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EDITORIAL*

ON THE ESTIMATION OF RECEPTOR OCCLUSION BY IRREVERSIBLE COMPETITIVE PHARMACOLOGICAL ANTAGONISTS†

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DRUG interactions are usually classified as chemical, physiological (functional) or pharmacological.

The pharmacological antagonisms, especially the simple reversible competitive case and the irreversible competitive case have been studied most, both because they are most accessible experimentally and because they allow some interpretation in chemical terms.

Measures of intensity of antagonism have been proposed for each model—the pA_2,[1] and the pD'_2[2] (or pA_h[3]). The latter index is not a null measurement with regard to level of response. Also, when the pD'_2 is interpreted in terms of a drug-receptor reaction, the method contains no internal checks on applicability of the interpretation. Arunlakshana and Schild[3] have a formula for the binding constant of a noncompetitive antagonist, but they do not expect to find a relevant pharmacological system very easily.

More recently, several authors have introduced a method for estimating the association constant of an agonist-receptor reaction.[4–8]

All three types of measurements are related and are just variants of the Langmuir adsorption isotherm. This note will point out the similarities and provide a null method for measuring activity of irreversible blocking agents.

The model used to describe drug behaviour in cases of competitive antagonism is the conventional[9] reaction, according to the law of mass action, between drug molecules and a specific chemical entity (receptor) in the tissue, i.e.

$$\underset{x}{\text{Drug}} + \underset{1\text{-}y}{\text{Receptor}} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{y}{\text{Drug-Receptor Complex}} \tag{1}$$

With x to represent concentration of the drug and y the (fractional) receptor occupancy, equilibrium may be represented by

$$y = \frac{x}{x + k_e} \tag{2}$$

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where $k_e = k_2/k_1$ is the dissociation constant of the drug-receptor complex.

Introducing an analytical unit of concentration $c = x/k_e$ gives

$$y = \frac{c}{c + 1} \tag{3}$$

showing that one is just dealing with a logistic function.[10]

The action of an antagonist may take many forms mathematically, but the simplest are that in the presence of the antagonist k_e is changed or the total occupancy possible is changed, i.e.

$$y = \frac{x}{x + ak_e} \tag{4}$$

or

$$y = \frac{x}{x + k_e} b \tag{5}$$

The first is the case of competitive antagonism (*cf.* the form of equation II of ref. 11) with the typical "parallel" shift of the dose-response curve. The second is the case of noncompetitive antagonism[12] or irreversible competitive antagonism (ref. 13, equation 5) where the maximum of the dose-response curve is "depressed".

Many other variants are obviously possible (i.e. a combination of the above two, or nonlinear cases), but the two described are probably the only ones worth considering. They are simple enough to be useful experimentally and they can be significant from a chemical point of view, yielding estimates of fractional receptor occlusion by the antagonist or of binding constants for the drug-receptor complex.

Experimental approaches to date have been varied. The most meaningful have been variants of Gaddum's "dose-ratio" approach.[14] Response to a stimulant is taken to be some function of y. The function has been chosen with varying degree of caution.[9,15–17] (Occupational and kinetic theories of drug action need not be distinguished in the present argument.) The minimal postulate, which will be used here, is to assume that a given response corresponds to a given fractional receptor occupancy by the stimulant. That is, comparisons made at the same response level are assumed to be made when the stimulant drug is occupying the same fraction of the receptor pool.

Equating responses before and after the antagonist then amounts to equating the right-hand side of equation 2 with that of equation 4 or 5, yielding

$$\frac{x_1}{x_1 + k_e} = \frac{x_2}{x_2 + ak_e} \tag{6}$$

or

$$\frac{x_1}{x_1 + k_e} = \frac{x_2}{x_2 + k_e} b \tag{7}$$

where the subscripts label concentrations of drug before and in the presence of the antagonist.

Rearranging gives

$$\frac{1}{x_1} = a \frac{1}{x_2} \tag{8}$$

and

$$\frac{1}{x_1} = \frac{1 - b}{b} \cdot \frac{1}{k_e} + \frac{1}{b} \cdot \frac{1}{x_2} \tag{9}$$

The pharmacological interpretation for the case of reversible competitive antagonism (equation 8) is straightforward; the parameter a corresponds to $x'/k_{e'} + 1$) where the primes refer to the antagonist. If $1/x_1$ is plotted against $1/x_2$, the slope (a) then will give a measure of the binding constant for the antagonist.

Interpretation of equation 9 can take two principal forms. If the antagonist is noncompetitive[11] and the effect of the antagonist is to reduce intrinsic activity in a fashion directly proportional to the number of molecules of antagonist bound to receptors, a measurement of the binding constant for the inhibitor-receptor reaction might be derived. In practice, however, the experimental situation is so ill-defined that such a chemical interpretation is unwarranted. The reciprocal plot (equation 9) can reasonably be used only to confirm that the dose-response curve is shifted as described empirically by equation 7.

Of more interest is the irreversible competitive antagonist. Here b is interpreted as the fraction of receptors not occluded by the antagonist. Then $1 - b$ is fractional receptor occupancy, a value with some chemical significance. The measurement is made without assumptions as to the shape of the dose-response curve, and linearity of the plot (equation 9) provides an internal check of applicability of the model. Maximal responses are not necessary, which is a decided advantage with a preparation such as the nictitating membrane which does not perform well after vigorous contraction.

In the case of the irreversible antagonist, the receptor occlusion is the relevant index as opposed to the binding constant estimated in the reversible competitive case. The dissociation rate constant is so low with a drug like phenoxybenzamine that a meaningful measurement of an equilibrium constant from dose-response curves is impractical.

With considerable spare receptor capacity, the receptor occlusion by an irreversible antagonist can be measured by the shift of the dose-response curve for the agonist, since deviation from parallelism is slight. However, the method based on equation 9 is applicable not only in this special case, but also when the peak response starts to decrease.

It is the intercept of the line of equation 9 that has been recognized as a measure of the binding constant for an agonist-receptor reaction.[4–8]

The discussion above has involved only the action of antagonists, that is, agents which reduce availability of receptors. Similar considerations are applicable, however, to the action of factors which might increase the absolute concentration of receptors. For example, decentralization of sympathetic postganglionic nerve fibres has been thought to produce an increased sensitivity to norepinephrine in the nictitating membrane of the cat by increasing the number of receptors per cell.[18]

An increase in that concentration of receptors would probably not alter the maximum responses obtained because of the existence of spare receptors[16] or spare receptor capacity;[17] i.e. receptor activation is not the weak link in the sequence from drug administration to response. Also, because of a spare receptor capacity, effective concentrations of agonists are probably small when expressed in analytical units of concentration (as in equation 3). When this is the case, y is directly proportional to c (put $c \ll 1$ in 3). A change in absolute concentration of receptors, without a change in the function relating response to an agonist, to the absolute concentration of agonist–receptor complexes, would therefore be expected to lead to a parallel shift of the dose-response curve to the left.

Suppose an antagonist is administered in such a preparation where the receptors are unchanged chemically (i.e. dissociation constants of drug-receptor complexes are unchanged) and the relation between response and absolute number of receptors occupied is unchanged. Equations 6–9 will still be applicable, since their form is unaffected by choice of absolute or relative receptor occupancy by agonist in equations 2, 4 and 5. The a of equation 8 will still measure $(x'/k_e' + 1)$ of a competitive antagonist. The $1 - b$ from equation 9 will still measure fractional receptor occlusion when an irreversible competitive antagonist has been used, and so, if the exposure (concentration at receptor $\times$ time) to the antagonist has been unchanged, $1-b$ will still reflect the association rate constant for the antagonist–receptor reaction. The analysis is independent of the relation between response to an agonist and receptor occupancy by the agonist whether the occupancy be pictured in absolute or relative terms.

This immunity is not shared by the pD'_2 which, defined in terms of half-maximal responses, is tied to the nature of the relation between absolute receptor occupancy by an agonist and the response it produces. If this relation is unchanged and the total number of receptors per cell increases, spare receptor capacity will increase. An irreversible blocking agent would then have to block a greater fraction of the receptor pool (greater b) to depress the maximum of the dose-response curve to any given level. Thus, even if the exposure, the response-receptor occupancy relation, and the chemical nature of the receptor and drug were all unchanged, the antagonist would appear less able to block receptors when viewed in the light of the pD'_2.

The presence of increased spare receptor capacity would not be missed when equation 9 is used. The $1/x_1$ intercept will give a measure of k_e for the agonist. From this and the doses used, y may be calculated. From these values of occupancy and the observed responses, the whole relationship between occupancy and response [including occupancy needed for maximal response, i.e. 1 — (spare receptor concentration)] may be plotted.

As mentioned earlier, a simple increase in total number of receptors per cell would shift the dose-response curve of an agonist to the left. The ratio of the increased concentration of receptors to the original concentration would equal the increase in sensitivity expressed as a dose-ratio. The ratio of y for maximal response to an agonist in the control situation to that after an increase in number of receptors per cell would also equal this dose-ratio. Thus, there exists an internal check of the hypothesis that a particular factor acts solely by increasing the absolute concentration of receptors in a tissue.

For simplicity, an increase in the number of receptors was used as an illustration of the alternative to a change in k_e as an explanation of increased sensitivity to an agonist. A change in efficacy[16] of the agonist would be another alternative.

The measurement of receptor occlusion by an arbitrarily selected dosage schedule of an irreversible competitive blocking agent can be more useful than might at first appear possible. For example, in the study of denervation supersensitivity, a change in the receptor for norepinephrine has been suggested. This has been examined recently in three investigations.[19–21] Receptor occlusion by a given dose of phenoxybenzamine on a normal nictitating membrane was compared with that on a chronically denervated or decentralized membrane. Use of the present analysis (equation 9) can facilitate quantitative interpretation of this sort of experiment. One no longer has to resort to phrases like "below and to the right" or "the shift is considerably less".

One cannot apply this sort of analysis mechanically, especially to a system such as the nictitating membrane *in vivo*. Furchgott and Bursztyn[8] examine pitfalls explicitly. Nevertheless, if a meaningful measurement is to be obtained, it will be an estimate of receptor occlusion rather than a pD'_2.

The plot associated with equation 9 should not be confused with the Lineweaver–Burke plot. In equation 9, both coordinates are reciprocal concentrations. The analysis in the case of enzymes is more direct, since one can assume that reaction velocity is proportional to the concentration of the enzyme-substrate complex. The corresponding assumption that response of a tissue is proportional to receptors occupied is usually incorrect. The analysis of equation 9 could be applied to enzymes, but the conventional approach is adequate and simpler.

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