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On the Analysis of Pharmacological Experiments in Terms of an Allosteric Receptor Model

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SUMMARY

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According to the usual drug-receptor model, developed by Clark, Gaddum, and Stephenson, one can determine drug-receptor dissociation equilibriun constants and relative efficacies experimentally, by analyzing the drug concentrations which cause equal responses under various conditions. Underlying this analysis is the concept of the "stimulus," which is directly proportional to the fraction of receptors activated, the proportionality constant being characteristic of each drug, and being known as the "efficacy." Equal biological responses are assumed to correspond to equal stimuli. Such experiments to determine equieffective drug concentrations circumvent the problem presented by the fact that there is no generally valid theory to interpret the shapes of concentration-response curves. This paper discusses the interpretation of these experiments according to the allosteric receptor model proposed by Karlin. In this model the receptor is assumed to have two conformational states, R and T, normally present in the ratio $T/R = L$. A drug A is assumed to combine reversibly with each of these two forms, with dissociation equilibrium constants K_{AR} and K_{AT}, respectively, and the biological response is assumed to depend on the proportion of receptors in the R form. The present analysis shows that for competitive drug interactions a "stimulus" can be defined which has exactly the same algebraic form as the "stimulus" in the Clark-Gaddum-Stephenson model; hence the two models are experimentally indistinguishable with respect to competitive drug interactions. The drug-receptor dissociation constant determined by these methods is K_{AT}, and the "efficacy" depend on the relative affinities of the drug for the R and T forms; i.e., the "efficacy" is

$$K_{AT}/K_{AR} - 1.$$

On the other hand, the experimental method utilizing partial irreversible receptor inactivation yields a different apparent dissociation constant, namely, $[K_{AT}^{-1} + (K_{AR}L)^{-1}]^{-1}$.

(Summary continued on following page)

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It has come to the author's attention that a rather similar analysis of the allosteric receptor model has been made independently by D. Colquhoun (personal communication), who presented it at the Symposium on Drug Receptors sponsored by the Biological Council and held in London in April 1972. It is to be published in the near future in a book edited by H. P. Rang.

SUMMARY (Continued)

By doing different kinds of experiments on two or more drugs, one can in principle determine all the equilibrium constants in the allosteric model. Both models allow for the possibilities of negative efficacy and of nonparallel log concentration-response curves for competitive antagonists with nonzero efficacy. Experimental evidence to date does not clearly rule out either model.

INTRODUCTION

Probably the most commonly used working hypothesis for drug-receptor interactions is the model developed by Clark (1, 2) and Gaddum (3), which assumes that drug combines with receptor in a reversible bimolecular reaction, and that occupation of the receptor by an active drug brings about the biological response. Many investigators have accepted Clark's implicit assumptions that the observed biological response to a drug is directly proportional to the number of receptors combined with it, and that a maximal response requires activation of all receptors. Clark himself, however, had doubts about the first of these assumptions (ref. 2, p. 64), and Stephenson (4) obtained evidence that neither of these assumptions was valid. He replaced them with the less restrictive assumption that the observed response was some unknown monotonic function of a quantity called the "stimulus." The "stimulus" caused by a drug was assumed to be directly proportional to the fraction of receptors occupied by the drug, the proportionality constant being characteristic of each drug and being known as the "efficacy." The "stimuli" caused by different drugs acting simultaneously on the same receptor were assumed to be additive.

According to the Stephenson model, the shapes of dose-response curves reflect in part the mathematical relation between "stimulus" and response, and since we have no way of determining this relation, the shapes of dose-response curves are of little or no use in providing insight into drug-receptor interactions. However, the Stephenson model asserts that equal responses correspond to equal "stimuli," and this assumption provides a basis for the analysis of equieffective drug concentrations under different experimental conditions. Experimental methods have been developed (4–11) which make use of this type of analysis to determine antagonist-receptor dissociation constants, agonist-receptor dissociation constants, and relative efficacies of agonists. Since all these fundamental parameters can be determined without recourse to Clark's highly dubious assumptions, there seems to be no reason to make those assumptions.

A few years ago Karlin (12) proposed an allosteric receptor model, which was a version of the allosteric protein model of Monod *et al.* (13). This is a very attractive receptor model because it gives a more clearly defined picture of molecular events than does the Clark-Gaddum-Stephenson model; however, much of Karlin's experimental support is based on analysis of the shapes of dose-response curves, and this requires unfounded assumptions about the mathematical relation between the number of activated receptors and the observed biological response. Karlin tried to keep these assumptions to a minimum, but he did assume that a maximal response requires maximal receptor activation, and that the slopes of the Hill plots of responses and of activated receptors are equal. That the first of these assumptions seems untenable as a generalization has already been noted. For the second, Karlin made a superficially plausible argument, but it appears to be fallacious. If the true activated receptor-response relation is not a certain type of rectangular hyperbola, then the slope of the Hill plot of responses will not in general be the same as the slope of the Hill plot of activated receptors. Karlin suggested that in such cases it is possible to approximate satisfactorily the true relation by a combination of segments of rectangular hyperbolae, but it is hard to see how this might be done. Any such combination of segments would necessarily be discontinuous, since the

hyperbolae are nonintersecting, and none of the segments would have the same slope as the corresponding part of the true activated receptor-response curve. Furthermore, if an average Hill plot slope over two or more segments is taken, this can no longer be shown to equal the slope of the Hill plot of activated receptors.

A disregard for the problem of the unknown activated receptor-response relation has characterized much of the literature on allosteric receptor models (12, 14–17). Rang (18) has pointed out the weakness of the resulting conclusions. It is quite possible, however, as with the Clark-Gaddum-Stephenson model, to place at least some conclusions on a firmer footing by basing the analysis on determinations of equieffective drug concentrations, on the assumption that equal responses correspond to equal degrees of receptor activation. Experimental results which have been analyzed and interpreted according to the Clark-Gaddum-Stephenson model can then be reanalyzed and reinterpreted according to the allosteric model.

A preliminary report of these studies has appeared elsewhere (19).

THEORY

There are three important equations, derived from the Clark-Gaddum-Stephenson model, which form the theoretical bases for three principal experimental methods for the determination of drug-receptor dissociation constants and relative efficacies. These are (a) the "dose ratio" equation for competitive antagonism (5, 6),

$$\frac{[A]}{[A]_0} = 1 + \frac{[B]}{K_B} \qquad (1)$$

(b) the equation for partial irreversible receptor inactivation (7–9),

$$\frac{1}{[A]} = \frac{1}{q}\frac{1}{[A]'} + \frac{(1-q)}{qK_A} \qquad (2)$$

and (c) the equation for comparing the dose-response curves of two agents when one of them is capable of causing its effects with negligible receptor occupancy (10, 11),

$$\frac{1}{[A]} = \frac{\epsilon_A K_B}{\epsilon_B K_A}\frac{1}{[B]} + \frac{\epsilon_A}{\epsilon_B K_A} \qquad (3)$$

In Eq. 1 $[A]$ is the concentration of drug A required to cause a certain effect in the presence of an antagonist B in the concentration $[B]$; $[A]_0$ is the concentration required to cause the same effect in the absence of B; and K_B is the drug B–receptor dissociation equilibrium constant. In Eq. 2 $[A]$ is the drug A concentration required to cause a certain effect; $[A]'$ is the concentration required to cause the same effect after irreversible inactivation of a fraction $(1 - q)$ of the receptors: and K_A is the drug A–receptor dissociation constant. In Eq. 3 $[A]$ is the drug A concentration required to cause a certain effect (with negligible receptor occupancy); $[B]$ is the drug B concentration required to cause the same effect; and ϵ_A, ϵ_B, K_A, and K_B are the respective Stephenson "efficacies" and drug-receptor dissociation constants.

Equation 1 can be solved directly for K_B in terms of experimentally determinable quantities; or K_B can be determined from the slope of a plot of $[A]/[A]_0$ vs. $[B]$; or log K_B can be determined from the abscissa intercept of a plot of log $([A]/[A]_0 - 1)$ vs. log $[B]$. Equation 2 permits computation of K_A from the slope and ordinate intercept of a plot of $1/[A]$ vs. $1/[A]'$; and Eq. 3 permits computation of K_B from the slope and intercept of a plot of $1/[A]$ vs. $1/[B]$.

A fourth equation gives the relative efficacy of two drugs A and B if the dissociation constants K_A and K_B are known (9):

$$\frac{\epsilon_B}{\epsilon_A} = \frac{[A](K_B + [B])}{[B](K_A + [A])} \qquad (4)$$

In this equation $[A]$ and $[B]$ are equieffective concentrations of A and B, respectively.

These equations are derived as follows. According to the Clark-Gaddum-Stephenson model we have, for drugs A and B and receptor R, the reactions

$$A + R \rightleftharpoons RA$$

and

$$B + R \rightleftharpoons RB$$

If y_A and y_B are the fractions of receptors occupied by A and B, respectively, then corresponding to these reactions we have the

chemical equilibria

$$K_A = \frac{[A](1 - y_A - y_B)}{y_A} \quad (5)$$

and

$$K_B = \frac{[B](1 - y_A - y_B)}{y_B} \quad (6)$$

Solving for y_A and y_B, we obtain

$$y_A = \frac{([A]/K_A)}{1 + ([A]/K_A) + ([B]/K_B)} \quad (7)$$

and

$$y_B = \frac{([B]/K_B)}{1 + ([A]/K_A) + ([B]/K_B)} \quad (8)$$

If ϵ_A and ϵ_B are the efficacies of RA and RB, respectively, then the stimulus S is equal to $\epsilon_A y_A + \epsilon_B y_B$. Substituting from Eqs. 7 and 8, we have

$$S = \frac{\epsilon_A([A]/K_A) + \epsilon_B([B]/K_B)}{1 + ([A]/K_A) + ([B]/K_B)} \quad (9)$$

If drug B is a competitive antagonist, then $\epsilon_B = 0$. Equieffective concentrations $[A]$ and $[A]_0$ in the presence and absence of B, respectively, produce equal stimuli; therefore, according to Eq. 9,

$$\frac{\epsilon_A([A]_0/K_A)}{1 + ([A]_0/K_A)} = \frac{\epsilon_A([A]/K_A)}{1 + ([A]/K_A) + ([B]/K_B)} \quad (10)$$

Solving this for $[A]/[A]_0$, we obtain Eq. 1.

If some fraction $(1 - q)$ of the receptors is inactivated, the stimulus will be proportional to the fraction q of receptors remaining functionally active. Equieffective concentrations $[A]$ and $[A]'$, before and after inactivation, respectively, produce equal stimuli; therefore, by Eq. 9,

$$\frac{\epsilon_A([A]/K_A)}{1+([A]/K_A)} = \frac{q\epsilon_A([A]'/K_A)}{1 + ([A]'/K_A)} \quad (11)$$

Solving this for $1/[A]$, we obtain Eq. 2.

If drug concentrations $[A]$ and $[B]$ are equieffective, we can again equate stimuli and obtain

$$\frac{\epsilon_A([A]/K_A)}{1 + ([A]/K_A)} = \frac{\epsilon_B([B]/K_B)}{1 + ([B]/K_B)} \quad (12)$$

If we make either of the two assumptions $\epsilon_A/\epsilon_B \gg 1$ or $[A]/K_A \ll 1$, and solve for $1/[A]$, we obtain Eq. 3. Equation 12 can also be solved to give Eq. 4.

We now consider the allosteric model. According to this model the receptor is capable of existing in either of two forms. Form R is the active form, which produces an observable biological response, while form T is inactive. In the untreated biological system these two forms are in equilibrium

$$T \rightleftharpoons R$$

and the equilibrium ratio of the proportions y_T and y_R of the two forms is $y_T/y_R = L$, where L is known as the allosteric constant (13). Forms R and T are each assumed to have n identical sites to which a drug A or B can become bound, in a reversible bimolecular reaction. This gives rise to a series of reactions which may be generalized as follows:

$$A + A_{i-1}B_jR \rightleftharpoons A_iB_jR$$

$$B + A_iB_{j-1}R \rightleftharpoons A_iB_jR$$

$$A + A_{i-1}B_jT \rightleftharpoons A_iB_jT$$

$$B + A_iB_{j-1}T \rightleftharpoons A_iB_jT$$

$$i + j \leqq n$$

If $y_{A_iB_jR}$ is the proportion of total receptor in the form A_iB_jR, then the proportion of total receptor sites occupied by A in the form A_iB_jR is $y_{A_iB_jR}\,(i/n)$. This is the proportion capable of dissociating to give the reaction $A_iB_jR \rightarrow A_{i-1}B_jR + A$. The proportion of total receptor sites unoccupied in the form $A_{i-1}B_jR$ (and hence capable of combining with A to give the reaction $A + A_{i-1}B_jR \rightarrow A_iB_jR$) is $y_{A_{i-1}B_jR}\,(n - i - j + 1)/n$. From these and similar considerations it follows that the generalized equilibrium equations for the reactions of R and T with A are

$$K_{AR} = \frac{(n - i - j + 1)y_{A_{i-1}B_jR}[A]}{iy_{A_iB_jR}} \quad (13a)$$

$$K_{AT} = \frac{(n - i - j + 1)y_{A_{i-1}B_jT}[A]}{iy_{A_iB_jT}} \quad (13b)$$

$$K_{BR} = \frac{(n - i - j + 1)y_{A_iB_{j-1}R}[B]}{jy_{A_iB_jR}} \quad (13c)$$

and

$$K_{BT} = \frac{(n - i - j + 1)y_{A_iB_{j-1}T}[B]}{jy_{A_iB_jT}} \quad (13d)$$

The allosteric model assumes that the biological response depends only on the total proportion of receptor in the R form, i.e., on

$$\begin{aligned} y_R &= y_R + y_{AR} + y_{BR} + \cdots \\ &\quad + y_{A_iB_jR} + \cdots + y_{A_nR} + y_{B_nR} \quad (14) \\ &= \sum_{j=0}^{n} \sum_{i=0}^{n-j} y_{A_iB_jR} \end{aligned}$$

To evaluate this expression, we note that iterative application of Eq. 13a gives

$$\begin{aligned} y_{A_iB_jR} &= \\ &\frac{(n - i - j + 1)[A]y_{A_{i-1}B_jR}}{iK_{AR}} \quad (15) \\ &= C_i^{n-j}\left(\frac{[A]}{K_{AR}}\right)^i y_{B_jR} \end{aligned}$$

where C_i^{n-j} is the binomial coefficient $(n - j)!/[i!(n - j - i)!]$. The factor y_{B_jR} on the right-hand side can be evaluated by a similar iterative application of Eq. 13c (with $i = 0$), and we have

$$\begin{aligned} y_{A_iB_jR} &= \\ &C_i^{n-j}\left(\frac{[A]}{K_{AR}}\right)^i C_j^n \left(\frac{[B]}{K_{BR}}\right)^j y_R \quad (16) \end{aligned}$$

Substituting in Eq. 14, we obtain

$$\begin{aligned} y_R &= \\ &y_R \sum_{j=0}^{n}\left[C_j^n \left(\frac{[B]}{K_{BR}}\right)^j \sum_{i=0}^{n-j} C_i^{n-j}\left(\frac{[A]}{K_{AR}}\right)^i\right] \\ &= y_R \sum_{j=0}^{n} C_j^n \left(\frac{[B]}{K_{BR}}\right)^j \left[1 \right. \quad (17) \\ &\quad \left. + \left(\frac{[A]}{K_{AR}}\right)\right]^{n-j} \\ &= y_R\left[1 + \left(\frac{[A]}{K_{AR}}\right) + \left(\frac{[B]}{K_{BR}}\right)\right]^n \end{aligned}$$

A similar derivation from Eq. 13b and c gives the total fraction of receptor in the T form:

$$y_T = y_T\left[1 + \left(\frac{[A]}{K_{AT}}\right) + \left(\frac{[B]}{K_{BT}}\right)\right]^n \quad (18)$$

Since $y_T = Ly_R$, Eqs. 17 and 18 can be substituted into $y_T + y_R = 1$ and the result solved for y_R, giving

$$\begin{aligned} y_R &= \left\{\left[1 + \left(\frac{[A]}{K_{AR}}\right) + \left(\frac{[B]}{K_{BR}}\right)\right]^n \right. \\ &\quad \left. + L\left[1 + \left(\frac{[A]}{K_{AT}}\right) + \left(\frac{[B]}{K_{BT}}\right)\right]^n\right\}^{-1} \quad (19) \end{aligned}$$

Substituting this into Eq. 17, we obtain

$$\begin{aligned} y_R &= \left\{1 + L \right. \\ &\left. \cdot \left[\frac{1 + ([A]/K_{AT}) + ([B]/K_{BT})}{1 + ([A]/K_{AR}) + ([B]/K_{BR})}\right]^n\right\}^{-1} \quad (20) \end{aligned}$$

Since response depends on y_R, it is natural to identify this quantity with the "stimulus" of Stephenson. However, the relation between the allosteric and Clark-Gaddum-Stephenson models can be shown more clearly if we choose a different definition, namely,

$$S' = \left|\left(\frac{Ly_R}{1 - y_R}\right)^{1/n}\right| - 1 \quad (21)$$

It is clear that equal values of y_R imply equal stimuli, by this definition. Substituting from Eq. 20 and rearranging, we obtain

$$\begin{aligned} S' &= \\ &\frac{(K_{AT}/K_{AR} - 1)([A]/K_{AT}) \\ &\quad + (K_{BT}/K_{BR} - 1)([B]/K_{BT})}{1 + ([A]/K_{AT}) + ([B]/K_{BT})} \quad (22) \end{aligned}$$

This is an expression of exactly the same form as Eq. 9, with ϵ_A replaced by $(K_{AT}/K_{AR} - 1)$ and ϵ_B by $(K_{BT}/K_{BR} - 1)$, and with K_A and K_B replaced by K_{AT} and K_{BT}, respectively. With these substitutions, therefore, Eqs. 1, 3, and 4 hold for the allosteric model as well as for the Clark-Gaddum-Stephenson model.

Concerning Eq. 2, however, the situation is somewhat different, because the stimulus, as defined in Eq. 21, is not directly proportional to the remaining fraction q of functionally active receptors. The fundamental relation for equieffective drug concentrations is $y_R = qy_R'$, which, on substituting from Eq.

20 (with $[B] = 0$), gives

$$\frac{1}{1 + L\left[\frac{1 + ([A]/K_{AT})}{1 + ([A]/K_{AR})}\right]^n} = \frac{q}{1 + L\left[\frac{1 + ([A]'/K_{AT})}{1 + ([A]'/K_{AR})}\right]^n} \tag{23}$$

This can be rearranged to the form

$$\left[\frac{1 + ([A]/K_{AT})}{1 + ([A]/K_{AR})}\right]^n = \frac{1}{q}\left[\frac{1 + ([A]'/K_{AT})}{1 + ([A]'/K_{AR})}\right]^n + \frac{1 - q}{qL} \tag{24}$$

A linear equation of the same form as Eq. 2 holds only if $n = 1$ and $[A]/K_{AR} \gg 1$ (the latter of course entails $[A]'/K_{AR} \gg 1$). With these assumptions Eq. 24 yields a linear equation (Eq. 26 below) of the same form as Eq. 2, but with the parameter K_A in Eq. 2 replaced by $[K_{AT}^{-1} + (K_{AR}L)^{-1}]^{-1}$.

To summarize, the four equations for the allosteric model corresponding to Eqs. 1–4 are

$$\frac{[A]}{[A]_0} = 1 + \frac{[B]}{K_{BT}} \tag{25}$$

$$\frac{1}{[A]} = \frac{1}{q}\left(\frac{1}{[A]'}\right) + \frac{1 - q}{q}\left(\frac{1}{K_{AT}} + \frac{1}{K_{AR}L}\right) \tag{26}$$

$$\frac{1}{[A]} = \frac{\epsilon_A K_{BT}}{\epsilon_B K_{AT}}\left(\frac{1}{[B]}\right) + \frac{\epsilon_A}{\epsilon_B K_{AT}} \tag{27}$$

and

$$\frac{\epsilon_B}{\epsilon_A} = \frac{[A](K_{BT} + [B])}{[B](K_{AT} + [A])} \tag{28}$$

In these equations $\epsilon_A = (K_{AT}/K_{AR} - 1)$ and $\epsilon_B = (K_{BT}/K_{BR} - 1)$, and Eq. 26 holds only for the special case in which $n = 1$ and $[A]/K_{AR} \gg 1$.

DISCUSSION

The fact that Eqs. 9 and 22 are of identical form means that the allosteric and Clark-Gaddum-Stephenson models are experimentally indistinguishable so far as competitive drug interactions are concerned. It is especially interesting that this holds for all n in the allosteric model and not just for $n = 1$. The formal identity of these two models has been missed by others and by myself (18, 19). The critical factor in recognizing this identity was the selection of an appropriate definition of "efficacy," namely, $K_{AT}/K_{AR} - 1$, instead of the more obvious choice, K_{AT}/K_{AR}.

The allosteric model offers a clear mechanistic explanation for a fundamental property of drugs, their ability to cause receptor activation. According to the allosteric model this property is due to the greater affinity of the drug for the active R form than for the inactive T form of the receptor. Previous authors (12, 14, 16, 17) have expressed this by saying that the "intrinsic activity" (20, 21) of a drug depends on its relative affinity for the two receptor forms. In the present analysis we have not used Ariëns' concept of "intrinsic activity," because as it was originally proposed (20), and as it is often used today, it assumes a knowledge of the activated receptor-response relation. We have used instead Stephenson's approach, which has from the start avoided this assumption. In this approach the ability of a drug to activate the receptor is reflected in its "efficacy," and, as the foregoing analysis shows, the "efficacy" is determined by the relative affinity for the active and inactive receptor forms.

With regard to the efficacy of antagonists, there is an interesting difference in bias between the allosteric and Clark-Gaddum-Stephenson models. Under the older model antagonists are usually assumed to have zero efficacy, whereas with the allosteric model antagonism is usually attributed to a preferential affinity for the inactive T form (15–17). This would give rise to a *negative* efficacy. This possibility of negative efficacy is not usually considered with the Clark-Gaddum-Stephenson model (although it can of course be introduced with no difficulty). On the other hand, discussions of the allosteric model have not emphasized the fact that a nonpreferential affinity—that is, an equal affinity—for the two forms of the receptor results in a fully effective competitive antagonist.

Since the allosteric model draws attention to the possibility of negative efficacy, it is of interest to know how this phenomenon might become apparent. Obviously a drug with negative efficacy might have a direct effect opposite to that of a drug with positive efficacy. However, negative efficacy need not always manifest itself in an observable negative drug effect. If $L \gg 1$, for example, almost all of the receptor is normally in the T form. In such a case the proportion of R form may be so low as to exert little effect, so that further reduction of the R form by a drug with negative efficacy would have no visible effect. It might even be argued that a marked preponderance of one form of an allosteric receptor at equilibrium in the absence of drugs is likely to be encountered more frequently than substantial proportions of each form. If so, systems capable of showing drug effects in both directions would be exceptional.

To look at the theoretical effects of a negative efficacy on the action of a drug as an antagonist, we rearrange Eq. 22 into the form

$$\left(\frac{\epsilon_A}{S'} - 1\right)\frac{[A]}{K_{AT}} + \left(\frac{\epsilon_B}{S'} - 1\right)\frac{[B]}{K_{BT}} = 1 \quad (29)$$

where $\epsilon_A = K_{AT}/K_{AR} - 1$ and $\epsilon_B = K_{BT}/K_{BR} - 1$. Setting $[B] = 0$, we find the corresponding $[A]_0 = K_{AT}/[(\epsilon_A/S') - 1]$, so that Eq. 29 can be solved for the dose ratio

$$\frac{[A]}{[A]_0} = 1 + \left(1 - \frac{\epsilon_B}{S'}\right)\frac{[B]}{K_{BT}} \quad (30)$$

This obviously reduces to Eq. 25 if $\epsilon_B = 0$ or, more generally, if $|\epsilon_B/S'| \ll 1$. Under other conditions, however, the apparent dissociation constant estimated from Eq. 30 would be $K_{BT}/[1 - (\epsilon_B/S')]$. This value is stimulus-dependent, and hence the log concentration-response curves would not be parallel.

This theoretical possibility of false apparent K_{BT} values and nonparallel log concentration-response curves is not likely to be important, however, except when the antagonist has detectable direct actions of its own. If the antagonist has no appreciable action of its own, the numerically largest stimulus it can produce, which equals the absolute value of ϵ_B, will not be an appreciable stimulus. For dose ratio determinations an appreciable stimulus is of course required, and therefore S' in Eq. 30 is likely to be numerically a good deal larger than ϵ_B, so that Eq. 30 reduces to Eq. 25. On the other hand, it is not *necessarily* true that an "appreciable" stimulus is substantially larger than a "negligible" stimulus. This depends on the stimulus-response relation; if this relation involves a rather sharp threshold, then an "appreciable" stimulus—one causing an appreciable response—may be only slightly larger numerically than a "negligible" stimulus.

Similar considerations apply with respect to the validity of Eq. 26 when an irreversible receptor inactivator is used. If (as is usually the case) the irreversible inactivator causes no detectable response, even when it is applied in high concentration for a prolonged period of time, this means that reduction or even abolition of the resting level of the R form has no effect. The simplest possible explanation for this would be that only a negligible proportion of receptor is in the R form at rest; i.e., L is large. If this is so, an agonist must produce a many-fold increase in the proportion of R form in order to cause a substantial response, and mathematically (Eq. 20) this requires a fairly high value of $[A]/K_{AR}$. It seems quite likely, therefore, that the assumption $[A]/K_{AR} - 1 \gg 1$ required for Eq. 26 will be true in many cases. Of course this is not absolutely certain, because it is conceivable that the proportion of R at rest is appreciable, but that there is a response threshold, such that decreases in R below the resting level have little detectable effect, while increases in R of comparable magnitude above the resting level have appreciable effects. This possibility does not seem especially likely, however.

With some drugs it has been possible (9, 11) to determine the dissociation constants by two or three of the experimental methods associated with Eqs. 1–3. Any agonist can be used as drug A in the method of Eq. 2, and if its efficacy is very low relative to some other agonist, it can be used as drug B in the method of Eq. 3 (with the higher-efficacy drug used as drug A) to obtain a second estimate of its dissociation constant. In addi-

tion, it may be possible to inactivate (by means of a β-haloalkylamine, for example) enough receptors to reduce the maximal response to a low-efficacy drug to a negligible level without abolishing the response to some other agonist with higher efficacy. If that is done, the low-efficacy drug will behave as a competitive antagonist to the higher-efficacy drug, and can be used as drug B in the method of Eq. 1 to obtain still another estimate of its dissociation constant.

An important difference between the allosteric and Clark-Gaddum-Stephenson models is that according to the older model (Eqs. 1–3) these three methods estimate the same parameter—the drug-receptor dissociation constant—whereas according to the allosteric model (Eqs. 25–27) the method of partial receptor inactivation (Eq. 26) estimates a different parameter—namely, $[K_{AT}^{-1} + (K_{AR}L)^{-1}]^{-1}$—from that estimated by the other two methods (i.e., the drug-T dissociation constant). The significance of this parameter can be deduced from Eq. 13a and b by using the relations $y_R = (y_R + y_T)/(1 + L)$ and $y_T = (y_R + y_T)L/(1 + L)$:

$$K_A = \left[\frac{1}{K_{AR}L} + \frac{1}{K_{AT}}\right]^{-1} = \left[\frac{y_{AR}}{Ly_R[A]} + \frac{y_{AT}}{y_T[A]}\right]^{-1} = \left(\frac{1 + L}{L}\right)\frac{(y_R + y_T)[A]}{(y_{AR} + y_{AT})} \quad (31)$$

Clearly K_A is directly proportional, with proportionality constant $(1 + L)/L$, to the over-all drug-receptor dissociation constant, with no distinction drawn between the R and T forms of the receptor.

The allosteric model would therefore predict that the K_A value determined when the drug is used as drug A in the method of Eq. 2 would be smaller than the K_B values determined when the drug is used as drug B in the methods of Eqs. 1 and 3. The data do indeed suggest a discrepancy (9, 11), but it is in the wrong direction: K_A values tend to be larger than K_B values. Since neither receptor model predicts such a discrepancy, these data do not support either model. It would be premature, however, to reject both models on the grounds of these data, since there seems a reasonable chance that the discrepancy may have come from some unrecognized source of experimental error.

If satisfactory experiments can be performed, it is in principle possible to determine experimentally all the important equilibrium constants in the allosteric model, at least for some drugs. Two drugs are required. For drug P the constant K_{PT} can be determined by using the drug as drug B in the methods of Eq. 25 or 27. The quantity $(1/K_{PT} + 1/K_{PR}L)$ comes from the method of Eq. 26, and from this and K_{PT} the quantity $K_{PR}L$ can be computed. In the same way the quantities K_{QT} and $K_{QR}L$ are determined for drug Q. For the two drugs the ordinate intercepts of Eq. 27 give $\epsilon_A/(\epsilon_P K_{AT})$ and $\epsilon_A/(\epsilon_Q K_{AT})$ (where ϵ_A and K_{AT} are the efficacy and T-dissociation constant for the strong agonist A employed in the method of Eq. 27), and the ratio of these ordinate intercepts gives

$$\frac{\epsilon_Q}{\epsilon_P} = \frac{(K_{QT}/K_{QR}) - 1)}{(K_{PT}/K_{PR}) - 1)} \quad (32)$$

Substituting $K_{PR}[K_{QR}L/(K_{PR}L)]$ for K_{QR} and solving for K_{PR}, we have

$$K_{PR} = \frac{K_{PT}(\epsilon_Q/\epsilon_P) - K_{QT}[(K_{PR}L)/(K_{QR}L)]}{(\epsilon_Q/\epsilon_P) - 1} \quad (33)$$

All the quantities on the right-hand side can be determined experimentally, so this allows computation of K_{PR}. A similar equation gives K_{QR}, and the value of L is determined from K_{PR} and $K_{PR}L$ or from K_{QR} and $K_{QR}L$.

This paper has concerned itself only with competitive drug interactions and not with allosteric effects. The latter, of course, are perhaps the most interesting feature of the allosteric model, and may provide a basis for analyzing the interactions of chemically unrelated drugs. We might hypothesize, for example, that two such drugs act at different sites on the same receptor. This proposal seems rather a long shot if we think of the receptor as a single polypeptide molecule, but it may seem more plausible if we consider the possibility that the several different receptor molecules in the membrane are associated together in a "protomer" (14), which

behaves as a single unit. If such a receptor or receptor protomer has n sites of one kind—say type 1—reacting with drug A and not with drug B, and m sites of a different kind (type 2), reacting with drug B and not with drug A, then the proportion of receptors or protomers in the active form is given (12) by

$$y_R = \left\{1 + L \cdot \left[\frac{1 + [B]/K_{BT}}{1 + [B]/K_{BR}}\right]^m \left[\frac{1 + [A]/K_{AT}}{1 + [A]/K_{AR}}\right]^n\right\}^{-1} \quad (34)$$

One can proceed here as for competitive interactions, perhaps again defining the "stimulus" as in Eq. 21; however, in this case the equations for equieffective drug concentrations do not seem to resolve themselves into any simple pattern. We nevertheless note one interesting point. If drug B has equal affinities for the R and T forms—that is, $K_{BR} = K_{BT}$—then it will not affect responses to drug A. However, drug B would be an effective competitive antagonist of other drugs acting at type 2 sites, as we have shown above. Therefore, even though the model postulates that type 1 and type 2 agonists act on the same receptor molecule or protomer, it allows for the possibility of completely selective competitive antagonists.

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