

## **Pharmacology Definitions**

### **Agonist**

An agonist is any molecule, large or small, that is able to act as a ligand and bind to a receptor by one of a variety of mechanisms, such as covalent linkage, ionic interaction, hydrogen bonding, Van der Waals forces, hydrophobic interactions or the zipper mechanism, which results in the receptor being taken from its resting state to an activated state which consequently results in a response from the cell. If the agonist can give a maximum response it is called a full agonist, whereas a partial agonist is not able to achieve the maximum response even when all the receptors are occupied. This is due to differences in efficacy. Agonists have both affinity for the receptor and efficacy with respect to a response.

### **Antagonist**

An antagonist is any molecule that is able to act as a ligand and bind to a receptor, by one of the methods mentioned above. The binding of an antagonist results in no response from the cell. The antagonist shows no selectivity for the activated state of the receptor and hence the receptor is not activated. Antagonists show affinity for the receptor binding site, having a complementary structure, however have zero efficacy and thus no response is ever achieved by its binding. There are several forms of antagonism, the main ones being reversible and irreversible competitive antagonism, and non-competitive antagonism.

### **Competitive binding**

Competitive binding describes the event whereby there are two different molecules which have very similar but not identical chemical structures. Both structures are complementary to, and will have affinity to bind to, an effector such as a receptor or enzyme, however normally only one will be able to produce a response. This situation can be considered using competitive antagonism as an example. A competitive antagonist, if present, will compete with agonist to bind to a receptor, without activating it – but in such a way to prevent the binding of the agonist. That is, the agonist and antagonist are in competition to bind to a receptor since the receptor can only bind to one of them at a time. At any agonist concentration, there will be reduced receptor occupancy by agonist in the presence of antagonist. However, because the two are in competition, raising the concentration of agonist will allow agonist occupancy to increase. This form of antagonism is surmountable and maximum response and occupancy can be achieved by raising the agonist concentration enough.

### **Non-competitive binding**

This describes the event where there are two molecules that are able to bind an effector molecule, such as a receptor or enzyme. The molecules need not be similar with respect to chemical structure, since their binding sites to that effector are not the same. That is, the two molecules bind to different positions on their effector. Normally one of the molecules is required to activate the effector, whilst the other is an inhibitor and acts to prevent the action of the effector. Hence when considering non-competitive antagonism as an example it would mean that the antagonist does not

bind to the receptors activation site and can bind either elsewhere on the receptor preventing, for example a conformation change from taking place and activating intracellular effectors, or may in fact have an effect to block some part of the downstream events from receptor activation between it and the response. So there is not competition between the molecules for binding the receptor. However, an agonist may bind to the receptor, but with no response since the antagonist is blocking the transformation to the activated state by binding elsewhere on the receptor. The distinguishing feature of non-competitive binding in comparison to competitive, is that non-competitive is non-surmountable and it is not possible for a maximum response to be achieved in the presence of a non-competitive antagonist.

### **Affinity**

Affinity describes the ability or tendency of an agonist to bind to its receptor to form a complex. It can be quantified in the form of the affinity constant. This is simply the rate constant for the equilibrium:



This is numerically  $[DR] / [D][R]$ . It represents a measure of the likelihood of the forward reaction taking place – that is, the formation of a drug – receptor complex from the individual components. Numerically, the affinity constant is the reciprocal of the drug/agonist concentration that produces 50% occupancy of receptors. The higher the affinity is, the lower this concentration will be, hence the higher the affinity constant.

### **Efficacy**

Efficacy describes the power or capacity of the agonist to produce an effect. Note there is no direct relationship between affinity and efficacy, and, for example, a 50% occupancy (relating to the affinity) does not result in a 50% response. Efficacy can also be defined thus: efficacy describes the ‘strength’ of a single drug-receptor complex in evoking a response of the tissue (a way of quantitatively comparing agonists efficacy – especially if they are for the same receptor – e.g. the different efficacies of full and partial agonists). A more specific example: consider a group of similar molecules with the ability to bind the receptor. Upon binding, the channel is opened, however the mean channel open time varies with agonist, and since the ion flux is constant, a different total amount of ion movement is observed. This results in different levels of response – that is, a way of defining efficacy of such agonists. Put in another way, efficacy is a property determined by the affinity of the agonist for the active state of its receptor.(NOT the affinity to bind to the receptor, however) – the greater the relative affinity for  $R^*$  with respect to  $R$ , the greater the efficacy of the agonist (part of the ‘Two State theory’).

## Discussion of some important equations

**Saturation Equation** 
$$\alpha = \frac{B}{B_{\max}} = \frac{k_1[D]}{1 + k_1[D]}$$

This equation is based on the simple relationship:  $[D] + [R] \rightleftharpoons [DR]$  where D represents the drug and R the receptor for which it is an agonist. The assumptions that are being made here are that i) the binding of D to R is in a 1 – 1 stoichiometry, and ii) that the concentration of D is much in excess than R so that there is little effect on [D] upon binding receptor.

By rearranging this to form the rate equation, a constant is introduced and that is represented by  $k_1$  which is known as the affinity constant and is defined as the reciprocal of the drug concentration that produces a 50% occupancy of the receptors. To get from the rate equation to the saturation equation, you substitute in a factor,  $\alpha$ , which is the fraction of receptors that combine with drug. Hence [DR] becomes  $\alpha$  and the [R] at equilibrium becomes  $(1 - \alpha)$ . Hence the resulting expression is:

$$k_1 = \frac{\alpha}{[D] + (1 - \alpha)}$$

Rearrangement gives the Saturation equation. This expression is a

hyperbolic function. By using a radiolabelled drug an experiment can be conducted to investigate the amount of specifically bound radioligand against the concentration of radioligand. If these data were plotted a hyperbolic curve would result which tended towards some maximum value for bound radioligand ( $B_{\max}$ ). Hence  $B / B_{\max}$  would be equal to the fraction of occupied receptors and hence  $\alpha$ . Since working with hyperbolic functions is not favourable some linear transformations have resulted, which allow linear graphs to be drawn which are easier to interpret. Two such transformations are the Double reciprocal plot and the Scatchard plot.

Such data and graphs are drawn in order to investigate  $B_{\max}$  which can not be easily and accurately identified from the hyperbolic curve. The other factor is the affinity constant (units  $M^{-1}$ ). As mentioned this is an important value to be known since it is a way of quantitatively considering the affinity of a drug for its receptor. This can be compared between drugs as a means of comparing quantitatively an aspect of their action. The reciprocal of the affinity constant is the dissociation constant which is defined as the concentration of drug that gives 50% receptor occupancy – so the higher the value the lower the affinity for the receptor.

If the affinity constant is known then the equation could be used to investigate the occupancy of receptors at different drug concentrations, this could be used to calculate a dose or drug concentration when the required occupancy of receptors for a specific response is known for, example.

**Schild equation**  $\frac{D_2}{D_1} = 1 + [A]k_2$

The question being considered is: how is the binding of drug D affected by the presence of a second drug, A, that binds to the same receptor. The rate equations for individual drugs are considered and by substituting in  $\alpha$  and  $\beta$  as the fraction of receptors occupied by D and A respectively where  $[R]$  is  $(1 - \alpha - \beta)$ , it can be seen that the two rate equations can be solved simultaneously. The resulting expression is:

$\alpha = \frac{[D]k_1}{1 + [D]k_1 + [A]k_2}$  where  $\alpha$  is the occupancy of receptors by drug D in the presence of drug A.

This analysis provides a means of determining the affinity constant of a competitive antagonist by exploiting its ability to produce a right shift in the concentration verses effect curve for an agonist.

Suppose a concentration,  $D_1$  of agonist occupies a fraction  $\alpha$  of receptors producing a particular response/effect. This will be equal to  $\alpha = \frac{k_1[D]}{1 + k_1[D]}$ .

Then consider that in the presence of a competitive antagonist a higher concentration,  $D_2$  of agonist will be needed to occupy the SAME fraction of receptors and hence produce the same effect. This is equal to the equation above for working out the occupancy of drug D in the presence of A. If you put the two expressions equalling, simplification results in the Schild equation shown above.

$D_2 / D_1$  is known as the Dose ratio and is independent of  $k_1$ . The dose ratio is defined as the ratio by which the agonist concentration has to be increased in the presence of the antagonist in order to restore a given level of response. This value can quite easily be found by carrying out experiments such that a log concentration agonist vs. effect plot can be plotted in the presence of a known amount of antagonist and without. Because there is a parallel shift of the curve, take any level of effect and find the concentration of agonist required in each cases to allow the dose response to be calculated. This makes it then possible to calculate the affinity constant for the antagonist – but it is not possible to use this method to calculate the affinity constant for the agonist.

This method for determining the affinity constant of the antagonist is successful because it does not make any assumptions about the relationship between response and occupancy – it supposes only that equal responses are produced by equal agonist occupancies, which is true.

The equation can be manipulated to achieve a linear, Schild, plot which allows the antagonists affinity constant to be derived from the x-intercept.

**Cheng Prusoff equation**  $k_u = \frac{1 + [L]k_l}{IC_{50}}$

This equation allows the binding of one radioligand to be used to determine the binding affinities of other unlabelled drugs that bind to the same receptor. Hence it allows that only one drug (per receptor type) need be radiolabelled which makes practical investigation less complicated.

It can be shown mathematically that in an experimental setup where you have labelled drug and then unlabelled drug is added, that there is preferential displacement of specifically bound drug and that the effect on non-specifically bound drug is negligible in comparison. Hence, if you have a setup whereby there is labelled drug allowed to achieve full receptor occupancy and that is the starting condition. Consequently the concentration of unlabelled drug is increased and at each step the occupancy by labelled drug is measured. A graph can be plotted of receptor occupancy by radioligand against the concentration of unlabelled drug. The graph shows a progressive fall to an asymptote, which can be considered to be the point where there is no further labelled drug specifically bound. This is not corresponding to zero readings from the recorder, since there is residual non – specifically bound radioligand.

The graph can then be used to find the point at which we displace half of the specifically bound labelled drug. The concentration of unlabelled drug that displaces 50% of the specifically bound radioligand is known as the  $IC_{50}$ .

To consider the derivation of the equation above, we need to consider the equation displayed before relating to the situation when you have two ligands binding the same receptor, where  $\alpha$  is the occupancy of drug D in the presence of drug A. If we consider that the drugs are U and L for unlabelled and labelled respectively. When the  $[U] = IC_{50}$  then statistically it can be shown that the occupancy of receptors by U is 50% (0.5). If these figures are substituted, then consequent rearranging of the equation leads to the Cheng Prusoff equation as shown above.

So experimentally this can be used to find out the affinity constants for other drugs that bind the same receptor as the labelled drug. The binding affinity and concentration are known from prior investigation. Hence upon finding the  $IC_{50}$  the affinity constant is revealed.