8

Pharmacology

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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Quick revision:

Receptors are Excellent Drug Targets

*Activated receptors directly, or indirectly, regulate cellular biochemical processes within and between cells to change cell functions.

*Recognition sites are precise molecular regions of receptor macromolecules to which the ligand binds.

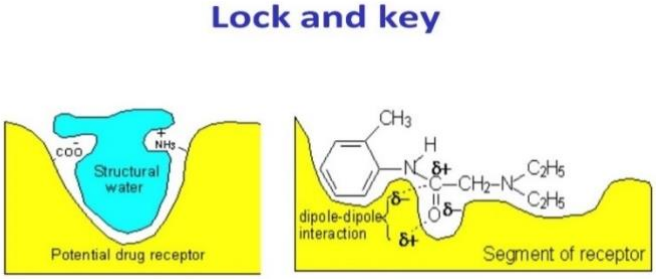
*Characteristics of Drug-Receptor Interactions:

- Chemical Bond: ionic, hydrogen, hydrophobic, Van der Waals, and covalent.
- Saturable.
- Competitive.
- Specific and Selective.
- Structure-activity relationships.
- Transduction mechanisms (sensitivity).

-We will talk about the 3**S**s (**S**pecificity, **S**electivity, and **S**ensitivity) a little more.

1-Specificity

This means that only certain drugs can bind to certain receptors, so when a drug enters a body, it will not bind to all receptors, instead only a subset of receptors will be targeted. This happens due to the complementarity of shape and charge (chemical structure) of the drug with the receptor. (lock and key theory)



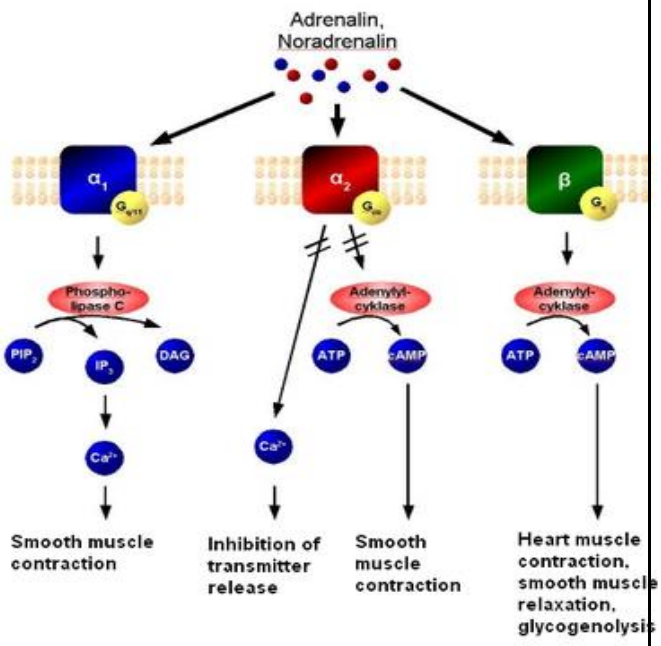
KEY: the precise fit of the ligand.

LOCK: the activation of the receptors, the opening of the lock.

2-Selectivity:

Selectivity is when we talk about a particular drug that is only able to bind to a certain **isoform** of the enzyme or the receptor. This is related to the different signaling components of the cell that get activated by this receptor.

For example: adrenergic receptors (alpha 1, alpha 2, beta 1, beta2, and beta 3).

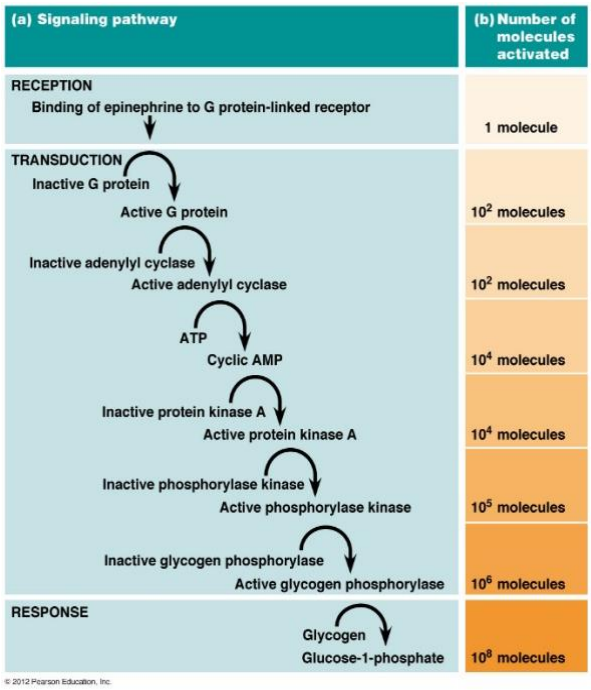


Adrenergic receptors (ANS receptors) have many types such as: alpha 1, alpha 2, beta 1, beta 2 and beta 3. Each type of these is located in different tissues of the body. for example alpha 1 receptors are located in vascular smooth muscle cells , while beta 1 receptors are located in the heart and beta 2 receptors are located in the lungs (bronchi). As we know adrenaline targets adrenergic receptors, so when adrenaline binds to alpha 1 receptors that are located in the smooth muscle cells of blood vessels, adrenaline causes contraction of the vascular smooth muscle cells leading to the constriction of the blood vessels and that's why adrenaline increases the blood pressure. However, when adrenaline binds to beta 2 receptors that are located on the lungs (bronchi) it will lead to bronchodilation (relaxation). So, the same hormone or neurotransmitter or endogenous compound (adrenaline) binds to the same receptor (adrenergic receptors) resulting in constriction in one area and dilation in another. How is that possible??

It's all about signal transduction. Receptors are coupled to specific signaling pathways, so the signal in the vascular smooth muscles is different than that in the smooth muscles of the bronchi (signals would be : increase in Camp , decrease in Camp , etc.). These differences in the signals from one subtype to another gives us different actions.

3-Sensitivity:

Sensitivity is all about amplification (intracellularly). G protein coupled receptors are a great example of this. Look at the figure, here we have one epinephrine molecule binding to its receptor this will give me a net effect of 10^8. The response here is the production of glucose-1-phosphate. So as can you see one receptor gave us 100 G proteins (10^2 amplification) and each one of these gave us 100 active adenylyl cyclases (10^3 amplification) and each one of those gave us 100 cAMP (10^4 amplification) so on until we get an amplification amplitude of 10^8. On the other hand, ion channels receptors give less amplification, so this property (sensitivity) is very important.



*Signal amplification:

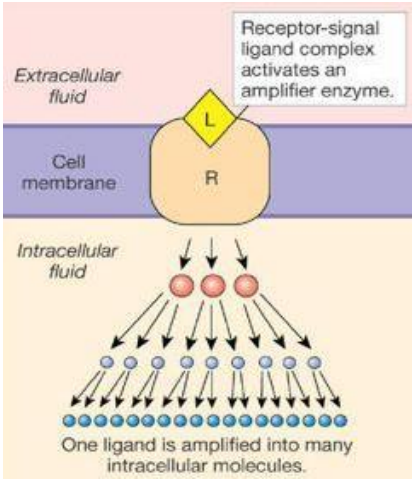
Signal amplification is related to the duration and intensity of binding.

Example: G protein coupled receptors.

-Phenomena that accounts for the amplification:

1. The receptor drug-complex interacts with many G proteins thereby multiplying the original signal many folds (intensity).

2. The activated G-protein persists for a longer duration than the original recepter-drug complex. A drug can bind to its receptor just for a few milliseconds but the subsequent activated G proteins may stay active for hundreds of milliseconds. That's because the signal transduction molecules are still on even tho the drug is not bound to the receptor. So, even though the drug dissociated from its receptor, we still have a response (duration).



Both of these effects (intensity and duration) of amplification can lead to the final clinical response amplification seen.

*Drug Receptors & Pharmacodynamics:

-Dose response relationships:

When a drug binds to a particular receptor, a drug-receptor complex is formed which produces a clinical effect. If we gradually increase the concentration of the drug we are going to see a gradual increase in clinical effect until we reach the maximum effect.

Receptors largely determine the quantitative relations between dose (or concentration) of the drug and the pharmacological effects.

The affinity between a drug and a particular receptor determines how much drug receptor complexes are formed.

The receptor's affinity for binding a drug determines the concentration of the drug required to form a significant number of drug-receptor complexes. So, a higher affinity means a lower concentration of the drug is required to form a significant number of drug-receptor complexes.

This mechanism is saturable, and the total number of receptors is usually much less than the amount of drug molecules. Therefore, the net effect is limited by the total number of drug-receptor complexes formed, which is limited by the total number of receptors present on that tissue. So, the total number of receptors limits the maximum effect the drug can produce.

*How do I determine the total number of a particular receptor?

We use radiolabeled ligands or substrates for that receptor and we incubate those ligands with a particular tissue that has the receptor we are studying. The radioactive substrates bind to their receptors and give a radioactive signal. We then measure the radioactivity we get; more radioactivity means more drug-receptor complexes are formed (more binding). By increasing the dose of the radioactive ligands to a very high concentration at which we expect all the receptors to be saturated and bound to the radioactive ligands, we can calculate the maximum number of receptors available on that tissue.

Nowadays we use new techniques such as genome sequencing, where we sequence the genome to determine the different proteins in the body. This technique led to the

discovery of proteins on the cell surface (receptors), for which no ligand has been discovered. These receptors are called orphan receptors.

Orphan receptors: receptors for which no ligand has been discovered, and whose function is still hypothesized. We need to study them further to figure out their ligand or what their effect on the body is.

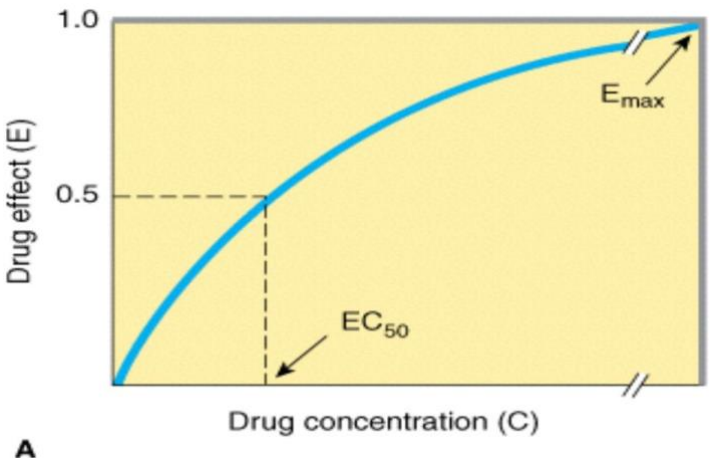
*Dose-response relationship: two different types:

- **Graded dose-response relationship**
- **Quantal dose-response curve.**

❖ Graded dose-response relationship:

As the concentration of a drug increases, its pharmacologic effect also gradually increases until all the receptors are occupied (the maximum effect). We study this relationship in an isolated tissue sample that we extract out of the body (in vitro) to make it easier and simpler; as inside the body we have many complexities that add to the pharmacological effect. So, for example if we want to study the effect of acetylcholine on muscle contraction, we extract a piece of muscle and attach it to a transducer that measures its stretching and then we add the drug (acetylcholine). We start with a small dose, which gives a certain degree of muscle contraction. Then we increase the dose, which gives us a greater degree of muscle contraction, until we reach the maximum effect where increasing the concentration of the drug (acetylcholine) won't give us any more muscle contraction. We call this the maximum effect (E-max) or the ceiling effect.

This is the example of a graded dose response where increasing the concentration of a particular drug gives us a greater response. Initially at low doses a small increase of concentration will result in a large increase in response i.e. the curve is steep. While close to E-max the dose—response curve plateaus (look at the figure). So, as the dose increases the response increment diminishes.



A

Look at the figure, it is a hyperbolic curve, in this curve we study the relationship between the concentration of the drug and the effect of that drug, so the concentration will regulate the effect at a certain dose.

$$E = \frac{E_{max} \times C}{C + EC_{50}}$$

E = The effect observed at concentration C
E_{max} = The maximal response that can be produced by the drug
EC_{50} = The concentration of drug that produces 50% of maximal effect. It depends on drug-receptor interaction, which means how much of the drug-receptor complex is formed.

So, E which is the effect of the drug at concentration C depends on

1- The maximum effect (E_{max})
2- The concentration of the drug (C)
3- EC_{50}

*Mass action law:

This law describes the interaction/affinity/association between 2 molecules in the universe or the environment. By assuming that the binding of one drug molecule does not alter the binding of subsequent molecules and applying the law of mass action, we can mathematically express the relationship between the percentage (or fraction) of bound receptors and the drug concentration:

Hill-Langmuir equation

$$B/B_{max} = \frac{[D]}{[D] + K_D}$$

$$B = \frac{B_{max} \times C}{C + K_d}$$

B = Drugs bound to receptors.
C = Concentration of free (unbound) drug.
B_{max} = The total concentration of receptor sites
K_d = The equilibrium dissociation constant.

From the equation, binding between 2 molecules in a particular environment (B) depends on:

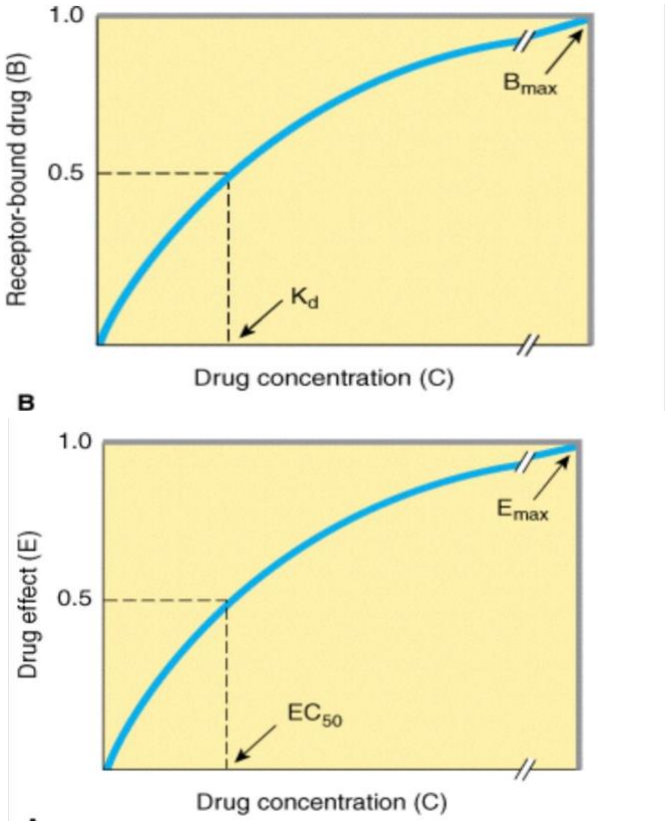
1- C - The concentration of free (unbound) drug
2- B_{max} - The maximum number of receptors that can be occupied by the drug.
3- K_d (equilibrium dissociation constant) - The concentration of a drug that gives me 50% of the maximum binding. The value of K_d can be used to determine the affinity of a drug for its receptor. Affinity describes the strength of interaction

(binding) between a ligand and its receptor. The higher the K_d value, the weaker the interaction and the lower the affinity, and vice versa.

$$D + R \xrightarrow{k} DR$$
$$DR \xrightarrow{k^{-1}} D + R$$

Note: EC_{50} and K_d may be identical, but **not necessarily**.

*What is the difference between these two curves??



They look alike, but they differ in B-max and E-max. In the first curve the y axis shows binding capacity but in the second curve it shows the effect of the drug. The shape of the curve and the parameters I'm studying are the same, so this indicates that EC_{50} (the concentration of the drug that will give me 50% of maximum effect) equals the concentration of the drug that gives me 50% of maximum binding. This is applicable if the effect of the drug only depends on the drug-receptor interaction (binding) and no other factors come into play (receptor-effector coupling).

*Receptor effector coupling:

When a receptor is occupied by an agonist, a conformational change occurs. This will lead to the activation of G protein, Adenylyl cyclase, cAMP and Protein kinase A. This cascade of events is called signal transduction, so we have a transduction process that transduces the drug occupancy of the receptor into a clinical effect. This is called coupling which is the transduction process that links a particular drug occupancy to its receptors with its pharmacological response. This is important because the efficacy of this coupling (occupancy response) is determined by the initial binding (initial conformational change of the receptor) and by the biochemical events that occur inside the cell (signaling molecules that are activated).

*Some drugs are said to have better efficacy in coupling than other drugs which brings us to the term full agonist and partial agonist.

Full agonists are more efficiently coupled to their receptors and are more effective in producing a clinical effect, stimulating a full response unlike partial agonist. The structure of the drug might affect the coupling which will affect signal transduction.

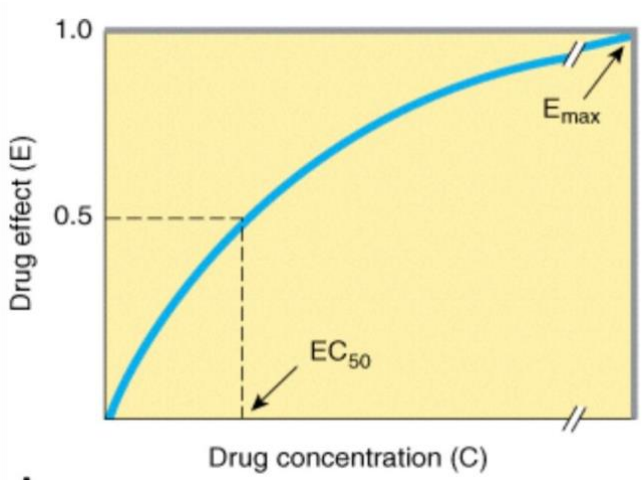
Coupling happens between drug receptor binding (conformational change) and the clinical effect. Some drugs (full agonists) are more effective at coupling than other drugs (partial agonists).

Therefore k_d doesn't always equal EC_{50}, as it depends on the coupling efficiency of the drug to the net effect not only on the drug-receptor binding.

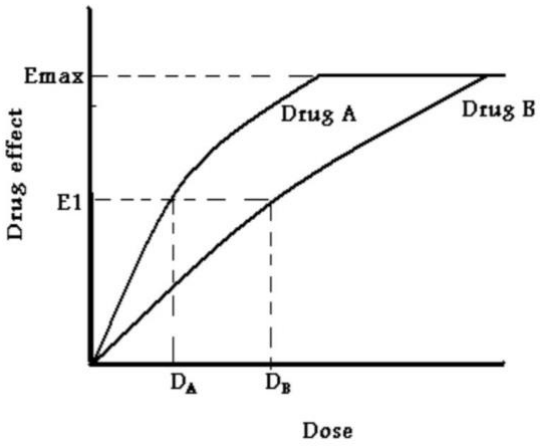
*Potency (affinity):

Potency is a measure of the amount of drug necessary to produce 50% of the maximum effect. The concentration of drug producing 50% of the maximum effect (EC_{50}) is usually used to determine potency. So, potency refers to the affinity of the drug to its receptor. A drug is more potent when we need less amount of the drug bound to the receptor to give 50% of the maximal effect (EC_{50}).

In other words, Potency refers to the affinity of a drug for its receptor or the concentration of drug required to produce a given effect. So low k_d means high potency. Potency refers to the amount or concentration of drug required to produce a response. It is measured on the X-axis of the dose-response curve.



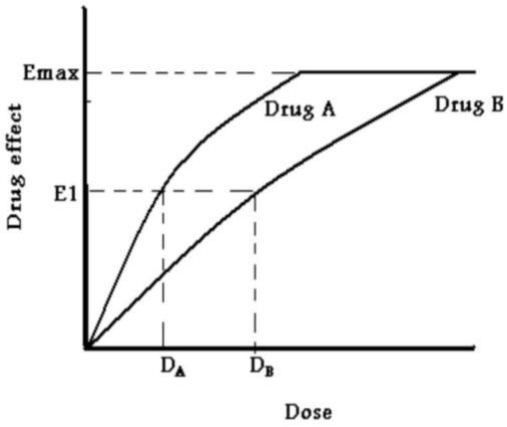
- **ED_{50} or EC_{50} or K_d** are all measures of drug potency
- E**D**$_{50}$: D refers to dose, which is the amount given to the patient.
- E**C**$_{50}$: C refers to concentration, which is the concentration of the drug measured in a certain part of the body such as the plasma.
- As seen in the figure, we have 2 drugs, drug A and drug B. drug A is more potent because we need less concentration of drug A to give me the same effect (E1) (EC_{50}).



*Efficacy (E_{max}):

Efficacy is the maximum response seen with a particular drug. Efficacy is dependent on the number of drug-receptor complexes formed and the intrinsic activity of the drug (its ability to activate the receptor and cause a cellular response). Maximal efficacy of a drug (E-max) assumes that all receptors are occupied by the drug, and no increase in response is observed if a higher concentration of drug is used.

Efficacy is calculated on the Y-axis of the dose-response curve.



Which drug on the figure has higher efficacy?
Both of them reach the same E-max, so their efficacy is the same.

Example: morphine is a painkiller that is used in surgeries and for cancer patients (very strong). The efficacy of morphine is higher than that of other painkillers such as Ibuprofen and aspirin. This is measured in relation to the clinical effect of the drugs, where morphine gives me 90% pain reduction while Ibuprofen only gives me 50% pain reduction so the maximum effect of morphine is higher than ibuprofen which is why it's used in severe conditions.

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*Spare receptors:

The maximum effect of a drug may be reached while there are empty receptors (spare receptors) with no ligands bound to them. In other words only a fraction of the total receptors for a specific ligand may need to be occupied to reach the maximum response and the rest of the receptors are called spare receptors. The number of spare receptors differ from one receptor to another. For example: insulin receptors have 99% spare receptors while beta adrenergic receptors on the heart have 5% spare receptors. This means we have a larger storage of insulin receptors in our bodies than adrenergic receptors. In normal conditions we need 1% of insulin receptors and the rest are spare, because we have a high variation in insulin needs (variation in dietary patterns/variation in glucose in my meals) so this reserve helps the body to cope with the high glucose levels, so sometimes we need to utilize these receptors but in normal conditions we don't need them. However, we have a low reserve of spare beta adrenergic receptors which is why in heart failure fast deterioration happens to the

patient (as we don't have a functional reserve of beta-adrenergic receptors to see an effect).

Spare receptors are found for only certain types of receptors.

Note: We know that we have spare receptors, but we still don't know why they are present. They might be reserves the body keeps until it needs them.

***Receptor occupancy theory: ''activation of membrane receptors and target cell responses is proportional to the degree of receptor occupancy''**

Which means the cell response is proportional to the number of receptors bound to a ligand.

*This theory was built on several **assumptions** that help us to study the drug receptor interaction in vitro (outside the body) such as:

-Association is limited by collision, orientation and energy.

-All receptors are equally accessible.

-All receptors are either free or bound, there is no partial binding.

-Neither drug or receptor are altered by binding.

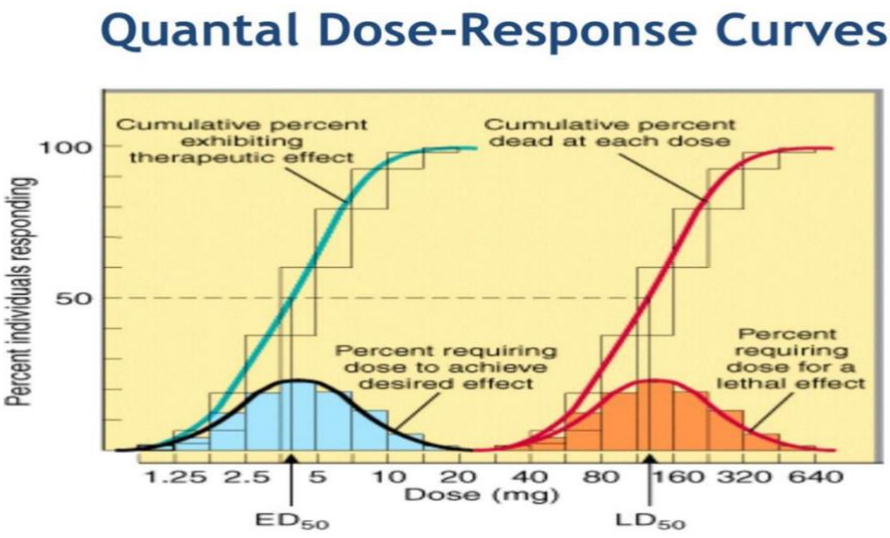
-Binding is reversible.

❖ Quantal dose response curve:

Another important dose–response relationship is between the dose of the drug and the proportion of a population that responds to it. These responses are known as quantal responses, because for any individual the effect either occurs or it doesn't. So it's an all or none study. Here we don't study how much a particular response increases (how much stretching of the muscle cell when acetylcholine is added) instead we study whether or not it happens (yes or no response)

For example, a group of patients were given the pain killer paracetamol. If the drug kills the pain that means that the drug gave us a response (YES), but if it doesn't kill the pain, then it didn't give us a response (NO). We then count how many of the patients said that they responded to the drug (YES), and we plot it. Look at the figure, the y axis presents the percent individuals responding, notice the blue curve is the therapeutic response. As you can see when the dose is increased, the number of individuals who are therapeutically effected (YES) is increasing.

Note: the blue columns indicate the number of individuals that respond to the drug when a certain dose is given, while the cumulative percent exhibiting therapeutic effect (the blue line) shows the sum of the number of individuals that respond to the drug on a certain dose and less doses.



The study is also performed for the toxic effect of drugs. Usually we don't study toxic effects on human beings, they are studied on animals. Using the same drug we will gradually increase the dose, measuring the percentage of animals dying at each dosage. Plotting the results will give us a curve that resembles the red curve. Increasing the dose of a drug results in toxicity or mortality.

From these two curves (red+blue) we can calculate what is the concentration of the drug where 50% of the individuals had a therapeutic response and the concentration of the drug where 50% of the experimental animals die. This helps us calculate the safety margin of the drug which is called the therapeutic index of the drug. It is the ratio between the dose of the drug that is killing 50% of the animals (LD_{50}) over the dose of the drug that treats 50% of the animals (ED_{50}).

The doctor said that the therapeutic index depends on the lethal dose but both the slides and the book say that it depends on the toxic dose.

$TI = TD_{50} / ED_{50}$

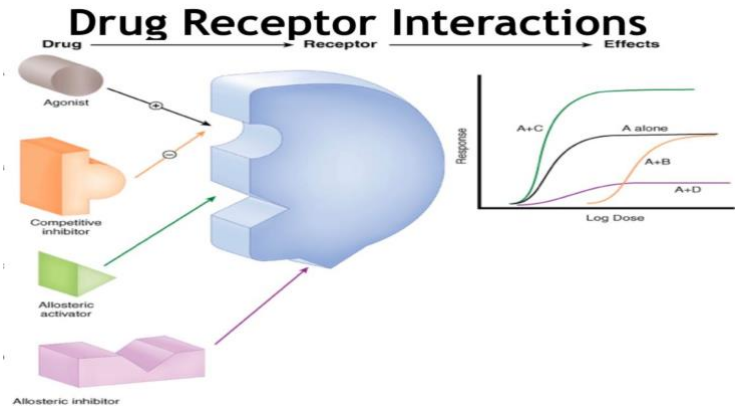
- TI: Therapeutic index
- Effective Dose (ED_{50}): The dose at which 50% of individuals exhibit the specified quantal effect.
- Toxic Dose (TD_{50}): The dose required to produce a particular toxic effect in 50% of animals.
- Lethal Dose (LD_{50}): The dose required to kill 50% of the of the animals.

The question here, how is the therapeutic index related to the safety of the drug??

For a drug to be safe, the therapeutic index must be high. The higher the therapeutic index the safer the drug is. From the equation if we want high TI, TD_{50} must be high while ED_{50} magnitude must be low. This is logical as we need the drug to give an effect

at low doses, and cause toxicity only on high doses. For more clarification; if a drug has a therapeutic index that is equal to 2 this means that if we double the dose of the drug, we will reach the toxic dose. Due to that drugs that have a low therapeutic index have to be monitored in the body to make sure we don't reach toxic levels in the patient's body. In addition we have to be careful with drug-drug interactions for these kinds of drugs, because simple drug-drug interactions may cause the concentration of the drug to increase to toxic levels. To conclude: A higher therapeutic index for a drug represents a safer drug.

Drugs can be either antagonist or agonists (look at figure):



***Agonists:** Bind to the agonist binding site where the endogenous compound binds and produces an action.

***Allosteric activators**: Act at separate sites to increase the efficacy of the agonist or its binding affinity. In other words, it binds to a different binding site which activates signaling events that will give me an effect.

***Competitive inhibitor (reversable):** Binds to the same site where the endogenous compound binds, to prevent the binding of the agonist, producing no action.

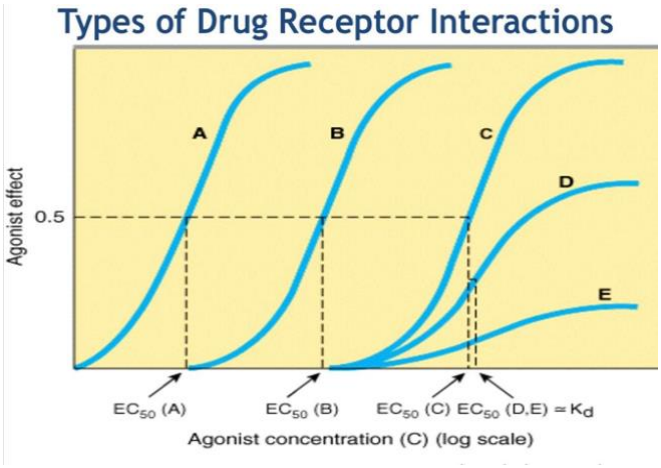
***Allosteric inhibitors:** Act at separate sites to decrease the efficacy of the agonist or its binding affinity.

*Possibilities of Drug Combinations:

- Antagonistic Effects
- Additive Effects.
- Synergistic Effects.
- No effect.

Types of Drug Receptor Interactions:

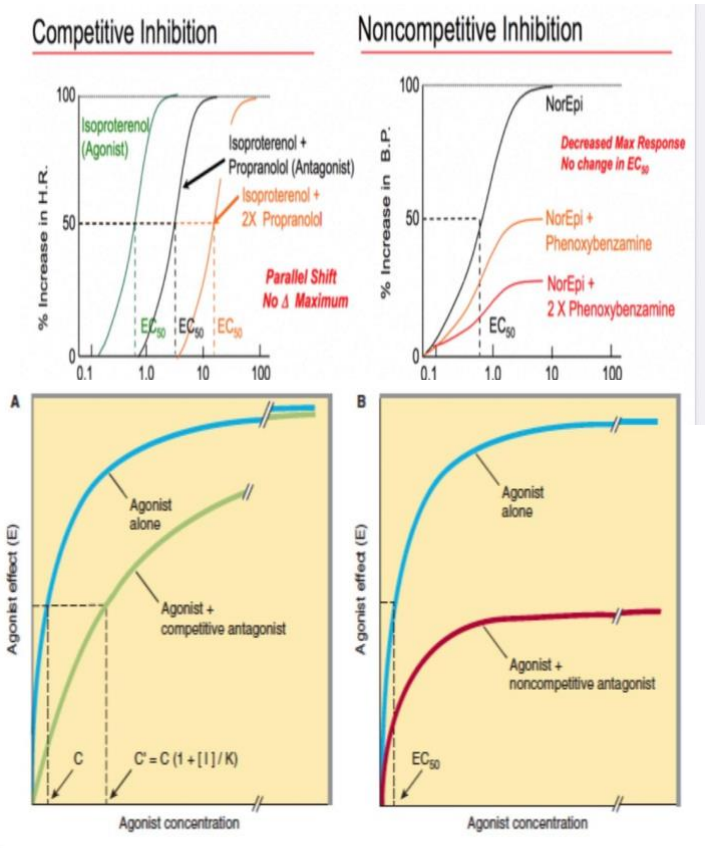
- Agonist.
- Antagonist.
- Partial Agonist = Partial Antagonist



- Some drugs give us a greater effect than other drugs even tho they act on the same receptor. What happens if I give the 2 drugs together??

1-Agonist with Competitive Antagonists: If both the antagonist and the agonist bind to the same site on the receptor in a reversible manner, they are said to be "competitive." The competitive antagonist prevents the agonist from binding to its receptor and maintains the receptor in its inactive state. However, this inhibition can be overcome by increasing the concentration of agonist relative to antagonist. Thus, competitive antagonists characteristically shift the agonist dose–response curve to the right (increased EC_{50}) without affecting E_{max}. So EC_{50} is increased as we don't have the same number of receptors sites available for the drug, and a higher concentration of the agonist is needed to occupy the receptor sites and stimulate the same effect.

Note: In some cases, if the drug concentration is increased to a very high concentration, all the receptors get blocked, causing a change in E-max as what happened to D and E in the figure above.



2-Agonist with a Aoncompetitive Antagonist (irreversible): An allosteric antagonist causes a downward shift of the E-max, with no change in the EC_{50} value of the agonist. This type of antagonist binds to a site ("allosteric site") other than the agonist-binding site and prevents the receptor from being activated by the agonist. So even if I increase the concentration of

the agonist the noncompetitive antagonists will still be bound to its receptor and inhibit the effect.

Drug Antagonism:

- Pharmacologic Antagonism:

– Competitive Antagonism.

– Noncompetitive antagonism.

- Physiologic Antagonism:

– Epinephrine in Anaphylaxis.

- Chemical Antagonism:

– Antacids in heartburn.

Note: These were written in the slides, but the doctor didn't refer to them.

Good luck …

"تلمَّحْ فجر الأجر؛ يهن ظلام التكليف"