Medicinal Chemistry
Professor Dr. Harinath Chakrapani
Department of Chemistry
Indian Institute of Science Education and Research, Pune
Receptor- Drug Interactions

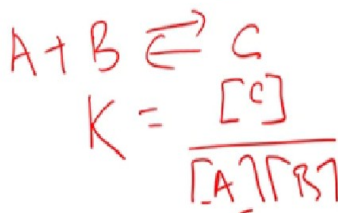
Welcome back, so in today's lecture we are going to look at some quantitative methods to understand the drug receptor interaction.

(Refer Slide Time: 0:24)

Drug-Receptor Interactions

This stability is commonly measured by how difficult it is for the complex to dissociate, which is represented by its K_d, the dissociation constant for the drug-receptor complex at equilibrium

$$K_d = \frac{[\text{drug}][\text{receptor}]}{[\text{drug - receptor complex}]}$$



So, nature of the drug receptor interactions can be understood by trying to figure out what the stability of the drug receptor complex looks like. So, the most stable the drug receptors complexes, the more difficult for it to dissociate. In order to understand this, we need to define a term know as dissociation constant. So the assumptions that we will be making, is the drug is in equilibrium with the receptor. What it means is that the drug interacts with the receptor and forms a receptors complex drug receptor complex and then it again dissociates to give you the drug and the receptor again.

So this much like a chemical equilibrium that were used to can be define by an equilibrium constant which is K_d. So K_d is nothing but the concentration of the drug multiplied by the concentration of the receptor, divided but the concentration of the receptor complex much like an equilibrium A plus B going to C, the equilibrium constant is define as concentration of C divided

by concentration of A and concentration of B. Now if you look at the reverse of this process is what you would get for the drug receptor complex.

(Refer Slide Time: 1:56)

$$K_d = \frac{[\text{drug}]\,[\text{receptor}]}{[\text{drug - receptor complex}]}$$

Low

High

The smaller the dissociation constant, the drug-receptor complex is in greater concentration... which also means that the drug has high affinity for the receptor!

The median dissociation constant for inhibitor drugs in the market is 20 nM

nM

Let us now look at the factors that determine the drug-receptor affinity...

Now, the smaller the dissociation constant K_d that means that the drug multiplied by the receptor concentration is much larger compare to the drug receptor complex concentration that means the this value is low and the this value is high. Then when you will divide this two, you will get a small number. That means that the drug receptor complex is extremely large in concentration, which also means the drug has very high affinity towards the receptor. So, the median dissociation constant for inhibitor drugs in the market is about twenty nano molar.

So what is a nano molar? Nano molar is a is consider molar and then if I divide this by thousand I get milli molar, which again I divide by thousand I get micro molar and then finally if I divided by thousand I get nano molar. So nano molar is an extremely a small number and therefore the dissociation constant for this drugs is quite low.

(Refer Slide Time: 3:22)

$$\Delta G^0 = -RT \ln K_{eq}$$

The spontaneous formation of a bond between atoms occurs with a decrease in free energy, that is, a non-covalent bond will occur only when there is a negative ΔG, which is the sum of an enthalpic term (ΔH) and an entropic term ($-T\Delta S$).

$$\Delta G = \Delta H - T\Delta S$$

Now let us look at some other that determine the affinity of the drug to the receptor. And before that let us define, since it is an equilibrium constant, K equilibrium as we have studied in fundamental thermodynamics. The equilibrium constant is related to the free energy by the following equation. Delta G knot equals to minuses RT ln K.

So the spontaneous formation of a bond between atoms occurs with the decrease in a free energy. And similarly when a drug binds to receptor, there is a non-covalent bond that will occur, which also must have a negative free energy term. And we already know that delta G is nothing but the sum of an enthalpic term and the entropic term.

That is delta G equals to delta H minus T delta S. So we will be not be going into too many details about the enthalpic components and the entropic components, but you can break it down into and try to understand that all of this factors are going to contribute to how well the non-covalent interactions are happening to make the drug bind to the receptor.

(Refer Slide Time: 4:33)

Receptor-Drug Interactions

The affinity of a drug for a receptor is a measure of how strongly that drug binds to the receptor.

Efficacy is a measure of the maximum biological effect that a drug can produce as a result of receptor binding.

It is important to appreciate the distinction between affinity and efficacy.

So, the affinity of a drug for a receptor is measure of how strongly the drug binds to the receptor. We shall define another term known as efficacy. Efficacy is a measurement of the maximum biological effect that a drug can produce as a result of receptor binding. So, we have looked that in detail in the past several lectures that once the ligand binds to the receptors its results in a conformation change which can activate certain a cascade of processes which results to signaling.

So, efficacy is how well these biological effects occur. Whereas, affinity is how well a drug binds to a receptor. So it is important to understand the differences between these two. Just because a drug has high affinity towards receptor, it does not mean it is going to have high efficacy.

(Refer Slide Time: 5:23)

Affinity, Efficacy and Potency

A compound with high affinity does not necessarily have high efficacy. For example, an antagonist can bind with high affinity but have no efficacy.

The potency of a drug refers to the amount of drug required to achieve a defined biological effect—the smaller the dose required, the more potent the drug. It is possible for a drug to be potent (i.e. active in small doses) but have a low efficacy.

So, for example an antagonist which can bind with high affinity but it can have no efficacy that means it may have no effect on the signaling cascade. Lastly we will look at what is known as potency. Potency of a drug refers to the amount of a drug required to achieve a defined biological effect.

So let us say that defined biological effect is innovation of proliferation that is how to slow down growth of cancer or decrease in pain or increase in healing or any process that we would define as a biological effect. So the potency refers to this and the smaller the dose required the more potent the drug. Off course you will appreciate that it is possible for a drug to be potent, but have very low efficacy.

(Refer Slide Time: 6:24)

Determination of Affinity

Affinity can be measured using a process known as radioligand labelling .

A known antagonist (or ligand) for the target receptor is labelled with radioactivity and is added to cells or tissue such that it can bind to the receptors present.

Once an equilibrium has been reached, the unbound ligands are removed by washing, filtration, or centrifugation.

Affinity offer drug to receptor can be measure using a process called as radio ligand labeling. So what we do in this process that we take a ligand and we label it with a radioactive isotope. And since the radioactive isotopes are easy to detect, it would be easy for us to understand how well the radio ligand is bound towards the receptor. So if we take an antagonist or a ligand and take one of the atoms in the antagonist of the ligand and label it with a radioactive isotope now when you add this to a cell in culture or tissue, now what would happen? Is that it would go and bind to this cells or tissue or receptors on this cells or tissues.

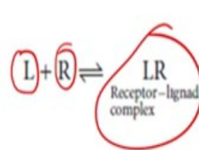
Now you wait for some time until an equilibrium has been reached. Then what we do is, we wash of the excess ligand, we filter it or centrifugation and then remove to a few washings and make sure that there is no unbound ligand that is present. Now if you measure the radioactivity that will give you an estimate of how much of drug is bound to the receptor in the cells or tissue.

(Refer Slide Time: 7:39)

Determination of Affinity

The extent of binding can then be measured by detecting the amount of radioactivity present in the cells or tissue, and the amount of radioactivity that was removed.

The equilibrium constant for bound versus unbound radioligand is defined as the dissociation binding constant (K_d) .



$$K_d = \frac{[L] \times [R]}{[LR]}$$

The extent of binding can be measured by detecting the amount of radioactive present in the cells and the amount of radioactivity that was removed because by washing of we know how much of ligand has been recovered. So based on this we can define an equilibrium that is ligand plus receptor going to a ligand receptor complex. And this equilibrium constant much like what we define earlier is the concentration of the ligand multiplied by the concentration of the receptor divided by the concentration of the ligand receptor or LR. And this is called as the dissociation binding constant (K_d).

(Refer Slide Time: 8:21)

[L] and [LR] can be found by measuring the radioactivity of unbound ligand and bound ligand, respectively, after correction for any background radiation...

However, it is not possible to measure [R], so we have to carry out some mathematical manipulations to remove [R] from the equation.

Now the concentration of ligand we can find because we know how much has been added and how much had been recovered. The concentration of LR can be found by measuring the radioactive of the bound ligand. And then off course we need to correct for any background radiations, but we have no clue as to what the concentrations of the receptors.

So therefore we have to use some mathematical manipulations to understand or to either measure R or to either remove R from this equation. So we will now look at how this is achieved.

(Refer Slide Time: 8:59)

The total number of receptors present must equal the number of receptors occupied by the ligand ([LR]) and those that are unoccupied ([R])

$$[R_{tot}] = [R] + [LR]$$

This means that the number of receptors unoccupied by a ligand is

$$K_d = \frac{[L] \times [R]}{[LR]} \qquad [R] = [R_{tot}] - [LR]$$

$$\frac{[\text{Bound ligand}]}{[\text{Free ligand}]} = \frac{[LR]}{[L]} = \frac{R_{tot} - [LR]}{K_d}$$

The total number of receptors present must be equal to the number of receptors occupied by the ligand that is concentration LR plus those that are unoccupied that is R. So we define a term which is R total which is the sum of R and LR. What this means is that, the number of receptors

that are unoccupied by ligand that is R is a difference between the total number of receptors and the ligand bound receptors.

(Refer Slide Time: 10:02)

The total number of receptors present must equal the number of receptors occupied by the ligand ([LR]) and those that are unoccupied ([R])

$$\frac{[\text{Bound ligand}]}{[\text{Free ligand}]} = \frac{[LR]}{[L]} = \frac{R_{tot} - [LR]}{K_d}$$

$$K_d = \frac{[L] \times [R]}{[LR]}$$

This means that the number of receptors unoccupied by a ligand is

$$[R] = [R_{tot}] - [LR]$$

And as we know from our previous equation K_d is defines as shown. Now doing a little bit of jugglery here, we can come up with this equation where the concentration of the bound ligand divided by concentration of free ligand that is LR divided by L is R total minus LR that the term I get from here divided by K_d and the total number of receptors present must equal therefore this sum of LR and R.

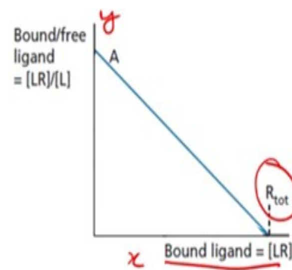
(Refer Slide Time: 10:11)

- We are still faced with the problem that K_d and R_{tot} cannot be measured directly.
- However, these terms can be determined by drawing a graph based on a number of experiments where different concentrations of a known radioligand are used.
- [LR] and [L] are measured in each case and a Scatchard plot is drawn which compares the ratio [LR]/[L] versus [LR].

So we are still faced with the problem that K_d and R total cannot be measure directly. However these terms can be determined by drawing a plot or a graph. So what we do we carry a number of experiments where we use different concentrations of a known radio ligand. And then we do what is known as a scatchard plot. A scatchard plot is nothing but a plot of LR and L and what we do is we plot the LR divided by L versus LR. Now let us look at what happens when we do this.

(Refer Slide Time: 10:46)

- This gives a straight line; the point where it meets the x-axis represents the total number of receptors available (R_{tot}).
- The slope is a measure of the radioligand's affinity for the receptor and allows K_d to be determined.

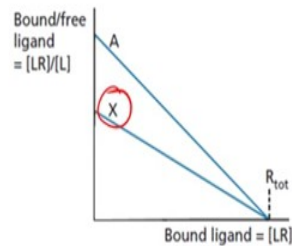


$$K_d = \frac{[L] \times [R]}{[LR]}$$

So on the y axis and this is the x axis, on the y axis is the concentration of the bound ligand that is LR divided by the concentration of the free ligand. And as we have looked at earlier, using this radioactive experiment we can find out the concentration of the bound ligand and the concentration of the free ligand. And if you plot that verses the concentration of the bound ligand alone, then you will expect us to see a straight line which is shown and the slope is the measure of K_d and the intercept here gives you an idea of total number of receptors that are present. So using this plot which is known as a scatchard plot, one can determine R total as well as K_d.

(Refer Slide Time: 11:40)

- This is done by repeating the radioligand experiments in the presence of the unlabelled drug.
- The drug competes with the radioligand for the receptor's binding sites and is called a displacer .
- The stronger the affinity of the drug, the more effectively it will compete for binding sites and the less radioactivity will be measured for [LR].
- This will result in a different line in the Scatchard plot.

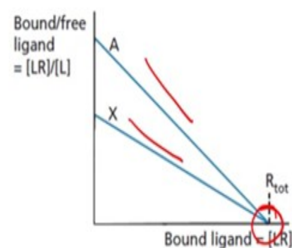


Now if you repeat this experiment in the presence of the unlabeled drug that is this is a new molecule that we want to design or we have designed or synthesized and we want to study the efficacy then if the drug competes with the radio ligand for the receptor's binding sites, it is called a displacer. Then what you would expect to see is a plot such this with X over here and you will see a decrease in the concentration of LR by L because you are competing for the sites.

The stronger the affinity of the drug, the more effectively it will compete for binding sites and the less radioactivity will be measured for LR. So what happens is that you get a different line in the scatchard plot.

(Refer Slide Time: 12:33)

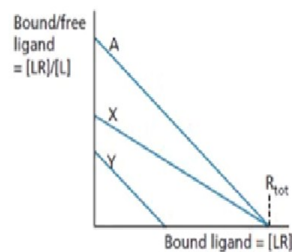
- If the drug competes directly with the radiolabelled ligand for the same binding site on the receptor, then the slope is decreased but the intercept on the x-axis remains the same (line X in the graph).
- In other words, if the radioligand concentration is much greater than the drug it will bind to all the receptors available.



If the drug competed directly with the radiolabelled ligand for the same binding site then the slope that we would see goes down, but then the intercept would remain the same because as you increase the concentration of the natural ligand, at some point you are going to displace all of the inhibitor and you are going to get a same concentration of R total. In other words, it the radio ligand concentration is much higher than the drug will bind to all of the receptors available. So you should now keep in mind, that this is very similar in perspective was applied to the enzyme inhibition as well.

(Refer Slide Time: 13:19)

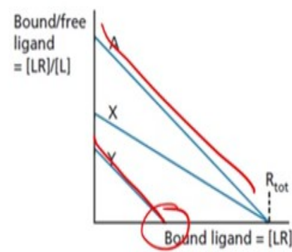
- Agents that bind to the receptor at an allosteric binding site do not compete with the radioligand for the same binding site and so cannot be displaced by high levels of radioligand.
- However, by binding to an allosteric site they make the normal binding site unrecognizable to the radioligand and so there are fewer receptors available.



Now agents that bind to the receptors at an allosteric do not compete with the radio ligand for the same binding site and therefore they cannot be displaced by high levels of radio ligand. However by binding to an allosteric site, they make the normal binding site unrecognizable for the radio ligand and so there are fewer receptors available.

(Refer Slide Time: 13:48)

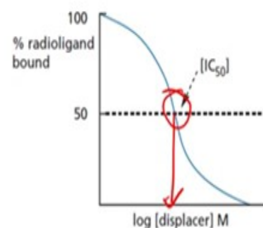
- This results in a line with an identical slope to line A, but crossing the x-axis at a different point, thus indicating a lower total number of available receptors



So since there are fewer receptors are available and the activity is happening the same way the slope of this remains unchanged. That means if you have a slope with respect to the original ligand the slope remains the same. But this concentration LR goes down.

(Refer Slide Time: 14:03)

- The data from these displacement experiments can be used to plot a different graph which compares the percentage of the radioligand that is bound to a receptor versus the concentration of the drug (or displacer).
- This results in a sigmoidal curve termed the displacement or inhibition curve , which can be used to identify the IC 50 value for the drug (i.e. the concentration of compound that prevents 50% of the radioactive ligand being bound).



The data from this displacement experiments can be used to plot a different graph from which we can calculate some important parameter. So what we do is, we plot the percentage of radiolabelled ligand bound versus the concentration of the new drug or displacer. So this and we use logarithmic term and when we plot this we get a plot as shown. Now this results in a sigmoidal curve and it is term as a displacement curve or inhibition curve.

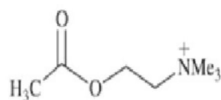
So from this we can get what is known as an IC_{50}. IC_{50} is nothing but the inhibitory concentration fifty percent, which means that it is the concentration of the compound that prevents fifty percent of the radioactive ligand from being bound. So that value is shown here and this value can be obtain by a mathematically fitting to a curve.

(Refer Slide Time: 15:03)

Hormones and neurotransmitters are important endogenous molecules that are responsible for the regulation of a myriad of physiological functions...

These molecules interact with a specific receptor in a tissue and elicit a specific characteristic response.

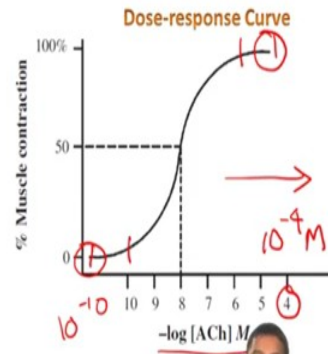
For example, the activation of a muscle by the CNS is mediated by release of the excitatory neurotransmitter acetylcholine



So hormones and neurotransmitters which we have looked at earlier are very important endogenous molecules which are responsible for the regulation of number of physiological functions. So this molecule interacts with specific receptors in a tissue and they elicit of a specific response.

For example we have looked at earlier; the neurotransmitter acetylcholine mediates muscle relaxations by through the central nervous system.

(Refer Slide Time: 15:33)



So if one would measure the percentage of muscle contraction as a function of dose of acetylcholine, what you would find that very low doses of acetylcholine you would find that there is very minimal response. And as the dose of acetylcholine goes up, you would see that the function or muscle contraction goes up.

Let me explain this plot a little bit more in detail. What we have done here on the x axis we have taken a negative log of concentration of acetylcholine. So in other words what this number four means is that, it is ten power minus four molar and here ten means ten power minus ten molar. So as we know ten power minus ten is a number which is significantly smaller than ten power minus four. So along the X axis if I go from left to right the concentration is going up.
So as the concentration goes up, the percentage muscle contraction is also going up. And you see that there is a region where there is not much of an effect and then after it goes up there is not much of an effect beyond a particular concentration. So it is likely at this top concentration the all the receptors sites are pretty much saturated and therefore no further muscle contraction possible.

Now as most of the receptors become occupied, the probability of additional drug receptors interactions goes down and the curve deviates some linearity and you get there a sigmoidal curve. Again using mathematical fitting we can obtain the very important terms that we can for example we can obtain an EC_{50} or an efficacy concentration fifty.

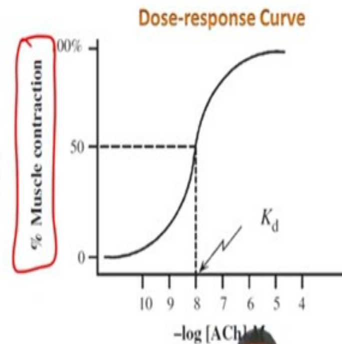
(Refer Slide Time: 17:32)

Kd = the concentration of test compound that gives half-maximal binding;

EC_{50} = the [effective] concentration of drug that elicits 50% of the total biological response

can be determined from the concentration–response data...

EC_{50} is a common standard measure for comparing potencies of compounds that interact with a receptor and elicit a particular biological response.



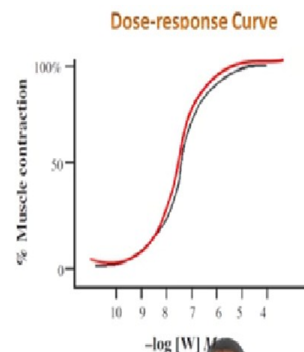
Now K_d as we have looked at earlier is the concentration of test compounds which gives the half maximal binding. EC_{50} is the effective concentration of drug that elicits fifty percent of the total biological response. So the biological response that we are looking at is muscle contraction. K_d we have looked at earlier as to how well it interacts with the receptors.

Therefore, from this concentration response data or dose response curve we can measure EC_{50} of the agonist or ligand.

(Refer Slide Time: 18:30)

For an agonist, when we plot % response versus concentration, we should get a curve that is similar to the natural ligand

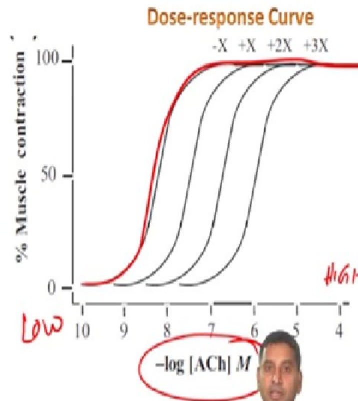
EC_{50} gives us an estimate of how good the compound is when compared with the natural ligand!



For an agonist when we plot percentage of response versus concentration, we should get a curve that is similar if not identical to the natural ligand. So here is a hypothetical plot of an agonist and the original curve is shown here in red. So as you can see there is a very good overlap of the

original natural ligand as well as the agonist. So if I determine the EC_{50} here, it should be very similar in number. So we will look at some examples in during problems.

(Refer Slide Time: 18:49)



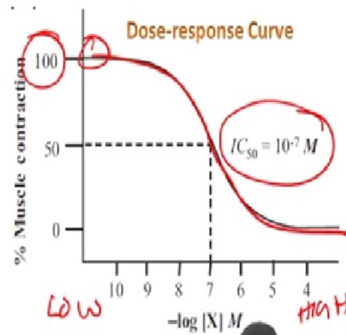
So now let us look at what happens when we have a competitive antagonist. So antagonist by its self will not show any response and when we add the natural ligand here when there is no antagonist involve you will see a curve like this which is identical to what you would see earlier. Now when you start adding a small amount of an antagonist X much like what we would see in a competitive inhibitor, you would see that this X starts to competes with the receptor binding sites.

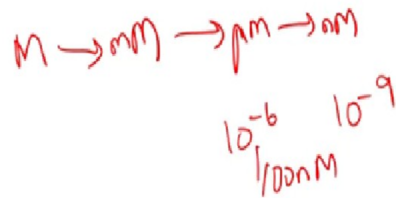
Therefore you would need a larger concentration of a natural ligand. Again keep in mind this is high concentration and this is low concentration. You would need a larger concentration of the natural ligand which is acetylcholine to see a response and therefore you would see a curve like this. Now as we add more and more of the antagonist you will find that the curve shifts towards the right.

Keep in mind since this is a competitive antagonist, at some concentration of acetylcholine you would expect that you would still see maximum response. That means that the top part of the curve remains more or less unaltered, except that the curve shifts towards the right.

(Refer Slide Time: 20:12)

The effect of varying concentration of a competitive antagonist X in the presence of a fixed, maximally effective concentration of agonist is studied... gives us an estimate of inhibitory potency



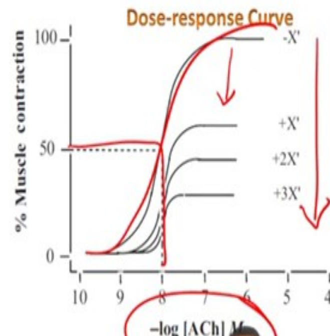


So we could also do this experiment in a different way. What we will do is we can take a single dose of acetylcholine and vary the concentration of the drug. So what we would see is that we take a very much high dose of acetylcholine and then when their concentration of the ligand of antagonist is very low, you would see no effect that means you will see hundred present muscle contractions.

Now as you slowly increase the concentration of the antagonist that means again here the concentration is low and this is high. As you increase the concentration of the antagonist you will find that at some point you will start seeing decrease in muscle contraction. And again as you go forward and you go to very high concentration of the antagonist, you will find that there is no muscle relaxation, because all the receptors sites are now being occupied by the antagonist. From the curve, again by curve fitting we can find out what the IC_{50} is.

So the IC_{50} value here is ten power minus seven molar. Like we looked at earlier, molar divide by thousand goes to milli molar, then it goes to micro molar, and then it goes to nano molar. So ten power minus nine is nano molar, ten power minus six is micro molar. So this compound is about between a micro molar and nano molar efficacy. So if you want to convert this, the value this should be hundred nano molar, which is not necessary a bad number.

(Refer Slide Time: 22:01)



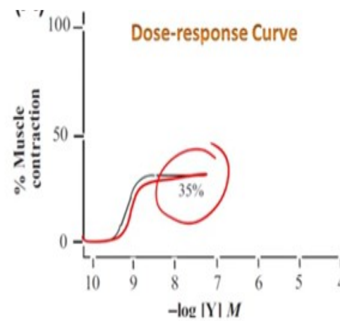
For non-competitive antagonist, what will happen is that, since it is we have already looked at the characteristics of a non-competitive antagonist. What would happen is that the maximal response will go down. So again you see percentage of muscle contraction with varying doses of acetylcholine, you will find a similar curve.

And when you add the non-competitive antagonist the maximal dose will go down, that is the maximal response will go down, because it is not competing for the binding site. So two different binding sites may actually be involved, so one where the non-competitive antagonist binds and because it an allosteric binding site, that the actual binding site of a acetylcholine is good to be altered and because of this alteration you will not see the same effect.

So when you have a non-competitive antagonist, what you will end up seeing the maximal dose of the or maximal response in this case muscle contraction will go down as you increase the concentration of the antagonist. But the IC_{50} here will remain the same, because what we are doing for IC_{50} is we are taking the maximum inhibitory concentration and then we are finding out fifty present of that value and so the IC_{50} will remain more or less the same.

(Refer Slide Time: 23:29)

For compound Y, when added, some response is elicited, but not a full response, regardless of how high a concentration of Y is used, then Y is called a partial agonist



A partial agonist has properties of both an agonist and an antagonist.

Now, when you see, take a hypothetical compound Y and when you add it and if you see a response such as this, where you see some increase in muscle contraction, then we would call this as a partial agonist. We have already looked at some of the aspects of some of a partial agonist before and partial agonist is somewhere between basically an agonist as well as the antagonist.

(Refer Slide Time: 23:56)

Sensitization and desensitization

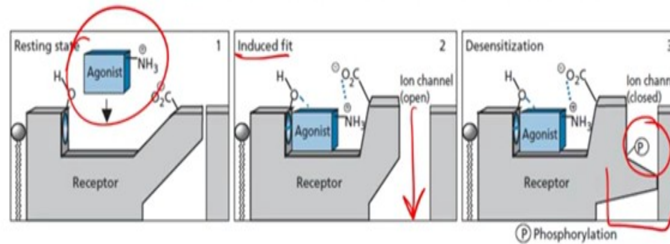
Desensitization can occur by a number of mechanisms.
Some drugs bind relatively strongly to a receptor and switch it on, but then subsequently block the receptor after a certain period of time.

Thus, they are acting as agonists, then antagonists.

Now finally let us look at this concept of sensitization and desensitization. So after the receptor has done his job, if there is a large concentration of a ligand, sometimes the receptors becomes insensitive to transmitting the signals. So this process is called as desensitization.

(Refer Slide Time: 24:31)

Sensitization and desensitization



The mechanism of how this takes place is not clear, but it is believed that prolonged binding of the agonist to the receptor results in phosphorylation of hydroxyl or phenolic groups in the receptor.

Desensitization can occur by number of mechanisms, so some of the mechanisms that are acting is by through phosphorylation of certain residues. So let us look at what happens here. So when let us say you have an agonist which is binding to a receptor site and there is a induce fit and you get the activity that we have is a ion channel that is open but now if the ion channel is opened for too long or if the agonist does not dissociate, then the cell response in a way to prevent further ions from getting in by doing post translational modification such as phosphorylation. Once this phosphorylation occurs that induces a conformational change which closes the channel.