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Introduction to Receptors: The Nicotinic Acetylcholine Receptor as a Model System
1/28/03

References

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3. Karlin, A. and Akabas, M.H. (1995). Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. Neuron 15, 1231-1244.

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DRUG-RECEPTOR INTERACTIONS

Suggested Reading: Goodman and Gilman (9th edition), Chapters 2 & 3, pp. 29-62.

I. DEFINITIONS

A. Pharmacodynamics is the study of the biochemical and physiological actions of drugs and the mechanisms for these actions.

B. Receptors are the macromolecular components of cells with which most drugs interact to cause their effects.

The concept that drugs act through combination with receptors is suggested by the following observations:

1. Many drugs are chemically inert.
2. Many drugs act at extremely low concentrations ($\leq 10^{-9}$M).
3. The action of many drugs shows a high degree of chemical specificity (e.g. stereospecific) and can be markedly altered by minor structural modifications in the drug molecule.
4. Drug action can be antagonized or mimicked by structural analogs.
5. The action of antagonists (substances that prevent drug action) also can be altered by structural modifications.
6. The actions of many drugs obey simple laws of Mass Action.

C. Many cellular proteins normally serve as receptors for endogenous hormones or neurotransmitters. Drugs that interact with one or another of these receptors and mimic the action of the endogenous substance are known as agonists. Drugs or chemicals that combine with the same receptors but are devoid of intrinsic activity are known as antagonists.

Structure-activity relationships are important in relation to drug-receptor interactions. The affinity of the drug for the receptor and the intrinsic activity of the drug depend on its structure.

Minor changes in structure may change an agonist into an antagonist or modify the relative magnitude of therapeutic (desired) to toxic effects. Changes in chemical structure also may strongly influence the pharmacokinetic properties of the drug.

D. The main functions of the receptor are ligand binding and message propagation. The latter usually depends on second messengers.

E. Receptors and their associated coupling proteins and effectors regulate the activity of cells and subcellular constituents. In addition receptors themselves are subject to regulation. Continued exposure to an agonist generally results in a decrease in responsiveness termed desensitization or down-regulation. A chronic decrease in exposure of a receptor to an endogenous agonist frequently results in supersensitivity or up-regulation.

F. Drug receptors are usually classified in terms of:

1. The relative potency of representative agonists.

2. The actions of selected antagonists.

Studies of this sort have led to the identification of many receptor subtypes. Detailed descriptions will be provided in subsequent lectures.

G. Drug actions not mediated by receptors

The actions of some drugs are not the result of interactions between the drug and a specific receptor. Some examples are:

1. Chelating agents (EDTA for lead poisoning).

2. Osmotic diuretics (mannitol).

3. Gastric antacids.

4. Volatile anesthetics.

II. CLASSIFICATION OF RECEPTORS

Receptors can be classified as protein or non-protein receptors. The non-protein receptors are, for the most part, DNA (e.g. many cancer chemotherapeutic drugs act by binding to DNA). Receptors can also be classified in terms of the ligand with which they interact.

A. Receptors for endogenous ligands, such as neurotransmitters and hormones.

1. Membrane receptors
   a. Ligand-gated ion channels
      These proteins consist of multiple subunits and function as receptor and effector, and include receptors for neurotransmitters (e.g., ACh, GABA and glycine).
   b. Single subunit receptor protein
      Many receptors for hormones and transmitters consist of 7 transmembrane alpha helices. The activated receptor interacts with a guanine nucleotide binding protein (G protein) which transduces the signal to an effector (i.e. production of a second messenger). Adrenergic & muscarinic ACh receptors are in this family.
   c. Tyrosine kinase
      The receptor itself functions as a protein kinase - it phosphorylates proteins on tyrosine residues. Receptors for growth factors and insulin are of this class.

2. Intracellular receptors
   Steroid hormones cross the cell membrane and bind to intracellular receptors; the hormone-receptor complex binds to DNA.

B. Receptors for exogenous ligands, such as drugs and toxins

1. Ion channels
   (e.g. Antiarrhythmic drugs and local anesthetics bind to and block ion channels.)

2. Enzymes
   Some drugs act by inhibiting enzymes (e.g. anticholinesterases inhibit AChE).

3. Ion transporters, pumps and carriers
   (e.g. Digitalis blocks the Na-K pump.)

4. Structural proteins
   (e.g. The vinca alkaloids, used to treat cancer, bind tubulin and prevent microtubule formation.)

III. QUANTITATION OF DRUG-RECEPTOR INTERACTIONS

Since much of our understanding of the relationship between drug dose and the response is based on the theory of drug-receptor interactions, we shall consider this subject in some detail.

A. It is assumed that drugs interact with macromolecular receptors in accordance with the Mass Action Law. For the sake of simplicity we assume:

1. Each receptor has a single binding site for a given drug.

2. All binding sites have the same affinity for the drug.

3. Binding to one site has no effect on other sites.

4. Binding of some drug to the receptor has no significant effect on the concentration of free drug.

5. The response is proportional to fraction of the receptor pool bound to drug (the *Occupancy Assumption*).

B. Now, let us consider the interaction of a drug, D, with its receptor, R:

$$D + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} DR \qquad (1)$$

where [DR] is the concentration of the drug-receptor complex, [D] the concentration of free drug, and [R] the concentration of free receptor. k_1 is the second order rate constant of drug association (with units of $Molar^{-1}sec^{-1}$) and k_2 is the first order rate constant for drug dissociation (with units of sec^{-1}). Thus the rate of formation of drug-receptor complex [DR] per unit time (i.e. [DR]/t) is given by the product $[D][R]k_1$ (units of Molar/sec). The rate at which DR breaks down to form D and free R is given by $[DR]k_2$. At equilibrium, the rate of formation of DR is equal to its rate of dissociation. Thus, at equilibrium, we can write:

$$[D][R]k_1 = [DR]k_2$$

or

$$K_d = \frac{k_2}{k_1} = \frac{[D][R]}{[DR]} \qquad (2)$$

where K_d is defined as the equilibrium dissociation constant (and has units of Molar). We now wish to determine how the concentration of drug-bound receptors [DR] will change as a function of free drug concentration [D]. The total concentration of receptors, R_T, is given by the sum of the free and bound receptor concentration:

$$R_T = [R] + [DR] \qquad (3)$$

By using the relation $[R] = R_T - [DR]$ from equation 3, we can eliminate [R] from equation 2, yielding:

$$K_d = \frac{k_2}{k_1} = \frac{[D]\,(R_T - [DR])}{[DR]}$$

By rearranging we get:

$$[DR] = \frac{R_T[D]}{[D] + K_d} \qquad (4)$$

In general, pharmacologists measure the response or effect, E, to a given drug (i.e. change in blood pressure) and do not directly measure the concentration of drug-bound receptors [DR] in equation 4. However, it is often assumed that the response to a drug is proportional to the number of drug bound receptors, i.e.:

$$E = k_3[DR] \qquad (5)$$

where k_3 is a proportionality constant. The maximum response to a saturating concentration of drug, E_{max}, is then given by:

$$E_{max} = k_3[R_T]$$

and using these relations we can rearrange equation 4 to obtain:

$$E = \frac{E_{max}[D]}{[D] + K_d} \qquad (6)$$

Notice that the derivation of equation (6) is identical to that for the Michaelis-Menten equation:

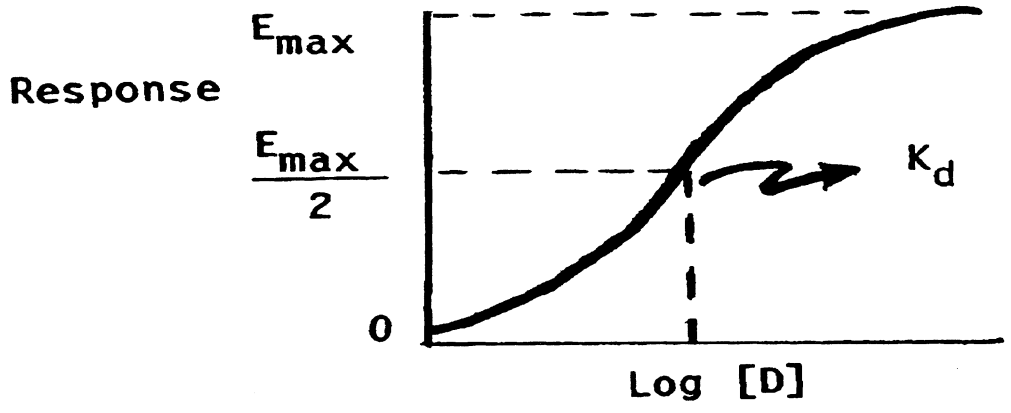
$$V = \frac{V_{max}[S]}{K_m + [S]}$$

where V is the velocity of an enzyme reaction as a function of the substrate concentration, S, the Michaelis-Menten constant, K_m, and the maximum velocity, V_{max}. Equation (6), thus, is generally applicable and subsumes enzyme-substrate and enzyme-inhibitor reactions as special cases.

IV. THE LOG-DOSE RESPONSE (LDR) CURVE

What information can be obtained from equation (6)? If we plot the response (E) against log [D], we obtain log-dose response (LDR) curve:

Figure 1



Clearly, from equation (6), E = 0 when [D] = 0, and E approaches E_{max} when [D] becomes very large.

From equation (6), the half-maximal response ($E = E_{max}/2$) is obtained when:

$$\frac{[D]}{K_d+[D]} = 1/2, \text{ or when } [D] = K_d.$$

This concentration is referred to as the ED_{50} (the effective dose yielding 50% maximal response).

From this it is possible to derive the dissociation constant of a drug without being able to measure the receptor concentration.

For a congeneric series of agonists, the lower the ED_{50}, the lower the dissociation constant (tighter binding to the receptor). However, these relationships are correct only when the occupancy assumption is correct.

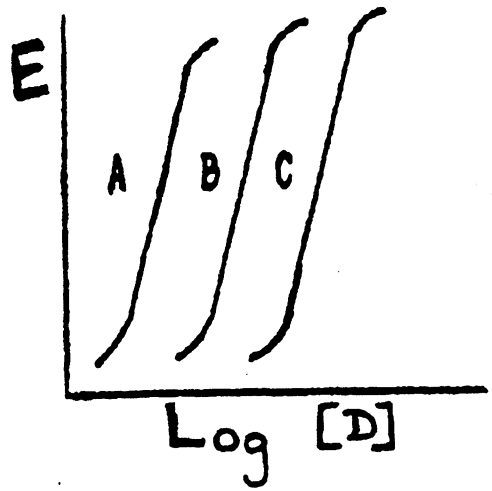
V. ANTAGONISTS

A. Competitive antagonists

An antagonist, I, is said to be competitive if it combines reversibly with the same binding site on the receptor as the agonist, D. I could occupy the same site as D, but not permit the events causing the response. For a receptor that must undergo a conformational change in response to a drug, I may occupy the binding site but not cause the conformational change. In the case of an enzyme, I may not undergo a catalytic transformation.

The effect of a competitive antagonist is to reduce the apparent affinity of D for R.

Figure 2



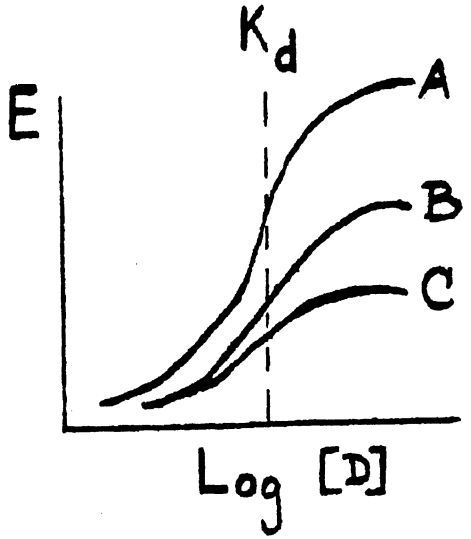
In the figure, the effect of an antagonist is shown using log dose-response plots. The curve is shifted to the right by antagonist; the slope and the maximum response do not change.

B. Noncompetitive antagonists

If an antagonist, I, interacts with the receptor such that the maximum response to the agonist (DR) is reduced, relative to the maximum response in the absence of antagonist, regardless of the concentration of D, I is said to be noncompetitive. For example, I could bind to the same site as D on R, but so tightly that it cannot be displaced by D. Alternatively, I could combine with R at a site distinct from the agonist binding site. Binding of I would reduce the ability of the agonist-bound receptor [DR] to elicit a response (i.e., I would decrease k_3 in occupancy theory, see equation 5). The effect of I may be reversible or nearly irreversible, but the agonist has no influence on reversibility or the extent of antagonism. The corresponding dose-response plots for the noncompetitive antagonists are shown in Figure 3.

Figure 3

Log Dose-Response Plot



Again, curve A is for the drug alone, curve B is that in the presence of a given concentration of the antagonist, and C in the presence of a still higher concentration of the antagonist.

The effects on the LDR curve are as follows:

a. The maximum response is decreased [since $E_{max} = k_3(R_T)$].
b. The position of the curve is not shifted. (There is no change in K_d.)
c. The slope will be reduced (a consequence of the Mass Action Law).

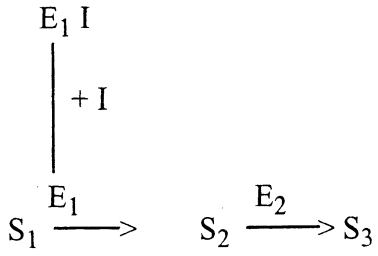
VI. ALTERNATIVES TO THE OCCUPANCY PRINCIPLE

The occupancy theory assumes that the magnitude of the response is proportional to the concentration of occupied receptors (i.e., $E = k_3[DR]$). In the simplest view, the proportionality constant k_3 is the same for all agonists. Different agonists may have different dissociation constants and thus may require different drug concentrations [D] to produce the same number of occupied receptors [DR]. However, there are important exceptions to this simple view.

A. Fractional Occupancy Threshold

In a metabolic sequence involving the receptor, if R is not rate-limiting for the process, then no drug effect may be seen until a minimum occupancy is exceeded. This alternative is known to occur in some instances with enzyme inhibition. For example, in the following reaction, enzyme E_1 converts substrate S_1 to product S_2 and enzyme E_2 converts S_2 to S_3:

Figure 5



Inhibitor I binds to and inactivates E_1.

If S_2 is present in excess and is not rate-limiting for production of S_3, some degree of inhibition of E_1 by I could be sustained without noticeable effect on the output, S_3. But, with increasing occupancy, eventually E_1 must become rate-limiting and the onset of the drug effect will be seen.

In the case of the LDR curve the effects of a fractional occupancy threshold are the following:

1. The slope is increased.

2. The response curve is shifted to the right of the receptor occupancy curve.

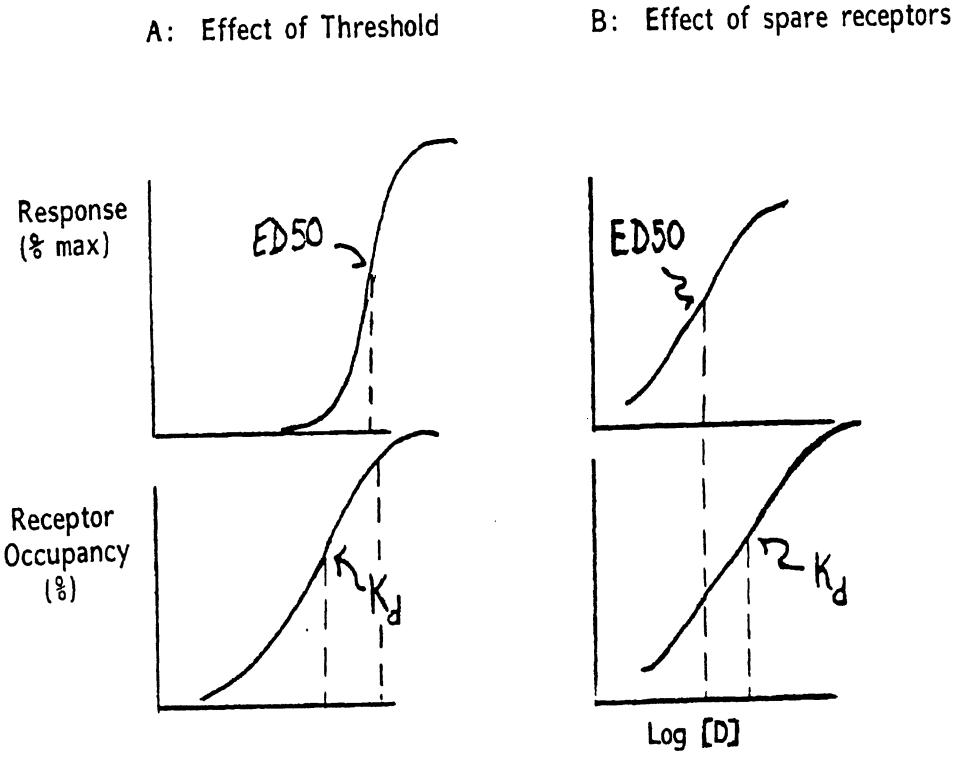
3. The ED_{50} overestimates the dissociation constant ($ED_{50} > K_d$).

These effects are illustrated in Figure 6A.

B. Spare Receptors

If occupancy of only a fraction of the total receptor population is sufficient to produce a maximal response, the remainder are called "spare" receptors. The postulate for spare receptors is equivalent to saying that the magnitude of the response is not limited by the drug-receptor interaction, but by some subsequent step. This is often true when there is a low threshold for observing a maximal response.

Figure 4



In the presence of spare receptors the LDR underestimates the dissociation constant: i.e.,

1. $ED_{50} < K_d$.

2. The LDR is shifted to the left of the receptor occupancy curve.

These effects are illustrated in Figure 4B.

Spare receptors are advantageous when a rapid onset and termination of response is needed, as for neurotransmitter action. If sensitivity were attained by a high affinity constant the dissociation of transmitter from receptor would be slow and so would termination of the response. Spare receptors allow the organism to obtain a full response at a low agonist concentration and with a high K_d; this results in rapid dissociation of drug from receptor and rapid termination of response.

VII. PARTIAL AGONISTS

A. A partial agonist is one that is incapable of causing a maximal response at any concentration. The behavior of partial agonists is explained by the concept of efficacy or intrinsic activity.

B. Affinity describes the binding forces between the drug and receptor. Efficacy (e) describes the physiological effectiveness of the drug receptor complex. According to simple occupancy theory, the response E is proportional to the number of agonist-bound receptors ([DR]), i.e., $E = k_3 [DR]$, where k_3 is a constant. To explain partial agonists, we introduce a factor e for efficacy, yielding:

$$\text{Response} \propto k_3 \varepsilon [DR]$$

The response thus depends in part on the amount of drug-receptor complex [DR] and, as before, this depends on the affinity and drug concentration. In addition, the response depends on the efficacy or intrinsic activity of the drug.

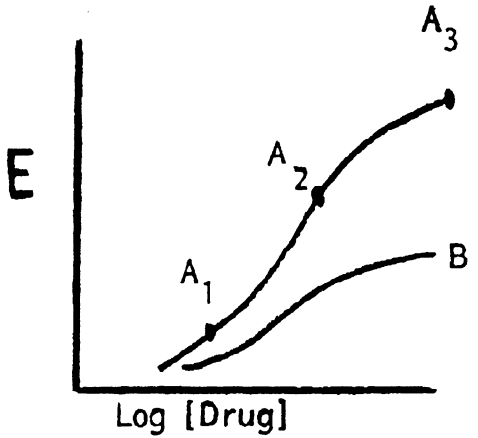
Antagonists have zero efficacy (ε=o); full agonists have high efficacy ($\varepsilon \approx 1$); for partial agonists efficacy is between zero and 1.

C. Partial Agonists as Antagonists

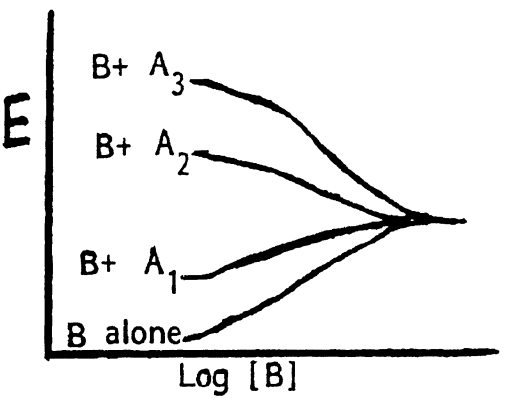
Partial agonists, since they can bind to receptors but have low intrinsic activity, can act as antagonists and reduce the response to full agonists. This is shown diagrammatically in the following figure, for a full agonist (A) and partial agonist (B).

Figure 5

Part I



Part II



Drugs A and B have the same affinity for a common receptor. A is a full agonist and B is a partial agonist. Part I of Figure 5 shows the LDR curves for drugs A and B. A_1, A_2, and A_3 are points on the LDR corresponding to three concentrations of drug A. In part II of the figure, the lower curve shows the effect of drug B alone. The second curve shows the effect of increasing concentrations of B in the presence of a set concentration, A_1, of drug A. Curves A_2 and A_3 show the effect of drug B at two higher set concentrations of A. Note that as B competes for binding with A, since it has lower efficacy, it behaves as an antagonist.

D. Modern view of agonist action

A fundamental weakness of the occupancy principle for agonist action is that it oversimplifies the underlying molecular events. We now believe that at least two reaction steps are required to explain agonist action. First, an agonist must bind to its receptor (recognition event); second, agonist binding must somehow lead to a conformational change in the receptor protein which activates it. For the case of the opening of the nicotinic ACh receptor ion channel by agonists, del Castillo and Katz proposed the following simple extension of the occupancy principle:

$$A + R \overset{K}{\longleftrightarrow} AR \overset{L}{\longleftrightarrow} AR^*$$

where A is an agonist molecule, R is the unliganded receptor-channel, AR is the liganded receptor-channel in the closed state, and AR^* is the liganded receptor-channel in the open state. This reaction is characterized by two equilibrium constants, K - the dissociation constant for agonist binding, and L - the equilibrium constant for the channel opening reaction:

$$\frac{[A][R]}{[AR]} = K \; ; \quad \frac{[AR]}{[AR^*]} = L$$

According to this scheme, for a full agonist $L<<1$: this means that the reaction between AR and AR^* proceeds almost completely to AR^*. This means that at saturating concentrations of agonist, almost all channels will open. For an antagonist, $L>>1$: this means that almost no channels will open, despite the fact that they may all be occupied by an antagonist. For a partial agonist, L assumes an intermediate value. Thus, a partial agonist will open some, but not all, channels. For example if $L=1$ for a given partial agonist, at a saturating concentration it will only open 50% of the channels (that is: $[AR] = [AR^*]$). According to the concept of efficacy that we considered above:

$$\varepsilon = \frac{1}{[1 + L]}$$

The view that agonist action involves at least two steps, a binding event and a conformational change which activates the receptor, applies to a wide range of receptors, not only ligand-gated ion channels. Even this view is greatly oversimplified. Many receptors (including the nicotinic ACh receptor) require the binding of more than one ligand. In addition, it is likely that a large macromolecule receptor can exist in many conformational states. However, for our purposes, the above model of del Castillo and Katz will suffice to understand most concepts of agonist action.

VIII. DOSE-RESPONSE FREQUENCY RELATIONSHIPS

Often we are interested in measuring a response in terms of the frequency or percent with which we observe a given effect (e.g. the percent of patients responding favorably to a drug).

A. Two typical LDR curves are shown in Figures 6 and 7.

Figure 6

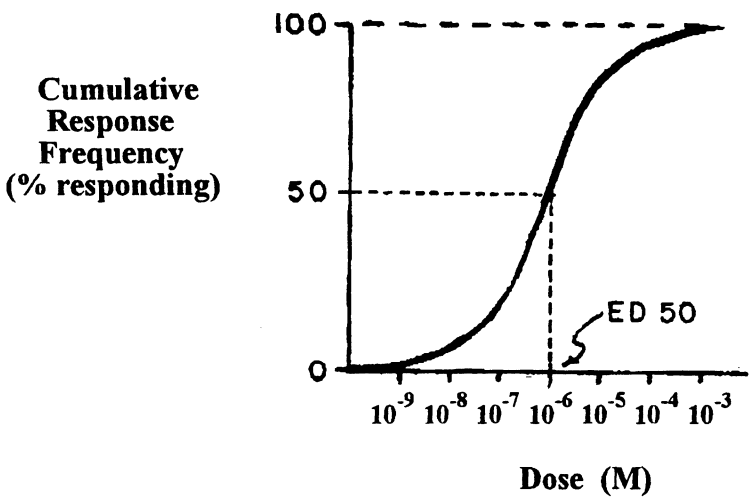
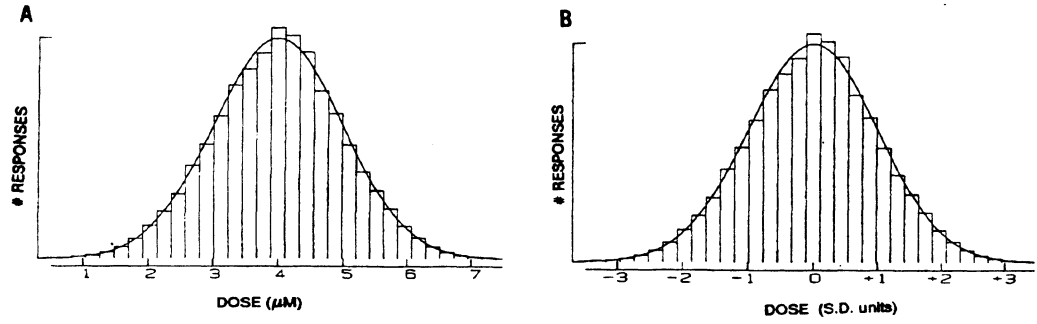


Figure 6 shows the cumulative response frequency as a function of log[dose]. This curve plots the total number (or percent) of individuals that show a response to a given dose of drug. For example, the ED_{50} of 10^{-6}M means that 50% of all patients will respond to this concentration of drug. At very high drug concentrations, nearly all patients respond (100%).

The cumulative LDR curve shows not only the ED_{50}, but also:

1. The maximal (or ceiling) effect.
2. The potency of the drug (i.e., the location of the curve on the dose axis and thus the ED_{50}).
3. The slope.
   This varies among drugs as a function of the mechanism of action of the drug and its interactions with receptors. In general, however, the slope indicates how steeply the intensity of drug effect varies with dose and, as for a drug that can cause sedation, sleep and coma, provides one indication of the margin of safety.

Figure 7



In addition to the cumulative dose-response frequency curve, we can also plot the non-cumulative dose-response frequency curve as shown in Figure 7A. This type of curve is constructed by determining the number of patients that first show a response within a defined drug concentration range (for example between 1 and 1.25 μM, 1.25 and 1.5 μM, 1.5 and 1.75 μM, etc.). We then divide up the concentration axis into a series of equally spaced intervals (e.g. in 0.25 μM steps in this example), and plot the number or frequency of patients first responding to a given drug concentration range vs. drug concentration. This yields a bar graph histogram as shown in Figure 7A.

It is often found that this histogram is bell-shaped and can be described by a Gaussian (or normal) distribution function (solid curve in Figure 7A). A Gaussian distribution is characterized by its mean value (i.e. the drug concentration at the mid-point of the curve) and its standard deviation (S.D.), a measure of the width or spread of the distribution. For a normal distribution, 50% of all individuals will first respond to a drug concentration equal to or greater than the mean while 50% respond to a concentration equal to or less than the mean. This means that for a Gaussian distribution the mean is equal to the Median Effective Dose (ED_{50}). For a Gaussian distribution, 68% of the population will require a drug concentration that falls within ± one S.D. of the mean. For example, if the mean were 4.5 μM and the standard deviation equal to 0.6 μM, 68% of the population will first respond to a drug in the concentration range between 3.9 and 5.1 μM. Sixteen percent of the population will require a drug concentration more than one S.D. above the mean, whereas 16% of the population will require a drug concentration less than one S.D. below the mean.

Often the non-cumulative dose-response frequency curves are only Gaussian when the concentration axis is on a logarithmic scale. Such distributions are said to be log normal. For a log normal distribution the concentration axis is divided up into steps that are equally spaced on a logarithmic scale (e.g. 0.1 μM, 1 μM, 10 μM, 100 μM).

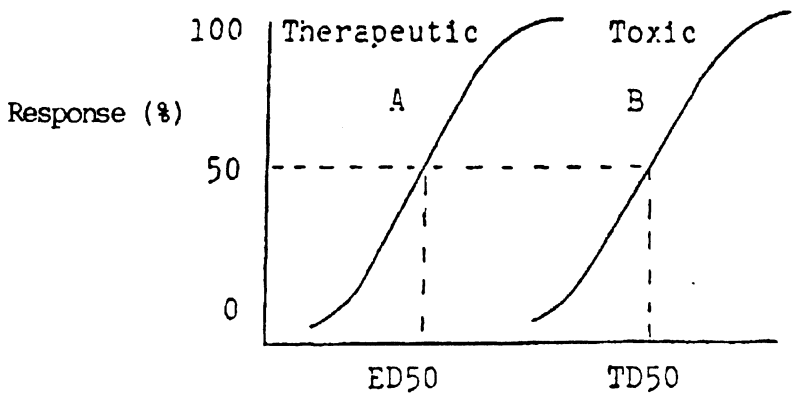
Finally, since all Gaussian distributions are completely described by only two parameters, the mean and S.D., we often will plot a "normalized" distribution, as shown in Figure 7B, where the mean concentration has been subtracted from each point on the concentration scale and then the result is divided by the observed S.D. (or the log [S.D.] for a log normal distribution). Thus, the value 0 on the X axis corresponds to the mean concentration, the value +1 equals the mean plus the S.D., the value +2 equals the mean plus 2 x (S.D.), etc.

B. Desired and Undesired Effects

All drugs have more than one effect. Usually one is the desired (therapeutic) action and the others are considered side effects or toxic effects. The toxic effects may be an extension of the therapeutic effect at excessively high dose (i.e., too great a decrease in systemic arterial pressure caused by a hypotensive agent) or an action unrelated to the desired effect.

The LDR curve can be used to quantify the dose-dependence of toxic effects as shown in Figure 8.

Figure 8



The TD_{50} is the dose at which 50% of individuals show a toxic response. When the toxic effect is lethal the term used is the LD_{50}.

The Therapeutic Index = TD_{50}/ED_{50} and measures the margin of safety with respect to a particular toxic action. Other toxic effects may well have quite different therapeutic ratios. At best the ratio is a crude indication of relative safety.

C. Variability

As indicated there is variability among normal individuals with respect to dose-response relationships. Additional variability may be a function of age, disease or the concurrent administration of other drugs.

1. Tachyphylaxis is a rapid decrease in the magnitude of response due to the action of a drug.

2. Tolerance is a decrease in response to a given dose that results from prolonged administration of a drug.

3. Some individuals are hyperreactive or hypersensitive to a drug and some may exhibit an idiosyncratic reaction to the drug.