological response.

EFFICACY of a drug is therefore more important clinically than

POTENCY since one could manipulate the drug dose to produce the desirable response in case of a drug with low potency but a drug with low efficacy could not be manipulated in the same way.



Spare Receptors: often times occupation of only a fraction of receptors is necessary for obtaining a maximal response. In this case the remaining unbound receptors are known as **spare receptors**. This means that there can be a reduction in receptors (up to a limit!!!) without a corresponding decrease in the maximal response.



4. Pharmacological Descriptors of Drug-Receptor Interactions

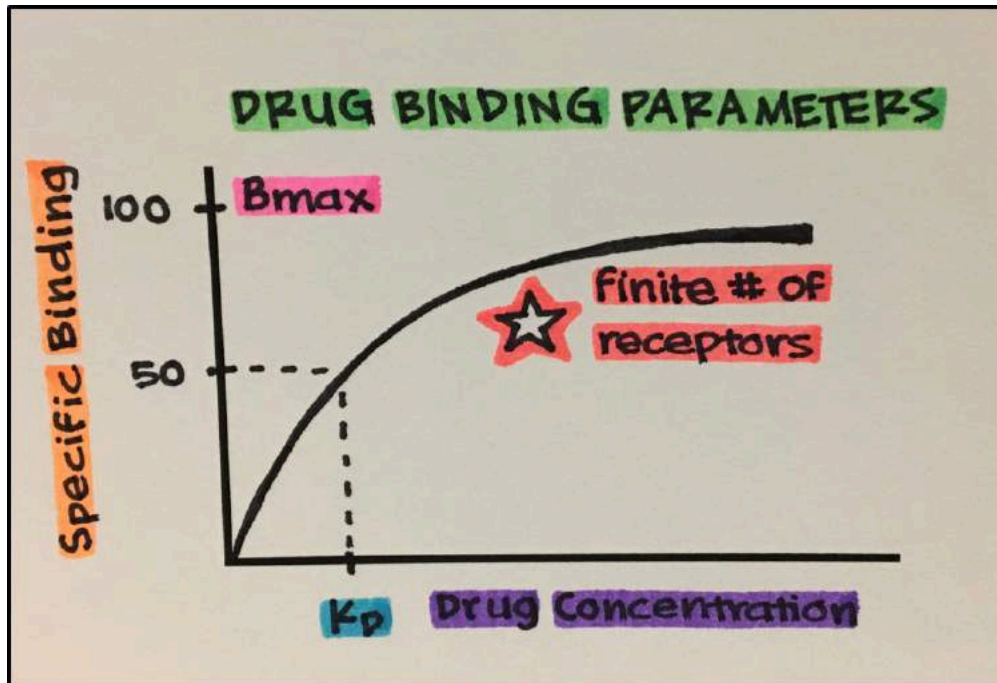
Pharmacological Descriptors of Drug-Receptor Interactions are used to describe and compare potency of drugs

- **K_D [equilibrium dissociation constant]:** indicates drug concentration at which 50% of the receptors are occupied by the drug

FYI: This is **constant** for a given drug-receptor system

- **B_{max} [maximal specific binding]:** represents the total number of receptors present in a cell or tissue

Reminder: a drug does not have to bind all available receptors in order to achieve the maximal effect (see [spare receptors](#))



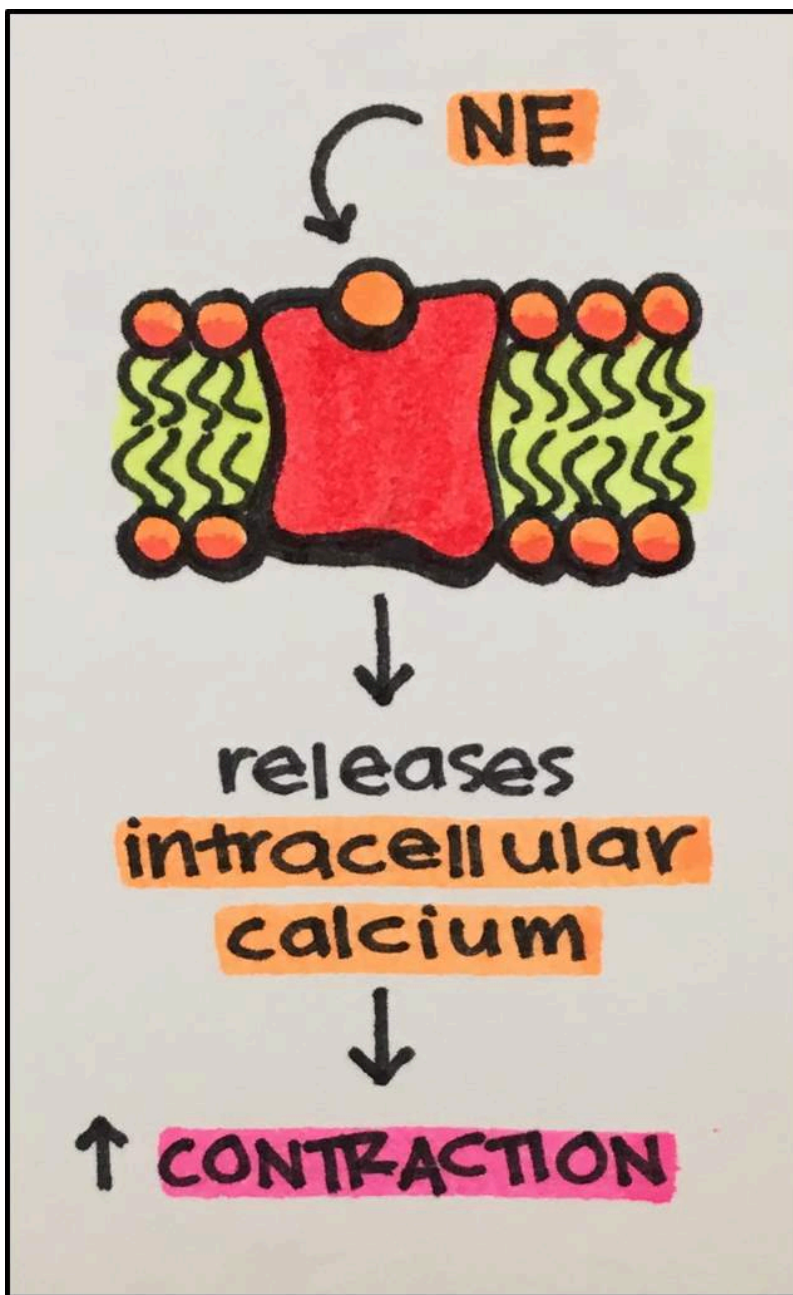
5. Drug Action vs. Drug Effect

Drug Action: drug-receptor interaction that results in a change in the tertiary structure of the receptor (conformational change) by agonists

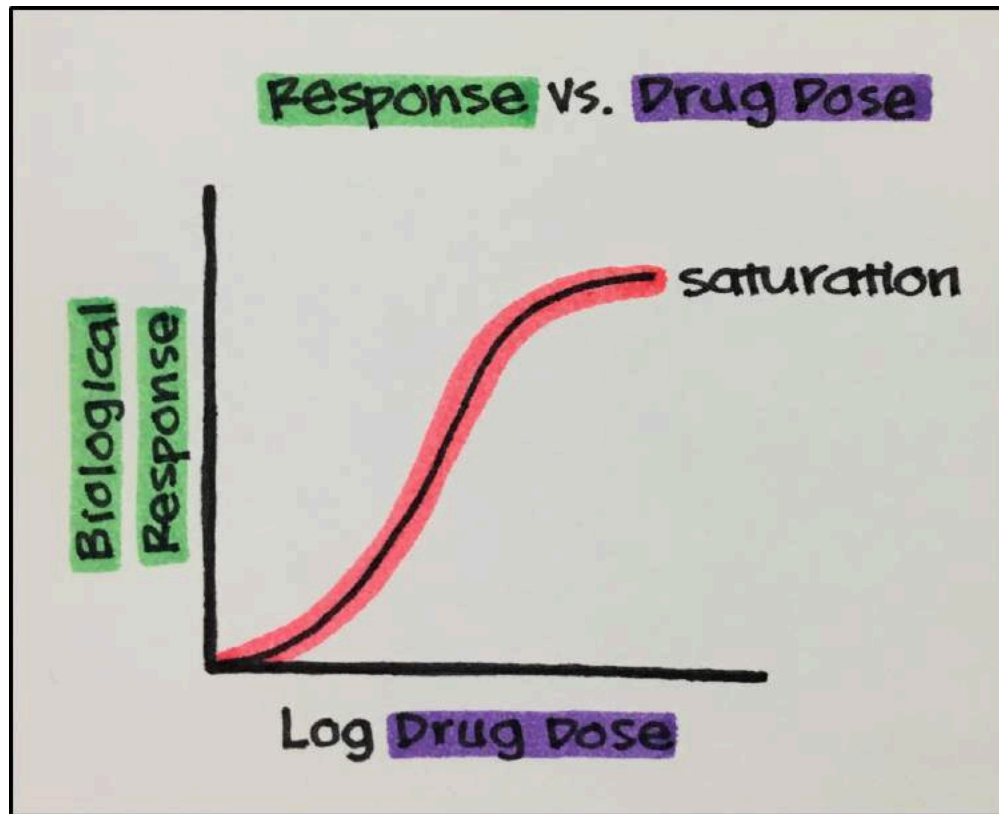
- See right: Norepinephrine [NE] acts as an agonist, activating its receptor to initiate a cascade of cellular events.

Drug/Pharmacological Effect: specific changes in physiological function as a result of drug interaction with a particular receptor.

- See right: the drug-receptor interaction leads to a cascade of events ultimately causing the heart to contract.

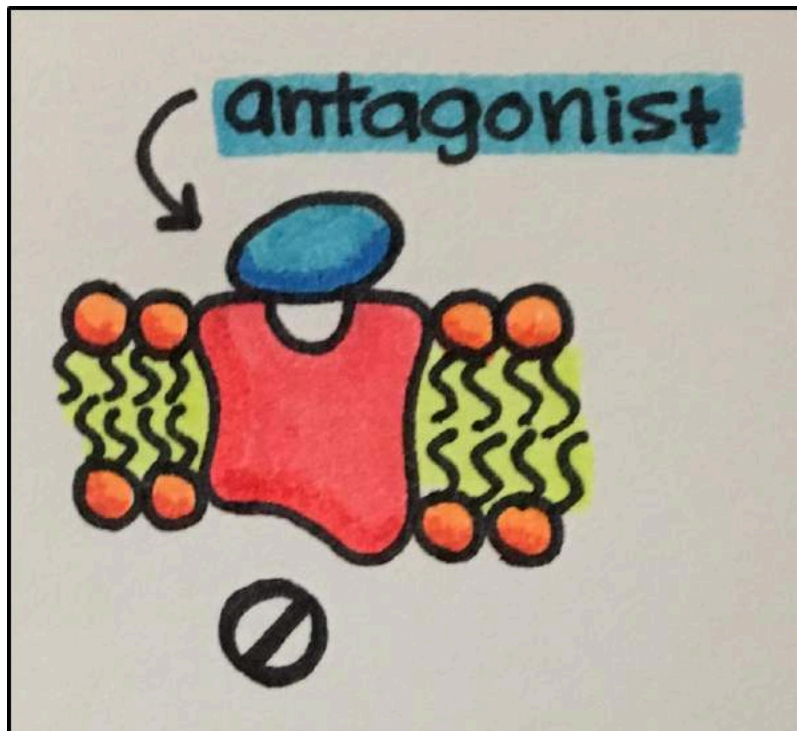
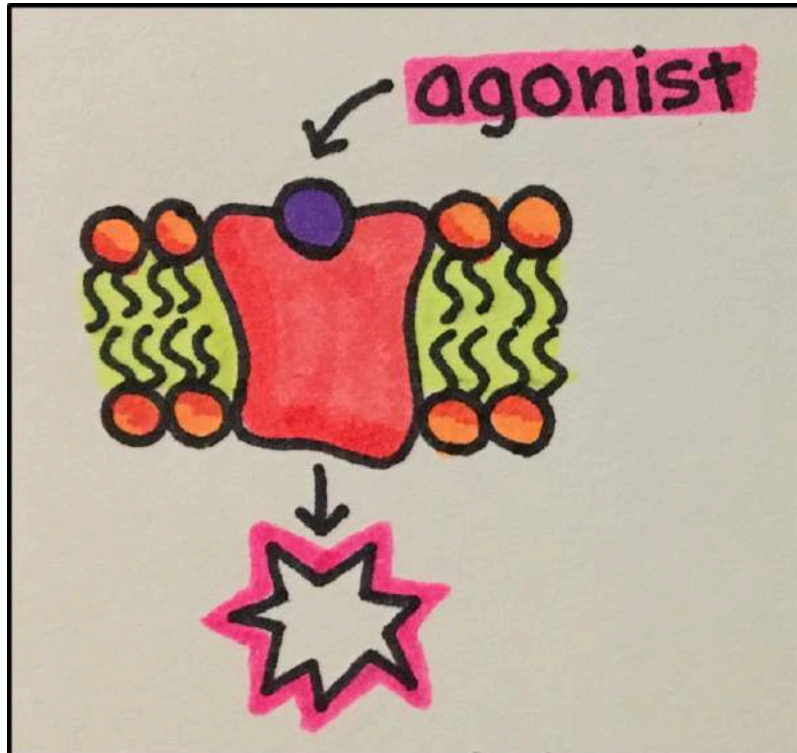


6. Characteristics of Drug-Receptor Interactions:



1. **Follow Mass-Action Law:** rate of reaction is **DIRECTLY** proportional to the concentration of the reactants, however, there is a limit. This limit is called the **point of saturation**
 - There are only so many receptors on each cell to which a drug can bind. **Point of saturation** refers to the point at which every receptor is bound.
2. **Selectivity:** every drug has a **preferred** receptor; however, it may bind to others with the same or lesser affinity than its preferred receptor. Side effects of drugs are a direct result of low selectivity because one drug can bind multiple receptors producing undesired biological responses.
 - Not to be confused with “**specificity**” meaning drug can only bind to one type of receptor, regardless of the drug dose: drugs that are “specific” are unheard of at this point
3. **Response is Proportional to Drug Dose [Concentration]** up to the **point of saturation**
4. **Binding Changes the Receptor:** when a drug binds a receptor it results in one of two changes as outlined below:
 - AGONIST:** results in an **active receptor conformation** to produce desired biological effect, i.e. intracellular signaling or changes in organ function

ANTAGONIST: a simple occupancy by the antagonist of the receptor to hinder access of an agonist to its binding site on the receptor, therefore, obliterating the response to the agonist.



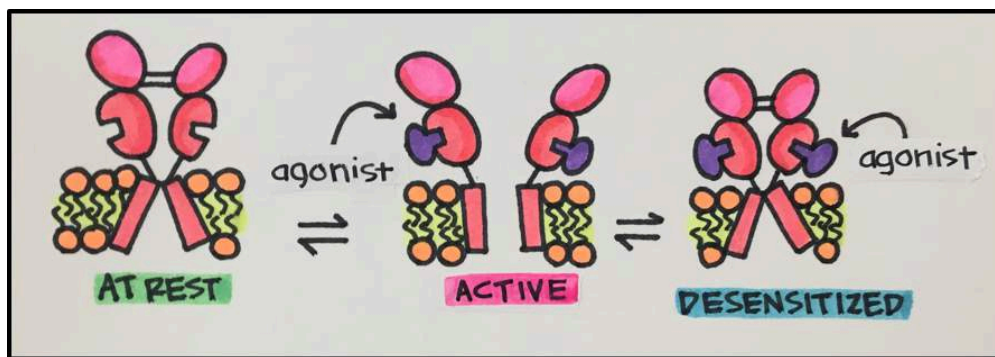
7. Response to Excessive and Reduced Stimulation of Receptors:

Excessive Receptor Activation can result in desensitization or down regulation.

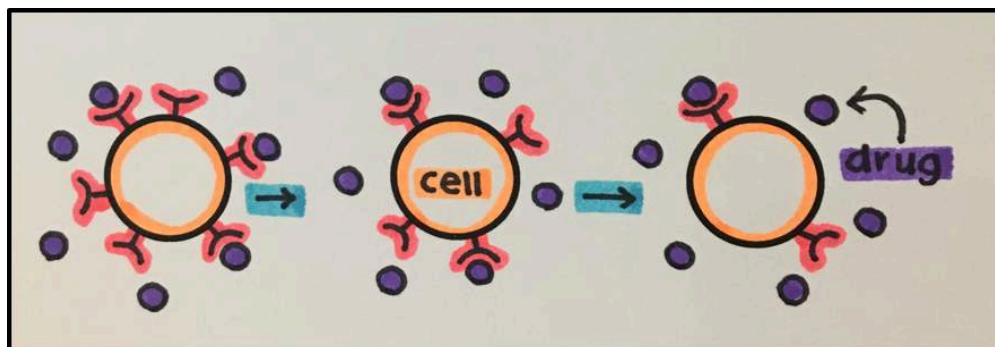
Desensitization: receptor becomes less responsive when exposed to a high concentration of an agonist drug for a long duration (several minutes).

What is an example of receptor desensitization?

Under normal physiological conditions acetylcholine stimulates nicotinic acetylcholine receptors on skeletal muscle to open an ion channel that allows calcium influx to cause muscle contraction. Acetylcholine is hydrolyzed rapidly by acetylcholinesterase; the channel closes and is ready to be opened again upon the arrival of new acetylcholine molecules. Pharmacological inhibitors of acetylcholine esterase cause accumulation of high concentrations of acetylcholine in the synaptic cleft. The ion channel on the receptor becomes insensitive to acetylcholine in attempt to protect the muscle from being overly activated.



Down Regulation: the number of receptors decreases (by proteolysis) upon exposure to a high concentration of agonist for a very long time (hours), making the cell less responsive to the same agonist or others that work at the same receptor.

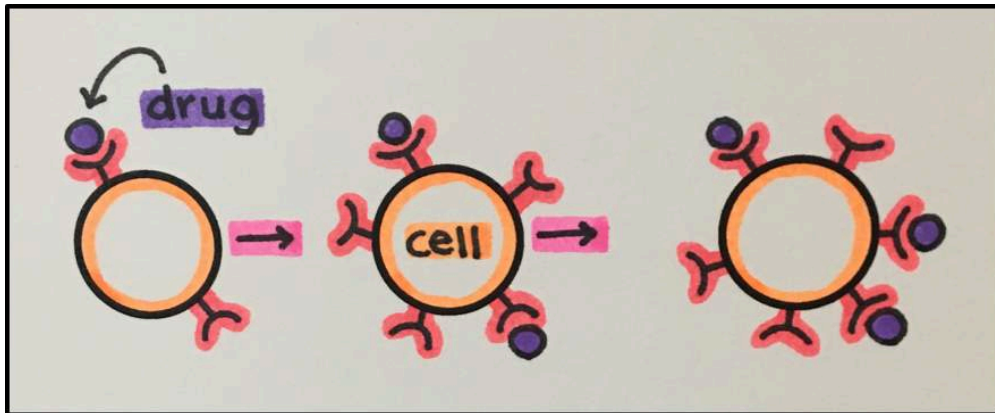


What is an example of downregulation?

Type 2 Diabetes: chronic increased levels of glucose leads to increased production of insulin. Over time, insulin receptors downregulate making the individual less sensitive to insulin.

Reduced Receptor stimulation: can result in receptor supersensitivity and upregulation.

Supersensitivity and Upregulation: occurs when a cell is deprived of its natural ligand for a long time, resulting in increased receptor expression over time and making the cell more sensitive to its ligand



What is an example of supersensitivity?

Reserpine depletes stores of catecholamines (e.g. norepinephrine) in neurons. Catecholamine receptors compensate by becoming more abundant and therefore more responsive to exogenous catecholamine-like drugs.

8. Two Main Classes of Receptor Ligands in Pharmacology: Agonists & Antagonists



Agonists: tend to be smaller molecules that effect activation of receptors.

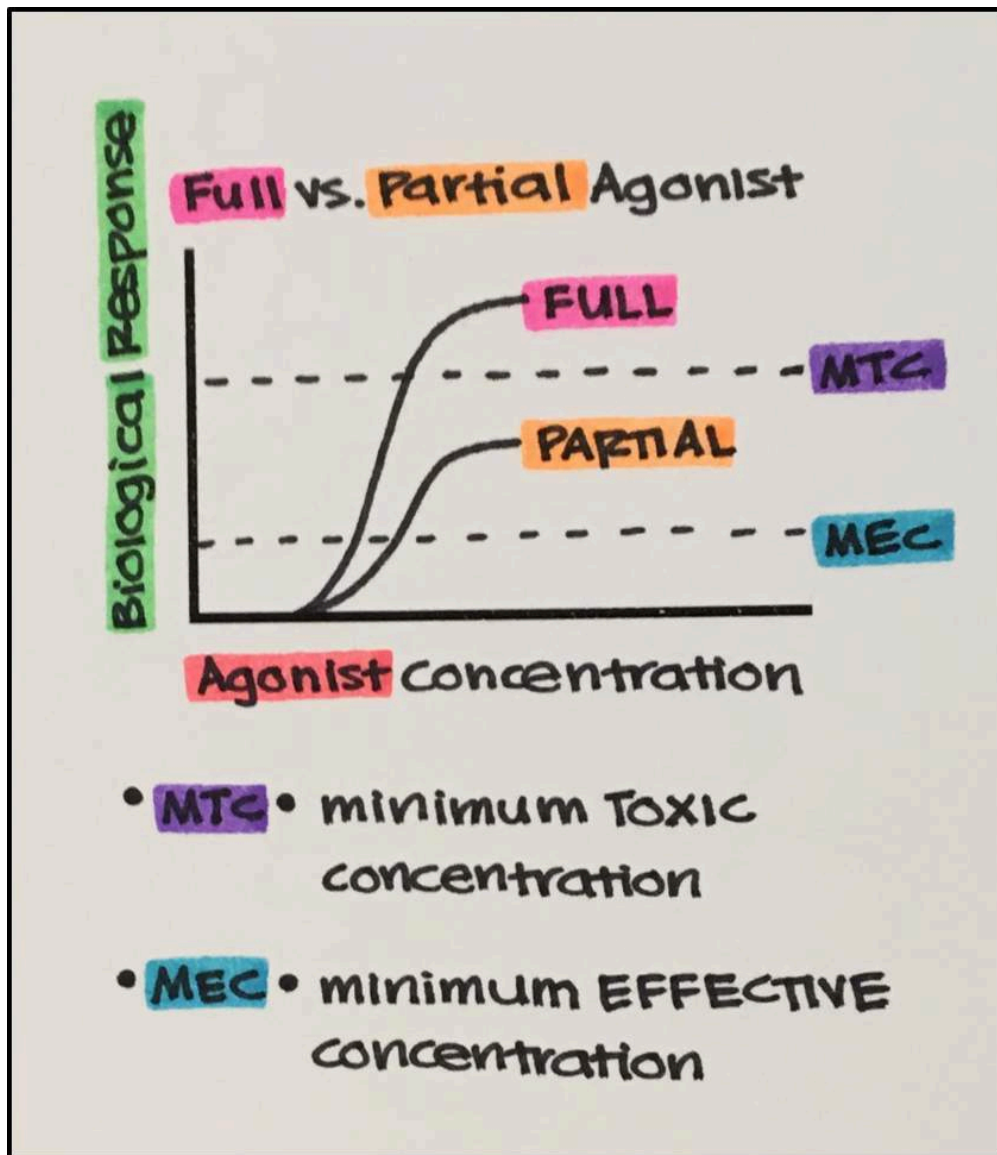
Note: This may result in stimulation or inhibition of cell and organ function

Full agonists: produces maximal biological response

- Has maximal **POSITIVE INTRINSIC** activity (**EFFICACY**)

Partial agonists: produces partial maximal biological response as compared to full agonist

- has lower **POSITIVE INTRINSIC** activity
- may **COMPETITIVELY INHIBIT** full agonist preventing maximal biological response
 - If the partial agonist is bound to the receptor, the full agonist cannot bind
- may have higher, lower or the same affinity (potency) for the receptor as the full agonist



Why use a partial agonist? Full agonist may cause too much activation resulting in toxicity or receptor adaptation on prolonged use (desensitization, downregulation). The lower efficacy of partial agonists minimizes these complications.

Inverse agonists: produces opposite biological response to that of the endogenous agonist/neurotransmitter

- has **NEGATIVE INTRINSIC** activity (**EFFICACY**)

Example: GABA^A receptor: agonists (benzodiazepines) produce a sedative effect whereas inverse agonists (i.e. Rho15-4513 — originally designed as alcohol antidote) produce anxiety-like effects.



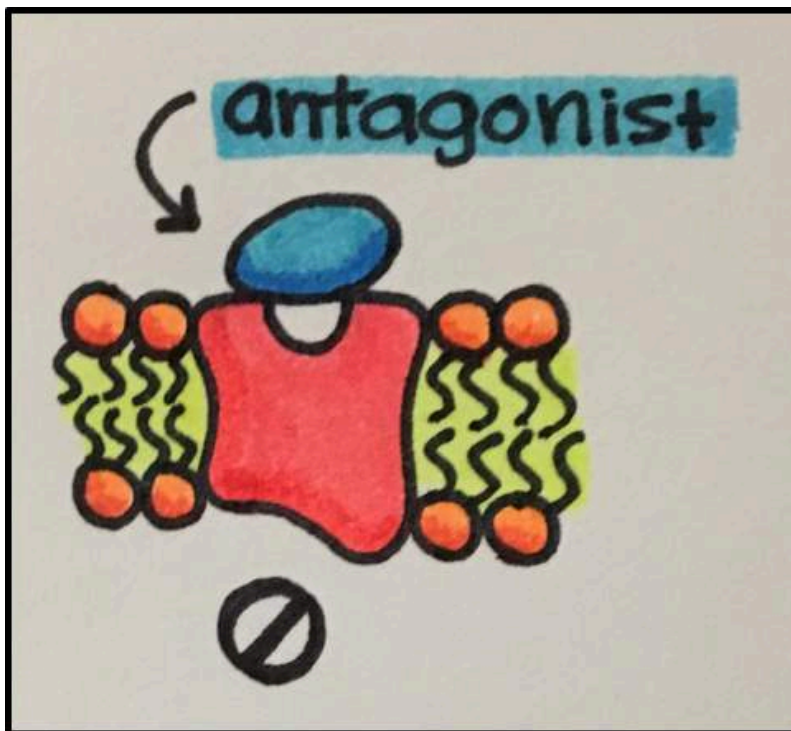
Antagonists: tend to be larger molecules producing **INHIBITORY** effect.

- there are antagonists that act at the receptor, also known as receptor antagonists,

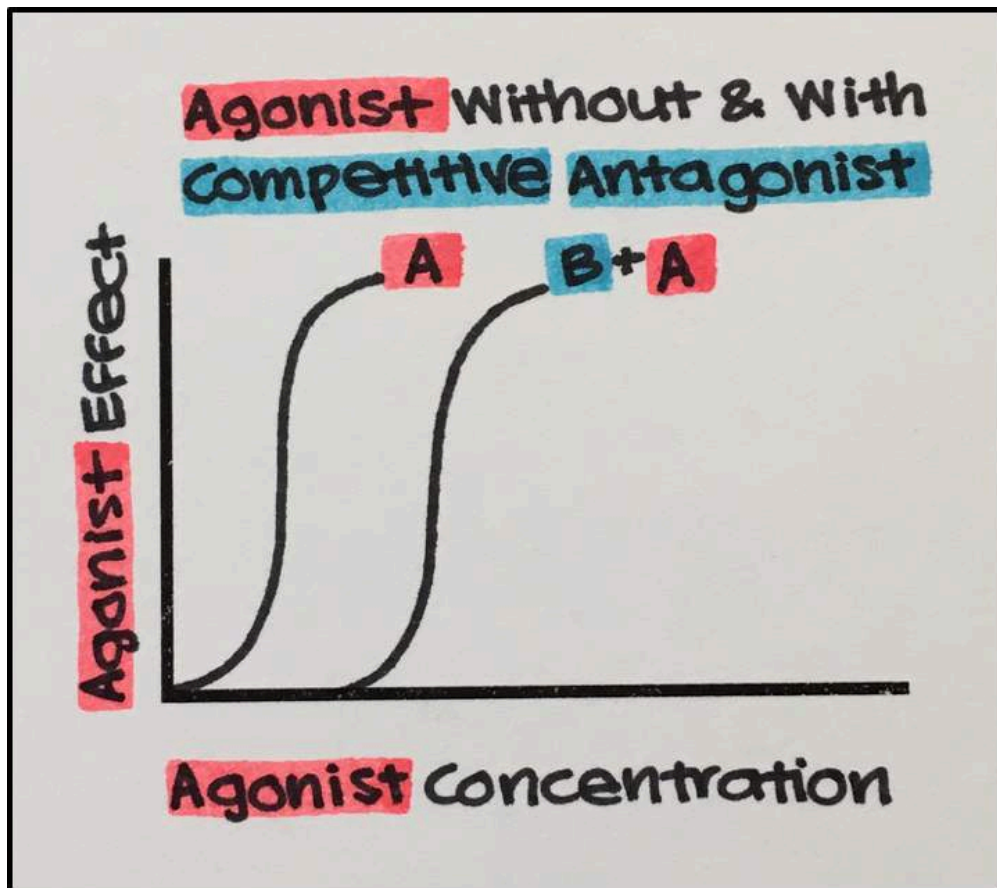
****Antagonists DO NOT have INTRINSIC ACTIVITY (EFFICACY):** reminder: antagonist simply block the agonist from binding. Furthermore, keep in mind that just because antagonists do not have intrinsic activity does not mean they do not have physiological consequences.

Example: While the beta-1 antagonist **DOES NOT** have **INTRINSIC** activity on its own, it still has pharmacological effects in vivo. Under normal circumstances, when a person is exercising norepinephrine is released binds to beta-1 receptors resulting in increased cardiac inotropy (contractility) as well as increased chronotropy (heart rate) to accommodate the increased demand for oxygen. If an individual were using a beta-1 antagonist (a.k.a. beta blocker), it would prevent norepinephrine from binding to beta-1 receptors thus causing reduced contractility and lower heart rate compared to someone who is not using a beta-1 antagonist. Labetalol is an example of a beta-1 antagonist used in individuals who have angina (chest pain) associated with increased heart rate. This drug enables them to exercise, while maintaining a lower heart rate so as to avoid chest pain.

Competitive Antagonist/Inhibitor: REVERSIBLE or SURMMOUNTABLE



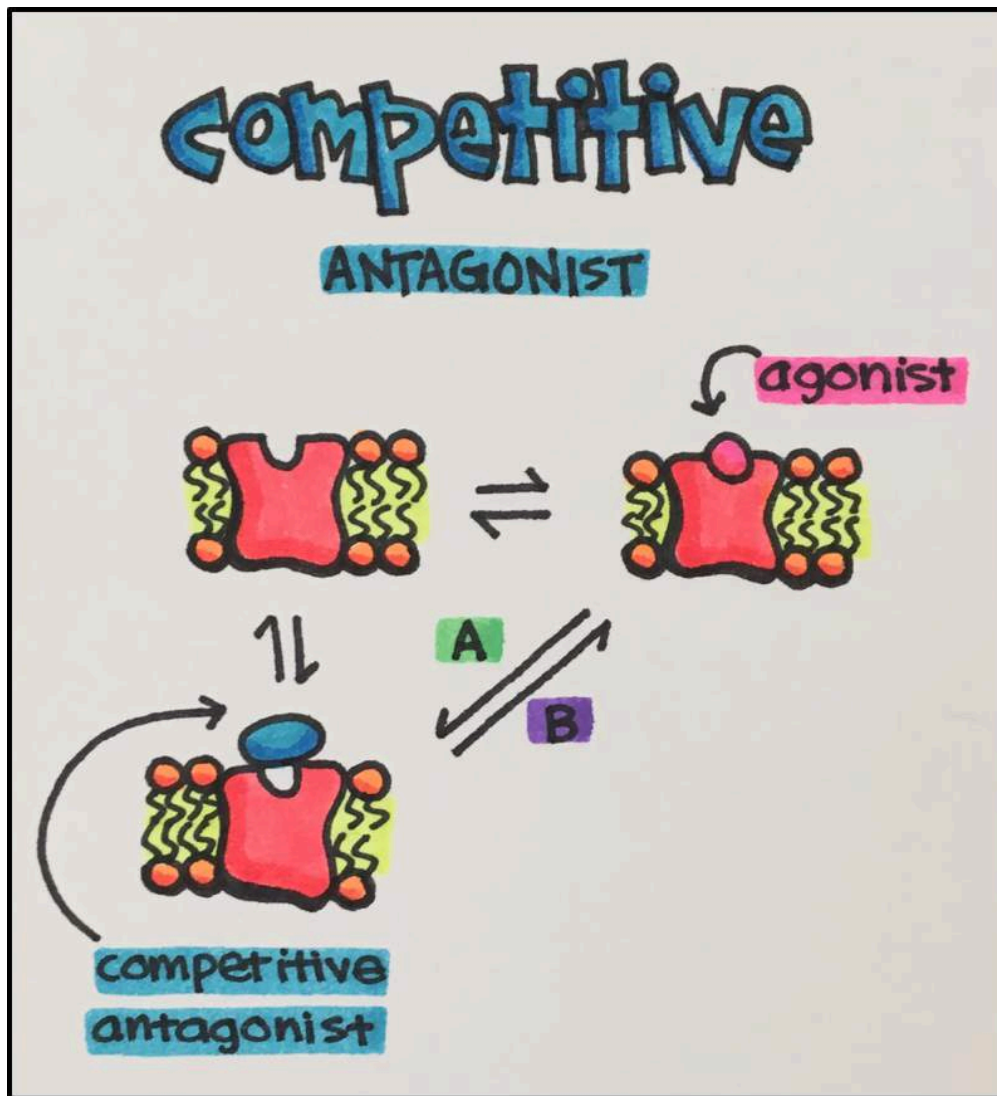
- Binds to a receptor at the same site as an endogenous or pharmacological agonist, blocking agonist binding and therefore receptor activation.



- Antagonism is reversed by increasing the amount/dose of agonist (surmountable). While competitive antagonists do not affect agonist efficacy (maximal response), they do decrease **AFFINITY** and **POTENCY**. Thus, a normal maximal response to the agonist may be attained in the presence of a competitive antagonist, albeit at higher agonist concentrations. Thus, agonist dose-response curve is therefore **SHIFTED TO THE RIGHT** (more agonist needed to produce the same response) in the presence of a competitive antagonist (REFER TO GRAPH).

In this graph:

- **A**: agonist
- **A + B**: agonist + competitive antagonist



The receptor can interact with the **agonist** OR the **competitive antagonist**, but not both, i.e. binding is mutually exclusive.

See above: When the agonist concentration/dose is gradually increased, the equilibrium shifts towards formation of agonist-receptor complex **[B]**. The opposite is true when the antagonist concentration is increased **[A]**.

- Competitive antagonists have an affinity (potency) for a receptor, however, do not have intrinsic activity: **REMINDER:** antagonists lack intrinsic activity

Non-Competitive Receptor Antagonists: UNSURMOUNTABLE

Two class:

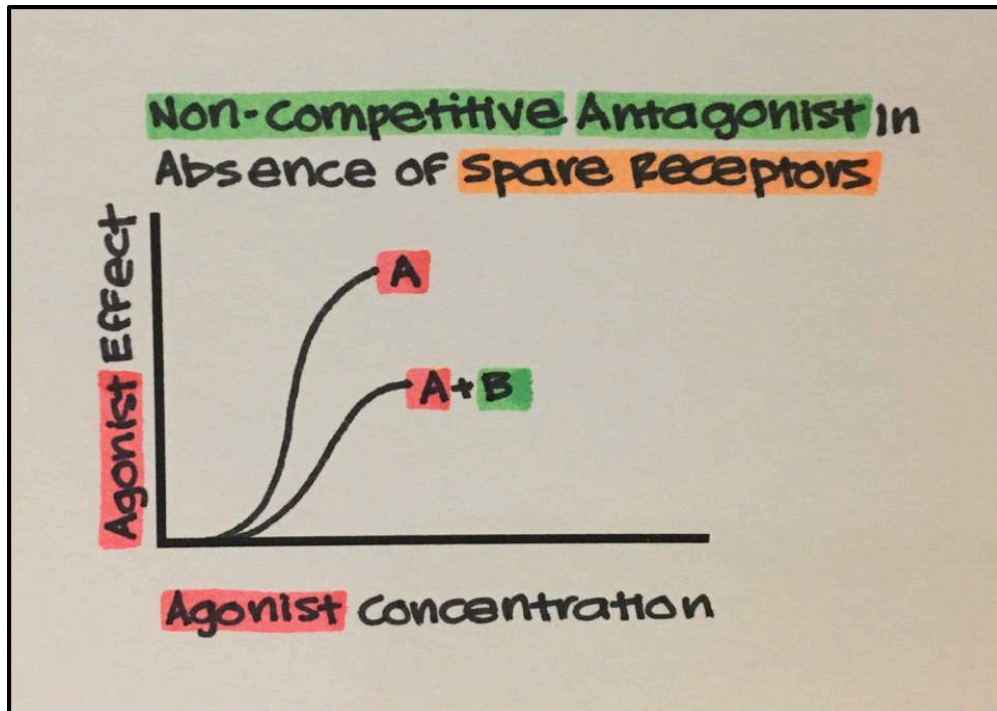
- IRREVERSIBLE
- ALLOSTERIC

A. Irreversible Non-Competitive Antagonists:

- Usually bind to the same site as the agonist, however, it is not readily displaced like

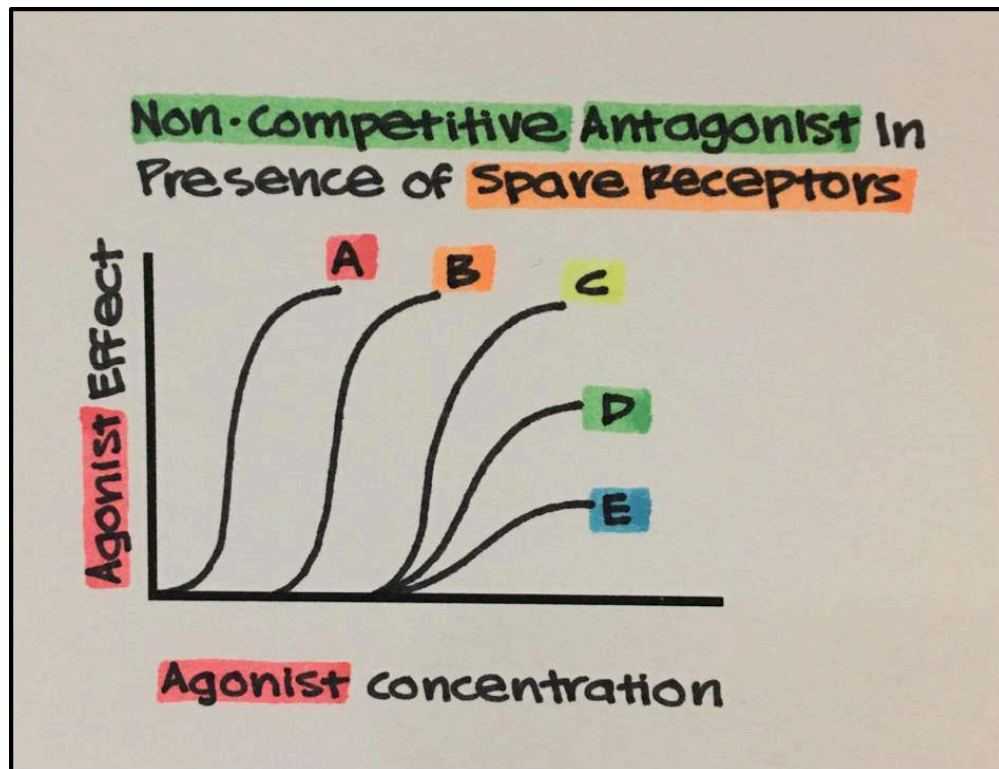
competitive antagonists.

- Generally irreversible due to the formation of a covalent bond between the antagonist and the receptor, in essence reducing the number of receptors available to the agonist.
- **In the ABSENCE of SPARE RECEPTORS:** The agonist dose-response curve will exhibit a lower maximum that is proportional to the dose of the non-competitive antagonist.



- **See above:** Unlike competitive antagonists, non-competitive antagonists reduce the actual number of receptors available to be activated, therefore, **EFFICACY** is **REDUCED**. The agonist exhibits a lower maximum response in the presence of the non-competitive inhibitor.
 - A agonist
 - **A + B** agonist with irreversible non-competitive antagonist

In the presence of spare receptors: Agonist dose-response curve will exhibit unaltered maximal response with increasing doses of the irreversible antagonist, until all spare receptors are exhausted. At this point, higher doses of the antagonist will cause dose-dependent reduction in the maximal response (reduced efficacy).



- See above: **A**, agonist alone exhibits maximal response (efficacy) and affinity (potency) for the receptor. **B**, agonist plus non-competitive antagonist: the agonist still exhibits maximal response because there are spare receptors, however, its affinity for the receptor is reduced. It is not until **D**, agonist plus higher concentration of non-competitive antagonist, that the agonist starts to exhibit a lower maximal response as all spare receptors have been eliminated by the irreversible antagonist.

B. Allosteric Non-Competitive Antagonists: A drug may bind to a site (allosteric site) on the receptor different from that where an agonist binds (primary or classical binding site). Binding to the allosteric site modifies the conformation of the primary site. In case of (negative) allosteric antagonists the altered conformation is less responsive to the agonist. Increasing agonist concentration does not displace the allosteric antagonist from the receptors since the two drugs bind to different sites, i.e. this type of antagonism is non-competitive and unsurmountable. See other types of allosteric modulators below.

Physiological Antagonist

- Molecules that do **NOT** bind the same receptor as the endogenous or a pharmacological agonist, but produce an effect that is opposite to the agonist effect.

Example: epinephrine is a physiological antagonist to histamine, even though they bind to their own specific receptors. When histamine binds to its receptor, arterial pressure decreases through vasodilation. However, when epinephrine binds to its receptor, arterial pressure increases through vasoconstriction, thus counteracting histamine or producing an antihistamine effect.

Chemical Antagonists:

- Drugs that do not interact with the agonist receptor but rather reduce the concentration of an agonist by forming a chemical complex; also known as chelating agents.

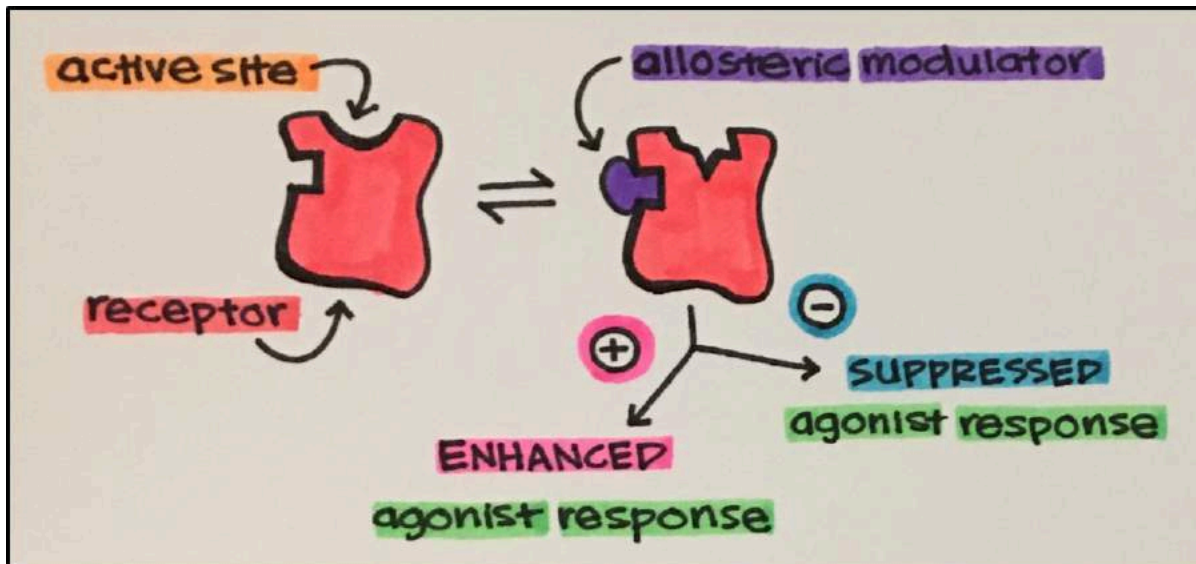
Example: Phosphate binders are used to prevent hyperphosphatemia in patients who have chronic kidney disease. Phosphate binders can act as a chemical antagonist with a number of medications including quinolone. Phosphate binders form a complex with quinolone reducing its effectiveness. Patients should be counseled to take a phosphate binder 3 hours before or after taking quinolone.

9. Receptor Allosteric Modulators

Allosteric modulators interact with sites on the receptor different from the primary agonist binding site. This results in modulation of the conformation of the agonist binding site to impart higher (allosteric enhancers) or lower (allosteric inhibitors) affinity for the agonist. Some allosteric modulators are able to induce an active conformation of the receptor primary binding site to result in receptor activation in the absence of an agonist.

Three Classes of Allosteric Modulators:

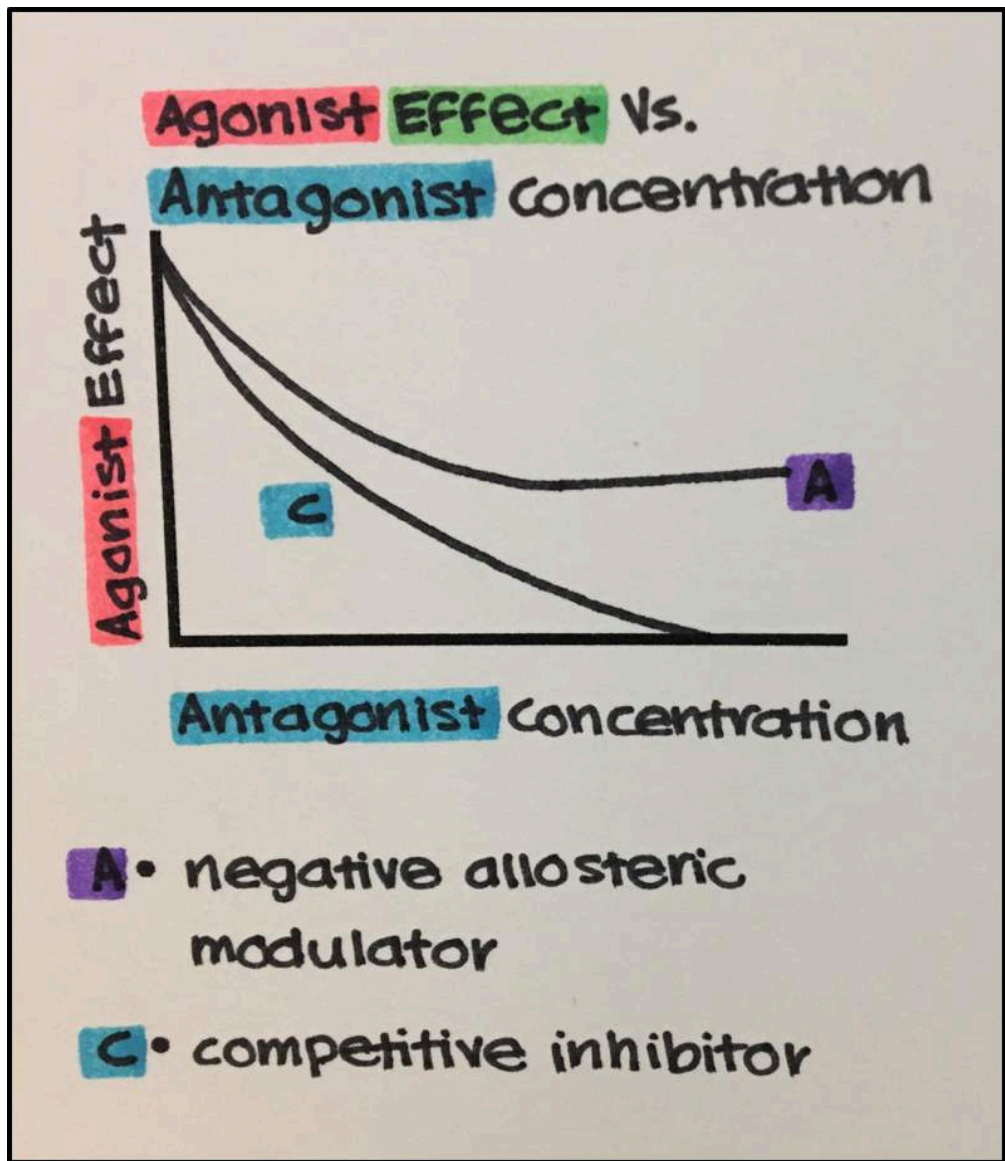
1. **Allosteric Agonists:** agonist by itself in absence of any other agonist
2. **Allosteric Enhancer (Agonist):** enhances (potentiates) agonist biological response
3. **Allosteric Inhibitors (Antagonist):** inhibits agonist biological response



As you can see in this image, allosteric modulators bind to an area/domain on the receptor that is topographically **DISTINCT** from the **PRIMARY AGONIST BINDING SITE**. Binding to this area results in modifications to the conformation of the primary site to either make it more or less favorable to bind the agonist (increase or decrease agonist affinity, respectively). This causes enhancement or dampening of agonist response.

10. Competitive Antagonist vs. Negative Allosteric Modulator

A competitive antagonist directly and physically blocks access of the agonist to the receptor, whereas a negative allosteric modulator indirectly changes agonist binding by interacting at a secondary site on the receptor to diminish the ability of the agonist to bind to the primary site. While a competitive antagonist shifts the dose-response curve of an agonist indefinitely by increasing antagonist dose, the shift caused by an allosteric antagonist (negative allosteric modulator) reaches a limit. This limit is dictated by the cooperativity factor that represents the maximal magnitude of interaction between the allosteric and primary binding sites. This limit makes negative allosteric modulators safer and more amenable to producing fine tuning of receptor function. This ceiling effect also ensures achieving the same response to a maximal dose of the antagonist regardless of different blood concentrations, e.g. due to differences in metabolism in one patient versus another. Another advantage of negative allosteric modulators is their higher organ selectivity as compared with competitive antagonists. This is because of uniqueness of the chemical nature of the allosteric site on the same receptor expressed in different tissues and organs. This is in contrast to the well conserved nature of the primary binding site. This results in better selectivity and less side effects of allosteric modulators.



This image above demonstrates the **ceiling effect** of a negative allosteric modulator/antagonist. The antagonist reduces the agonist response in a dose-dependent manner. However, this antagonism reaches a plateau even at higher doses. This is in contrast to complete inhibition of the agonist response by a competitive antagonist.

Why does this matter? There is need to reduce certain biological responses without completely shutting them down. For example, a person who has gastrointestinal spasms needs a modulator that returns the hyperactive state of gastric and intestinal muscles to normal, without causing complete paralysis of the gastrointestinal tract.

To summarize, **ADVANTAGES** of **negative allosteric modulators** over competitive antagonists include:

Ceiling Effect: can only inhibit agonist response to a certain point, protecting patient from excessive inhibition that could have serious consequences.

Fine-Tune ability: allosteric modulators have greater potential for receptor subtype selectivity than do competitive antagonists, and this minimizes side effects.

11. Types of Drug-Drug Interactions

Additivity: when the effect of two drugs given in combination equals the mathematical summation of their effects when given alone.

Synergism: when the combine effect of two drugs is greater than the sum of their effects when given separately.

Potentiation: when one drug does not elicit a response on its own but enhances the response to another drug.

Examples of Drug-Drug Interactions: given responses to 4 drugs

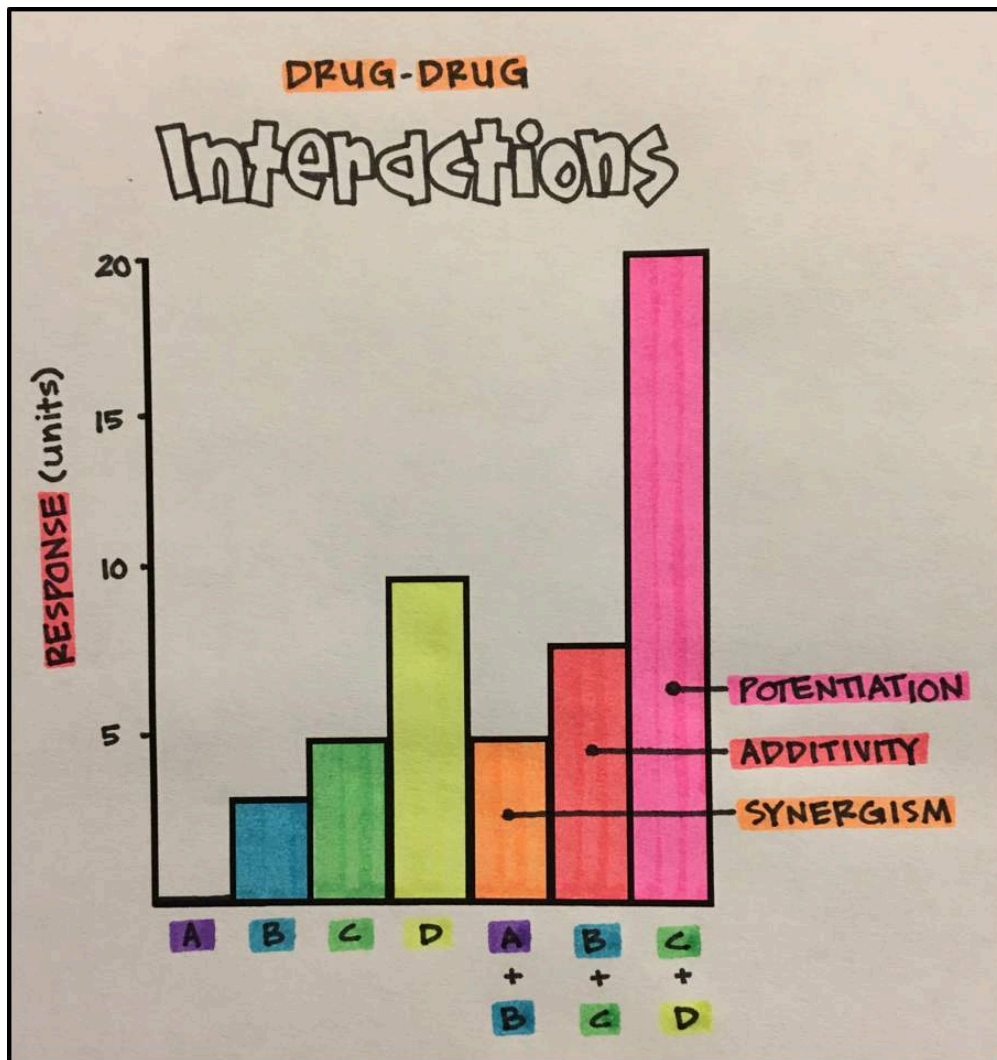
- **Drug A = 0 units response**
- Drug B = 3 units response
- Drug C = 5 units response
- Drug D = 10 units response

See Below

Drug A + **Drug B** = 5 units >> **potentiation**

Drug B + **Drug C** = 8 unites >> **additivity**

Drug C + **Drug D** = 20 units >> **synergism**



12. Introduction to Signal Transduction

Signal Transduction • transmission of molecular signals from outside the cell into the cell via cell-surface receptors. Signal transmission is caused either by:

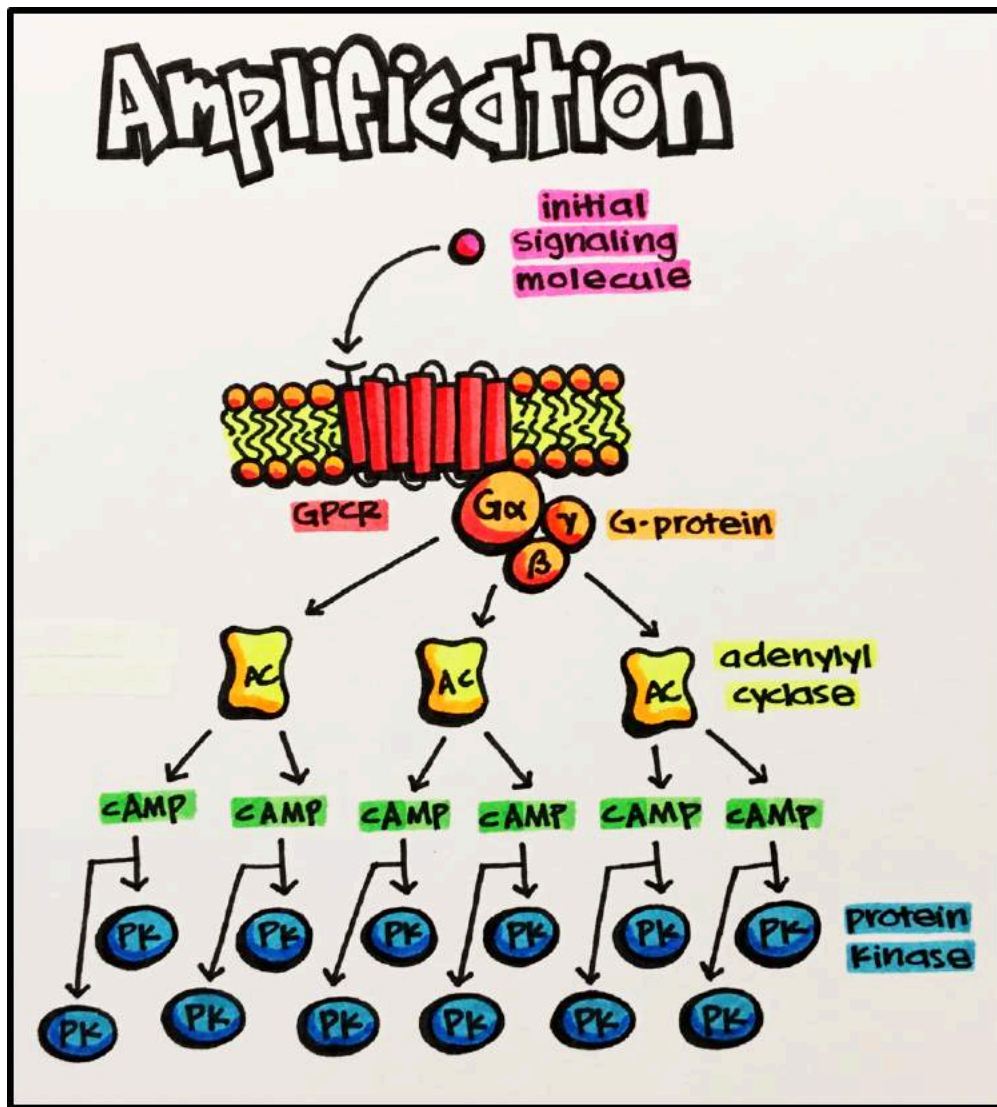
- a cascade of events or biochemical changes within the cell
 - o receptors that initiate biochemical changes accomplish this either by intrinsic enzymatic activities (within the receptor itself) or by activating intracellular messenger molecules
- modification of the cell membrane potential initiated by the movement of ions into or out of the cell

Why is this important? Identifying the cellular events that take place upon receptor activation is necessary for designing pharmacological agents that potentiate (increase) or diminish (decrease) signaling. For example, certain signaling pathways become overly active in cancerous cells. Suppression of these exaggerated signals may be one way to reduce cell proliferation in cancer.

Purpose of Signal Transduction

- **Signal amplification** • increasing a signal so that minimal receptor occupation by small amounts of neurotransmitters in the synapse produces significant cellular responses.

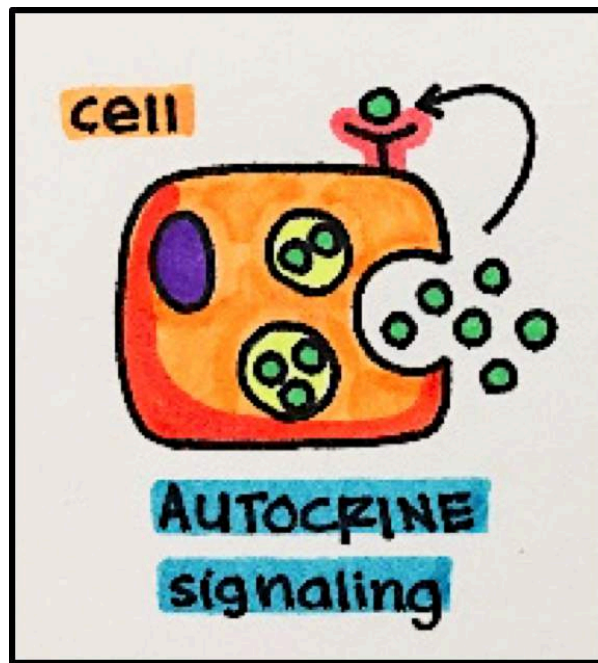
<https://highered.mheducation.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120069/bio08.swf::Signal%20Amplification>



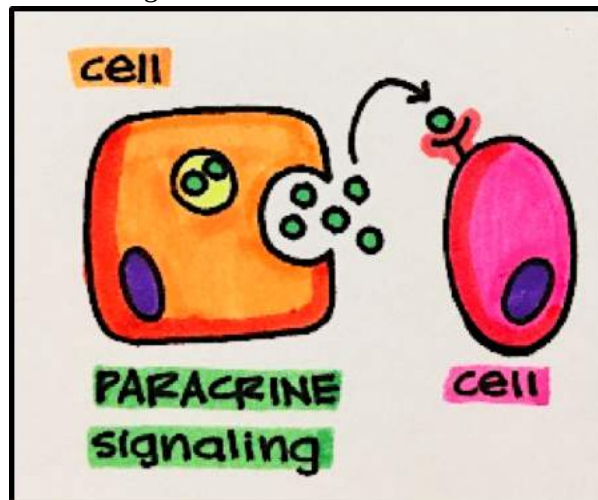
- **Signal dampening** • decreasing an abnormally high signal to maintain physiological homeostasis. An example is how receptors on the presynaptic nerve ending respond to high levels of a neurotransmitter in the synapse by decreasing further neurotransmitter release (auto receptors)
 - **Feedback inhibition:**
Output of signal transduction is used as a signal to decrease the signaling process itself (which will usually limit the production of intracellular messengers)

Types of Signal Transduction

- **Autocrine**
- **Paracrine**
- **Endocrine**

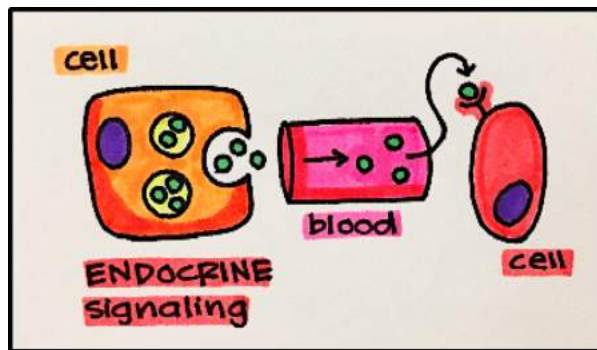


Autocrine • cell secretes chemical messenger or hormone that will then activate receptors on that same cell.



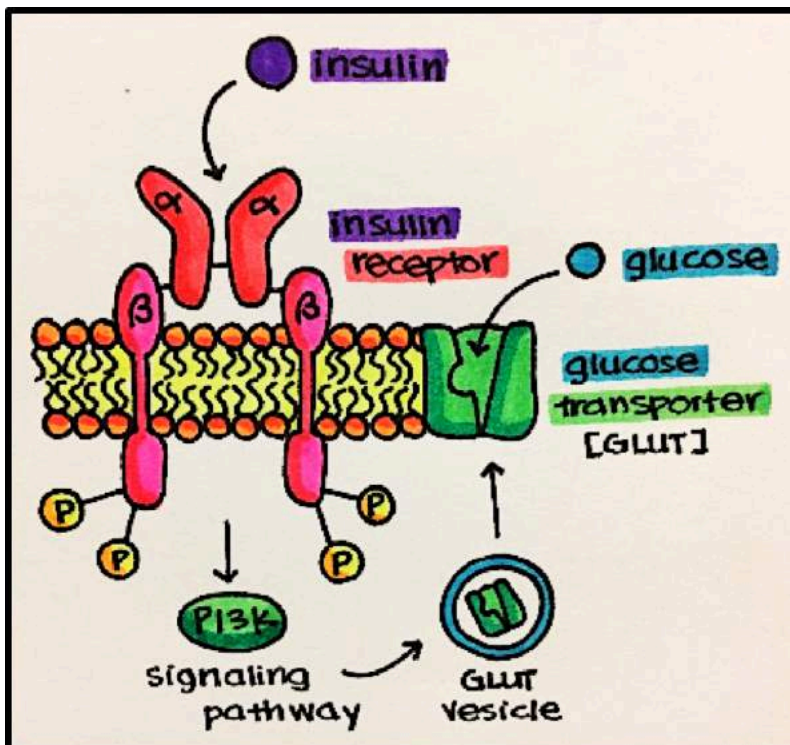
Paracrine • cell secretes chemical messenger or hormone that will then activate cell-surface receptors on a cell nearby.

- A good example is synaptic transmission, which is how neurons communicate through neurotransmitters.



Endocrine • cell secretes chemical messenger or hormone that needs to be transported via the blood stream to reach a distant target cell.

- **Neuroendocrine** cells receive neuronal input (neurotransmitters released by nerve cells or neurosecretory cells) and, as a consequence of this input, release message molecules (hormones) to the bloodstream.



Example • insulin is synthesized and secreted by pancreatic beta cells, released into the blood and transported to cells all over the body. Insulin then stimulates its receptors on these cells to initiate a cascade of events leading to expression of glucose transporters to allow cells to take glucose in for energy utilization.

Signal Transduction Pathways are not always linear.

- **Convergence** • when signals from a variety of unrelated receptors can **converge** and activate common effectors.
- **Divergence** • when a signal from a single receptor can activate multiple effectors.

- **Crosstalk** • when different signals increase or decrease the production of other signals

Effector • The eventual target of signaling. For example, the heart is an effector of norepinephrine released from nerve terminals.

Types of Signal Transducing Messengers

1. **First Messengers** • agonists (i.e. hormones, neurotransmitters, pharmacological agonists)
2. **Second Messengers** • molecules that transmit signals received at receptors (i.e., cAMP, cGMP, DNA binding, ions)
3. **Third Messengers** • (i.e., ions, protein kinases)

Types of Receptors

1. **Carriers** (Transporters)
2. **Signal Transduction Receptors**
 1. Enzyme-linked
 2. G-protein-coupled
 3. Ligand-gated channels
 4. Intracellular
3. **Enzymes**

Receptors Responsible for Signal Transduction

There are 4 Classes of Signal Transducing Receptors:

1. **Enzyme-Linked Receptors** (Receptor Kinases) • have intrinsic enzymatic activity or are associated with an intracellular enzyme
2. **GPCRs** (G-Protein-Coupled Receptors) • couple to GTP binding proteins (G-proteins) inside the cell to activate them. Active G proteins modulate the activity of various ion channels and enzymes.
3. **Ligand-Gated Ion Channels** • allow specific ions to flow into or out of the cell in response to binding of a chemical messenger
4. **Intracellular Receptors** (Cytoplasmic & Nuclear Receptors) • activation of these receptors located within a cell results in directly altered gene transcription

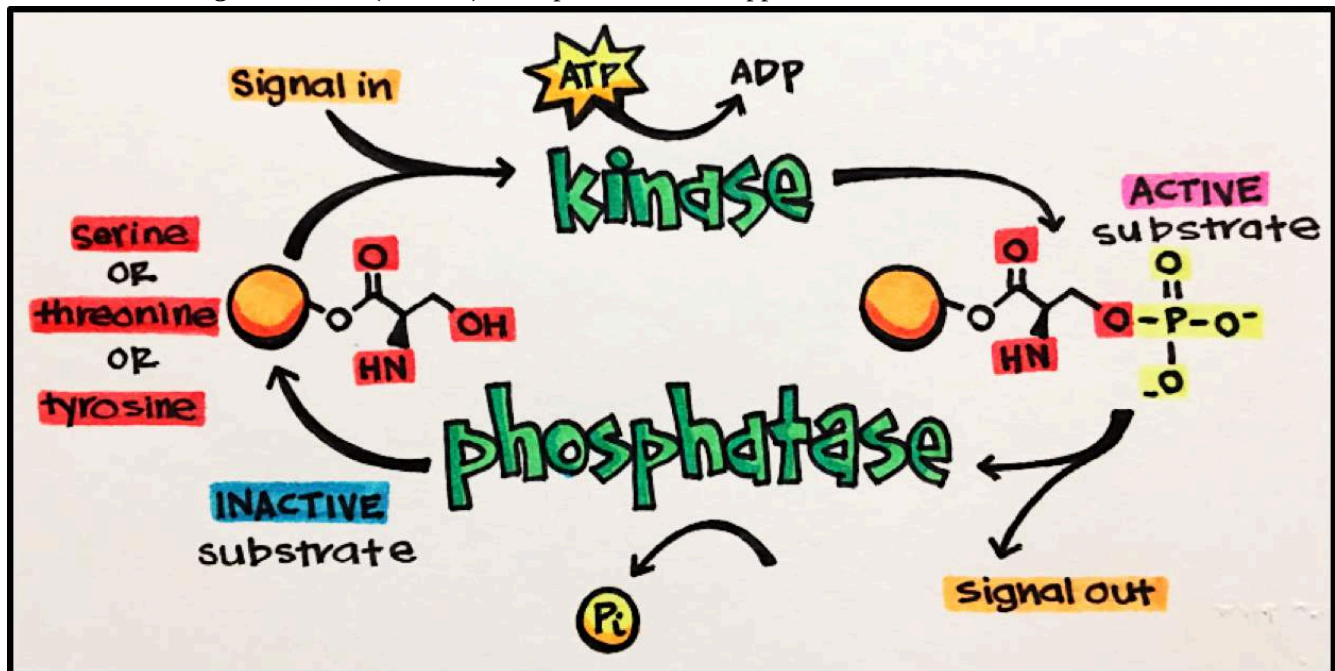
13. Enzyme-Linked Receptors

Enzyme-Linked Receptors • have intrinsic enzymatic activity or are associated with an enzyme (usually a kinase) • play a role in apoptosis, cell differentiation, cell division, cell growth, immune response, inflammation, and tissue repair.

Kinases (Protein Kinases [PKs]) • enzymes that catalyze the phosphorylation of target molecules to cause their activation. In other words, they add a phosphate group to a molecule/protein/another kinase.

- **Protein Kinase Inhibitors** (PKIs): stop kinase activity, important class of molecules used for cancer treatment

Phosphatases • enzymes that catalyze the dephosphorylation of target molecules. This dephosphorylation usually inactivates the target molecule (effector). Phosphatases act in opposition to kinases.

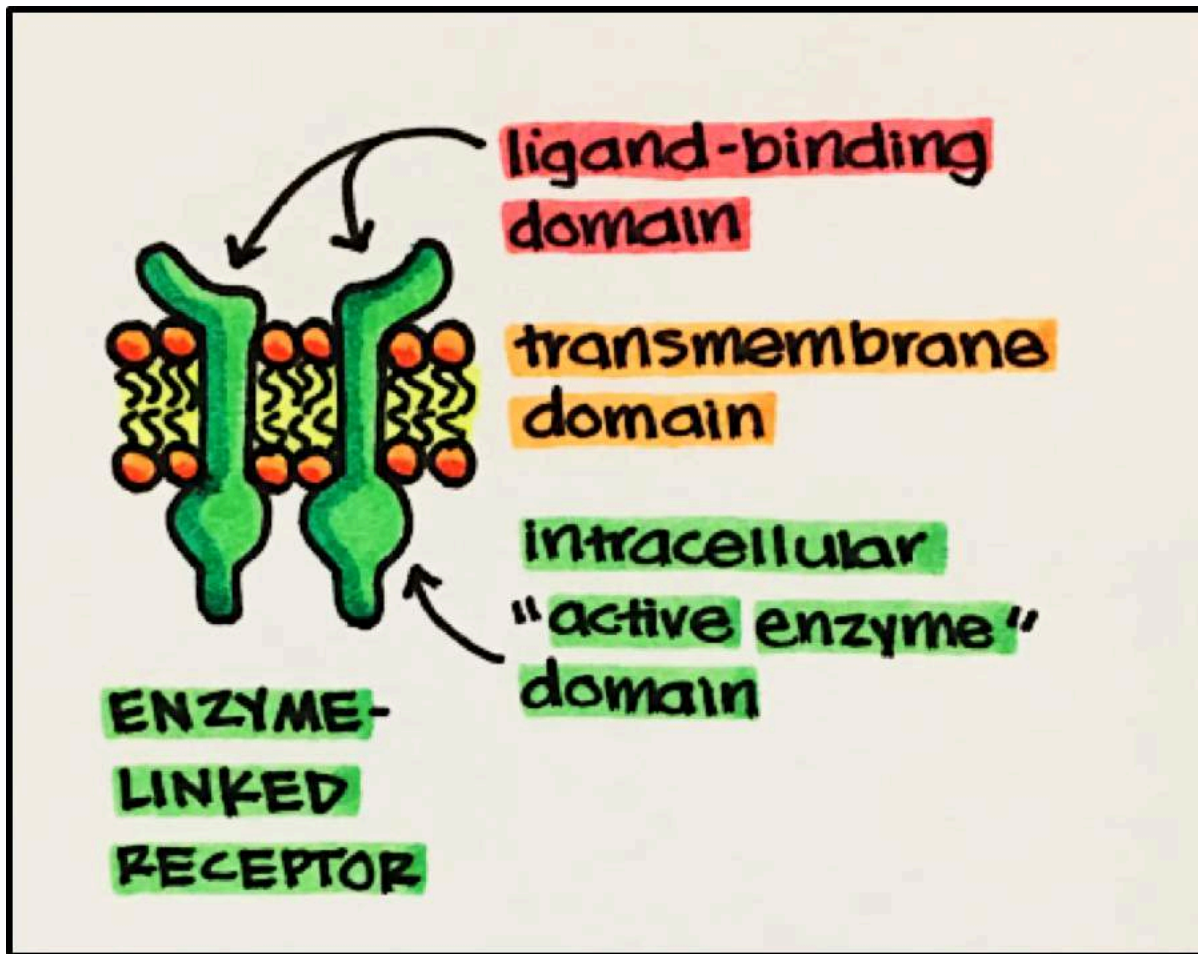


It is the balanced activity of kinases and phosphatases that results in effective, and fast, signaling events to allow for the desired biological effect and enable our bodies to maintain homeostasis.

Enzyme Linked Receptor Structure • composed of three key domains

- **Ligand-Binding Domain** • often has a large **EXTRACELLULAR** ligand-binding domain allowing for easy access and activation to the receptor.
- **Transmembrane Domain** • composed of a series of hydrophobic amino acids (reminder: inner membrane is lipophilic/hydrophobic) that tethers the receptor to the cell membrane.
- **Intracellular “Active Enzyme” Domain** • either intrinsic to the receptor or

tightly bound to the transmembrane domain. The majority of the “active enzyme” domains are kinases that phosphorylate the amino acids serine, threonine and tyrosine of proteins.

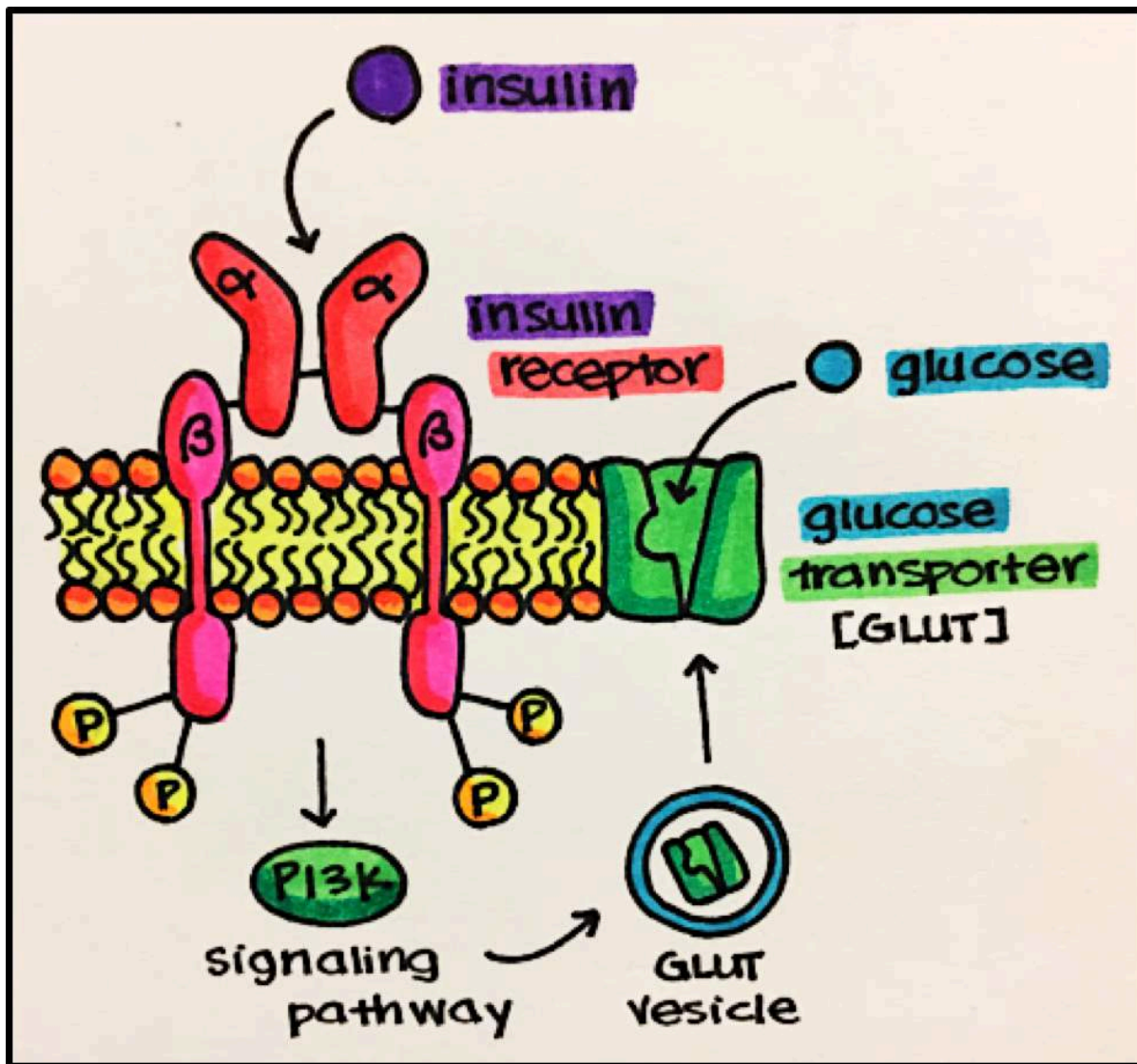


Example • Insulin Receptor • ligand is insulin, which stimulates carbohydrate (glucose) utilization and protein synthesis.

Ligand-binding domain = alpha domain

Transmembrane domain = runs through the membrane anchoring it to the membrane

“Active enzyme” domain = tyrosine kinase domain (intracellular component) activated by phosphorylation



Once activated, the insulin receptor leads to a cascade of events eventually resulting in expression of glucose transporters (GLUTs) on the surface of a cell to allow it to bring in glucose for energy utilization.

Signal Transduction by Enzyme-Linked Receptors

1. Ligand binding leads to dimerization of two neighboring receptors.
 2. Neighboring dimerized receptors auto phosphorylate one another
 3. SH2-domain proteins bind to the phosphorylated receptors and are then phosphorylated enabling the continuation of the signal eventually leading to gene transcription.
- **SH2-domain** [Scr homology 2] • protein domain composed of about 100 amino acid residues. SH2-domains most commonly play a role in the signal transduction by receptor tyrosine kinase pathways as you will see later on in this section. They interact with specific target molecules (peptides) with a phosphorylated tyrosine residue.

3 Main Types of Enzyme-Linked Receptors •

- **Receptor Tyrosine Kinases [RTK]** • make up the majority of enzyme-linked receptors. Signal transduction through RTK results in specific phosphorylation of **tyrosine** residues on target proteins and subsequent increase in gene transcription and regulation of cell growth, differentiation and survival.
- **Serine/Threonine Kinases** • specifically phosphorylate the hydroxyl side chains of **serine or threonine** amino acid residues. These kinases may have a role in cell proliferation, differentiation, apoptosis and even embryonic development.
- **Tyrosine Kinase-Associated Receptors** [“mixed”] • do not have a tyrosine kinase domain, rather act through cytoplasmic tyrosine kinases.

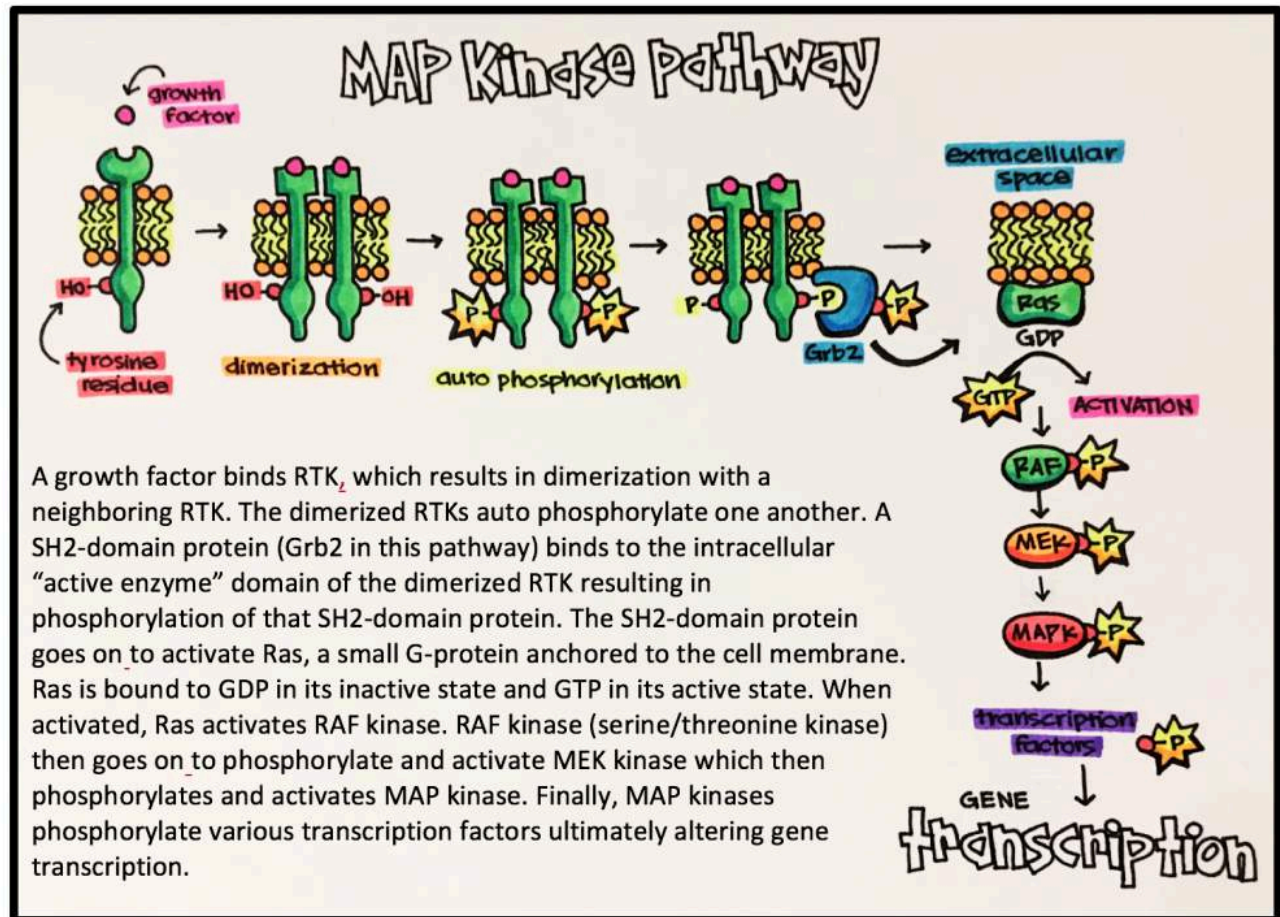
Receptor Tyrosine Kinases [RTK] • This class of receptors are also considered enzymes that have intrinsic enzymatic activity. When RTK agonists bind to these receptors, their intrinsic enzymatic activity is stimulated. RTKs bind growth factors to signal processes that result in the regulation of cell growth, differentiation and survival through gene transcription. Subclasses include:

- **Epidermal Growth Factor Receptors [EGFR, ErbB1, HER1]** • This RTK subclass is activated by epidermal growth factor resulting in cell division that leads to cell growth, proliferation and differentiation. These receptors are found in abnormally high levels on the surface of a number of types of cancer cells allowing these cells to multiply excessively.
- **Nerve Growth Factor Receptors [NGF]** • This RTK subclass is activated by nerve growth factors, more specifically neurotrophins; a family of proteins involved with development, survival and function of neurons.
- **Insulin Receptors** • This RTK subclass is activated by insulin resulting in expression of glucose transporters [GLUT] and allow cells to accumulate glucose from the blood.
- **Toll Like Receptors [TLRs]** • This RTK subclass is activated by pathogen-derived molecules allowing the body to detect unwanted pathogens early on as well as sense “danger” signals leading to the eventual destruction of those pathogens.

When RTKs are activated by an agonist, they form cross-linked dimers resulting in the activation of the tyrosine kinase by phosphorylation. Remember, kinases specifically act to catalyze the phosphorylation of a target molecule, which in this case is a neighboring RTK. The dimerized RTKs phosphorylate each other multiple times to result in signal amplification. This process is known as cross-phosphorylation. RTK cross-phosphorylation then leads to the phosphorylation of other proteins that will eventually result in modulation of gene transcription.

Key Pathway • Ras/RAF/Mitogen-Activated Protein [MAP] Kinase Pathway

Key Pathway • Ras/RAF/Mitogen-Activated Protein [MAP] Kinase Pathway



Ras = G-protein specific to this pathway

RAF = proto-oncogene serine/threonine kinase

MEK = mitogen-activated protein kinase

MAPK = mitogen-activated protein kinase

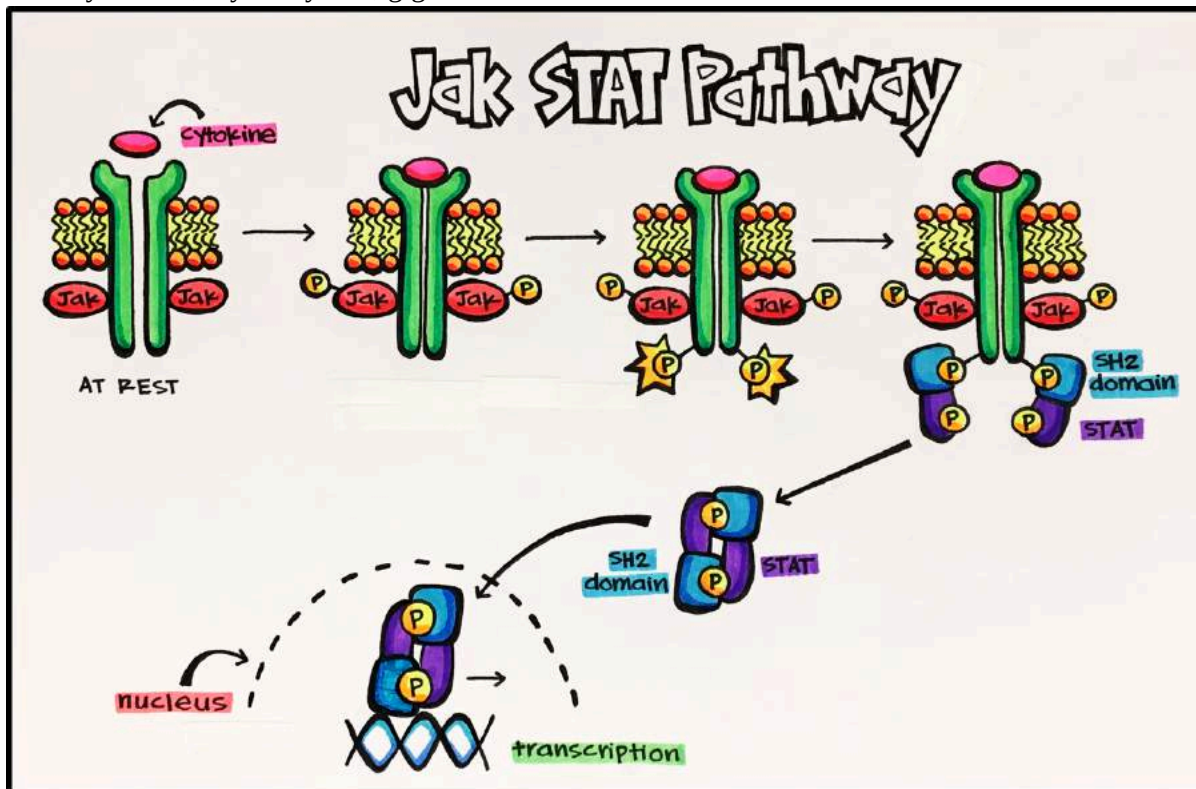
Ras • a small G protein (GTPase) involved in signal transduction leading to cell division and proliferation. If not regulated properly, Ras proteins can lead to uncontrolled cell division that eventually results in tumor formation.

RAF [Rapidly Accelerated Fibrosarcoma] • family of protein kinases that are involved with retroviral oncogenes (genes that can potentially cause cancer).

Tyrosine-Kinase Associated Receptors • associate with intracellular proteins that have tyrosine kinase activity. These receptors lack the tyrosine kinase domain that was discussed earlier and, therefore, accomplish tyrosine phosphorylation by cytoplasmic tyrosine kinases instead.

Cytokine receptors make up the largest family of receptors that relay signals into the cell by cytoplasmic tyrosine kinases. These particular receptors are associated with the cytoplasmic kinase, Jak (Janus kinase). Jak will go on to activate a gene regulatory protein called STAT (signal transducers and activators of transcription). The pathway is described and depicted below:

Key Pathway • Jak/Stat pathway (Janus kinase/signal transducers and activators of transcription) • The Jak/Stat pathway is the principal pathway for cytokines and growth factors in humans. This pathway is activated by a number of cytokines (most commonly interferons) and growth factors. Activation stimulates cell proliferation, differentiation, migration and apoptosis. Furthermore, cytokines control the synthesis and release of a number of inflammatory mediators. When a cytokine binds to its enzyme-linked receptor it results in a conformational change leading to phosphorylation of the intracellular active-enzyme domain, eventually leading to the transcription of inflammatory mediators. As with all signal transduction mechanisms, homeostasis is reliant on proper regulation of all these different pathways. Lack of proper regulation of the JAK pathway can cause inflammatory disease, erythrocytosis, gigantism and leukemias.

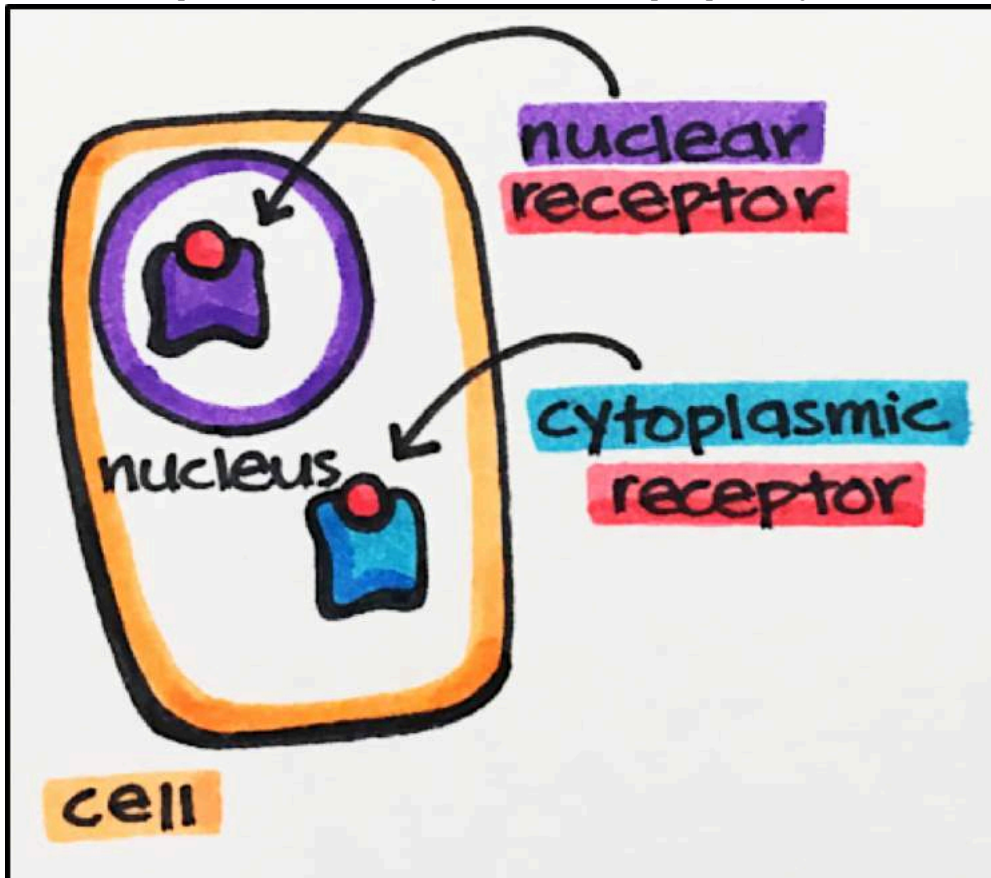


**For more information on enzyme-linked receptors, see this link below:

<https://www.ncbi.nlm.nih.gov/books/NBK26822/>

15. Nuclear Receptors

Nuclear receptors are receptors located inside the cell. These receptors are found either in the cytoplasm (Type I) or the nucleus (Type II) of a cell. Examples include: estrogen, glucocorticoids, thyroid hormone T3 or vitamins D and A. Receptor stimulation of any intracellular receptor primarily results in altered gene transcription.



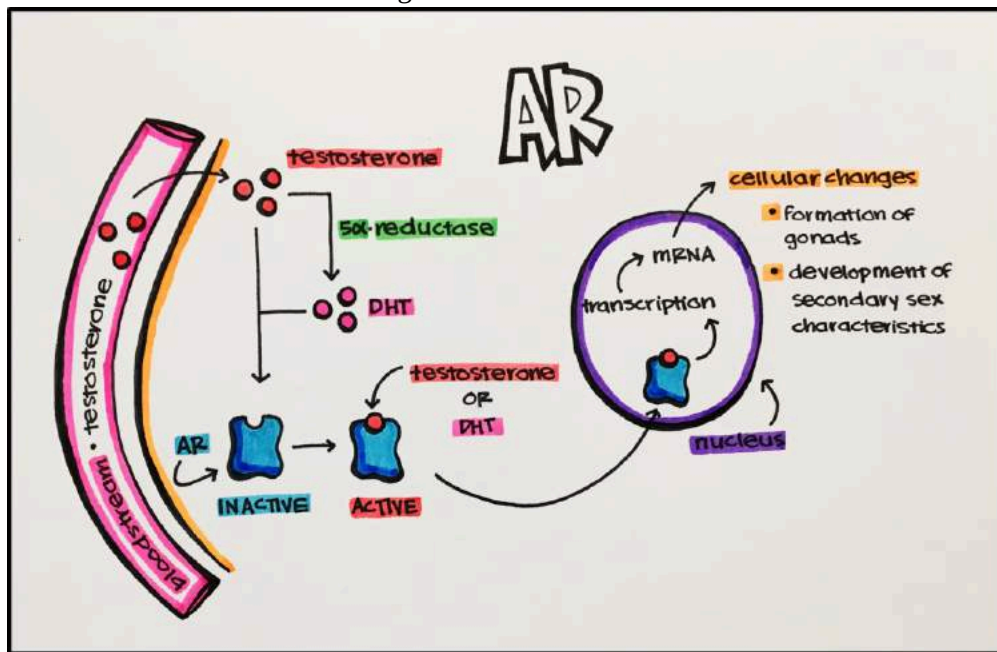
- **Type I** • intracellular receptors located in the cytoplasm of a cell. These receptors are translocated to the nucleus after stimulation by an agonist.
 - **Examples** androgen [AR], glucocorticoid [GR α], mineralocorticoid [MR], and progesterone receptors [PR].
- **Type II** • intracellular receptors located in the nucleus of a cell, even in the absence of agonists.
 - **Examples** • peroxisome proliferator [PPAR α, β, γ , sigma], retinoic acid [RAR α, β, γ], thyroid receptors [TR α, β].

Nuclear Receptors • a subtype (Type II) of intracellular receptors that directly alter gene transcription. Nuclear receptors [NR] are a superfamily of transcription factors. They are modulated by and bind small lipophilic molecules (partial or mixed agonists) with low affinity. Some NRs do not have a known ligand at this point in time and are therefore known as **Orphan Receptors**.

Structure • monomeric proteins with similar general structure

- N-terminal Domain • Activation function 1 [AF1] site binds other transcription factors to modify the binding or activity of the receptor itself. These transcription factors are specific to a given cell.
- Core Domain • It is at this domain that DNA recognition and binding of hormone response elements [HREs] occur. The domain is made of two zinc fingers (cysteine/histidine rich loops in the amino acid chain that are held in a particular conformation by zinc ions).
 - HREs • portion of gene regulated by NRs that also result in receptor dimerization.

There are a number of nuclear receptors that produce a wide range of physiological functions. These receptors and their resultant functions will be discussed throughout the curriculum.



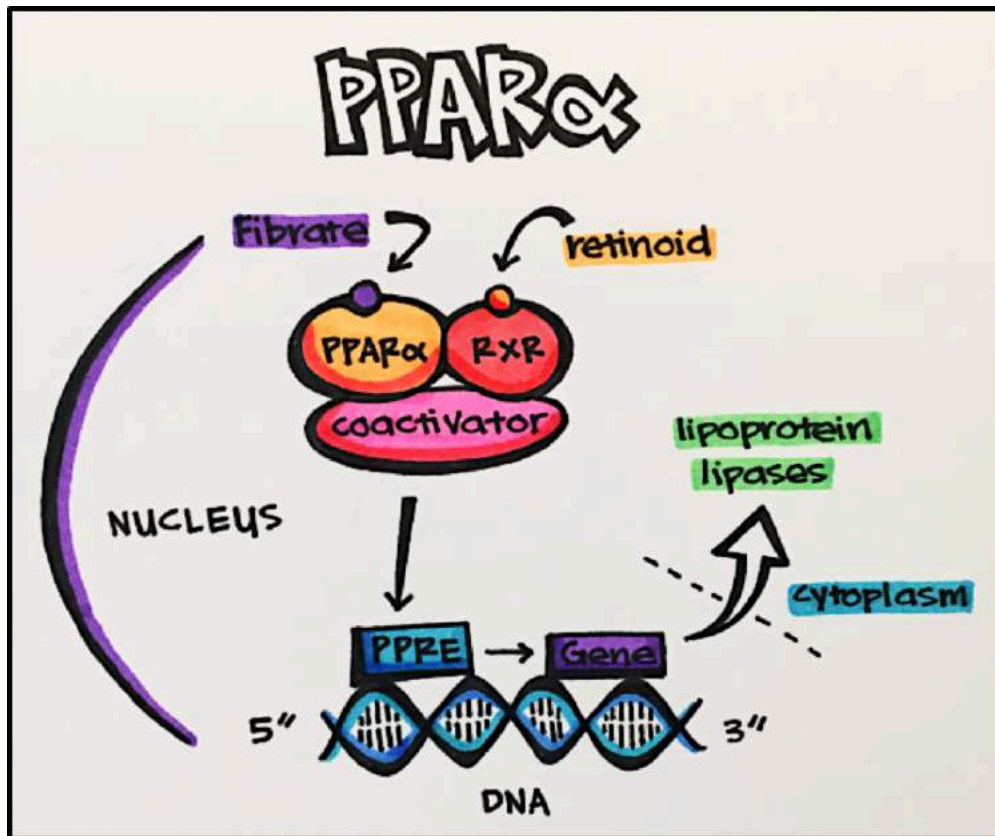
Type I Nuclear Receptors

- **Androgen Receptors [AR]** Endogenous AR agonists include testosterone and dihydrotestosterone [DHT]. These ligands bind AR in the cytosol of a cell and then translocate to the nucleus in order to alter transcription leading to protein synthesis, cell growth, formation of gonads, development of secondary sex characteristics and more.
- **Glucocorticoid Receptors [GR]** Cortisol is one of the most a common pharmaceutical targets as a endogenous GR agonist. Cortisol and other glucocorticoid hormones are stress hormones essential to maintaining homeostasis for a number of physiological processes such as metabolism, immune function, skeletal growth, cardiovascular function, reproduction and cognition. Upon binding, GR agonists can result in either activation or repression of transcription (depending on the particular isoform). For more details, see the link below: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4084612/>
- **Progesterone Receptors [PR]** Upon binding PR in the cytosol, progesterone results in activation of transcription affecting a number of biological processes

including the menstrual cycle.

Type II Nuclear Receptors

- Peroxisome Proliferator-Activated Receptor Alpha Receptors [PPAR].** Upon stimulation, these receptors lead to an increase in lipoprotein lipase (an enzyme that catalyzes the breakdown of lipoproteins) resulting in decreased hepatic triglyceride [TG] production. Fibrates, a drug class that targets these receptors, are PPARa agonists. Their primary action is to decrease TG blood levels, however, they also help increase HDL (“good fat”), lower LDL (“bad fat”), improving a patient’s lipid profile and providing improved cardiovascular protection.

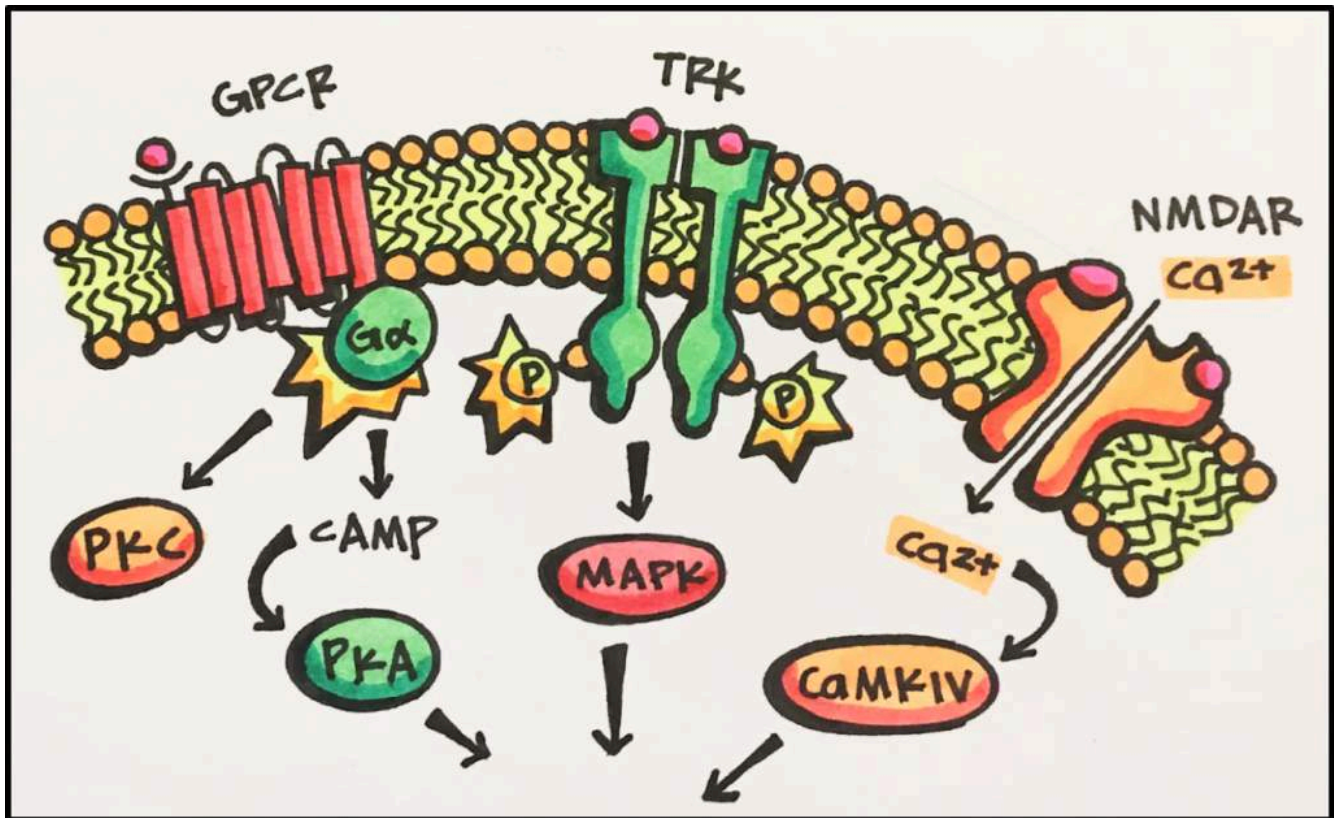


RXR • Retinoid X Receptor

PPRE • Peroxisome Proliferator Response Element

- Thyroid Hormone Receptors [THR α,β]** Upon stimulation, these receptors play a role in the rate of metabolism, neural development and cholesterol metabolism. Hypothyroidism, reduced TR stimulation, leads to slowed metabolism and presents as general fatigue and lethargy, weight gain, slowed heart rate, feeling cold and more. Conversely, hyperthyroidism, increases TR stimulation, leads to increased metabolism and presents as weight loss, increased heart rate, feeling hot and more. Nuclear Receptor-mediated transcriptional regulation can be

modulated by several signaling pathways; this is a great example of **convergence**. Convergence is when multiple pathways lead to the same effect and in the case of nuclear receptors the effect is transcription. These pathways can be initiated by either both external and internal signals involving GPCRs, ion channels, TRKs as well as several other receptors.



nuclear receptor-mediated

transcriptional

regulation

Overview of Receptor Types

Enzyme-Linked Receptors

Location cell membrane

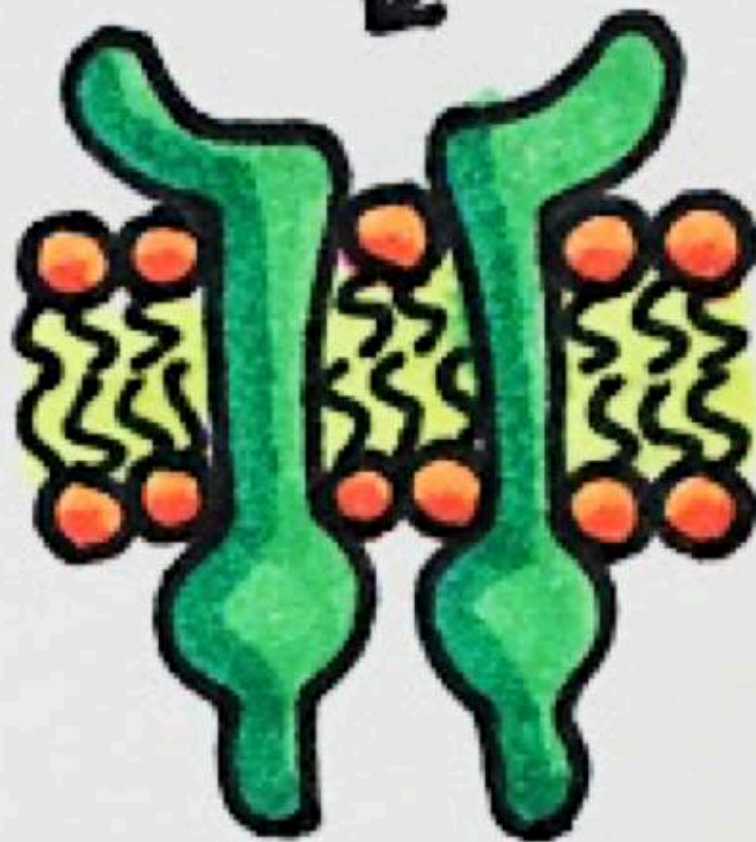
Effector protein kinases

direct

Coupling

Structure single transmembrane helix linking extracellular receptor domain to intracellular enzyme (often kinases) domain.

ligand-binding site



intracellular
"active enzyme"
domain

Hormones bind to a receptor dimer to result in activation.

Examples insulin, growth factors, cytokine receptors

16. Receptor Regulation

Overview

- **Intrinsic Regulation** • receptors initiate regulation of a variety of events and are themselves subject to regulatory and homeostatic controls.
- **Disease States** • disease states can alter the number, function, and/or activity of receptors.
- **Drugs** • drugs can act as agonist, antagonists and allosteric modulators all of which can alter receptor function and number.

Intrinsic Regulation

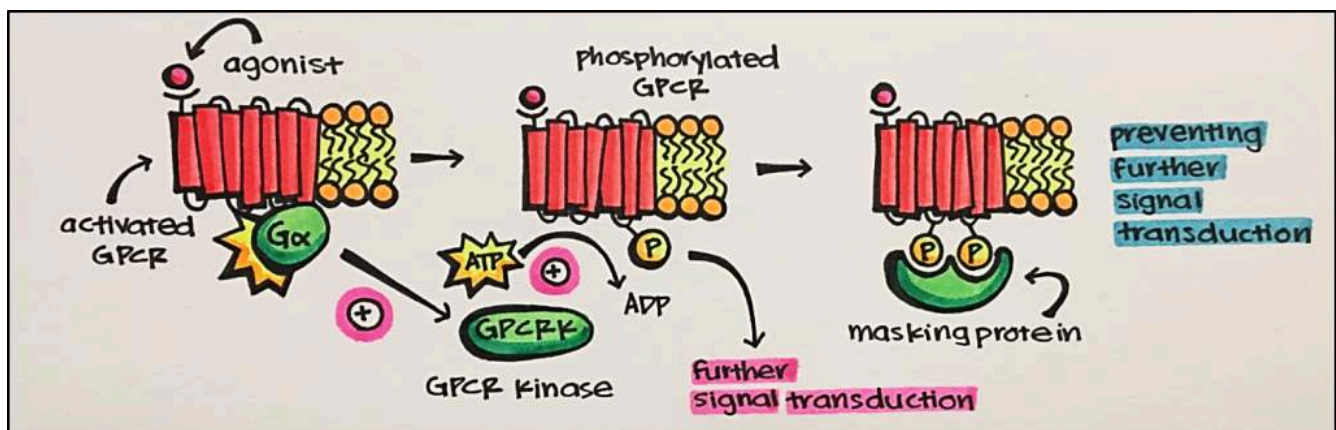
Intrinsic Regulation • receptors are subject to regulation and homeostatic control. Left unregulated, receptors may be over stimulated, which may lead to pathological changes, including apoptosis. Therefore, regulation helps protect cells. There are times when an increased response is needed and others when a decreased response is preferred.

Cellular mechanisms of Increased Response

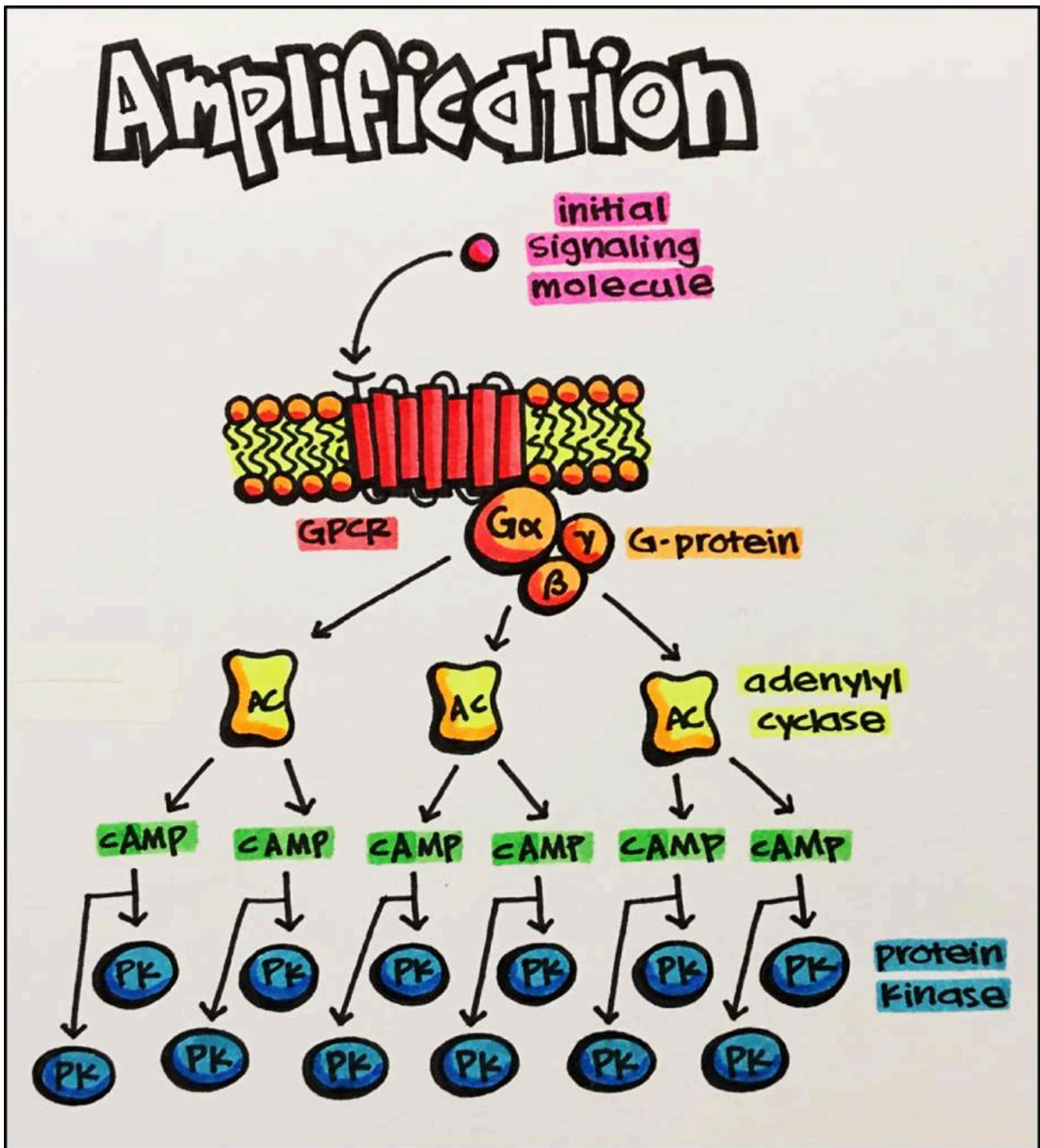
- Super/Hyper Sensitivity
- Synergism
- Upregulation

Super/Hypersensitivity • Super or hypersensitivity refers to an enhanced response to an agonist. Hypersensitivity may occur as a result of unmasking of receptors or accentuation of signal amplification.

- **Unmasking of Receptors** • some receptors have a masking protein covering the intracellular portion of a receptor, like the GPCR seen below for example. This masking protein prevents signal transduction and leads to decreased activity. When a masking protein is removed, activity increases.

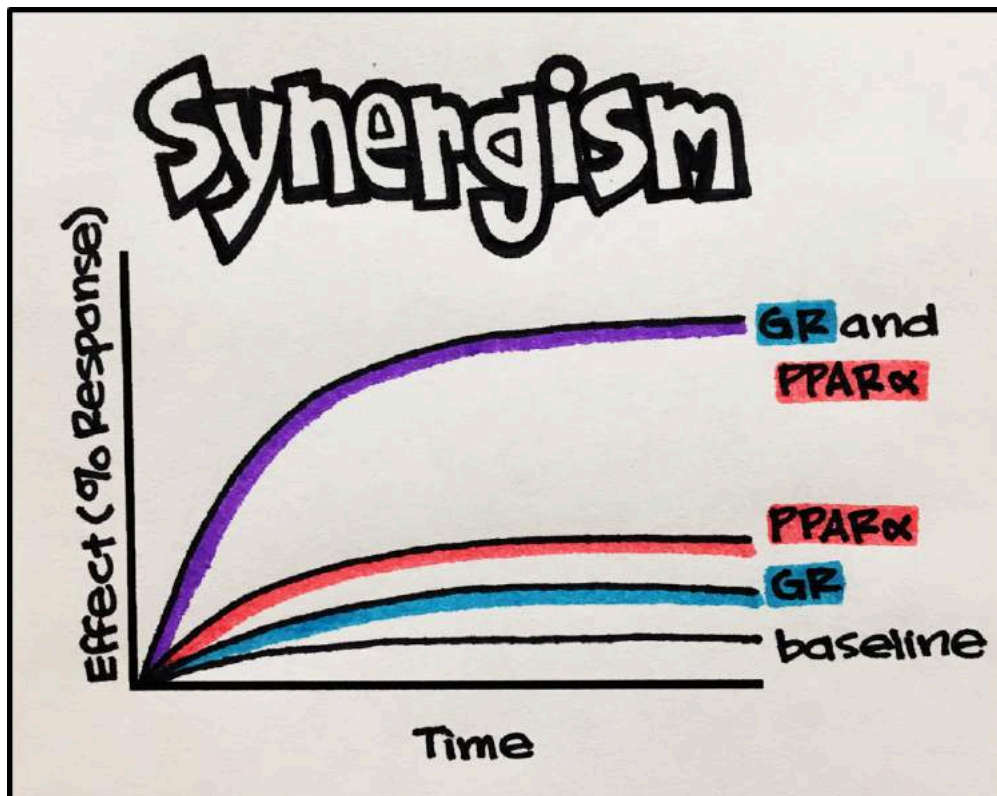


- **Accentuation of Signal Amplification** • this may occur with an increase in receptor phosphorylation or increased enzymatic activity. As was discussed earlier, phosphorylation can lead to the activation of a chain of second messengers and signal amplification. Therefore, as phosphorylation increases, so does signal transduction and amplification. Likewise, there are enzymes that catalyze components in a signal transduction pathway leading to increased signal transduction and amplification as well. The image below shows how signal amplification can be initiated by a single molecule. This particular example leads to the activation of protein kinases, which goes on to phosphorylate specific target molecules. As the signal is amplified, so is the amount of phosphorylation leading to an accentuation of signal amplification. If these kinases are not balanced by phosphatases, enzymes that reverse phosphorylation, phosphorylation can predominate and lead to further accentuation of the original signal.



Synergism • when two receptors produce a combined effect that is greater than the sum of their individual effect.

Example • Glucocorticoids stimulate **glucocorticoid receptors** [GR], a type of nuclear receptor, resulting in increased erythroid cell (series of cell leading to red blood cells) production. When **peroxisome proliferator-activated receptor alpha** [PPAR α] and GRs are stimulated simultaneously by their respective agonists, the resultant erythroid cell production is greater than the sum of production by each receptor individually. Together, PPAR α and GR stimulation result in an enhanced response.

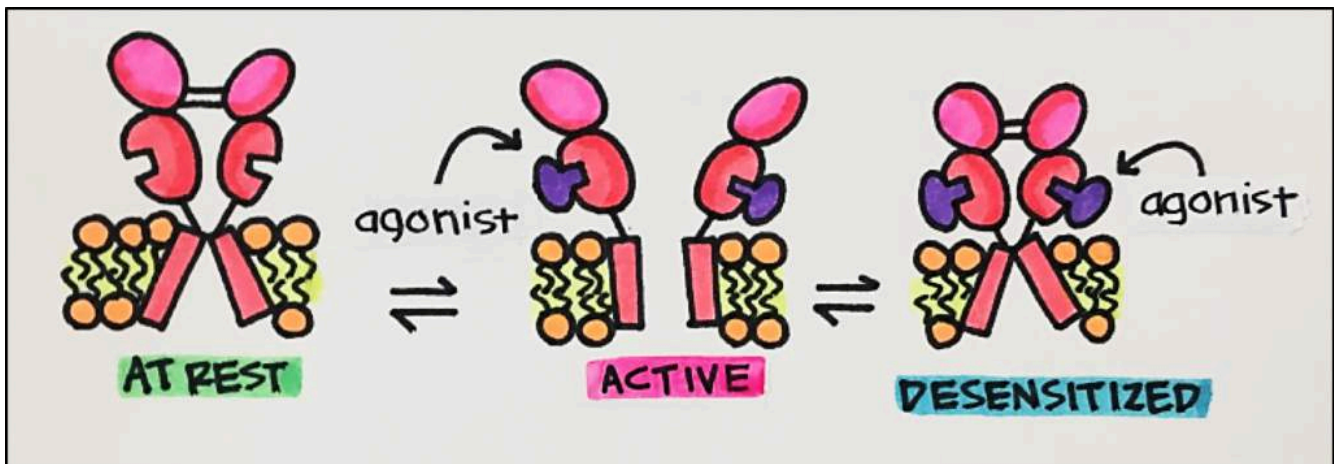


Upregulation • Upregulation refers to an increase in the number of receptors due to prolonged deprivation of receptors of interacting with their physiological neurotransmitter (e.g. by denervation of chronic use of a receptor antagonist). By expressing more receptors, there is a greater probability that a hormone will bump into and stimulate its receptor.

Cellular mechanisms of Decreased Response

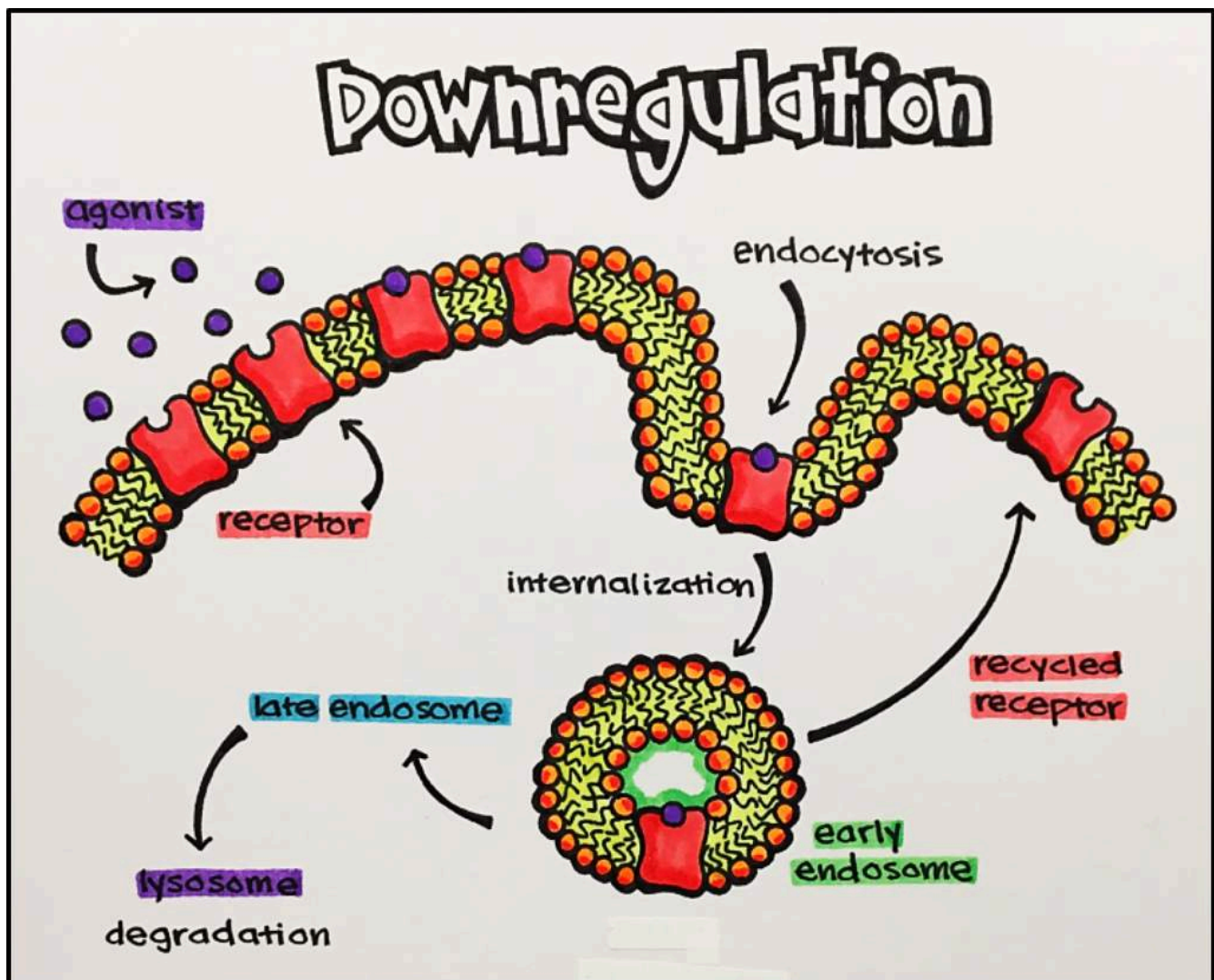
- Desensitization
- Downregulation
- Tachyphylaxis
- Tolerance

Desensitization • Desensitization refers to a reduced response to an agonist drug due to over activation of a receptor (high doses, prolonged exposure to agonist). There is a number of mechanisms of desensitization including: loss of receptor function through a decrease in receptor-coupled signaling components (e.g., G-proteins). Receptor desensitization may occur in the absence of significant changes in the number of receptors.



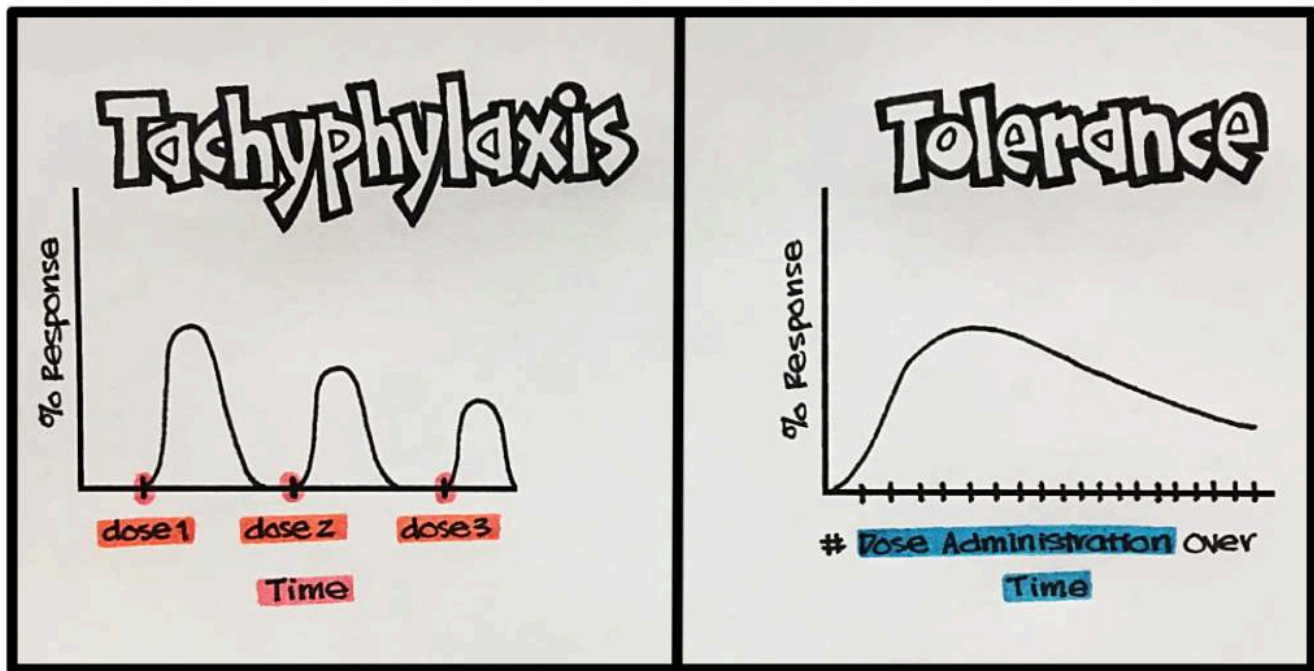
This example here demonstrates **loss of receptor function**; one of several types of desensitization. As you can see on the far right, the agonist is bound to the receptor, however, this does not result in the channel opening.

Downregulation • Downregulation specifically refers to a reduction in the total number of receptors available to be stimulated due to prolonged receptor activation (e.g. by chronic treatment with a pharmacological agonist drug or prolonged inhibition of metabolism of a neurotransmitter). This reduction in receptors in turn will decrease the cell's sensitivity to an agonist or drug. Downregulation occurs through endocytosis. Internalized receptors may either be degraded in the lysosomes or recycled back to the membrane surface later.



As the image shows, endocytosis involved the internalization of a receptor preventing it from carrying out signal transduction. A receptor may either go to the endosome then lysosome to be degraded or recycled back to the membrane surface to once again carry out signal transduction.

Tachyphylaxis • Tachyphylaxis is a **rapid decrease** in response to an agonist drug following repeated administration within a brief period; an acute form of desensitization. This can occur without a change in the number of receptors or the ability of a receptor to affect the downstream signaling molecules. Increasing the dose of the drug will not improve the response.



Tolerance • Tolerance refers to a **gradual decreased** response to a drug, requiring a higher dose of drug to achieve the same initial response. Tolerance is different from tachyphylaxis because it develops over a long period of time, whereas, tachyphylaxis is an acute event. In addition, tolerance can be overcome by increasing the dose, unlike tachyphylaxis.

Example • A great example of tolerance is in the story of Brett Favre, a Hall of Fame quarterback who played for the Green Bay Packers 1991-2010. In the midst of his career he developed an addiction to Vicodin (an analgesic medication that contains hydrocodone, an agonist of opiate receptors). He started taking two tablets to treat his sports-related injuries/pain and eventually worked his way up to 15 tablets due to a development of tolerance to Vicodin. At his worst, he was taking 14-days' worth of Vicodin each day in order to achieve the same effect he originally had with 2 tablets, which contributed to his streak of 297 consecutive games played; currently the longest streak in NFL history by 27 games. You may read more about it at the link below:

<http://www.cbssports.com/nfl/news/brett-favre-says-he-used-to-take-a-months-worth-of-painkillers-in-two-days/>

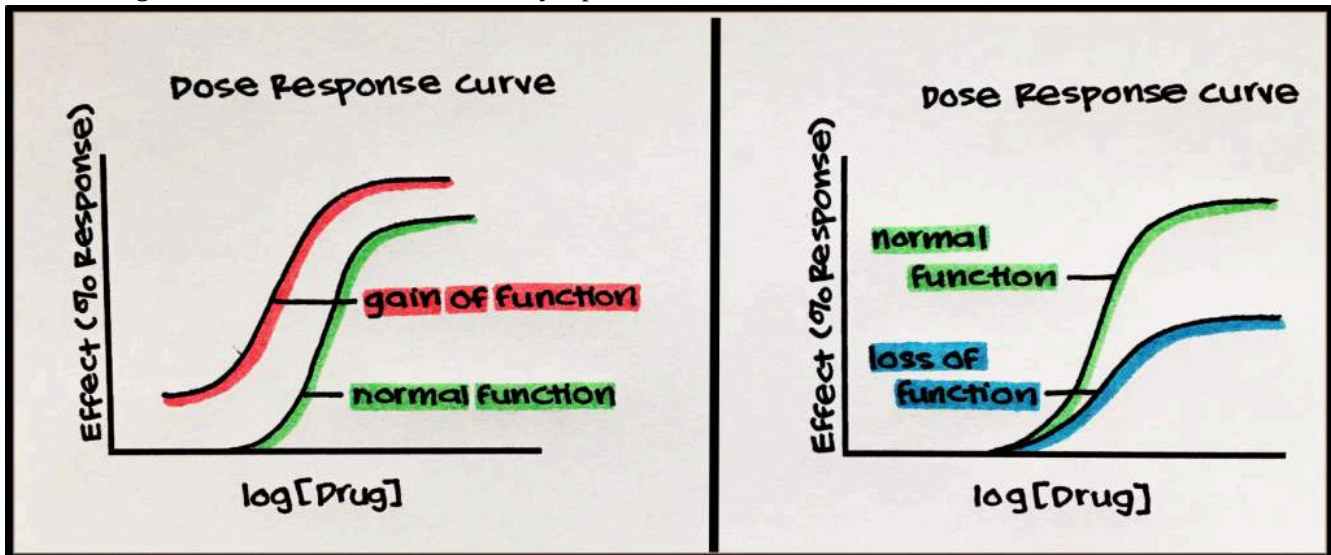
Changes in receptor response by disease states

Disease states may **alter the number of available receptors**, which can alter the sensitivity and response of a given cell or tissue. Disease states may therefore **alter the actual function or activity** of those receptors; either through loss or gain of function.

Example • Loss of Receptors

Myasthenia Gravis is an autoimmune disorder in which antibodies destroy nicotinic acetylcholine receptors [nAChR] located in skeletal muscle. nAChRs help communicate signals resulting in muscle contraction. Thus, Myasthenia Gravis causes muscle weakness, droopy eyes and even difficulty in swallowing.

Myasthenia Gravis is treated with immunosuppressants to decrease the production of antibodies that destroy nAChRs as well as with acetylcholine esterase inhibitors [AChEIs] that prevent the breakdown of acetylcholine, a nAChR agonist, to increase its level in the synapse.



Example • **Loss of Function** • Androgen Receptors [AR]

Androgen receptors have variants caused by genetic mutations. These variants have varied levels of function, ranging from partial to complete loss of function. Individuals who have complete AR insensitivity exhibit **Complete Androgen Insensitivity Syndrome [CAIS]**, and those who have partial AR insensitivity suffer from **Partial Androgen Insensitivity Syndrome [PAIS]**. Both syndromes cause a loss of receptor function.

Treatment for these syndromes includes hormone therapy; testosterone and/or dihydrotestosterone [DHT]. One great advantage of DHT over testosterone is that cannot be aromatized to estrogen, eliminating possible side effects associated with estrogen exposure.

Example • **Gain-of-Function** • A number of endocrine diseases are caused by gain-of-function mutations of GPCRs.

Type 2 Diabetes Mellitus [DM2] • DM2 can be associated with a gain-of-function mutation, resulting in **increased expression of the α_2A -adrenergic receptor**; a GPCR that prevents or suppresses the secretion of insulin. As you can imagine, a patient with this gain-of-function mutation will have elevated blood glucose, potentially leading to type II diabetes mellitus.

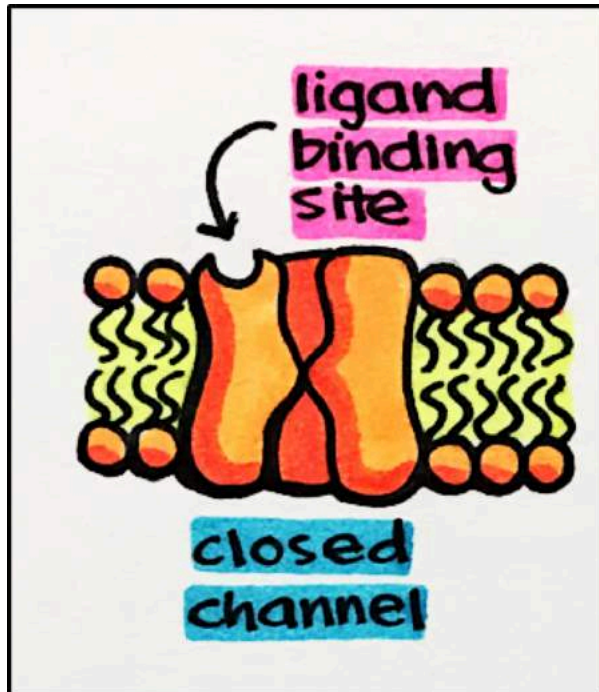
Familial Hypocalcemia Hypocalciuria • This disease involves a gain-of-function mutation of the **calcium-sensing receptor [CaSR]**; a GPCR that allows the body to monitor and regulate the amount of calcium in the blood. This gain-of-function leads to increased sensitivity to calcium. Because CaSR maintains calcium homeostasis, its exaggerated response to calcium tells the body to excrete more calcium. This leads to decreased calcium levels in the blood (hypocalcemia) by suppressing the secretion of parathyroid hormone and increased renal excretion of calcium (hypercalciuria).

17. Ion Channels

pore-forming membrane proteins that allow ions to pass through a channel pore

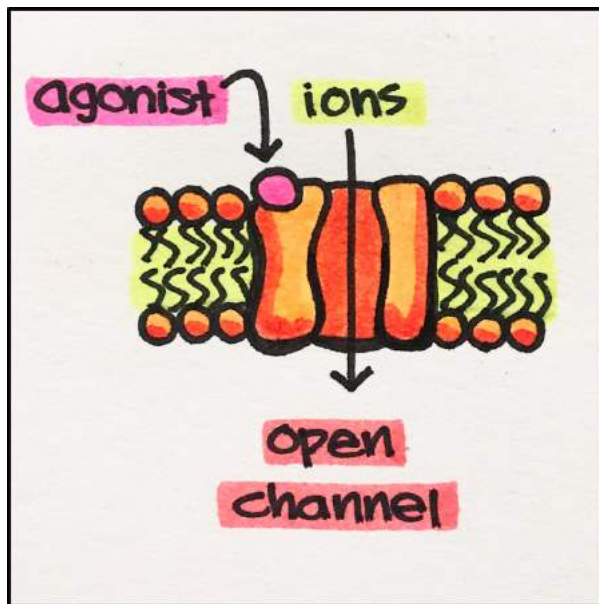
- **Ligan-Gated Ion Channels (aka ionotropic)**

- Ion channels that open in response to specific ligand molecule(s) binding to the receptor protein



- **Voltage-Gated Ion Channels**

- Ion channels that open and close in response to changes in membrane potential



Ligand-Gated Ion Channels

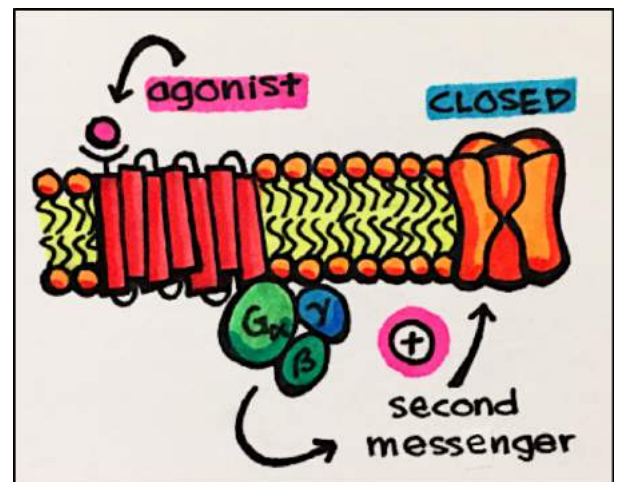
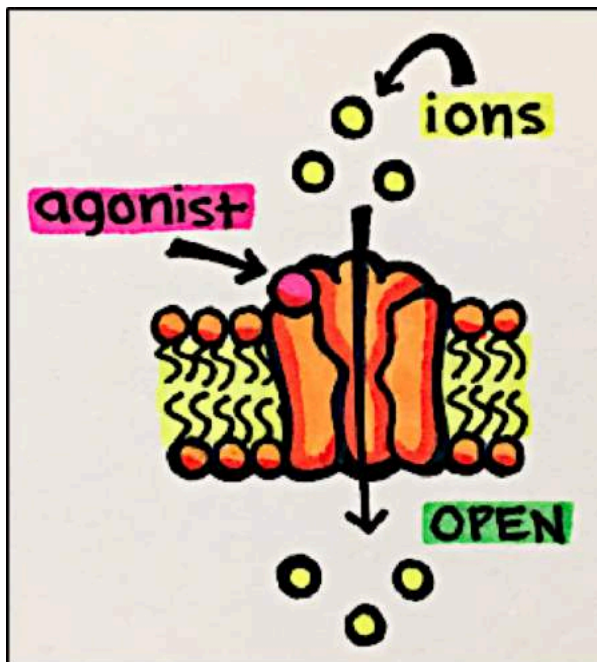
Ligand-Gated Ion Channels – allow ions to flow into or out of the cell in response to the binding of a chemical messenger to their respective receptors.

- **Acetylcholine Receptors (nicotinic)**
- **GABA_A Receptors**
- **Glutamate Receptors (NMDAA, AMPA)**

Receptor Stimulation – Ligand binds to the receptor →> causes receptor conformational change →> channel opens allowing **SPECIFIC** ions to pass through.

IONOTROPIC vs METABOTROPIC Receptors

	Ionotropic Receptor	Metabotropic Receptor
Receptor Type	ligand-gated transmembrane protein	ligand-gated transmembrane protein
Receptor Structure	ion channel	no ion channel
Time to Action	fast acting	slow acting
Result of Ligand Binding	ligand directly opens ion channel	ligand activates G-protein leading to a cascade of events that may eventually result in opening an ion channel

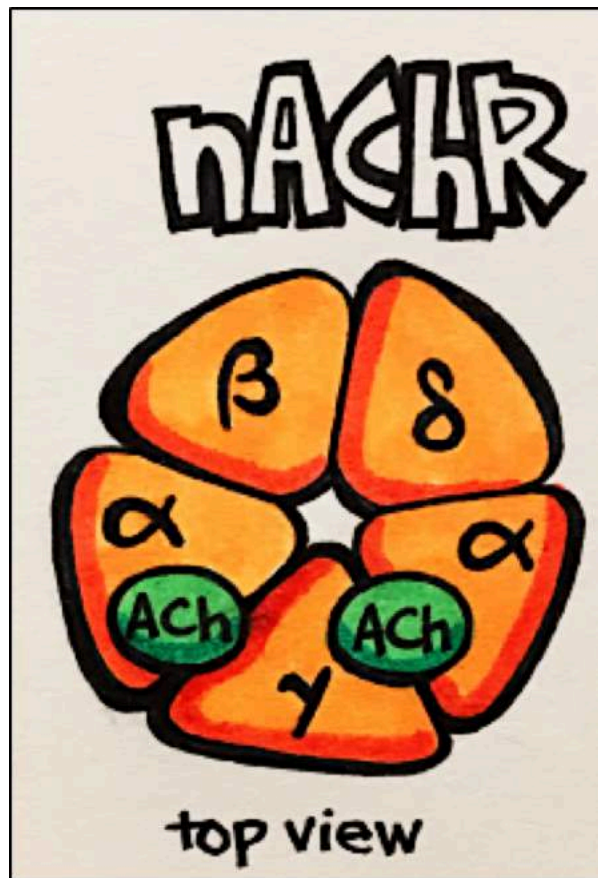


ACETYLCHOLINE RECEPTORS

Acetylcholine Receptors – There are two families of acetylcholine receptors; muscarinic and nicotinic. Both families are found **pre-synaptically** (neurons that send neurotransmitter signals) as well as **post-synaptically** (neurons that receive neurotransmitter signals). They are widely distributed throughout the central nervous system (CNS) and peripheral organs. Certain neurodegenerative diseases, especially Alzheimer's dementia, are associated with reduction in the number of cholinergic neurons (neurons that synthesize and release acetylcholine).

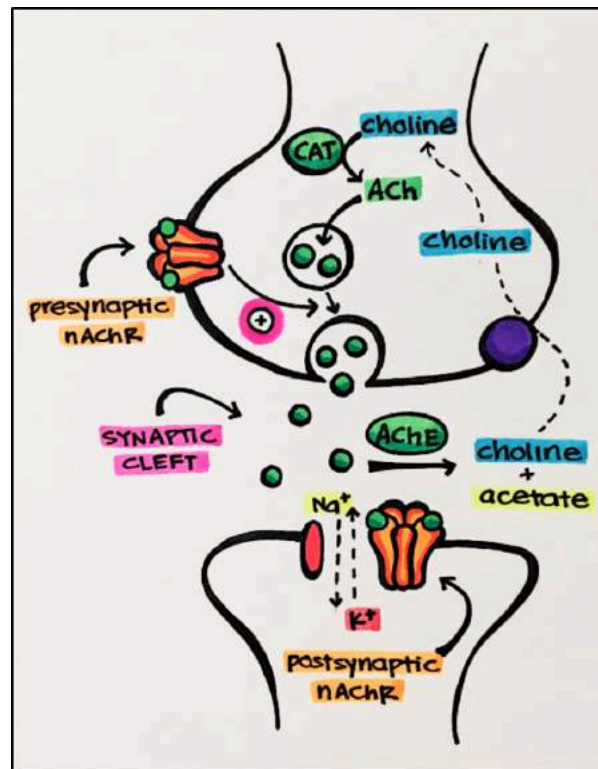
Nicotinic Acetylcholine Receptors [nAChR] – these channels are pentamers composed of multiple subunits, these channels are directly coupled to cation channels and mediate **FAST EXCITATORY** synaptic transmission at the **NEUROMUSCULAR** junction, autonomic ganglia and various sites in the CNS.

- **nAChR** has 5 subunits and requires two molecules of acetylcholine [ACh] to bind to open the channel (see below).



Muscarinic Acetylcholine Receptors [mAChRs] – mAChRs are G protein coupled receptors [GPCRs] and, therefore, will not be discussed in detail in this section.

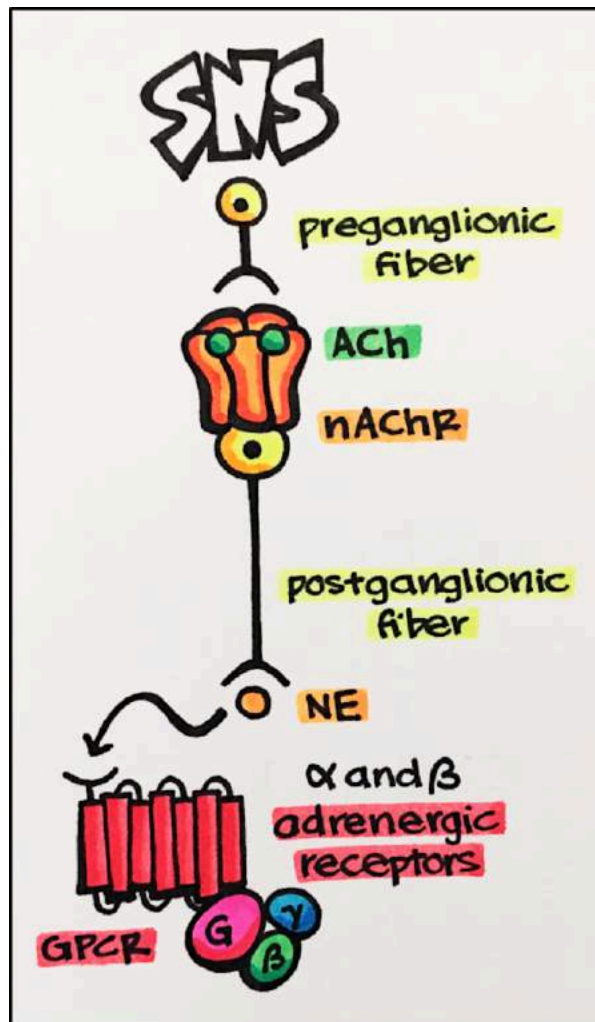
nAChR Synapse – nAChRs are found in **neuromuscular** and **ganglionic** synapses. **Reminder** – a synapse is the gap through which electrical or chemical signals are transmitted from a nerve to a muscle or another nerve. Presynaptic nAChRs facilitate ACh release during synaptic activity. Excess neurotransmitter is recycled and broken down by an enzyme called acetylcholinesterase [AChE], rendering ACh inactive as a neurotransmitter.



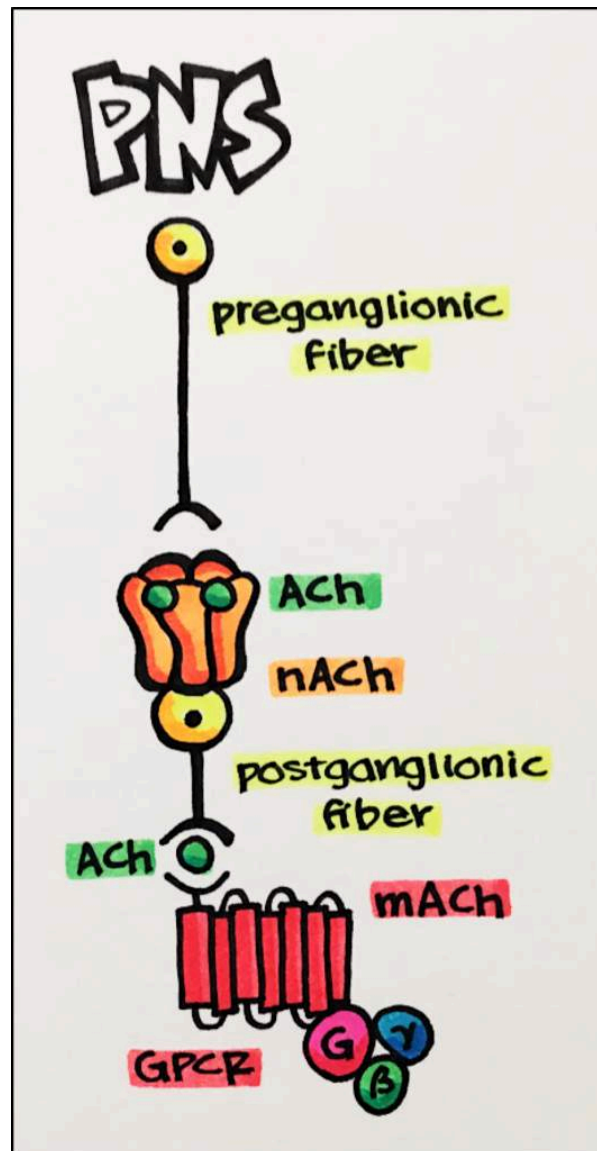
ACh (seen in the synaptic cleft above) is an important drug target. By inhibiting AChE, ACh is not broken down, the amount of ACh in the synaptic cleft increases, allowing for increased stimulation of synaptic receptors by ACh.

The autonomic system is responsible for controlling those functions we do not consciously control such as breathing, food digestion, heart rate, etc. The autonomic nervous system can be divided into two branches: the sympathetic nervous system [SNS] and the parasympathetic nervous system [PNS]. Nicotinic acetylcholine receptors play an important role in both the SNS as well as the PNS.

Below – In this image, the nicotinic acetylcholine receptor [nAChR] functions to regulate the release of the neurotransmitters [NT] norepinephrine [NE]. Two acetylcholine [ACh] molecules bind the nAChR resulting in the release of NE from the postganglionic fiber. NE will bind to alpha and beta receptors [GPCRs] located on peripheral tissues producing a number of effects associated with a “fight or flight” response such as increased heart rate and contractility, and bronchiole dilation.



Below – In this image, the nAChR also functions to regulate the release of the NT ACh from the postganglionic fiber. ACh goes on to stimulate muscarinic acetylcholine receptors [mAChR; GPCR] located on peripheral tissues producing a number of effects associated with “resting and digesting” such as reduced heart rate and contractility and increased intestinal activity.



GABA (gamma aminobutyric acid) RECEPTORS

GABA_A Receptors – these receptors are pentamers (i.e. five subunits) that include a GABA binding site, a channel through which ions (chloride) can travel, as well as a variety of modulatory sites.

GABA is an endogenous agonist synthesized from glutamate

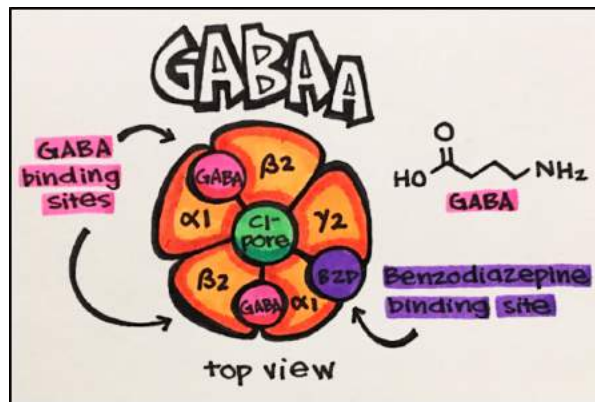
GABA is the main **INHIBITORY** transmitter in the brain. It acts on both **GABA_A receptors** (GPCRs).

GABA_A receptors are ionotropic receptors that allow for chloride ions to flow into a cell upon agonist (GABA) binding. Chloride ions moving into a cell typically decrease second messenger signaling and produce inhibitory effects.

Pharmacological modulators of GABA_A receptors include alcohol, barbiturates, benzodiazepines, and neurosteroids (steroids that alter neuronal activity).

Example – Endogenous neurosteroids are synthesized in the brain, adrenals and gonads. These neurosteroids are metabolites of steroids such as progesterone and testosterone; some are positive modulators (agonists) and others are negative modulators (antagonists) of GABA_A receptors.

GABA_A receptors provide one of the best examples of the application of allosteric modulators. For example, benzodiazepines (e.g., valium) bind to a receptor subunit different from (i.e., allosteric) that where GABA binds. This binding results in altering the conformation of the GABA binding site to a state that has higher affinity for GABA (positive allosteric modulation). This results in potentiating the CNS suppression induced by GABA and explains the effectiveness of benzodiazepines in the treatment of anxiety.



GABA_A Antagonists – Decrease central nervous system activity, sometimes used with anesthesia in an inpatient setting. Sedatives, hypnotics, muscle relaxants and anti-convulsants.

GLUTAMATE RECEPTORS

Glutamate Receptors – composed of homo- or hetero-tetramers (four subunits). Found in the CNS on presynaptic neurons that control the release of neurotransmitters. Some glutamate receptors are coupled to G proteins (**metabotropic**) while others are coupled to ion channels (**ionotropic**).

Types of ionotropic Glutamate Receptors

- AMPA Receptors – Expressed on astrocytes as well on neurons and mediate fast excitatory synaptic transmission.
- Kainate Receptors – Expressed on presynaptic neurons as well as nerve terminals. No known drug targets currently available for this particular receptor.
- **NMDA Receptors** – Expressed on presynaptic and postsynaptic neurons as well as nerve terminals.

NMDA RECEPTORS

NMDA [N-methyl-D-aspartate] receptors are glutamate-gated cation channels. Once activated, these receptors are

highly permeable to sodium and calcium. As mentioned above, NMDA receptors are expressed on presynaptic and postsynaptic neurons as well as nerve terminals. It is at these nerve terminals that glutamate acts on NMDA receptors to alter neurotransmitter release; mainly enhancing it. Furthermore, postsynaptic receptors contribute a slow component to the excitatory synaptic potential.

NMDA receptors have a number of important roles such as in the development of the CNS, rhythmic breathing as well as learning and memory.

Agonists – NMDA receptors need both glutamate and glycine to bind to the receptor in order to produce a biological effect.

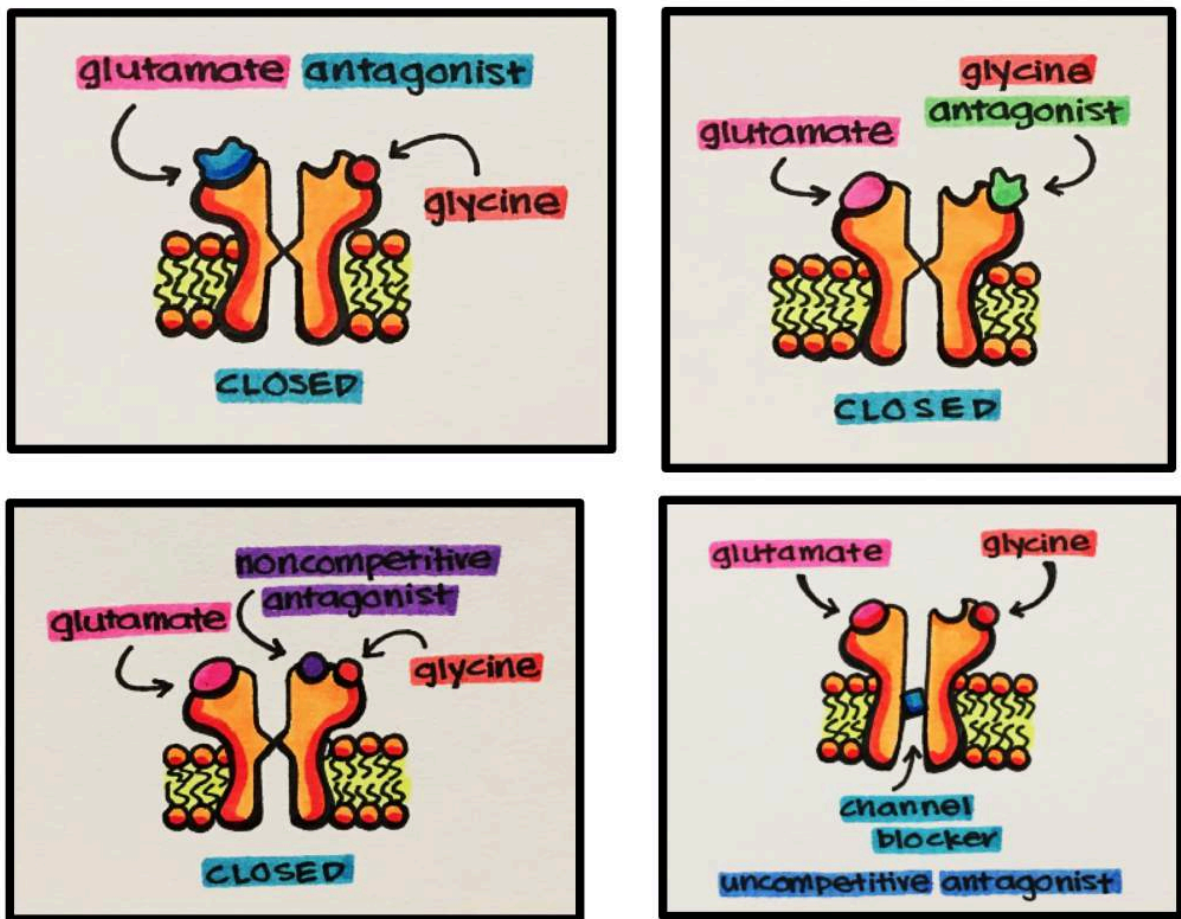
Actions – increased Na^+ and Ca^{2+} influx

Structure – each subunit of the NMDA Receptor has an **extracellular N-terminus** with 3 transmembrane domains, an intramembrane reentrant “**p-loop**” between the 1st and 3rd transmembrane domains and an **intracellular C-terminus**.

- **Active Site** – site at which agonist (glutamate) binds to stimulate a biological effect or response
- **Glycine Binding Site** – a specific modulatory site at which glycine binds to the receptor.
 - Glycine is a **co-agonist**. This means that both glutamate and glycine must simultaneously bind and stimulate the receptor in order to produce a physiological effect.
- **Allosteric Site** – secondary site at which drugs bind to modulate the binding of the agonist to the receptor primary binding site to either enhance (positive allosterism) or reduce (negative allosterism) its effects.
- **Channel-blocking Site** – site at which drugs bind to block the movement of Na^+ and Ca^{2+} ions into the cell

NMDA Antagonists –

- **Competitive NMDA Antagonists** – block glutamate from binding to the active site
- **Glycine Antagonists** – bind to the glycine site to block glycine binding, which is necessary for receptor activation
- **Noncompetitive Antagonists** – inhibit glutamate binding indirectly by binding at negative allosteric sites
- **Uncompetitive Antagonists** – bind inside the channel pore to block the ion channel and prevent ion movement into and out of a cell



Examples – drugs and their indications

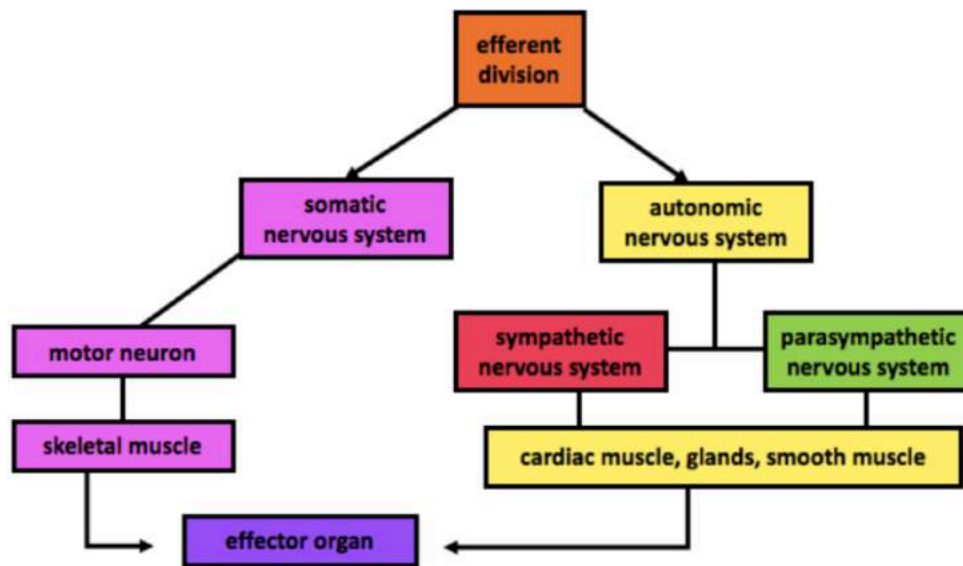
- **Anesthetics** – dizoclipine (MK801 ®), **ketamine (Ketaject ®)**, phencyclidine
 - **Ketamine** is an anesthetic adjunct. It is indicated for surgical or diagnostic procedures; ideally short procedures. Ketamine is a noncompetitive NMDA receptor antagonist, which means this drug acts at an allosteric site (site other than ligand-binding site) to inhibit receptor function.
- **Anti-tussives** (cough suppressants) – dextromethorphan/dextrophan is a noncompetitive NMDA receptor antagonist. It is the main active ingredient in Robitussin ®, a widely used cough suppressant
- **Alzheimer’s Disease** – memantine (Namenda ®)
 - Neurodegenerative disorders such as Alzheimer’s disease are caused by abnormally high neuronal excitation (excitotoxicity). Memantine is a noncompetitive NMDA receptor antagonist that binds to the NMDA receptor cation channel to prevent Ca^{2+} influx. It is therefore indicated for Alzheimer’s disease.

21. Agents and Actions of the Autonomic Nervous System: Parasympathetic Nervous System

Parasympathetic Nervous System

Parasympathetic Nervous System [PNS] – “rest and digest”

The PNS can also be thought of as the “D” division – defecation, digestion, and diuresis
Most organs are innervated with parasympathetic nerve ganglions.



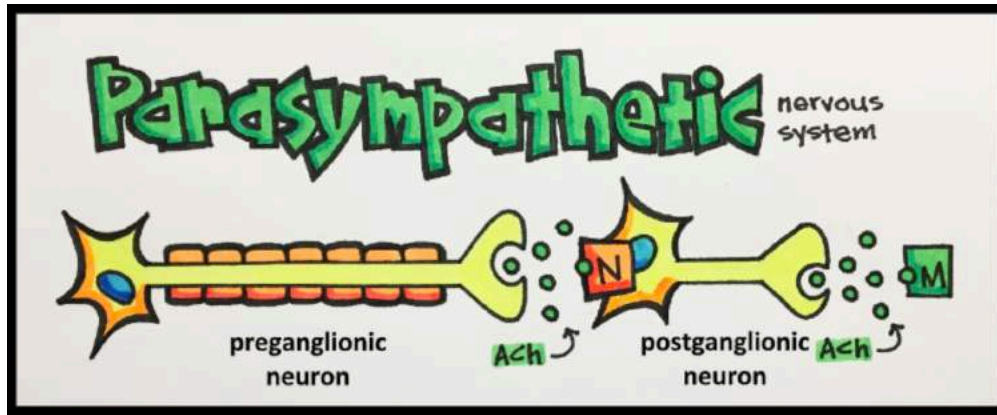
EXCEPTION – the **ciliary smooth muscle** of the eye only has parasympathetic innervation

What does your body need when at rest?

- **Decreased cardiac output** (compared to sympathetic) – lower oxygen demand when at rest
- **Energy storage** (glycogenesis, lipogenesis) – lower energy demand at rest
- **Increased digestion** – increased GI motility and secretions
- **Waste elimination** – defecation and urination

Parasympathetic Neurons

Pre-ganglionic and post-ganglionic parasympathetic neurons release acetylcholine [ACh].



Acetylcholine

Acetylcholine interacts with two types of receptors:

1. **Nicotinic receptors** – located on ganglion
2. **Muscarinic receptors** – located on effector organ/tissue
 1. **M1** –
 2. **M2** –
 3. **M3** –
 4. **M4** –
 5. **M5** –

Physiological Effects of PNS on Organs/Tissue & Respective Receptors

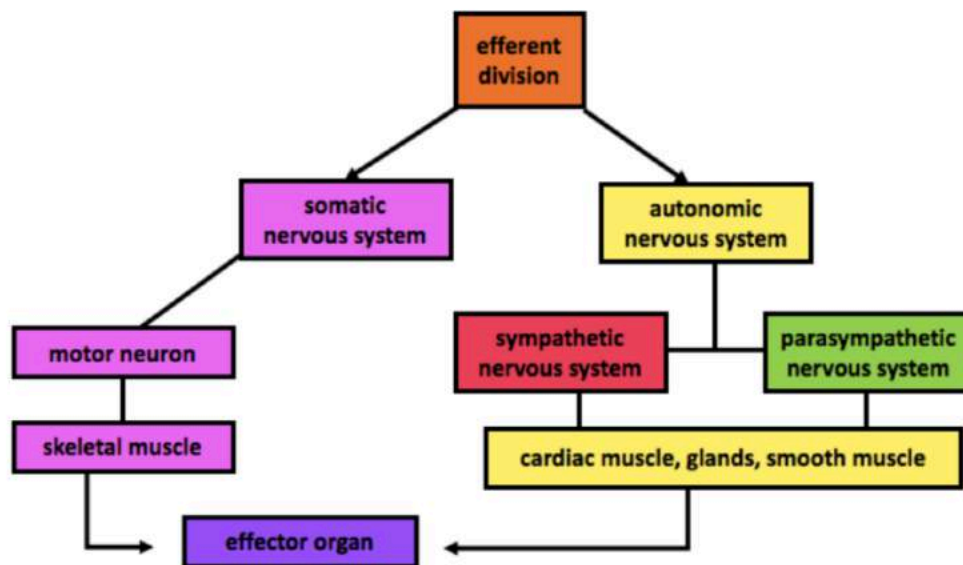
Organ/Tissue	Receptor Subtype	Physiological Effect	
BLADDER – detrusor	M3	contracts	promotes urination
BLADDER – sphincter	M3	relaxes	promotes urination
EYE – ciliary muscle	M3	contracts	improves near-sighted vision
EYE – pupil	M3	contracts	
GASTROINTESTINAL – glands	M3	promotes secretion	enhances digestion
GASTROINTESTINAL – smooth muscle	M3	contracts	enhances digestion & motility
GASTROINTESTINAL – sphincter	M3	relaxes	promotes defecation
HEART – AV node	M2	decreases conduction	
HEART – cardiac muscle	M2	decreases contractility	negative ionotropic effect [atria only]
HEART – cardiac output	M2	decreases	decreases blood delivery
HEART – SV node	M2	decreased heart rate	negative chronotropic effect – decreases oxygen delivery
LUNG – smooth muscle (bronchioles & trachea)	M3	contracts	
SALIVARY GLAND	M3	stimulates watery secretions	enhances digestion
VASCULATURE – smooth muscle	no PNS innervation		

22. Agents and Actions of the Autonomic Nervous System: Sympathetic Nervous System

Sympathetic Nervous System

Sympathetic Nervous System [SNS] – “fight or flight”

The SNS can also be thought of as the “E division” – embarrassment, emergency, exercise, excitement



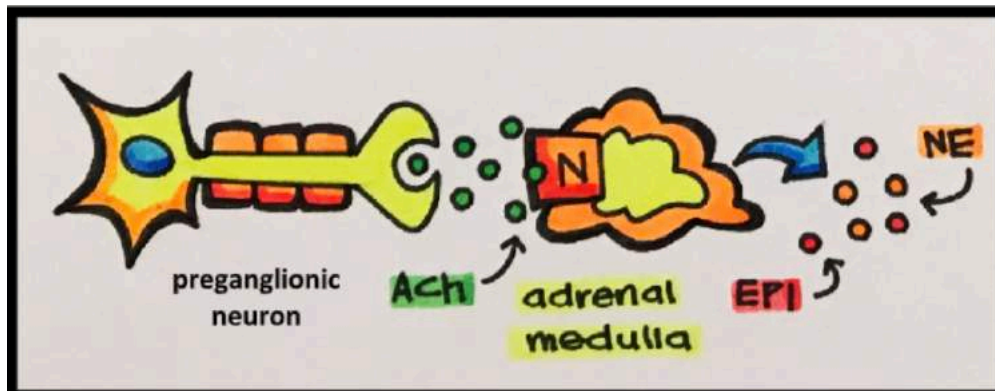
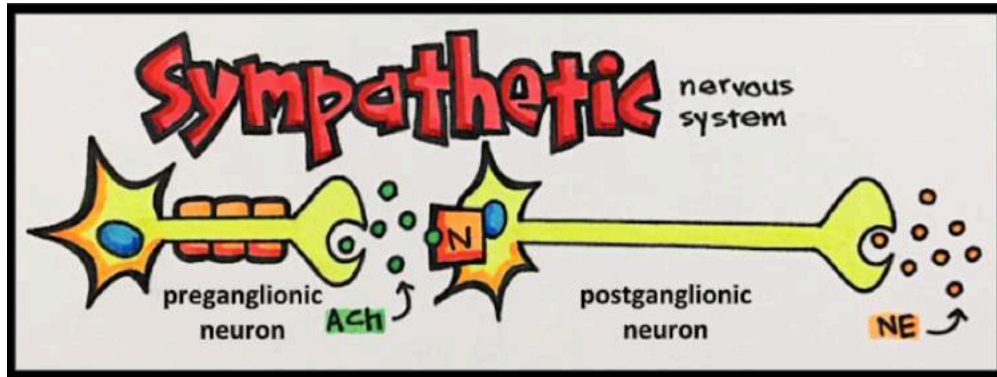
What do you want your body to do when in fight or flight situation?

- **Alertness**
- **Bronchodilation** – increased oxygen necessary for brain and muscles to function well
- **Blood shunted to organs and muscles** – need organs and muscles to function well
- **Decreased digestion** – body does not need to exert energy on digestion in emergency
- **Increased cardiac output** – improved blood and oxygen delivery
- **Production of energy** (fatty acid release, glycogenolysis) – important for muscles to perform
- **Prevention of waste elimination** – body does not need to exert energy on elimination in emergency
- **Sweat** – help maintain homeostasis

EXCEPTIONS – most **blood vessels** and all **sweat glands** only have sympathetic innervation

Sympathetic Neurons

Pre-ganglionic sympathetic neurons release **acetylcholine [ACh]** and **post-ganglionic** sympathetic neurons release **norepinephrine [NE]**. ACh will then go onto stimulate the post-ganglion sympathetic neurons at **muscarinic receptors** releasing NE and then NE will go onto stimulate **adrenoceptors** located on a variety of **effector organs and tissue** such as cardiac muscle, smooth muscle, and glands.



EXCEPTIONS – acetylcholine [ACh] is released by post-ganglionic sympathetic neurons innervating sweat glands, which means ACh receptors (as opposed to adrenoceptors) have to be blocked in order to decrease sweating as

Norepinephrine

Norepinephrine [NE] interacts with two types of receptors:

1. **Alpha-adrenergic receptors** [alpha adrenoceptors]
 1. **Alpha-1** – blood vessels
 2. **Alpha-2** – CNS (presynaptic)
2. **Beta-adrenergic receptors** [beta adrenoceptors]
 1. **Beta-1** – heart
 2. **Beta-2** – lungs
 3. **Beta-3** – bladder

Physiological Effects of SNS on Organs/Tissue & Respective Receptors

Organ/Tissue	Receptor Subtype	Physiological Effect	
BLADDER – smooth muscle	Beta-3	relaxes	prevents urination
BLADDER – sphincter	Beta-3	contracts	prevents urination
EYE – ciliary muscle	no SNS innervation	relaxes	improves far-sighted vision
EYE – pupil	Beta-	dilation/relaxation	mydriasis
GASTROINTESTINAL – glands	Beta-2	inhibits secretion	prevents digestion
GASTROINTESTINAL – smooth muscle	Alpha-2 Beta-2	relaxes smooth muscle	prevents digestion
GASTROINTESTINAL – sphincter	Alpha-1	contracts	prevents defecation
HEART – AV node	Beta-1	increased conduction	
Heart – cardiac muscle	Beta-1	increased contractility	positive ionotropic effect – enhances blood delivery
Heart – cardiac output	Beta-1	increases	enhances blood delivery
Heart – SV node	Beta-1	increased heart rate	positive chronotropic effect – increases oxygen delivery
Lung – smooth muscle (bronchioles & trachea)	Beta-2	dilation/relaxation	enhances breathing
Salivary Gland	Beta-2	stimulates viscous secretions	
Sweat Gland		stimulates sweat secretion	regulates body temperature
Vasculature – smooth muscle	Alpha-1 Beta-2	contracts relaxes coronary arteries	shunt blood to vital organs (brain, heart, etc), enhance cardiac output