DRUG-RECEPTOR INTERACTIONS

OBJECTIVES: **This lecture will introduce the qualitative and quantitative vocabulary of pharmacology. Drugs interact with specific receptors to produce or block biochemical and physiological effects. These interactions can be modeled by applying the principle of mass-action to agonist and antagonist dose-response relationships. The concept of a full agonist, a partial agonist, a reversible antagonist, and an irreversible antagonist will be explored.**

The identification of receptor molecules by structural, functional, and ligand binding criteria will also be discussed.

I. Introduction

A drug receptor is any biological component capable of binding a drug molecule and generating a cellular effect. When P. Erlich introduced the term "receptor" around 1900, he was considering the mechanism of action of agents toxic to invading microorganisms but not to the host. Even before Ehrlich's studies, pharmacologists and physiologists examining the effect of poisons on animals and man concluded that specific receptors probably exist that mediate information transfer from nerve to muscle. Between 1880 and 1900 J. N. Langley and others examined the actions on the vertebrate peripheral nervous system of a variety of plant alkaloids. In one study Langley examined the effects on skeletal muscle of nicotine and of tubocurarine. Nicotine applied to the neuromuscular junction caused a muscle contraction similar to that elicited by stimulation of the nerve, while tubocurarine blocked the action of both applied nicotine and of nerve stimulation. Langley concluded that there was probably a "receptive substance" for nicotine and for turocurarine.

Since both agents affected muscle contraction in the absence of nerve, he concluded that the function of the nerve was closely related or identical to stimulation of the "nicotine receptive substance" of muscle.

In initial studies the existence of agents that mimic nerve (agonists) and of agents that block agonist action (antagonists) was crucial. The identity of the neurotransmitter substances and receptors themselves was unknown. A clue concerning the chemical nature of those receptors was found in a comparison of the activity of substances that exist as pairs of optically active stereoisomers: L-epinephrine was found to be at least 15 times as potent as D-epinephrine in controlling the rate of

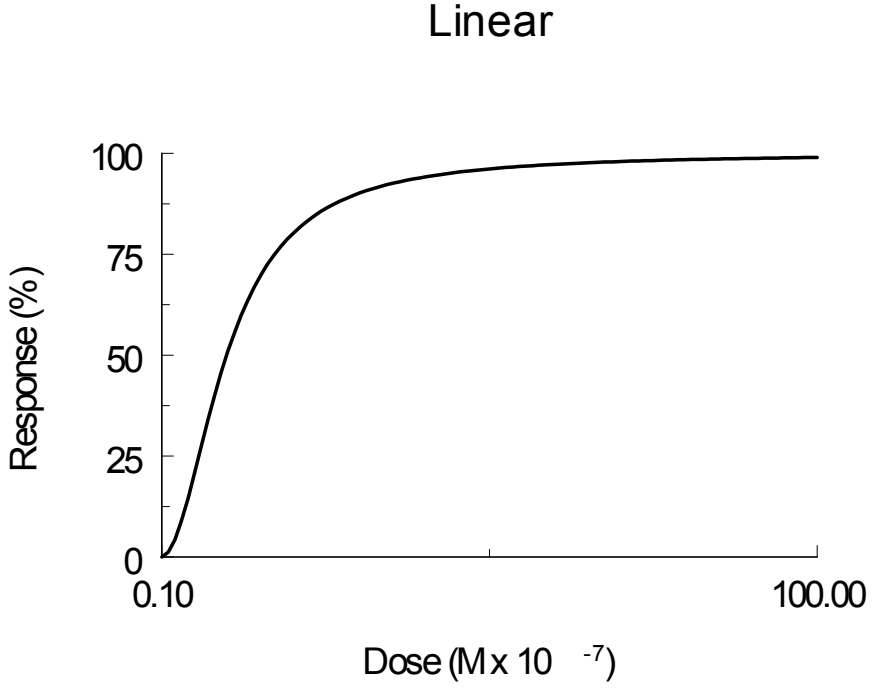
beating of an isolated heart; the analgesic activity of morphine derivatives also demonstrated stereospecificity. The stereospecificity of the action of many agonists and antagonists suggested that the binding sites of receptors would be similar to the active sites of enzymes. The analysis of drug actions in terms of specifc receptors depends upon the systematic analysis of the dose-dependence of agonist and antagonist actions. In the following lectures we will consider families or classes of drugs that exert their therapeutic action as a result of interactions with specific receptors.

In this lecture we introduce the general principals of agonist and antagonist **dose-response relations**, as well as the biochemical criteria used to identify drug receptors.

II. Agonist Dose-Response Relations

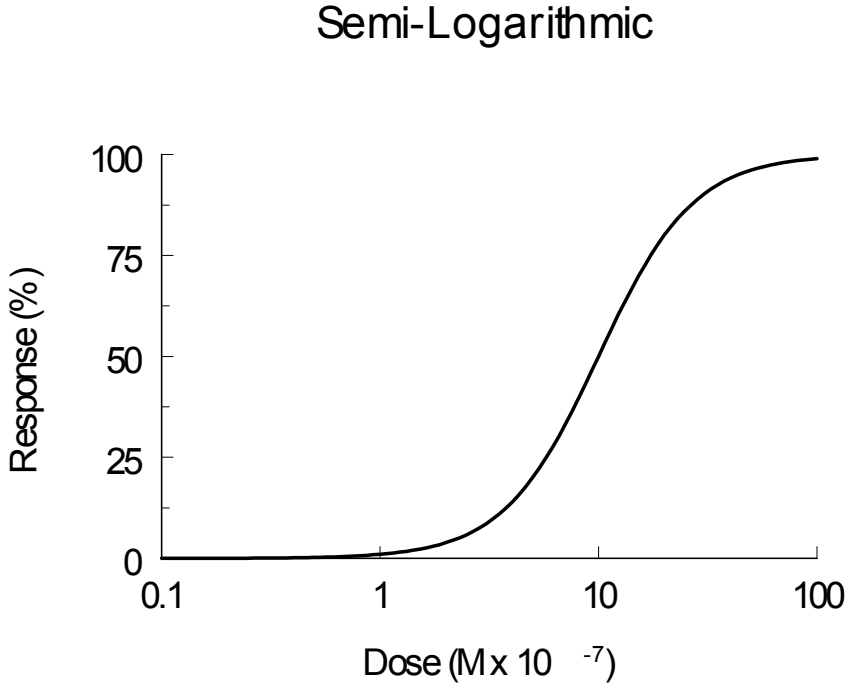
A. Dose-response relations can be established whenever a drug produces a graded response, for example, a change in heart rate or systemic blood pressure. Doses can be expressed as mg drug/kg body weight or, for isolated organ preparations, directly in terms of concentration (moles drug/liter; M). Responses can be plotted as a linear function of agonist dose:

Fig 1



Because responses occur for drug concentrations that vary over many orders of magnitude, it is more common to present dose-response data in a semi-logarithmic plot

Fig 2



B. Dose-response relations can also be established when an individual response is **all- or-none** (quantal). For a hypnotic (sleep inducing) drug, the individual response is sleep/awake, and an undesirable response might be the presence or absence of convulsions. A relationship between dose and response is established by administering fixed doses of drug to a group of recipients. For each dose, the response is the proportion (%) of the group responding. The response is thus graded over the population rather than the individual.

C. <u>Empirical definitions</u>

ED_{50} - dose that produces half-maximal effect or desired response in 50% of recipients.

TD_{50} - dose producing a toxic response in 50% of recipients (or Ld_{50}, the lethal dose).

Therapeutic Index (TI) = TD_{50}/Ed_{50}. A measure of drug safety.

D. A model based on the principle of Mass-Action

A. J. Clark (1926) noted that many dose-response curves are hyperbolic (see Fig 1), and this suggested to him that the response is proportional to receptor occupancy. If a drug D binds reversibly to a receptor R, and if each "event" is independent of others, then

$$\mathbf{(1)} \qquad D + R \underset{k-1}{\overset{k+1}{\rightleftharpoons}} DR$$

Where D, R, And DR are the instantaneous concentrations of drug, free receptor and bound receptor, respectively.

At equilibrium:

$$\mathbf{(2)} \qquad \frac{DR}{R_o} = \frac{D}{D + K_D}$$

where R_o is the concentration of total receptors (R_o = R + DR) and $K_D = k_{-1}/k_{+1}$ is the equilibrium dissociation constant (1/affinity constant), i.e., the drug concentration at which half of the receptors are bound.

Eq. 2 describes the simplest binding of drugs to receptors, and additional assumptions are necessary to relate drug binding to the response being measured. By far the most common assumption is that drug effects are some function of the occupancy of receptors (as opposed to the rate of association or dissociation). If an effect is simply proportional to the fraction of occupied receptors (effect = αDR), then the factor α on the left side of eq. (2) is unity (equals 1). However, effects are usually complex functions of receptor occupancy so, to emphasize the fact that the exact relation between occupancy and response is generally unknown, we rewrite eq. (2):

$$\mathbf{(3)} \qquad \frac{\text{Effect}}{\text{MaximumEffect}} = \frac{D}{D + K_{AP}}$$

Where K_{Ap} is the apparent dissociation constant, i.e., the drug concentration that elicits a half-maximal effect.

Note the formal identity between eqs. (2) and (3) and the Michaelis-Menten equation:

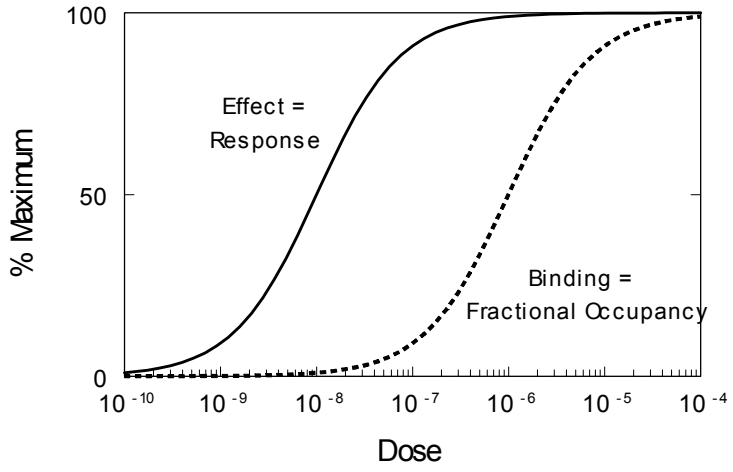
$$\frac{\mathbf{V}}{\mathbf{V_{max}}} = \frac{\mathbf{S}}{\mathbf{S} + \mathbf{K_m}}$$

Where V = reaction velocity
V_{max} = maximum reaction velocity
S = substrate concentration
K_m = the S at which $V = \frac{1}{2} V_{max}$

The same linear transformations used to study enzyme kinetics can therefore be applied to drug-receptor interactions (see Appendix).

It may be plausible that response should be directly proportional to receptor occupancy, but consider a simple counter example: What if half-maximal contraction of a muscle could be caused by occupation of 1% of the total number of receptors (R_o) by an agonist that binds with an equilibrium constant K_D?

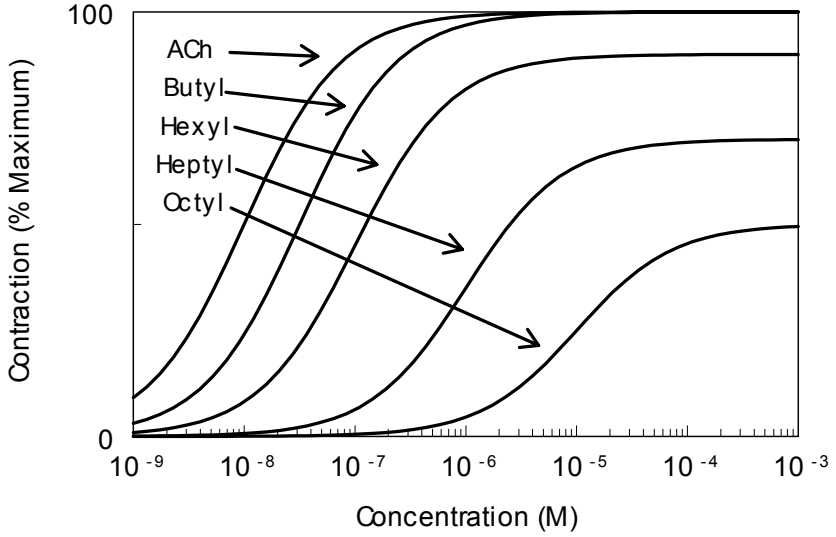
Since $DR/R_o = D/(D + K_D)$, then $DR/R_o = 0.01$ when $D \cong 0.01\ K_D$.
If $K_D = 10^{-6}$ M, 1% occupancy would occur when $D = 10^{-8}$ M, and, hence, $K_{Ap} = 10^{-8}$ M.



This is an example of spare receptors. The notion is important because it predicts that it is the relationship between occupancy and response, and not solely binding (K_D), that determines K_{Ap}. The responses generated by Ach binding to identical acetylcholine receptors present on the surfaces of a cardiac muscle cell (heart beat) and a gland cell (secretion) might be characterized by different K_{Ap}'s for Ach.

E. Partial agonists and efficacy

There are many instances in which drugs presumed to act on the same receptor produce very different maximum effects. For example, various alkyl derivatives of trimethylammonium stimulate muscarinic receptors in the gut, but they produce different maximum responses (in this case measured as muscle contraction) when all receptors are occupied.



To compare the dose-response relations for each compound it is necessary to specify not only drug potencies (K_{Ap} or ED_{50}) but also the maximal response (efficacy). In terms of receptor occupancy theory, eq. 1 can be generalized:

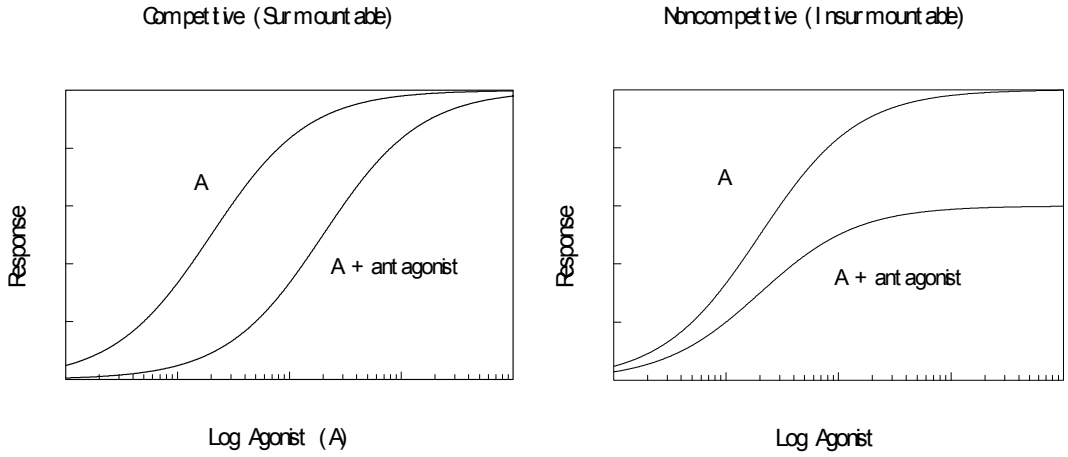
$$D + R \underset{k-1}{\overset{k+1}{\rightleftharpoons}} DR \underset{k-2}{\overset{k+2}{\rightleftharpoons}} DR^*$$

where DR* is the active complex. Efficacy depends upon the ratio DR*/DR which alone depends on k_2/k_{-2}, i.e., efficacy depends upon the specific drug for a single receptor.

In the preceding example, it was asserted that all the compounds interact with muscarinic receptors. To demonstrate that the compounds interact with that receptor, it is necessary to characterize the potencies of drugs that block their action, i.e., of antagonists.

III. Pharmacological Antagonism—An Overview

A. Reversible antagonists



Competitive antagonists do not themselves produce an effect, but they decrease in a reversible manner the apparent potency of agonists (i.e., they increase K_{Ap}). The hallmark of competitive antagonism is the fact that the same maximum effect can be achieved by increasing the agonist concentration at a fixed antagonist concentration.

Noncompetitive antagonists reduce the maximum response (efficacy). In effect, some receptors are eliminated. The affinity of those receptors that remain may not be altered. Noncompetitive antagonists are usually less specific than competitive antagonists and have little use in clinical medicine.

The model for competitive antagonism is based on the principle of mass action. It is assumed that agonist (D) and Antagonist (A) compete for the same receptor (R).

$$\mathbf{D + R \rightleftarrows DR;\ K_D = Agonist\ \ Dissociati\ \ on\ Constant}$$

$$A + R \rightleftarrows AR;\ K_A = \text{Antagonist Dissociation Constant}$$

At equilibrium:

$$(4) \qquad \frac{DR}{R_o} = \frac{D}{D + K_D\left(1 + \frac{A}{K_A}\right)}$$

Eq. 4 has the same form as eq. (2) describing agonist binding alone,

$$\frac{DR}{R_o} = \frac{D}{D(D + K_D)}$$

except that the concentration of D now required to bind half of the receptors is given by $K_D(1 + A/K_A)$. The affinity (potency) is decreased by a factor that depends on A and K_A.

We discussed above the fact the K_D cannot be simply estimated from the agonist dose-response curve. The precise relation between effect and receptor occupancy is usually not known. If one assumes only that equal responses are associated with equal receptor occupancies, however, then it <u>is</u> possible to obtain an accurate estimate of <u>antagonist</u> dissociation constants from dose-response data. If agonist dose D' produces the same effect in the presence of antagonist concentration A as does D in the absence of antagonist, then the fraction of receptors occupied by agonist (DR/R_o) must be the same and can be equated.

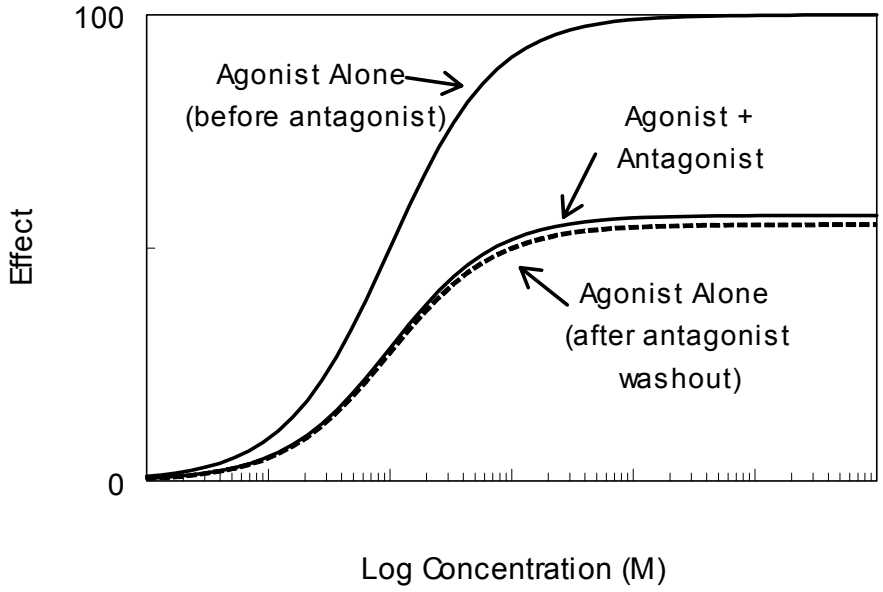
$$(5) \qquad \frac{D}{D + K_D} = \frac{D'}{D' + K_D\left[1 + \frac{A}{K_A}\right]}$$

Algebraic manipulation of eq. (5) results in the <u>dose-ratio equation</u>:

$$(6) \qquad \frac{D'}{D} - 1 = \frac{A}{K_A}$$

Thus, a linear relation should exist between D'/D and A for all concentrations of A, and the slope of such a line is $1/K_A$.

B. Irreversible antagonists



The change in agonist dose-response relation is like that for a noncompetitive antagonist - a fraction of the receptors are lost. The difference is that, in this case, the lost receptors don't "reappear" after the antagonist is washed out.

C. An example

Acetylcholine and histamine both cause contraction of intestinal muscle. Atropine can block the action of both compounds, but quantitative analyses of the antagonism by atropine indicates that its K_A for antagonisms of ACh is 10^{-9} M whereas for histamine it is 10^{-6} M.

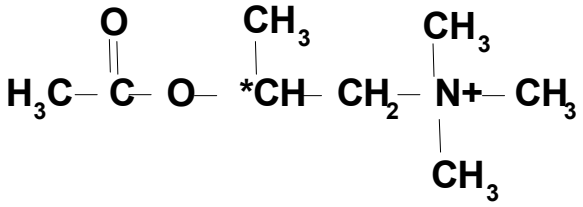
Furthermore, the K_A characterizing the atropine antagonism of each of the alkyltrimethylammonium compounds is 10^{-9} M. Thus it would appear that ACh, atropine and trimethylammonium compounds all interact with one receptor, whereas histamine interacts with a different receptor. Atropine also antagonizes the action of ACh in cardiac muscle and in many gland cells, and in each case the K_A is 10^{-9} M. This result indicates that there is likely to be a similar or identical ACh receptor present in all of those different organs.

IV. Identification of Receptor Molecules

Based upon the previous discussion, the following creiteria constitute reasonable evidence for specific drug receptors.

A. Minor changes in agonist or antagonist structure result in large changes in potency.

Where applicable the "smallest" change in structure is that to the mirror image of a molecule containing an asymmetric carbon (stereospecificity). A molecule as simple as Ach has no asymmetric carbon but acetyl-β-methylcholine



is a potent muscarinic agonist that is 25-fold more potent than its stereoisomer acetyl-α-methylcholine.

B. Antagonist potencies define a common class of receptors in different tissues and species.

For tubocurare acting at skeletal neuromuscular junctions, $K_A = 10^{-9}$ M in blocking both the excitatory action of Ach on the gut and the inhibitory action at cardiac muscle (a 100-fold higher concentration is necessary to block Ach action at the skeletal neuromuscular junction). Propranolol inhibits the action of norepinephrine with $K_A \sim 3 \times 10^{-8}$ M at both heart and intestinal muscle (and in turkey erythrocytes, for that matter!). The absolute values of K_A are not important, but the fact that classes of receptors in different tissues and species can be defined by the same antagonists potencies does lend credence to t he concept of specific receptors. An elabooration of this correlation is found in the **rank order of potency** of a series of agonist or antagonist molecules.

C. Binding of radioactive ligands

A. J. Clark (1933) noted that 2×10^{-8} gm Ach (10^{-10} moles) per gm of tissues caused a 50% reduction in the rate of beating of the heart. He estimated that if all the Ach were bound on the cell surfaces, it would

cover about 0.01% of the surface area. This suggested that if unique molecules (receptors) are present on the target cell, there need not be many of them. Many early attempts to identify receptors by the binding of radioactive ligands were unsuccessful because the ligands did not contain enough radioactivity to detect small quantities of receptor.

High specific activity radioactive ligands are now available for rmany of the receptors to be discussed in this course, e.g. cholinergic, adrenergic, dopaminergic, opiate, benzodiazepine.

It is possible to study drug binding to whole tissue or to tissue homogenates. The latter is preferable because there are fewer diffusion barriers and equilibrium is thus reached more rapidly. To study binding it is necessary to separate the free from the bound drug. Several different procedures have been used to accomplish this. If the ligand is tightly bound to the receptor and the receptor is part of a membrane, then it is possible to separate bound from free by a filtration or centrifugtation assay. Once the particulate matter is trapped on a filter, it is possible to wash the filter to remove residual free drug (this will work only if the drug remains bound to the receptor for a time longer than the duration of washing). Another procedure is equilibrium dialysis. The receptor is confined within a bag that is permeable to the drug but not to larger macromolecules or particles. At equilibrium, the drug concentration outside the bag is that of free drug only, while that inside the bag is the total (i.e., free plus bound) concentration.

Ligands adhere nonspecifically to most tissues. Based on the criteria outlined above, demonstration of specific binding sites requires that binding exhibit:

1. Saturation, i.e. a finite, presumably small, number of sites.

2. Pharmacological Specificity—ligands should bind stereospecifically, and appropriate antagonists and agonists should inhibit the binding.

3. Physiological Relevance—the amount of specific binding in different tissues should correlate with the ability of the ligand to produce a response (or prevent one) in those tissues. The time course of binding must be appropriate (i.e. it should precede or coincide with the response).

The following curves illustrate binding of the potent muscarinic antagonist
[^{3}H]propylbenzoylcholine (PrBCh) to brain tissue (synaptosomes).

(See Hulm, E. C. et al. (1978) Molec. Pharmacol. 14:737-750).

Circles in (a) show the total amount of drug bound as a function of free [^{3}H]PrBCh concentration. This curve does not saturate. Squares in the same graph show the amount bound in the presence of a high concentration of atropine (10^{-6} M). The difference between the two curves in (a) is shown in (b). This curve is a hyperbola that can be fit by the mass action equation, equation (2), above. From this kind of data, it can be assumed that the first curve (circles) contains two components: a small number of high affinity specific receptors and a very large number of low affinity nonspecific sites (not inhibited by atropine). The "concentration" of specific sites is equivalent to 50 x 10^{-11} moles/gm of tissue. The muscarinic receptor comprises about 0.01% of the protein in the tissue homogenate.

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APPENDIX

The same linear transformations use to study enzyme kinetics can be applied to drug receptor interactions (eq. 2 or 3). Two of the more common ones are:

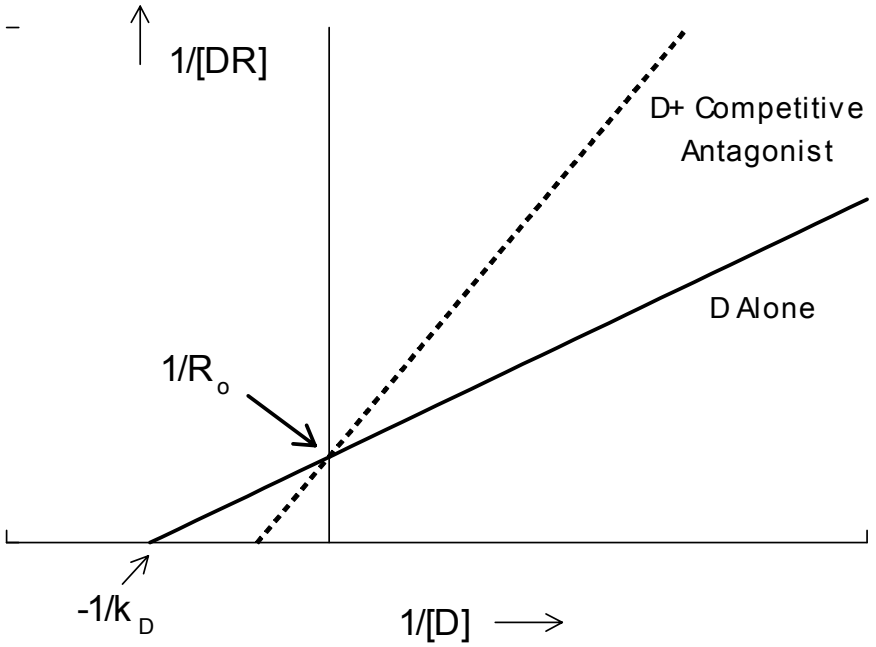
Lineweaver-Burke (Double Reciprocal) Plot

$R_o/DR = (D + K_D)/D = 1 + K_D/D$

and in the presence of a competitive inhibitor (A)

$R_o/DR = 1 + K_D(1 + A/K_A)/D$

A plot of 1/DR (or 1/response) vs 1/D will yield a straight line. The extrapolated y intercept is $1/R_o$ (or 1/Max response) and the extrapolated x intercept is $-1/K_D$ (or $-1/K_{Ap}$).



Scatchard Plot

Since $KD = D \times (R_o - DR)/DR$

Then $DR/D = (R_o - DR)/K_D = R_o/K_D - DR/K_D$

or, in the presence of a competitive inhibitor (A)

$DR/D = (R_o\text{-}DR)/K_D(1 + A/K_A)$

A plot of DR/D (or bound/free) vs DR (bound) will yield a straight line with the x intercept equal to R_o(or Max Response) and the slope equal to $-1/K_D$ (or $1/K_{Ap}$).