BASIC PRINCIPLES AND TECHNIQUES FOR RECEPTOR BINDING

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Introduction

Receptor binding studies are used to determine the affinity of various drugs for a receptor as well as the binding site density (B_{max}) of receptor families and their subtypes in different tissues or samples. These studies thus help to determine whether a drug will have therapeutic or adverse effects at different subtypes. Receptor binding studies help in mapping the distribution of receptors in different areas of the body, as well as the effects of physiological and pathological conditions on the expression of the receptors.

There are two basic types of receptor binding experiments: saturation and competition. Saturation experiments are used to determine the affinity ($1/K_d$) of a radioactive ligand for a receptor and the B_{max} of the receptor in specific tissues or samples. Competition studies are used for measuring the affinity ($1/K_i$) of unlabeled ligands for receptors. By measuring the affinity of numerous unlabeled ligands for a receptor it is possible to identify subtypes of the receptors as well as the presence of these subtypes in various tissues using correlation plots of the pK_i values.

Basic Concepts in Receptor Binding Studies

The basis of the receptor binding study is the binding of ligand (L) to the receptor (R) to form a ligand-receptor complex (LR). The ligand-receptor complex is classically referred to as Bound, meaning the amount of ligand that is bound to the receptor. The unbound ligand (L) is referred to as Free, meaning the amount of ligand that is free and able to interact with the receptor.

$$\text{Receptor (R)} + \text{Ligand (L)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Ligand-Receptor Complex (LR)}$$

$$\text{Receptor (R)} + \text{Free (F)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Bound (B)}$$

The parameter measured is the amount of radioactive ligand that is bound to the receptor. This requires separation of the free ligand from the bound ligand after the reaction has reached steady-state conditions. K_d is a measure of the affinity of a ligand (drug) for a receptor, and is equal to k_2/k_1, where k_1 is the association rate constant and k_2 is the dissociation rate constant. K_d is the concentration of radioactive ligand required to occupy 50% of the receptors. B_{max} is a measure of the density of the receptor in a tissue and is equivalent to Bound when all of the receptors are occupied by radioactive ligand (Figure 1). The equations, given on the following pages, that are used to analyze the results of binding experiments, are based on the assumption that the binding of the ligand to the receptor fits this model.

Basic Steps in Receptor Binding Studies

STEPS IN RECEPTOR BINDING

Binding of Radioactive Ligand (±Nonradioactive Ligand) to the Receptor
↓
Separation of Bound from Free
↓
Measurement of the Amount of Radioactivity Bound
↓
Analysis of the Results

Binding of the radioactive ligand to the receptor
The source of the receptor can be cell membranes, intact cells, organ tissue slices or a solubilized receptor preparation. It is important to wash the receptor preparation to remove the endogenous ligands and other small molecules that might interfere with the binding of the radioactive ligand to the

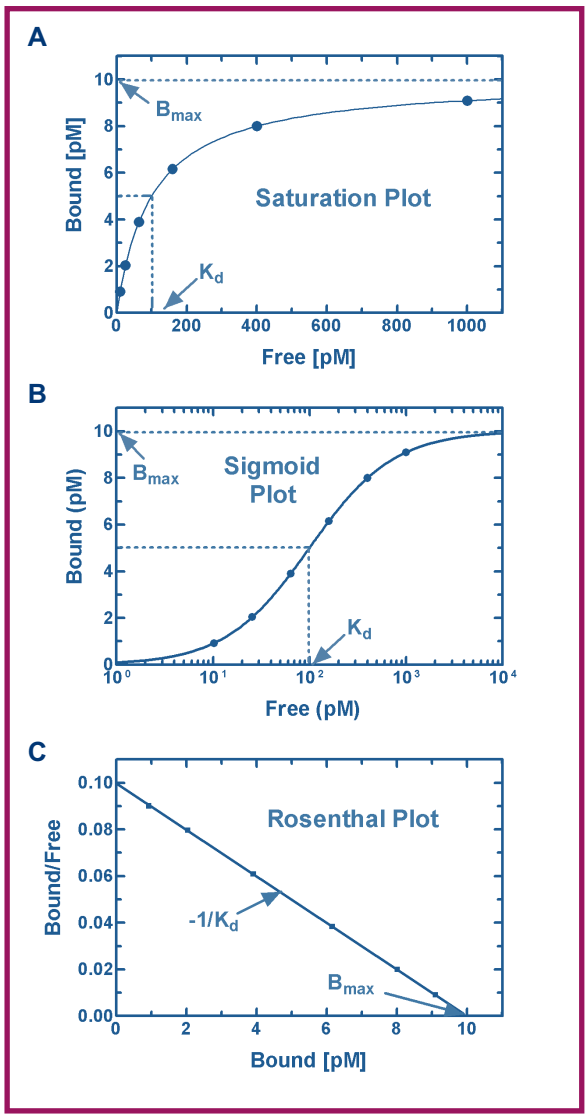

Figure 1. A comparison of saturation, sigmoid and Rosenthal plots of a saturation binding experiment
A. The results of the saturation experiment can be plotted with Bound (the concentration of radioactive ligand that is bound to the receptor) on the Y-axis and Free (the concentration of free radioactive ligand) on the X-axis. As the concentration of radioligand increases the amount bound increases until a point is reached where no matter how much more radioactive ligand is added, the amount bound does not increase further. The resulting graph is a hyperbola and is called a saturation curve. B_{max} is the maximal binding which is approached asymptotically as radioligand concentration is increased. B_{max} is the density of the receptor in the tissue being studied. K_d is the concentration of ligand required to occupy 50% of the binding sites. A non-linear regression analysis of the data in this form is used to determine K_d and B_{max} values.

B. A plot of the Bound vs. the log of the Free concentration of the drug gives a sigmoid curve. This graph is useful for visualizing data over large concentration ranges.

C. The results of a saturation experiment can be plotted as Bound vs. Bound/Free to obtain a Rosenthal Plot. The K_d value is -1/slope and the B_{max} value is the x-intercept. This graph is used for visualizing changes in K_d and not for analyzing the data. It is often mistakenly called a Scatchard plot.

receptor. The radioactive ligand used should have a high affinity (nanomolar or higher) for the receptor being studied, low affinity for other receptors and a high specific radioactivity (generally 30 Ci/mmol or higher). The radioactive ligand is usually an antagonist as these compounds generally have a higher affinity for receptors than agonists. The radioactive isotope is usually ^{3}H or ^{125}I.

The amount of receptor preparation used in the assay is based on the affinity of the radioligand for the receptor, the specific activity of the radioligand and the density of the receptor in the tissue. Ideally, 100 to 500 counts per minute (cpm) should be bound to the receptor of interest at the lowest concentration of radioligand used in the assay. The amount of radioligand that binds can be enhanced by either increasing the tissue (receptor) concentration or using a radioactive ligand with a higher specific activity, such as an ^{125}I-labeled ligand. Less than 10% of the radioligand should be bound to the tissue because the equations for analysis of a saturation experiment (Equation 1) are based on the assumption that the free concentration of ligand does not change.

The tissue is incubated with the radioactive ligand until steady-state conditions are reached (Figure 2). The time required to reach steady-state may be minutes to hours. The incubations can be done on ice, at room temperature or at 37°C depending on the stability of the radioactive ligand and tissue. Most investigators seem to prefer a temperature of 22-25°C (room temperature). It should be noted that the affinity of the receptor for the radioligand may be temperature-dependent.

Separation of the bound ligand from the free ligand

Once steady-state conditions have been reached, the bound radioactive ligand is separated from the free ligand using filtration or centrifugation techniques. The important principle to keep in mind is that the method needs to be rapid (usually less than 10 seconds) so that the bound ligand does not dissociate from the receptor. If the ligand dissociates from the receptor during separation, then the observed binding will be lower than the actual binding.

The filtration method functions by trapping the receptor-ligand complex on the filter, whilst allowing the free ligand to pass through. The amount of receptor-ligand complex formed (Bound) can be estimated by measuring the amount of radioactivity on the filter. Cell harvester-type filter machines are often used for filtering tissue homogenates because 24, 48 or 94 samples can be filtered at one time and the filters can be quickly rinsed to remove residual free ligand. Difficulties that can arise with the filtration assay are that (a) the ligands may bind tightly to the filter, and (b) the filter may become clogged (if high concentrations of protein are used) causing filtration to be too slow. If the binding of the radioligand to the filter itself is too high, different types of filters can be tried or the filter can be soaked in a 0.1% polyethyleneimine solution.[1]

Measurement of the amount of radioactive ligand bound to the tissue

The amount of radioactive ligand bound can be determined using a scintillation counter or gamma

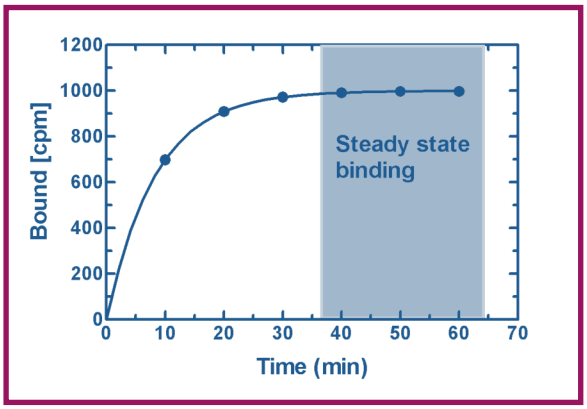

Figure 2. Time course for the binding of radioactive ligand to the receptor
Equilibrium occurs when the rate of association of the radioligand is equal to the rate of dissociation. Experimentally, it is usually sufficient to determine the time after which the Bound no longer increases, a condition called steady-state. Steady-state conditions are determined by measuring the amount of ligand bound to the receptor at various times after the start of the incubation. The data can be plotted on a graph where the X-axis is time and the Y-axis is the amount bound. When the amount of radioligand bound to the receptor no longer increases with time, steady-state has been reached. For the experiment shown, steady-state is between 40 and 60 minutes.

counter, depending on the isotope. When using filtration to separate bound from free, the filter is simply placed in a counting vial and scintillation fluid added if the isotope is tritium. Centrifugation assays are usually done using microfuge tubes. The tip of the tube containing the pellet is cut off and placed in the vial. If the isotope used is tritium, then it is important to let the sample incubate with scintillation fluid long enough for the tritium to diffuse out of the pellet or filter and interact with the scintillator.

Analysis of the results

Receptor binding data should be analyzed by non-linear regression analysis using a computer program such as Prism from GraphPad Software, Inc. (www.graphpad.com). Different types of analysis are used for saturation and competition experiments and are discussed in more detail in the next two sections.

Saturation Experiments

In a saturation experiment, various concentrations of the radioligand are incubated with the receptor, producing increasing concentrations of ligand-receptor complex (Figure 1). The equation for the resulting hyperbola is:

$$\text{Bound} = \frac{B_{max} \times \text{Free}}{K_d + \text{Free}}$$

Equation 1

K_d is the concentration where 50% of the receptors are occupied by radioactive ligand. As the concentration of radioactive ligand increases, a point is reached where the amount of ligand bound no longer increases. This is the B_{max} value and is a measure of the density of the receptor in that tissue preparation.

Specific versus non-specific binding

Unfortunately, radioactive ligands usually bind to more than one type of binding site. The site that is being studied is referred to as the specific site, or specific binding. All other binding sites are referred to as non-specific sites. Non-specific binding sites may be receptors in the same family or in other receptor families which recognize similar chemical structures. For example, the Tocris radioligand [^{3}H]-LY 341495 binds to both $mGlu_2$ and $mGlu_3$ (metabotropic glutamate) receptors. If the $mGlu_2$ receptor is being studied, then binding of [^{3}H]-LY 341495 to $mGlu_2$ receptor would be considered specific binding and binding to $mGlu_3$ receptor would be considered non-specific binding. Non-specific binding sites may also be other constituents of the tissue or even sites on test-tubes or glass fiber filters. Some non-specific binding sites are present in finite (saturable) concentrations and others are present in essentially infinite concentrations. In order to distinguish binding to specific sites from binding to non-specific sites, a second set of incubations is run simultaneously using radioactive ligand and an unlabeled ligand at a concentration sufficient to block the binding of the radioactive ligand to the specific sites, but not the non-specific sites. The amount of radioactive ligand bound in the absence of unlabeled ligand is referred to as total binding. Binding in the presence of the unlabeled ligand is referred to as non-specific binding. The difference between the two is specific binding.

Total bound - Non-Specific Bound = Specific Bound

Several factors should be considered when choosing the unlabeled ligand for defining non-specific binding. The ligand should be a competitive inhibitor that has a high affinity for the site of interest and a low affinity for non-specific binding sites. It is recommended that the unlabeled form of the radioligand not be used to determine non-specific binding as this will bind to all of the same sites as the radioactive ligand, and some of these sites will be non-specific sites.

Experimental design

In a saturation experiment, the incubation tubes are usually set up in quadruplicate with two tubes being used for total binding and two for non-specific binding at each concentration of radioligand. The same amount of buffer and tissue is added to all of the tubes. In addition, the unlabeled ligand used to block specific binding is added to the non-specific tubes.

Duplicate Total Binding Tubes	Duplicate Non-specific Binding Tubes
Buffer Tissue Radioactive ligand	Buffer Tissue Radioactive ligand Unlabeled ligand

A typical saturation experiment for a single binding site contains six concentrations of radioactive ligand, three below the K_d and three above the K_d. The highest concentration is usually 10 times the K_d. Serial 1:2.5 dilutions of the radioactive ligand are frequently used.

Tube number	Duplicate Total Binding Tubes	Duplicate Non-Specific Binding Tubes
1-4	0.1024 x K_d	0.1024 x K_d
5-8	0.256 x K_d	0.256 x K_d
9-12	0.64 x K_d	0.64 x K_d
13-16	1.6 x K_d	1.6 x K_d
17-20	4 x K_d	4 x K_d
21-24	10 x K_d	10 x K_d

As described above, the ligand is allowed to bind to the receptor until steady-state conditions are reached. The free ligand is then separated from bound ligand and the bound radioactive ligand is counted in a scintillation counter.

Analysis of data

Analysis of the data requires the subtraction of non-specific binding from total binding to give specific binding. The cpm values obtained from the scintillation counter are converted to molar units by correcting for the counting efficiency of the scintillation counter, the specific activity of the radioligand, conversion of disintegrations per minute

$$\frac{\text{cpm}}{\text{ml}} \times \frac{\text{dpm}}{\text{cpm}} \times \frac{\text{Ci}}{2.2\text{x}10^{12}\text{dpm}} \times \frac{\text{mmol}}{\text{Ci}} \times \frac{1000\text{ ml}}{\text{L}} \times \frac{1\text{ mol}}{1000\text{ mmol}} = \frac{\text{mol}}{\text{L}}$$

cpm/ Assay Tube — *Counting Efficiency* — *Specific Activity of Radioligand* — *Bound (M)*

This can be reduced to:

$$\frac{\text{cpm}}{\text{ml}} \times \frac{1}{\text{eff}} \times \frac{1}{2.2} \times \frac{1}{\text{Specific Activity}} = \text{pM Bound}$$

Equation 2

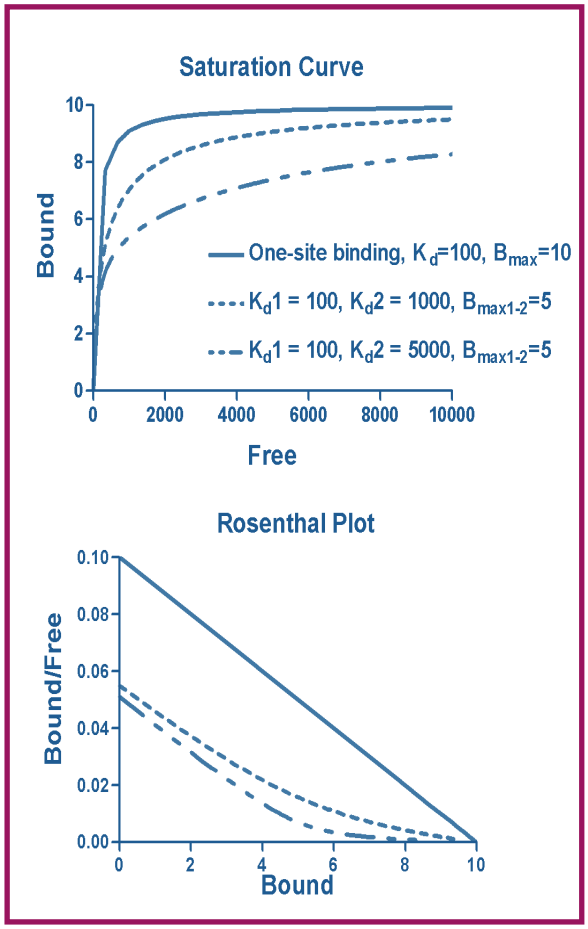

Figure 3. Comparison of saturation curve and Rosenthal plot showing one- and two-site binding
Shown are the changes in the saturation curve and Rosenthal plot for binding to a single site compared to two sites with 10- and 50-fold differences in the K_d values. As the difference in K_d and/or B_{max} for the two sites increases, the curvature of the Rosenthal plot increases. (The theoretical curves were generated using the Prism computer program for one- and two-site binding hyperbolas.)

(dpm) to Ci and conversion of ml and mmol to liters and moles, as shown in Equation 2.

The bound values are usually given in concentration units of fM or pM values. The data are plotted as Bound versus Free (Figure 1).

The K_d and B_{max} values are obtained by analyzing the data using non-linear regression techniques with the equation for a hyperbola (Equation 1). Both the K_d and B_{max} values obtained from this analysis will be in molar (M) units. The B_{max} values are converted to mol/mg of protein or mol/wet weight of tissue by dividing the B_{max} (mol/assay tube) by the concentration of protein or wet weight of tissue in the assay tube.

An alternative way to visualize the data is to plot it as Bound/Free vs Bound as a Rosenthal Plot (Figure 2).[2] If the radioactive ligand only binds to a single site a straight line is obtained. The slope of the line is equal to $-1/K_d$ and the x-intercept is B_{max}. The data analysis is not as accurate using this plot because the Bound value is in both axes thus amplifying any error in the Bound measurement. However, this graph helps one to visualize changes in K_d (changes in the slope) or B_{max} (changes in the x-intercept) that are produced by different experimental conditions. In addition, a non-linear Rosenthal plot suggests the possibility of two (or more) binding sites. This type of plot is often mistakenly called a Scatchard plot,[3,4] particularly by those who have not read Scatchard's paper.[5] The Scatchard derivation assumes a single species of binding macromolecule of known molecular weight and concentration. When these values are not known, as is the case in most receptor binding studies, it is appropriate to reference the Rosenthal derivation.[2]

Two-site binding
At times, the radioactive ligand will bind to two saturable binding sites. This occurs when there are two receptors (or other binding sites) in the tissue to which the ligand binds with different affinities. It is difficult to detect two-site binding by looking at a saturation curve. However, the Rosenthal plot of two-site binding sites will be curvilinear (Figure 3). The equation for the two-site model is:

$$\text{Bound} = \frac{B_{max}^{1} \times \text{Free}}{K_d^{1} + \text{Free}} + \frac{B_{max}^{2} \times \text{Free}}{K_d^{2} + \text{Free}}$$

Equation 3

Competition Experiments

Most receptor ligands are not available in a radioactive form, therefore there is no way to directly measure their affinity for a receptor. The affinity of the unlabeled ligand for the receptor can be determined indirectly by measuring its ability to compete with, and thus inhibit, the binding of a radioactive ligand to its receptor. In a competition experiment, various concentrations of an unlabeled ligand compete with a fixed concentration of a radiolabeled ligand for binding to the receptor. As the concentration of unlabeled ligand increases, the amount of radioligand bound to the receptor decreases, as illustrated in Figure 4. The binding parameter obtained from this experiment is the concentration of unlabeled ligand that inhibits the binding of the radioactive ligand by 50% (IC_{50} value). The dissociation constant for the unlabeled ligand for the receptor is often referred to as the K_i rather than K_d because it is obtained from inhibition experiments rather than saturation experiments. The K_i value for the unlabeled ligand can be obtained from the IC_{50} value using the Cheng-

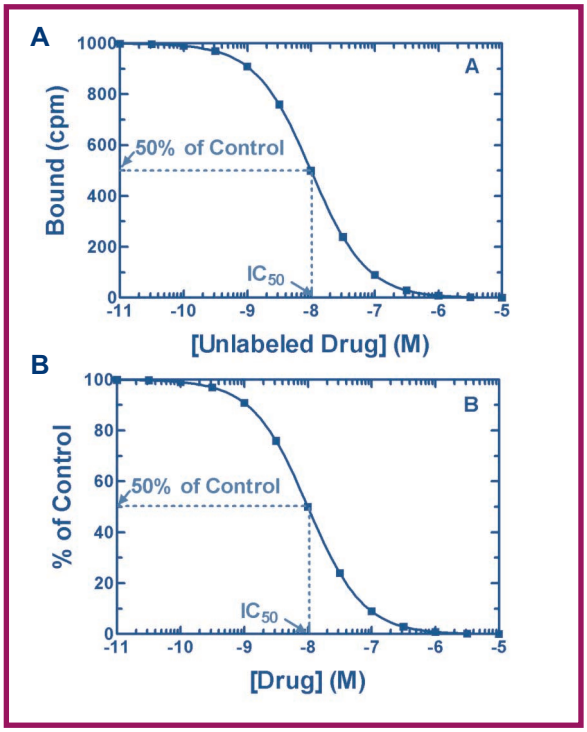

Figure 4. Competition plots
Various concentrations of unlabeled ligand are incubated with the receptor in the presence of a fixed concentration of radioactive ligand. The IC_{50} value is the concentration of unlabeled ligand that inhibits the binding of 50% of the radioactive ligand to the specific binding sites. A. The units for Y-axis are Bound (cpm). B. The units for the Y-axis are Percent of Control where the percent of control is the amount bound in the presence of a specific concentration of unlabeled ligand divided by the amount bound in the absence of unlabeled ligand x 100%.

Prusoff equation (Equation 4)[6] where L is the concentration of radioactive ligand used and K_d is the affinity of the radioactive ligand for the receptor.

$$K_i = \frac{IC_{50}}{1 + \frac{L}{K_d}}$$

Equation 4

Experimental design

The concentration of radioactive ligand should be approximately 0.8 x the K_d. If a higher concentration of radioactive ligand is used, then higher concentrations of unlabeled ligand will be required to inhibit the binding of the radioactive ligand, and the Cheng-Prusoff correction to the IC_{50} value will become quite large. At lower radioligand concentrations, there may not be sufficient binding to obtain reliable data. K_d values are affected by buffers,[7] metal ions and properties of the tissue, therefore the K_d values for the radioactive ligand should be determined using the same experimental conditions as those used for the competition experiments.

Competition assays are usually carried out using duplicate assay tubes, each containing the same amount of radioactive ligand, tissue (receptor) and buffer. The first two tubes are used as control tubes and they contain no unlabeled ligand. The last two tubes often contain the unlabeled ligand that is used to determine non-specific binding in saturation experiments. Ten concentrations of unlabeled ligand are usually sufficient to define an inhibition curve for a ligand that binds to a single site. The data are plotted on a log rather than linear plot, therefore it is helpful to use a series of 10- and 30-fold dilutions of the unlabeled ligand over a 10^5-fold concentration range, as shown in Figure 4.

As with the saturation experiments, the competition experiments are terminated after steady-state conditions have been reached. The free radioactive ligand is separated from bound ligand in the same manner as for the saturation experiments, and the samples are counted.

In some experiments, the labeled and unlabeled ligands used in the competition experiments are the same and have the same affinity for the receptor. This is sometimes called a homologous binding experiment, and is conceptually identical to a saturation curve where the specific activity of the radioligand is different at each concentration.[8] If a very large concentration range in saturation

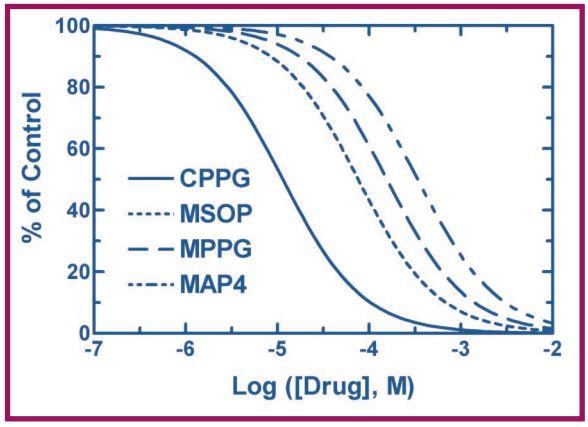

Figure 5. Simulation of competition curves with various inhibitors

Curves for competition of **CPPG, MSOP, MPPG and MAP4** with **[^{3}H]-LY 341495** for the human $mGlu_6$ are shown. (These curves were generated using the K_i values reported by Wright *et al* (2000)[10], a concentration of 25.3 nM for **[^{3}H]-LY 341495** and a K_d for **[^{3}H]-LY 341495** of 31.6 nM).

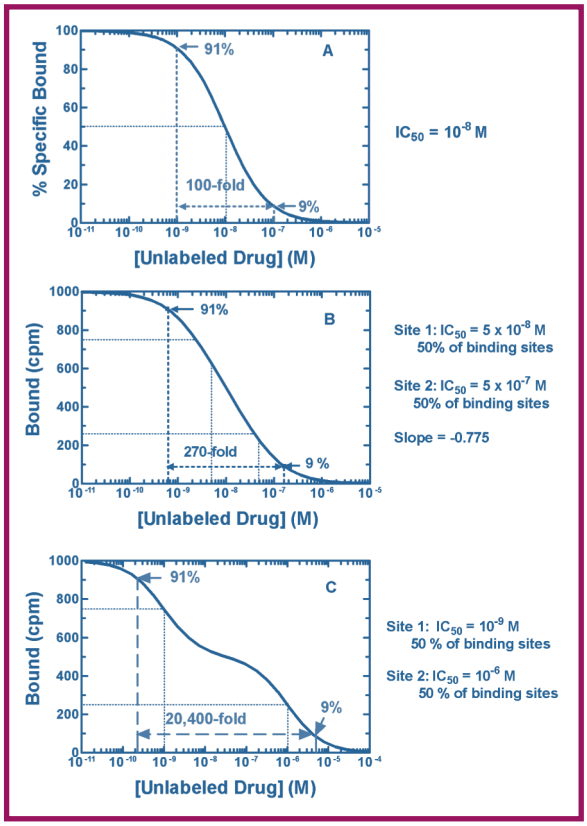

Figure 6. Comparison of one- and two-site competition curves

In one-site binding there is a 100-fold difference between concentrations of the inhibitor required to inhibit the binding of 9 to 91% of the radioactive ligand (A). In two-site binding the difference will be higher. A quick method for detecting two-site binding is to look at the difference between the concentration of inhibitor required to displace 9 and 91% of the radioactive ligand from the receptor. A difference greater than 100-fold suggests two-site binding. Another method is to look at the slope of the line. For one-site binding the slope should be one and for two-site binding it will be lower. In Figure 6B there is only a 10-fold difference in the affinity of the unlabeled ligand for the two sites. However, in Figure 6C there is a 1000-fold difference, resulting in a plateau between two slopes. The presence of a plateau is a clear indication that two-site binding is present.

Dotted lines are used in the graphs to indicate the theoretical IC_{50} values and the point of half-maximal binding to a specific site. With one-site binding these dashed lines intercept on the curve (A). However, with two-site binding (B) the lines do not intercept on the curve because binding to the low affinity and high affinity sites overlap. This is why it is important to use non-linear regression analysis to anlayze the data. In Figure C there is a 1000-fold difference in the affinity of the two sites and, consequently, there is little overlap in binding of the ligands to the two sites. (These theoretical curves were obtained using a one- and two-site competition non-linear regression analysis with the Prism computer program.)

experiment is desired, a standard saturation protocol coupled with a homologous competition experiment is useful.[9]

Analysis of the data

To visualize the results, the data are plotted on a semilog graph so that a sigmoid plot is obtained. The Y-axis can be cpm bound (Figure 4A) or percent of control (Figure 4B). Percent of control is determined by dividing the amount bound at each unlabeled ligand concentration by the amount bound in the absence of unlabeled ligand (control values). The data are then analyzed using non-linear regression analysis of the equation for a sigmoid plot (Equation 5):

$$Y = \text{Bottom} \times \frac{\text{Top-Bottom}}{1 + 10^{x\text{-Log}IC_{50}}}$$

Equation 5

where Bottom is the bottom of the curve, Top is the top of the Curve and Y is the amount of radioactive ligand bound at any concentration of unlabeled

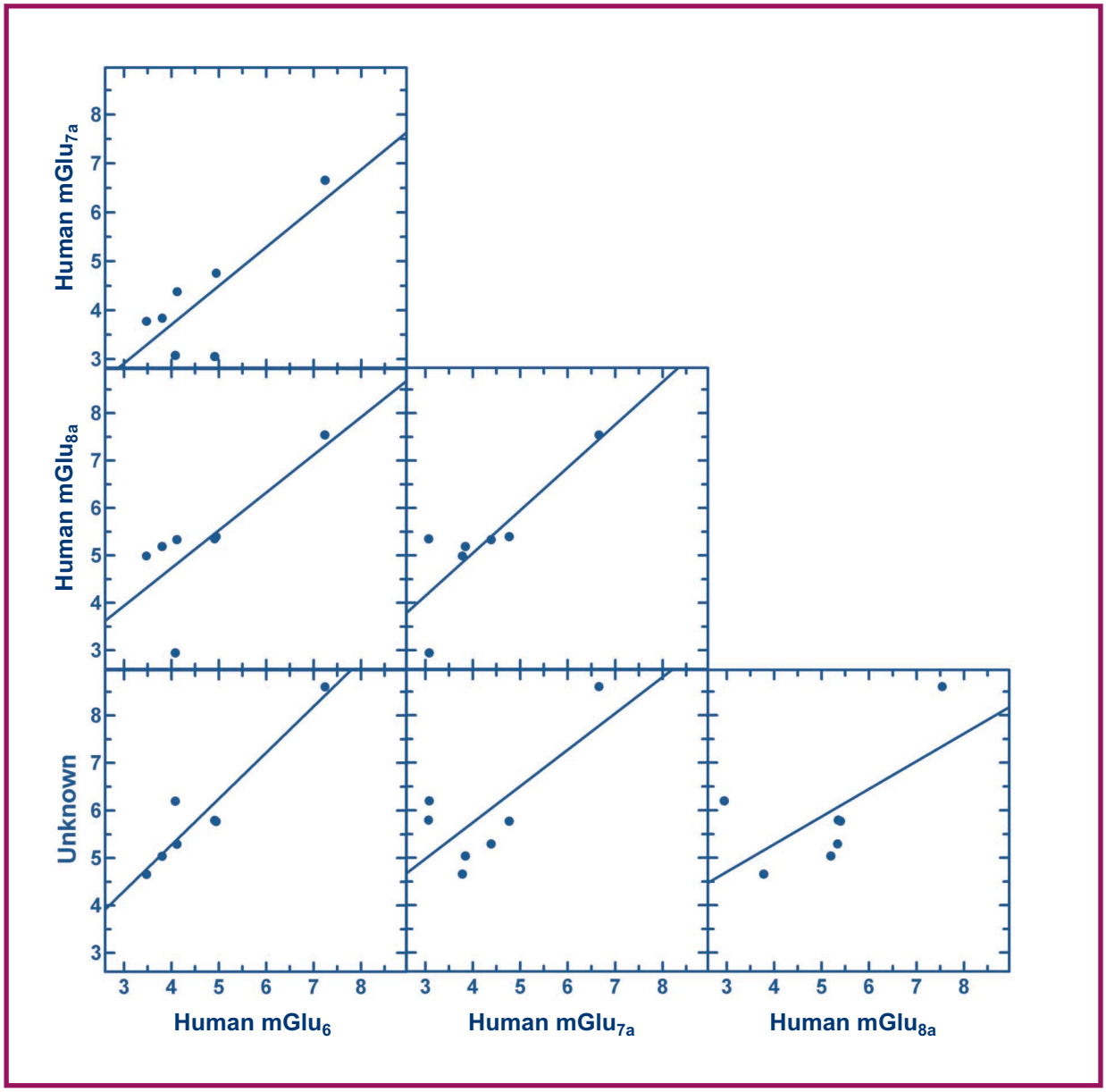

Figure 7. Correlation plots for determination of mGlu subtype in an unknown tissue
The pK_i values for **MAP4, MSOP, MPPG, CPPG, LY 341495, ACPD** and glutamate binding to human $mGlu_6$, $mGlu_{7a}$, $mGlu_{8a}$ and rat tissue were determined using competition experiments with **[^{3}H]-LY 341495** as shown in Figure 5. The pK_i values for the four antagonists for the rat tissue are compared to the pK_i obtained for $mGlu_6$, $mGlu_{7a}$, and $mGlu_{8a}$. The slopes (upper right numbers) and correlation coefficients (r; lower left numbers) determined for each graph are shown below.

Slope / r	Human $mGlu_6$	Human $mGlu_{7a}$	Human $mGlu_{8a}$	Unknown
Human $mGlu_6$		0.79	0.80	0.97
Human $mGlu_7$	0.81		0.90	0.77
Human $mGlu_8$	0.48	0.83		0.58
Unknown	0.94	0.73	0.65	

The unknown tissue appears to have $mGlu_6$ receptors because both the correlation coefficient and the slope for the correlation between the unknown tissue and $mGlu_6$ are close to unity. (The K_i values for the human mGlu subtypes used for this simulation were taken from Wright *et al* (2000)[10]).

ligand. The affinity of a large number of drugs for a specific receptor can be determined very quickly using competition experiments, as shown in Figure 5.

Two-site binding

It is not uncommon for a radioligand to bind with essentially equal affinity to two sites (two receptor subtypes, for example), and an unlabeled ligand that has different affinities for the two sites. Competition plots with two binding sites will have slopes that are less than one, or have an initial slope followed by a plateau and then another slope, as shown in Figure 6. Twenty to thirty concentrations of unlabeled ligand may be required to clearly define the curve. Competition data of binding to two sites are best visualized by using a plot of bound vs. bound times inhibitor concentration.[8] This plot is linear for a single binding site, but is markedly non-linear for two sites. The slope of the line is $-1/IC_{50}$, and thus is comparable to the Rosenthal plot where the slope is $-1/K_d$.

Correlation plots for determining the identity of receptor subtypes in different tissues

Receptor subtypes are usually defined pharmacologically, i.e. by the rank order of potency of agonists and antagonists that bind to the receptor. Therefore, the K_i values obtained from competition experiments can be used to identify the subtype of a specific receptor in a given tissue. The first step is to compile data from competition experiments using

various unlabeled antagonists, against radioligand binding, e.g. [^{3}H]-LY 341495 binding for the $mGlu_6$ receptor (see Figure 5). Similar competition experiments are conducted using tissue that contains other known subtypes of mGlu receptor as well as tissue containing an unknown subtype. The IC_{50} values for the different drugs for the different receptor preparations are converted to pK_i (negative log of the K_i) values to give a pK_i profile for each receptor or tissue preparation. A convenient method of comparing the pK_i profile from the different preparations is to plot the pK_i values for each preparation pair-wise against each other. The correlation coefficients (r) and slopes of the plots are then obtained using linear regression analysis. In a perfect match, the slope and correlation coefficients are equal to one. The greater the deviations of the slope or correlation coefficient are from one, the less likely the two receptor subtypes are the same. An example of how the identity of mGlu receptor subtypes in an unknown tissue can be identified using correlation plots is shown in Figure 7. Based on the data in this figure, it would be reasonable to conclude that the unknown tissue contains predominately the $mGlu_6$ receptor subtype rather than the $mGlu_{7a}$ or the $mGlu_{8a}$ receptor subtypes.

Conclusion

Receptor binding studies are relatively easy to conduct, and they provide a wealth of knowledge about receptor subtypes and the affinity of drugs for those receptors. It is critical that appropriate binding conditions are used. The analysis of the data is based on equilibrium conditions being present when free drug is separated from bound drug and the free drug concentration remaining constant. The conformation of receptor subtypes can be altered by agents such as metal ions and GTP. Thus it is essential to make sure that similar assay conditions are used when comparing one study to another. More details on how to conduct and interpret receptor binding studies can be found in the suggested reading given below.

Suggested Reading for More Detail on Receptor Binding Techniques

Web Sites:

Receptor Binding Tutorial (www.unmc.edu/Pharmacology/receptortutorial). This site was designed to simulate the steps required for doing receptor binding experiments using an interactive process. The basic aspects of the different experiments are presented and the user is lead, in an interactive manner, through the steps required for the designing of experiments, preparation of reagents, addition of reagents to test tubes and analysis and interpretation of the results.

Data Analysis Resource Center (www.graphpad.com) gives lots of information on the statistical analyses of receptor binding data as well as a free download of the booklet “The GraphPad Guide to Radioligand Binding”.

Articles:

Bylund and Toews (1993) Radioligand binding methods: practical guide and tips. Am.J.Physiol. ***265*** L421.

Books:

Bylund and Yamamura (1990) Methods for receptor binding, in Methods in Neurotransmitter Receptor Analysis. Eds.Yamamura *et al*, pp 1-35, Raven Press.

McKinney (1998) Practical Aspects of Radioligand Binding, In Current Protocols in Pharmacology. Eds. S.J. Enna and M. Williams, John Wiley & Sons, Unit 1.3.

Definition of Terms

Affinity: a measure of how tightly a drug binds to a receptor. Mathematically, affinity is $1/K_d$. The higher the affinity (lower the K_d) the tighter the drug binds to the receptor. Usually the higher the affinity the slower the ligand dissociates from the receptor.

B_{max}: the density of the receptor site in a particular preparation. It is usually determined in a saturation experiment as the maximum number of receptors occupied at saturating concentrations of radioligand. The units of B_{max} are mol/g of protein or tissue.

IC_{50}: is the concentration of the inhibitor required to inhibit the binding of a radioligand by 50%.

K_d: the dissociation equilibrium constant of a drug for a receptor. K_d is a measure of the affinity of a drug for a receptor. The lower the K_d the tighter the drug binds to the receptor and the higher the affinity of the drug for the receptor.

K_i: the equilibrium dissociation constant for a competitive inhibitor of the receptor. Note: The competitive inhibitor can be an agonist or an antagonist. It is called a competitive inhibitor because its value is determined by measuring the ability of the unlabeled drug to compete with a radiolabeled drug for the receptor. In simple terms, the K_i value for an unlabeled drug should be the same as the K_d value obtained for the same drug in radiolabeled form.

References

1. **Bylund and Toews** (1993) Radioligand binding methods: practical guide and tips. Am.J.Physiol. ***265L*** 421.
2. **Rosenthal** (1967) Graphical method for the determination and presentation of binding parameters in a complex system. Anal.Biochem. ***20*** 525.
3. **Bylund and Martinez** (1980) Alpha2-Adrenergic receptors appear in rat salivary glands after reserpine treatment. Nature ***285*** 229.
4. **Limbird** (1996) Cell Surface Receptors: A short course on theory and methods. Kluwer Academic, Boston pp 84-87.
5. **Scatchard** (1949) The attractions of proteins for small molecules and ions. Ann.N.Y.Acad.Sci. ***51*** 660.
6. **Cheng and Prusoff** (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem.Pharmacol. ***22*** 3099.
7. **Deupree** *et al* (1996) Buffers differentially alter the binding of [^{3}H]rauwolscine and [^{3}H]RX821002 to the alpha-2 adrenergic receptor subtypes. J.Pharmacol.Exp.Ther. ***278*** 1215.
8. **Bylund** (1986) Graphic presentation and analysis of inhibition data from ligand-binding experiments. Anal.Biochem. ***159*** 50.
9. **Bylund and Murrin** (2000) Radioligand saturation binding experiments over large concentration ranges. Life Sci. ***67*** 2897.
10. **Wright** *et al* (2000) Binding of [^{3}H](2S,1´S,2´S)-2-(9-xanthylmethyl-2-(2´-carboxycyclopropyl) glycine ([^{3}H]LY341495) to cell membranes expressing recombinant human group III metabotropic glutamate receptor subtypes. Naunyn-Schmied.Arch.Pharmacol. ***362*** 546.