THE RELATION BETWEEN CLASSICAL AND COOPERATIVE MODELS FOR DRUG ACTION

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It is not my purpose to advocate any particular model for drug action as being the truth. And, *a fortiori*, the particular values of parameters used here in numerical examples are chosen for illustration only, though they are chosen to be not grossly incompatible with experimental results (see p. 163). My intention is merely to discuss the extent to which some new observations are compatible with the substantial body of quantitative evidence that is consistent with the classical ideas about drug action. These new observations suggest that some form of cooperative step is involved in the response to certain agonists, whereas no cooperativity was postulated in the classical ideas about drug action.

I. Classical ideas about drug action

The classical theory of drug antagonism was developed by Gaddum (1937), Schild (1949) and Arunlakshana & Schild (1959), on the basis of the work of Langley (1905), Hill (1909) and Clark (1933, 1937). The classical theory of the action of agonist drugs was developed mainly by Stephenson (1956), alternative models being proposed by Ariens *et al.* (1964) whose group subsequently adopted, in essence, Stephenson's view (van Rossum, 1966).

In all of this work the drug was assumed to react with identical, independent binding sites, the receptors, and occupation of the receptor by an agonist was supposed to activate it, the activation ceasing when the drug dissociated. The reaction may be written

$$A + R \rightleftharpoons AR \qquad (1)$$

where A represents the drug, R the receptor, and AR the complex. The

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model implies that binding at equilibrium will follow the simple hyperbolic Langmuir (1918) curve (Fig. 1), which was first described by Hill (1909). The Langmuir equation is

$$p_A = \frac{x_A}{x_A + K_A} = \frac{c_A}{c_A + 1} \tag{2}$$

where the subscript indicates the drug referred to, and

p = fraction of receptors occupied (the *occupancy*)
x = drug concentration
K = equilibrium dissociation constant (with the dimensions of concentration)
$c = x/K$ the normalized (dimensionless) concentration; concentration expressed as a multiple of the equilibrium constant. (3)

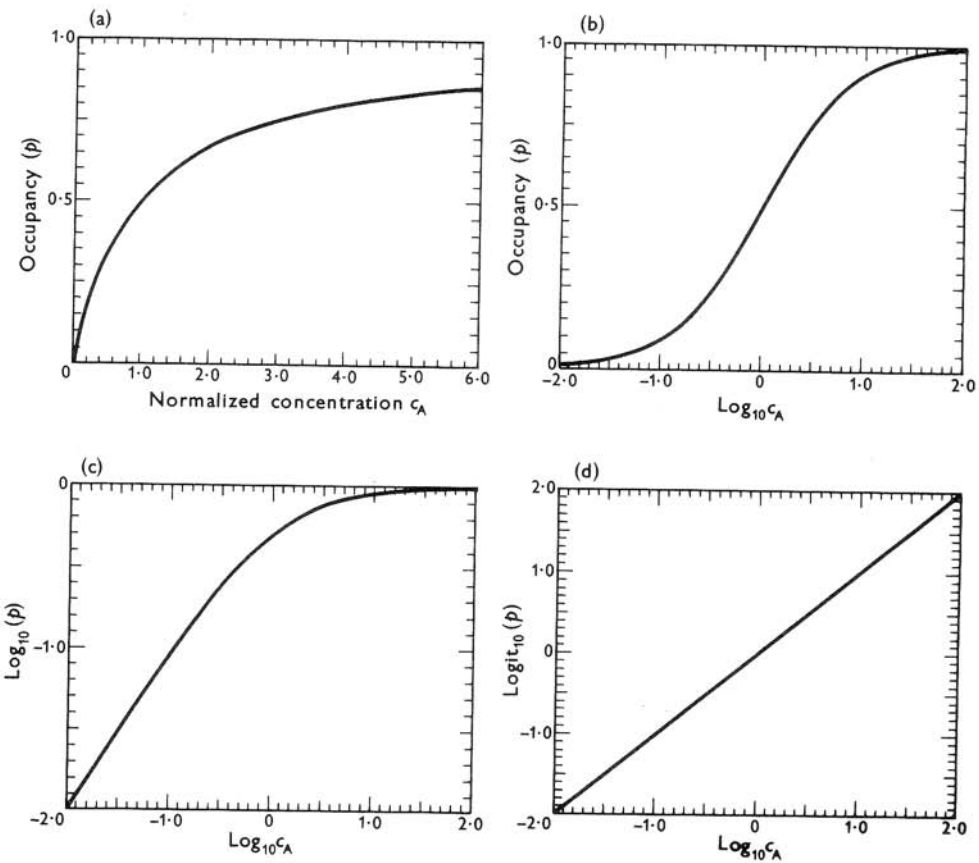

FIG. 1. The Langmuir curve (equation 1) plotted in various ways. (a), Occupancy, p, against normalized concentration, c_A. (b), p against log c_A. (c), $\log_{10} p$ against $\log_{10} c_A$. (d), Hill plot, that is, logit $(p) \equiv \log [(p(c)-p(0))/(p(\infty)-p(0))]$ against log c_A.

If several drugs are allowed to equilibrate simultaneously with the binding sites we get

$$p_A = \frac{c_A}{1+\sum c} \qquad \text{where} \quad \sum c = c_A + c_B + c_C \ldots \text{etc.} \tag{4}$$

Efficacy

The effectiveness of a competitive antagonist (at equilibrium) is described by its equilibrium constant alone. In the case of agonists, Stephenson (1956) postulated that, in addition to the equilibrium constant, one more drug-dependent variable, the efficacy, e, was needed to account for their action. Efficacy is an empirical constant, ranging from 0 (for an antagonist) upwards, and may be regarded as a measure of the effectiveness with which the drug, once combined, activates the receptor. Stephenson defined a *stimulus*, $S = e \times p$, and postulated that any drug producing a given value of the stimulus would produce the same tissue response, that is, the relationship between stimulus and response is characteristic of the tissue and does not depend on the drug used. Drugs with high efficacy can produce the maximum response of which the tissue is capable while occupying only a small fraction of receptors. In this case there is said to be a substantial number of spare receptors. The stimulus–response relationship is, in general, unknown, and calculations of drug affinities and efficacies depend on comparisons of drug concentrations producing equal responses, that is, by the use of a *null method.* This means that one needs to assume only that if an agonist produces a certain stimulus (or opens a certain fraction of ion channels in the models discussed below), the same response will always result, regardless of whether, for example, some other receptors are occupied by an antagonist.

Various null methods have been devised, within the framework of the classical theory, for the estimation of efficacies and equilibrium constants for antagonists, partial agonists and full agonists. The four main approaches used are the following:

1. Analysis of competitive reversible antagonism (Gaddum, 1937; Schild, 1949; Arunlakshana & Schild, 1959). The usual procedure is to measure the agonist dose ratio as a function of antagonist concentration. In most cases, the predicted linear relationship is obeyed with great accuracy (see Rang, 1971 and Fig. 8b) enabling the equilibrium constant to be calculated for the antagonist.
2. Comparison of concentration–effect curves for agonists of differing efficacies (Stephenson, 1956). If equiactive concentrations of drugs of different efficacy are plotted against each other as reciprocals, a linear relationship is predicted, from which (provided the difference in their efficacies is large) the equilibrium constant for the drug of lower efficacy can be calculated (for examples see Barlow, Scott & Stephenson, 1967; Mackay, 1966; Waud, 1969).

3. Interaction between drugs of different efficacy (Stephenson, 1956). In this type of test, the concentration of an agonist tested on its own is compared with the concentration that gives the same effect when applied together with a known concentration of a second agonist of lower efficacy. In a variant of this approach Furchgott & Bursztyn (1967) used an irreversible blocking agent to obtund completely the stimulation by the weaker agonist, and then measured its equilibrium constant as a competitive antagonist of the more efficacious drug.
4. The use of irreversible antagonists (Stephenson, 1966; Furchgott, 1966; van Rossum, 1966). In this method the concentrations of an agonist giving equal responses before and after irreversible block of a proportion of the receptors are compared and plotted on a reciprocal scale. The predicted linear reciprocal plot is usually obtained, and the resulting estimates of equilibrium constants agree quite well with those obtained by other methods (Furchgott & Bursztyn, 1967; Parker, 1972).

In addition to these measurements at equilibrium, kinetic experiments on the rate of approach to equilibrium (for example, Hill, 1909; Paton, 1961; Paton & Rang, 1965; Rang, 1966; Stephenson & Ginsborg, 1969; Colquhoun, 1968; Colquhoun & Ritchie, 1972b; Colquhoun, Henderson & Ritchie, 1972) also gave results that were in striking agreement with the predictions of the classical theories. However, the increasing realization that diffusion in the presence of binding may closely mimic the classical kinetic behaviour of the drug–receptor interaction has complicated the interpretation of kinetic experiments (see, for example, Rang, 1966; Thron & Waud, 1968; Waud, 1968; Colquhoun, Henderson & Ritchie, 1972).

More direct approaches

None of the experiments referred to so far give *direct* information about whether the relationship between drug concentration and occupancy (or number of receptors activated) in fact follows the postulated Langmuir curve in Fig. 1, though they are consistent with this model. Unfortunately, as is well known (for example, Stephenson, 1956; Waud, 1968; Rang, 1971), the complexity of the events between drug binding and response prevents any detailed analysis of the shape of the concentration–effect curve, when complex responses such as muscle tension are measured. The most fundamental response that can be measured at the moment is the ionic conductance change produced in postsynaptic membranes by transmitter analogues, where presumably this change is *directly* proportional to the number of ionic channels opened by the drug. It is from such measurements that the main difficulties for the classical model have arisen; these difficulties have led to the proposal of alternative cooperative models.

II. Reasons for modifying the classical ideas

Evidence for cooperativity in the relation between concentration and conductance increase

It was noticed by Katz & Thesleff (1957) and Jenkinson (1960) that the depolarization produced by carbachol at the frog neuromuscular junction was not related to concentration by the simple hyperbolic Langmuir curve, which the classical ideas would predict if occupation of a receptor opened a channel, but by a distinctly sigmoid curve. This sigmoidicity is accompanied by values for the slope of the Hill plot in the region of 2, rather than 1·0 expected for the Langmuir curve (Figs. 1 and 2). Similar sigmoid depolarization curves have been observed in the electroplax of the electric eel, in response to drugs such as carbachol, decamethonium and phenyltrimethylammonium (Karlin, 1967; Changeux & Podleski, 1968).

The sigmoid curve is still observed when the primary phenomenon, conductance increase, is measured in voltage-clamped preparations. This has been shown for the action of γ-aminobutyric acid (GABA) on insect muscle (Werman & Brookes, 1969) and crayfish muscle (Takeuchi & Takeuchi, 1969; Feltz, 1971), as illustrated in Fig. 2. (In this case the voltage change is small because GABA increases the chloride conductance and the chloride equilibrium potential is close to the resting potential.) Similar results were found at the voltage-clamped frog neuromuscular junction by Rang (1973), who observed sigmoid concentration–effect curves for cholinergic agonists. It does not seem that the sigmoidicity can be attributed to an artifact of the electrical measurement method, for Kasai & Changeux (1971a) obtained very similar results in measurements of the efflux of radiosodium from isolated sacs of membrane prepared from eel electroplax cells.

Can the receptor exist in only two conformations?

Many explanations for the observed cooperativity are, of course, possible. The choice could be narrowed a little if it were known whether or not the response to all agonists were qualitatively similar. For example, cholinergic agonists are known to increase the conductance of the postsynaptic membrane to both sodium and potassium. If the relative conductance increased to the two ions were not the same for all drugs, then it is obvious that more than one variable, in addition to an equilibrium constant, would be necessary to describe the differences between various agonists. So the qualitative similarity of responses was implicit in the classical theory which postulated a single variable, the efficacy. It has been shown by Rang (1972), at the frog neuromuscular junction, that the current flow induced by the drug is related to the voltage at which the membrane is clamped, in just the same way for a number of cholinergic agonists. The current–voltage curves could be superimposed by merely altering the drug concentration. There is evidence that

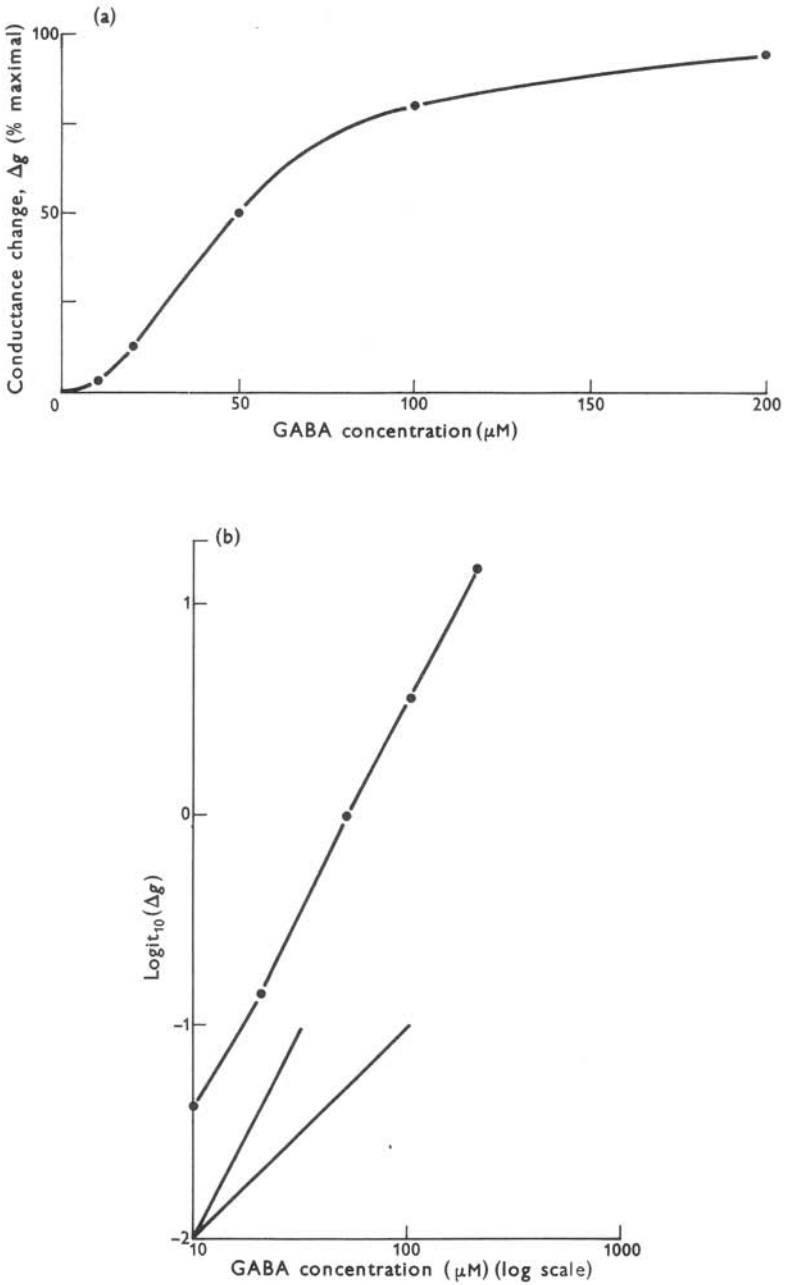

FIG. 2. Experimental sigmoid concentration–effect curves. (a), Conductance change at the crustacean neuromuscular junction produced by γ-aminobutyric acid (GABA). (b), Hill plot (defined in Fig. 1) of the same results. Replotted from Takeuchi & Takeuchi (1967). The lines at the foot have slopes equal to 1·0 and 2·0.

this relation extends up to the voltage at which no current flows; that is, that the reversal potentials, and hence the relative conductance increases to sodium and potassium, are the same for different cholinergic agonists (Manalis & Werman, 1969; Koester & Nastuk, 1970; Feltz & Mallart, 1971). In eel electroplax cells, Changeux & Podleski (1968) found that altering the external potassium concentration produced an equal modification of the depolarization caused by carbachol and decamethonium, which also suggests that both drugs act by the same ionic mechanism. These observations suggest that all the drugs tested produce channels with the same ionic conductance properties, namely, that the 'activated' receptor is the same, even though the efficacies of the drugs tested vary considerably. In other words, it now seems reasonable to postulate that the channel may exist in only two states,* open or shut, which are the same whatever drug is used. Only the length of time for which it is open and shut are dependent on the drug. This is in contrast with the classical view in which efficacy was usually thought of as representing a more-or-less continuously variable extent of opening of the channel, according to the nature of the ligand used. It is implicit in the rate theory of drug action proposed by Paton (1961) that the elementary quantum of stimulus to the tissue was independent of the drug used (see Furchgott, 1964; Paton & Rang, 1965; Waud, 1968). However, this model does not account for cooperativity: some two-state models that do will now be mentioned.

III. Explanations for the observed cooperativity

The sigmoid curves shown in Fig. 2 bear an obvious resemblance to the sigmoid oxygen binding curves seen with haemoglobin, or the sigmoid velocity/substrate concentration curve seen with enzymes such as aspartate transcarbamylase. A similar sigmoid relationship would result if several *independent* subunits had to be simultaneously in the correct conformation in order to open a channel; this sort of mechanism was used by Hodgkin & Huxley (1952) to describe the potassium channel in squid axon (in this case the equilibrium between different subunit conformations is controlled by membrane potential rather than drug concentration). These resemblances suggest certain simple models for drug action which are generally called allosteric though the term is rather ambiguous. The interaction between the binding site and the ionophore can, in a rather trivial sense, be described as allosteric, but homotropic and heterotropic interactions between separate binding sites have also been suggested, and these mechanisms closely resemble those postulated for allosteric enzymes. The present discussion will be restricted to drugs that act at the same site, or at least sufficiently nearly the

* The phenomenon of desensitization may require a third conformational state, in addition to the resting and activated states of the receptor (see Katz & Thesleff, 1957; Rang & Ritter, 1970a,b) but this added complication is not discussed further in this article.

same site for their binding to be mutually exclusive. A large part of the experimental evidence is most economically accounted for by this sort of mechanism, without having to postulate actions at more than one sort of site.

Two-state models in general

It will be found in Sections IV–VI that a large class of two-state models is compatible with the experimental results, that is, this class predicts results of the same form as the classical model. This phenomenon is a direct consequence of the fact that the experiments have, necessarily, been done using null methods (see above and p. 167). The models to be discussed all suppose that the receptor consists of one or more protomers. Each protomer bears a drug binding site, and can exist in only two conformations, for which the affinity of drug molecules may be different. The conformations corresponding to the open and closed states of the ion channel will be denoted R and T, respectively. The class of models compatible with the type of experimental observation referred to earlier (p. 153) includes all of those for which the fraction of open channels at equilibrium, in the presence of any number of drugs which all compete for the same binding site, is given by an expression of the form

$$p_{open}=f\left(\frac{1+\sum c}{1+\sum Mc}\right) \tag{5}$$

where $\sum$ indicates summation of the values for each sort of ligand present, and

p_{open} = fraction of channels in the open state.
f = is any monotonic increasing function.
$c=x/K_R$. The normalized concentration; the concentration, x, expressed as a multiple of the microscopic equilibrium constant for the interaction of the drug with binding site on a protomer in the R (open) conformation. (6)
$M=K_R/K_T$. The affinity of the drug for the binding site on the T conformation of a protomer, relative to that for the R conformation. This will be small for drugs which favour the R conformation, and thus tend to open the channels. Likewise, $Mc=x/K_T$, is concentration normalized with respect to the equilibrium constant for the T conformation.

The argument in parentheses in equation (5) is very closely related to Stephenson's 'stimulus'*. Some examples of mechanisms of this class will

* If the right-hand side of equation (5) is written for a single drug as

$$f\left(\frac{1+c}{1+Mc}-1\right)$$

now be considered in detail, and then the relation between this class of model and the classical model, and the relation of both to experimental results, will be discussed. Equation (5) implies that relations similar to the classical ones will be predicted if all of the variables that change during the experiment are included in the argument in parentheses. This is not so for the method in which concentration–effect curves are compared before and after an irreversible antagonist is applied. Therefore predictions for this type of experiment are less simple, and will be discussed separately (section VII).

The independent subunit model

The explanation of the observed cooperativity need not involve any interaction between binding sites. Following the analogy of the Hodgkin–Huxley (1952) model for the axonal potassium channel (see also Hill & Chen, 1971), we could postulate that in order for a channel to open a certain number (n say), of associated subunits must all be in the R conformation, and that the conformation (and hence affinity for drug) of each subunit is independent of that of all the others. Following Monod, Wyman & Changeux (1965), the reaction postulated, with a ligand A, for a single subunit is

$$\begin{array}{ccc} & L & \\ \mathrm{T} & \rightleftharpoons & \mathrm{R} \\ K_{\mathrm{AT}} \uparrow\downarrow & & \uparrow\downarrow K_{\mathrm{AR}} \\ \mathrm{TA} & & \mathrm{RA} \end{array} \qquad (7)$$

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the argument can be written as

$$\left(\frac{1}{M}-1\right)\left(\frac{Mc}{Mc+1}\right)$$

or

$$\left(\frac{1}{M}-1\right)\left(\frac{x}{x+K_{\mathrm{T}}}\right).$$

This is analogous with Stephenson's model

$$\text{Response}=f(S)=f(e\times p),$$

with the factor

$$\left(\frac{1}{M}-1\right)$$

corresponding with e, and the occupancy, p, corresponding with

$$\frac{x}{x+K_{\mathrm{T}}}$$

which is a Langmuir occupancy function, though it is at best only an approximation to the actual occupancy in cooperative models. For the two-state model, K_{T} represents the equilibrium constant for the T conformation, and it will be shown (p. 180) that experimental measurements of equilibrium constants do in fact give, approximately, estimates of K_{T}.

where $L=[T]/[R]$ = equilibrium constant for the T⇌R transition. The other symbols are defined in (6) above. Notice that L will be large when most channels are closed in the resting state. It makes no difference to predictions at equilibrium whether the TA⇌RA transition takes place at a finite rate or not, because the equilibrium constant for this transition is defined by the other equilibrium constants, being LM. The fraction of protomers in the R conformation in the presence of a number of ligands that compete for the same binding site will be, at equilibrium,

$$p_R = \frac{1}{1+L\left(\frac{1+\sum Mc}{1+\sum c}\right)} \tag{8}$$

If we assume that a channel opens only when all of its n protomers are in the R conformation, then the fraction of channels that are open, p_{open} is equal to the fraction of sets of n protomers that are all in the R conformation. This fraction follows from the multiplication rule of probability (see, for example, Colquhoun, 1971, pp. 20, 380–85). Because of the independence of subunits it is

$$p_{open} = p_R^n = \left(\frac{1}{1+L\left(\frac{1+\sum Mc}{1+\sum c}\right)}\right)^n \tag{9}$$

This is seen to be an example of equation (5).

As illustrated in Fig. 3, this model shows sigmoidicity and Hill slopes greater than 1·0, as found experimentally (Fig. 2).

The fraction of open channels in the absence of ligand ($c=0$) is, of course, in general, not zero, but from (9),

$$p_{open}(0) = \left(\frac{1}{1+L}\right)^n \tag{10}$$

and the maximum fraction of channels that can be opened by a large concentration of drug A ($c_A \to \infty$) is, in general, less than one, and is

$$p_{open}(\infty) = \left(\frac{1}{1+LM_A}\right)^n \tag{11}$$

The fraction of sites occupied by drug molecules as a function of drug concentration deviates from equation (9); it follows a hyperbolic curve with an apparent equilibrium constant of $K_R(L+1)/(LM+1)$, which approximates to LK_R for a strong agonist ($M \ll 1$) and to K_T for an antagonist ($M \geqslant 1$). It is clear from (10) and (11) that if L is very large $p_{open}(0) \simeq 0$, that is, hardly any channels are open in the resting state, and if M is very small (that is, the ligand combines only with the R conformation, and $LM \ll 1$), $p_{open}(\infty) \simeq 1$, that is, all the channels can be opened by a sufficient drug concentration. In

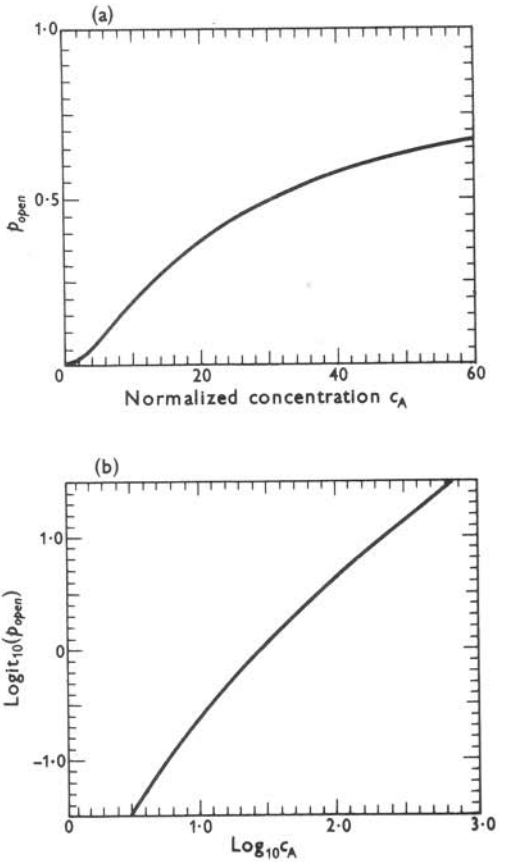


FIG. 3. Theoretical sigmoid concentration–effect curves. Independent model, $n=4$. Fraction of channels open in resting state $p_{open}(0)=0\cdot5\times10^{-3}$; fraction opened by very high drug concentration, $p_{open}(\infty)=0\cdot96$ ($L=5\cdot6$, $M=0\cdot0018$). (a), p_{open} against c (see equation 6); (b), Hill plot (maximum slope in range plotted is 1·8).

this special case only, virtually all the protomers in the R conformation will be occupied by a drug molecule, and virtually all those in the T conformation will be vacant. It, therefore, *looks as though* the act of occupancy has induced the conformation change. This special case is, therefore, referred to as the *induced fit case*. In this case, considering a single ligand for simplicity, equation (9) reduces to

$$p_{open}=\left(\frac{x}{x+LK_R}\right)^n \tag{12}$$

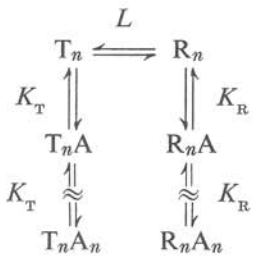
and the binding to individual subunits follows the simple Langmuir equation with an apparent equilibrium constant LK_R, which, because L is large, is

much higher than the real equilibrium constant, K_R; that is, the affinity is underestimated.

Furthermore, models that postulate that it is necessary to have m or more of the n independent subunits in the R conformation in order to open a channel are also special cases of equation (5) and, therefore, predict the same sort of behaviour, described below, in experiments based on null methods. This model, having an additional arbitrary parameter, can describe an even wider range of behaviour than equation (9).

The Monod–Wyman–Changeux (MWC) model

Monod, Wyman & Changeux (1965) proposed a model to account for the cooperative behaviour of haemoglobin and certain enzymes. Its application to drug receptors has been discussed by Karlin (1967) and Podleski & Changeux (1970). This model also postulates the existence of n subunits (protomers) of the sort symbolized in (7), but instead of the subunits being independent, they are linked in such a way that all n are constrained to adopt the same conformation, so there are still only two states, R_n (open) or T_n (shut). The transition between these states is referred to as a concerted transition. The reactions with a ligand, A, will be



where $L=[T_n]/[R_n]$ is the equilibrium constant for $T_n \rightleftharpoons R_n$ transition in the absence of ligand (which will, as before, be large if most channels are closed at rest). K_R and K_T are the *microscopic* equilibrium constants for the interaction with binding sites on protomers in the R and T conformations.

As in the independent case, it makes no difference to predictions at equilibrium whether the transitions $T_nA_i \rightleftharpoons R_nA_i$ $(i>0)$ take place at a finite rate, because the equilibrium constants for these reactions are defined by the other equilibrium constants, being LM^i.

The application of this model to drug receptors has been discussed by Karlin (1967). The fraction of channels in the open state (that is, R_n, R_nA, . . . R_nA_n) is

$$p_{open}=p_R=\frac{1}{1+L\left(\frac{1+\sum Mc}{1+\sum c}\right)^n} \qquad (13)$$

which is once again an example of the general case, equation (5). This model, also, predicts sigmoid binding curves and Hill plots* of slope greater than 1 as illustrated in Fig. 4. As in the independent case, a ligand that binds preferentially to the R state ($M<1$) will tend to shift the equilibrium towards

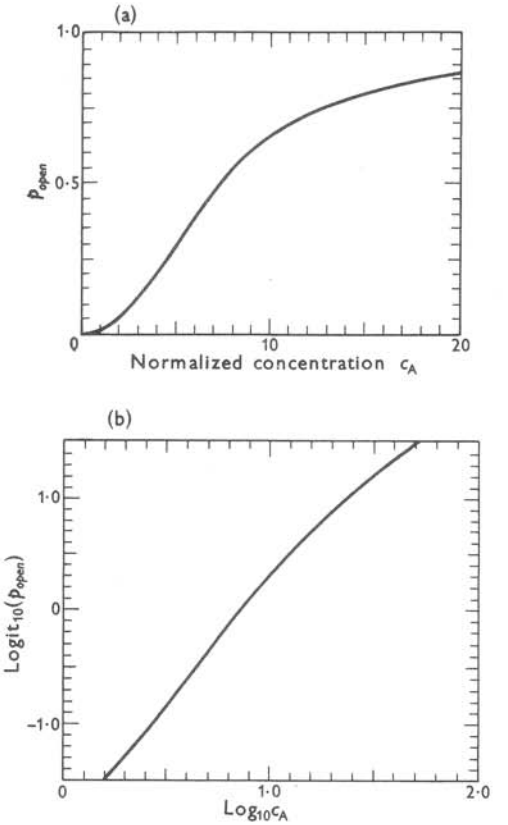


FIG. 4. Theoretical sigmoid concentration–effect curves. MWC model, $n=4$. $p_{open}(0)=10^{-3}$, $p_{open}(\infty)=0{\cdot}98$ ($L=1000$, $M=0{\cdot}067$). (a), p_{open} against c (see equation 6); (b), Hill plot (maximum slope in range plotted is 2·1).

* As with other models the Hill plot is not, in general, straight. The maximum slope is not more than n, being

$$n\sum_{i=0}^{n-1} M^i \Big/ \left(\sum_{i=0}^{n-1} M^{i/2}\right)^2$$

This maximum occurs at

$$c_R = 1/\sqrt{M}$$

and

$$p_R = 1/(1+LM^{n/2})$$

a point which may be outside the observable range of p_R.

this state. In this case, the concerted change of all n protomers means that this transition generates new high affinity (R) sites, which accounts for the cooperativity. In the absence of ligand

$$p_{open}(0)=p_{\mathrm{R}}(0)=\frac{1}{1+L} \tag{14}$$

and at very high concentrations

$$p_{open}(\infty)=p_{\mathrm{R}}(\infty)=\frac{1}{1+LM^n} \tag{15}$$

As in the independent case, the fraction of sites occupied will, in general, be different from p_{open}. But, again as in the independent case, when M is near zero (the ligand combines only with the open state) and L is very large, we approach a situation in which the receptors are either in the closed, vacant state (T_n) or in the open, occupied state ($\mathrm{R}_n\mathrm{A}_n$). Under these 'induced-fit' conditions

$$p_{open}=p_{\mathrm{R}}=\frac{x^n}{x^n+LK_{\mathrm{R}}^n} \tag{16}$$

which is the form of the Hill equation, proposed by Brown & Hill (1923) to account for the sigmoid binding of oxygen to haemoglobin. It has a linear Hill plot with a slope of n. The apparent equilibrium constant is seen to be increased (that is, affinity decreased) by a factor of $L^{1/n}$. Because of the slightly different definitions (cf. equations 10 and 14), this factor will have about the same numerical value as the factor L encountered in the independent case.

The lattice model

Changeux, Thiéry, Tung & Kittel (1967) proposed a model for cooperativity based on the proposition that the excitable membrane resembles a two-dimensional crystalline lattice made up of protomers (subunits), each with one or more binding sites. Suppose each protomer incorporates a channel, and can exist in R (open) and T (shut) conformations, with equilibrium constants K_{R} and K_{T} for the ligand. The equilibrium constant for the $\mathrm{R}\rightleftharpoons\mathrm{T}$ transition must as before be quite large in order that most channels are shut in the absence of ligand. For a protomer whose neighbours are all in the T conformation this equilibrium constant is defined as $L_0=[\mathrm{T}]/[\mathrm{R}]$. The origin of cooperativity in this model lies in the postulate that the energy needed for promotion of any protomer from the T to the R conformation decreases according to the number of neighbouring protomers in the R conformation. A simple assumption about the nature of the interaction between

protomers then gives the equilibrium constant between unoccupied R and T protomers as

$$L = L_0 \Lambda^{p_R} \tag{17}$$

where Λ is a measure of the interaction between protomers (Changeux *et al.*, 1967). The reaction scheme is thus exactly like (7), except that L is no longer a constant, but now depends on the fraction of protomers in the R conformation. This fraction is accordingly

$$p_R = \frac{1}{1 + L_0 \Lambda^{p_R} \left(\frac{1 + \sum Mc}{1 + \sum c} \right)} \tag{18}$$

This reduces to (8) when there is no interaction between protomers ($\Lambda = 1$). When $\Lambda < 1$ it predicts sigmoid curves and steep Hill plots like the other models (Changeux *et al.*, 1967). Although (18) is a transcendental equation, which has to be solved numerically for p_R, it is nevertheless of the general form of equation (5),* and so is consistent with the experimental observations.

Distinction between models, and estimation of parameters

No attempt has been made to distinguish between the models mentioned. This seems wise in view of the considerable controversy that still surrounds the basis of the cooperativity in such a well-studied molecule as haemoglobin (see, for example, Perutz, 1970; Edelstein, 1971; Minton, 1971; Hewitt, Kilmartin, Ten Eyck & Perutz, 1972; Ogata & McConnell, 1972). As shown below, a large part of the evidence is compatible with all of them. Of techniques in use at present, two are clearly potentially useful in distinguishing between models.

The first is the measurement of the conductance changes in response to various combinations of drugs. This has given useful results, but is limited by the technical difficulty of measuring large conductance changes. This often means that Hill plots can be obtained only for weak agonists, where the maximum response is in the observable range of conductance. It also means that it is not known whether even the most potent agonists can open *all* channels, so M cannot be estimated accurately. For numerical examples, values of M producing $p_{open}(\infty) = 0{\cdot}8 - 1{\cdot}0$ have been used for full agonists, and $p_{open}(\infty) \simeq 0{\cdot}05$ for partial agonists, these values being plausible guesses for the values at the frog neuromuscular junction. At the frog neuromuscular junction, it appears (Rang, personal communication) that no more than about 0·1 % of channels can be open at rest, so L must be at least 1000 in

* As long as Λ is not so small that the membrane undergoes an all-or-nothing phase transition (Changeux *et al.*, 1967). Such transitions are not seen in the experiments under discussion.

the MWC model, or $1000^{1/n}$ in the independent model. This may be compared with haemoglobin A for which L (MWC model) is thought to be at least 3000 and possibly much greater (see references above). Appropriate values of n are also uncertain. Numerical calculations have been done with $n=2$ and $n=4$, the latter being used here, arbitrarily, for numerical illustrations.

The second experimental approach is to measure the binding of labelled drug. The binding curve should show no sigmoidicity for the independent model, whereas for the MWC model and the lattice model, it could show sigmoidicity, as haemoglobin does (Monod *et al.*, 1965; Changeux *et al.*, 1967). Moderately precise results are available for binding of some antag-

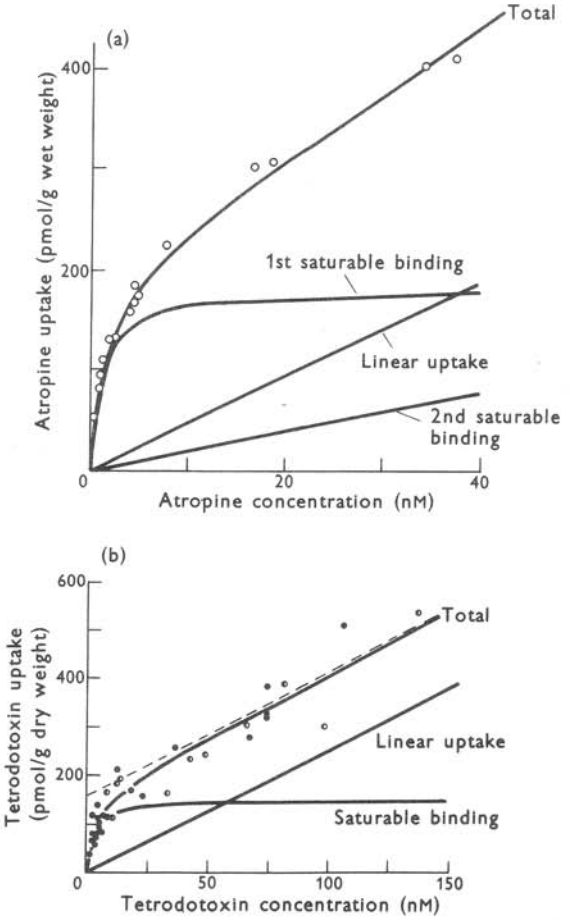


FIG. 5. Binding to intact tissues. (a), Binding of labelled atropine to smooth muscle of guinea-pig ileum, with postulated components of binding (Paton & Rang, 1965). (b), Binding of tetrodotoxin to rabbit vagus nerve, with postulated components of binding (Colquhoun, Henderson & Ritchie, 1972).

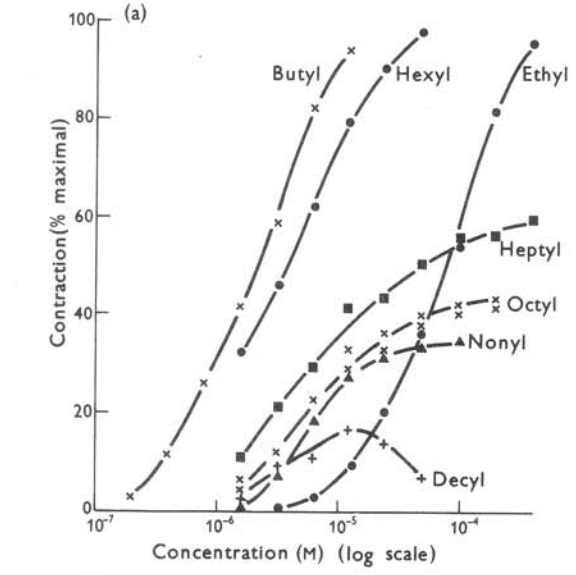
onist drugs. As shown in Fig. 5, no sigmoidicity is visible, but even according to the cooperative models, little would be expected for antagonists; with large L values, roughly hyperbolic binding, with equilibrium constant K_T would be expected. Unfortunately, there are still no precise enough experiments on the binding of agonists for a clear distinction to be made (see, for example, Paton & Rang, 1965; Kasai & Changeux, 1971b).

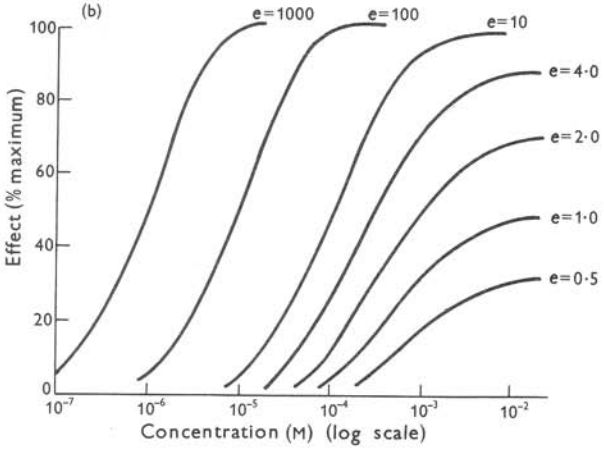
IV. Efficacy as selectivity

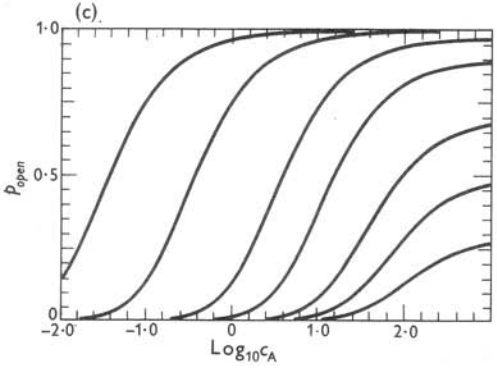
Fig. 6a shows the experimental results of Stephenson (1956), which show the transition from partial to full agonist in the alkyltrimethylammonium series of compounds acting on the guinea-pig ileum. Stephenson postulated that variation of a single drug-dependent parameter, efficacy, could account for the type of effect—antagonist, partial agonist, full agonist—produced by any particular drug, and showed that his results were quantitatively consistent with this hypothesis. Figure 6b shows theoretical curves calculated by Stephenson (1956) to illustrate the effect of changing efficacy, e, when the equilibrium constant (affinity) for the drug–receptor interaction was kept constant.

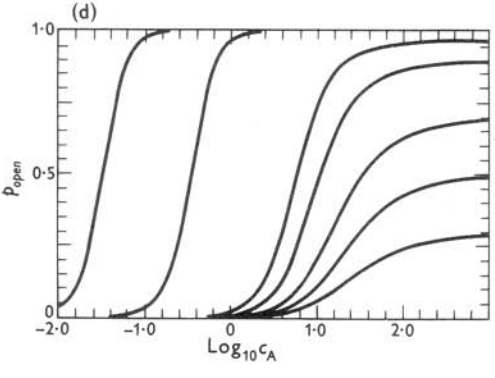
In the cooperative models discussed above, the selectivity, or relative affinity, M, of a ligand for the T and R conformations plays the role of efficacy (Changeux & Podleski, 1968), and unlike the original arbitrary parameter, this has a simple physical interpretation. Fig. 6 shows curves calculated for various values of M. Because it is shown below that the affinity (in classical terms) is much the same as K_T (in cooperative models), reducing M at constant affinity, means reducing K_R (that is, increasing the affinity for the open state), with K_T constant. The curves resemble those from the classical model. Clearly, the same general pattern will result from all two-state models of the class defined in (5). The maximum response, $f(1/M)$, will decrease as M increases, as in Fig. 6. Moreover, for a potent agonist (M very small), (5) reduces to $p_R \simeq f(1+x/K_R)$ so reducing K_R reduces the concentration for a given response by the same factor, thus producing the parallel shift to the left of the response–log concentration curve seen in Fig. 6.

The arguments in Section V, and in the footnote on pp. 156–7, suggest that the nearest that can be got to an analogue of the efficacy, e, is the quantity $(1/M)-1$. This becomes infinite for the most potent agonists, that is, when M becomes zero, as expected. And, again as expected, it is zero when $M=1$, so that the ligand occupies sites but has no effect on the open–shut equilibrium, that is, it is an ideal competitive antagonist. In the cooperative model a ligand could have M greater than 1 (so $1/M-1$ could be as little as -1). In this case the ligand would actively *close* channels, a sort of antagonist not included in the classical model (see Section V).


(a)
Butyl
Hexyl
Ethyl
Heptyl
Octyl
Nonyl
Decyl
Contraction (% maximal)
Concentration (M) (log scale)


(b)
e=1000
e=100
e=10
e=4·0
e=2·0
e=1·0
e=0·5
Effect (% maximum)
Concentration (M) (log scale)


(c)
p_{open}
$Log_{10}c_A$


(d)
p_{open}
$Log_{10}c_A$

V. Interaction of full agonists with partial agonists and antagonists acting at the same site

Methods have been devised for measuring, in the framework of classical theory, the equilibrium constants for antagonists (Gaddum, Hameed, Hathway & Stephens, 1955; Schild, 1949; Arunlakshana & Schild, 1959), and for measuring the equilibrium constants and relative efficacies of partial agonists (Stephenson, 1956). All of these methods are necessarily null methods (see Section I). They involve measuring the concentrations of agonist, A, needed to produce *equal responses* in the absence and presence of the antagonist or partial agonist.

Partial agonists

If equal responses are produced by concentration x_A of a full agonist on its own, and by concentration x'_A in the presence of the partial agonist at concentration x_B, then the classical theory indicates that, the following relationship should hold (Stephenson, 1956)

$$x_A = x'_A \left(\frac{1}{\frac{x_B}{K_B}\left(1 - \frac{e_B}{e_A}\right) + 1} \right) + e_B K_A x_B \qquad (19)$$

where e_A and e_B are the efficacies for the full and partial agonists. This predicts that a plot of x_A against x'_A should be linear, and the experimental results in Fig. 7 show that this is the case.

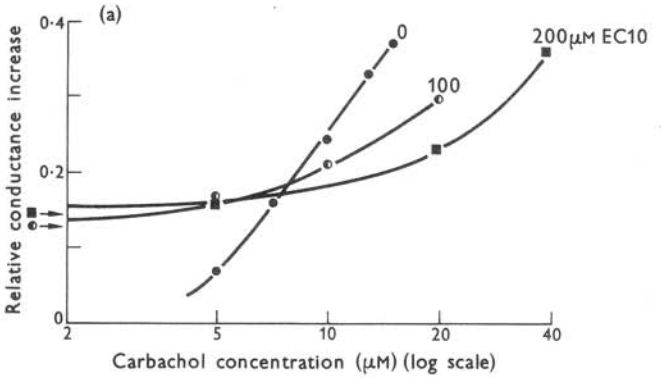
Provided that the efficacy of the full agonist is much greater than that of the partial agonist ($e_A \gg e_B$), K_B can be estimated from the slope of the plot as

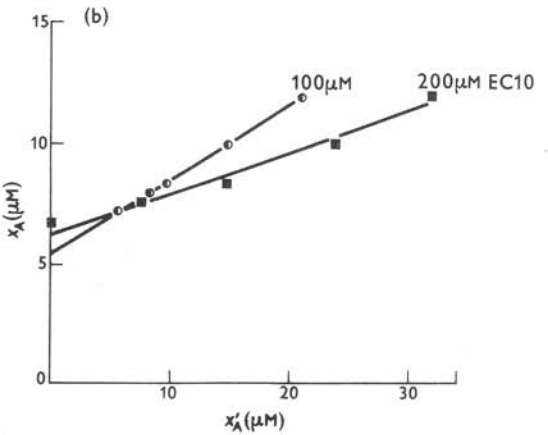
$$K_B \approx K_{est} = \frac{x_B}{\frac{1}{slope} - 1} \qquad (20)$$

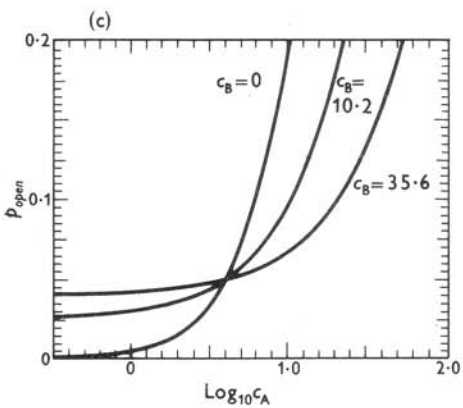
K_{est} actually estimates $K_B/(1 - e_B/e_A)$, which is close to K_B if $e_A \gg e_B$. This analysis is equivalent to the method described by Stephenson (1956).

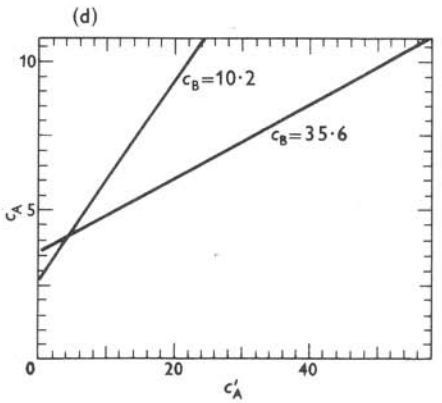
It is shown in Fig. 7 (c and d) that behaviour similar to that observed is predicted by the MWC model. According to any cooperative model of the class defined by equation (5) (examples of these were discussed in Section III), assuming as usual that equal responses to A in the presence and absence of B

FIG. 6. The interpretation of efficacy. (a), Concentration–effect curves for alkyltrimethylammonium compounds on guinea-pig ileum from Stephenson (1956). (b) Theoretical curves for the classical model found by changing efficacy with constant equilibrium constant (10^{-3} M for all curves). From Stephenson (1956). (c), Independent model, $n=4$, $L=5{\cdot}6$. $K_T=100$ for all curves, K_R reduced such that (from right to left) $M=0{\cdot}063$, $0{\cdot}034$, $0{\cdot}017$, $0{\cdot}0048$, $0{\cdot}0013$, $0{\cdot}00013$, $0{\cdot}000013$. (d), MWC model, $n=4$, $L=1000$. $K_T=10$ for all curves, K_R reduced such that (from right to left) $M=0{\cdot}22$, $0{\cdot}18$, $0{\cdot}14$, $0{\cdot}10$, $0{\cdot}075$, $0{\cdot}0075$, $0{\cdot}00075$.


(a)
0·4
0·2
0
Relative conductance increase
0
100
200μM EC10
2
5
10
20
40
Carbachol concentration (μM) (log scale)


(b)
15
10
5
0
x_A(μM)
100μM
200μM EC10
10
20
30
x'_A(μM)


(c)
0·2
0·1
0
p_{open}
c_B=0
c_B=
10·2
c_B=35·6
0
1·0
2·0
$Log_{10}c_A$


(d)
10
5
0
c_A
c_B=10·2
c_B=35·6
20
40
c'_A

correspond to equal values of p_{open}, it is found that the relation between x_A and x'_A is given by

$$x_A = x'_A \left[\frac{1}{1+\frac{x_B}{K_{BT}}\left(\frac{1-M_A/M_B}{1-M_A}\right)} \right] + \left[\frac{K_{AR}\frac{x_B}{K_{BT}}\left(\frac{1/M_B-1}{1-M_A}\right)}{1+\frac{x_B}{K_{BT}}\left(\frac{1-M_A/M_B}{1-M_A}\right)} \right] \tag{21}$$

which is again a straight line as illustrated in Fig. 7d. And application of equation (20) to get an estimate of the equilibrium constant gives simply

$$K_{est} = K_{BT}\left(\frac{1-M_A}{1-M_A/M_B}\right) \tag{22}$$

This is very like the classical result, and with the similar assumption that the agonist, A, is much more efficacious than the partial agonist, that is, that A is more selective for the R conformation, so $M_A \ll M_B$, it is seen that $K_{est} = K_{BT}$. It should be noticed that, because values of M_A cannot be accurately estimated (see p. 163), it is by no means certain that the factor $(1-M_A)/(1-M_A/M_B)$ is negligible.

Thus, the cooperative models yield similar predictions to the classical model, and making similar approximations, the former models all yield an estimate of the equilibrium constant for interaction of the partial agonist for the T conformation, which is the conformation associated with the shut channel.

Comparison of the classical and cooperative models shows that the ratio of efficacies in the classical model, e_A/e_B, is replaced, on the cooperative models, by

$$\frac{1/M_A-1}{1/M_B-1} \tag{23}$$

as discussed in Section IV, and footnote on pp. 156–7.

FIG. 7. Interaction method for partial agonists. (a), Interaction between carbachol (CCh) and two concentrations of decamethylene-*bis*(ethyldimethylammonium) (EC10) at the voltage-clamped frog neuromuscular junction (Rang, unpublished). The responses produced by EC10 alone are marked on the ordinate. (b), Plot of equieffective concentrations of CCh with (x'_A) and without (x_A) EC10, from Fig. 7(a). (c), Independent model, $n=4$, $L=5{\cdot}6$ (so, $p_{open}(0)=0{\cdot}5\times10^{-3}$). Full agonist has $M=0{\cdot}001$ (so, $p_{open}(\infty)=0{\cdot}98$). Partial agonist has $M=0{\cdot}2$ (so, $p_{open}(\infty)=0{\cdot}05$). All lines cross just as in the classical theory, at a point the ordinate of which is the maximum response of which the partial agonist is capable, that is, $p_{open}(\infty)=0{\cdot}05$ in this example. The normalized concentrations, c_B, of partial agonist supposed present, which are marked on the curves, are such that in the absence of the full agonist they will open 2·5% and 4% of the channels (that is, 50% and 80% of the maximum response that the partial agonist is capable of producing). (d), Plot of equieffective concentrations as in Fig. 7b. The lines are straight as in the classical theory, and intercept the ordinate above the origin. Either of these lines gives a good estimate of K_T (from equations (19) and (22)) because, in this example, $(1-M_A)/(1-M_A/M_B)=0{\cdot}996$, nearly unity.

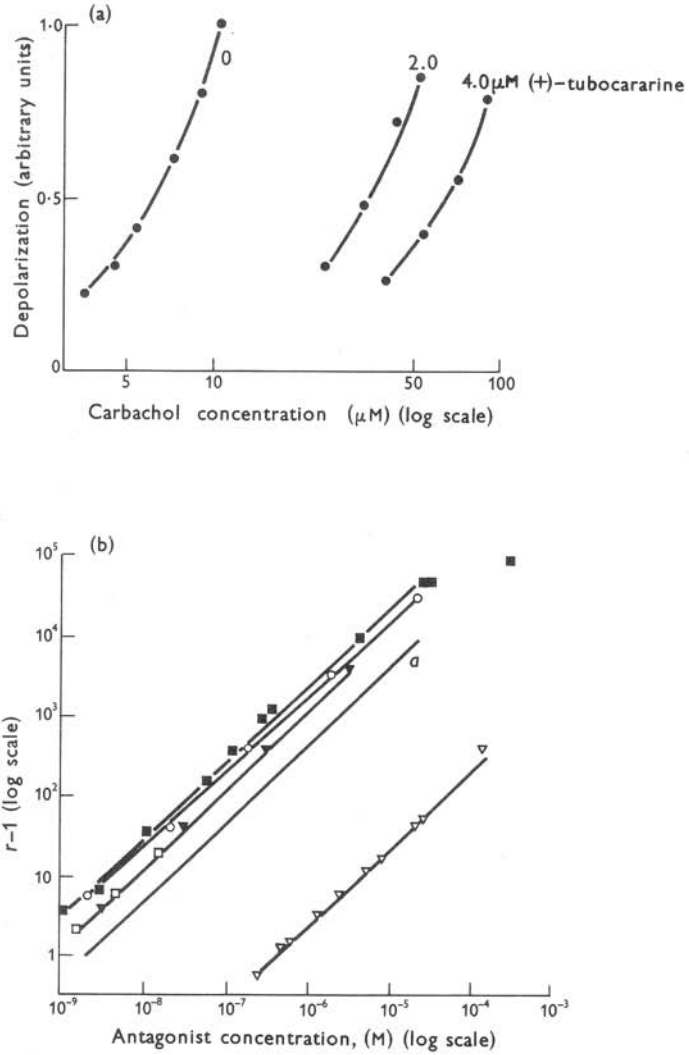


FIG. 8. Experimental results with antagonists. (a), An example of parallel shift of effect–log concentration curves by an antagonist. Depolarization (arbitrary units) of the frog neuromuscular junction by carbachol. (+)-Tubocurarine concentration: left curve, 0; middle curve, 2×10^{-6} M; right curve, 4×10^{-6} M. (From Jenkinson, 1960.) (b), Schild dose–ratio plots. Log $(r-1)$ is plotted against log x_B (antagonist concentration). (■), Hyoscine as antagonist of acetylcholine on guinea-pig ileum (Paton, 1961). (○), Atropine as antagonist of carbachol on guinea-pig atria (Thron & Waud, 1968). (□), Mepyramine as antagonist of histamine on guinea-pig ileum (Paton, unpublished). (▼), Atropine as antagonist of acetylcholine on guinea-pig ileum (Arunlakshana & Schild, 1959). (*a*), Propranolol as antagonist of (−)-isoprenaline on guinea-pig atria (Blinks, 1967). (▽), (+)-Tubocurarine as antagonist of acetylcholine on frog toe muscle (Jenkinson, 1960). In each case the slope is close to 1·0. Data replotted from the papers indicated. From Rang & Ritter (1971), by courtesy of the authors and Oslo University Press.

Antagonists

It has frequently been observed (see Fig. 8) that in the presence of a competitive antagonist, the log concentration–response curve is shifted in a parallel fashion to the right by a distance, defined as log r, where the dose ratio, r, is x'_A/x_A, the relative concentrations of agonist (A) producing the same response after and before adding the agonist (B). According to the classical theory of drug antagonism (Arunlakshana & Schild, 1959)

$$r=1+x_B/K_B \tag{24}$$

a result which may appropriately be called the Schild equation. This predicts that r is constant, regardless of the response level chosen, which is consistent with the observed parallelism. It also predicts that a plot of log $(r-1)$ against log x_B should be a straight line with unit slope. This prediction has been repeatedly confirmed with considerable accuracy, over a large range of concentrations, and for many drugs. Some of these results are shown in Fig. 8. The equilibrium constant can be estimated from the intercept of this plot, log K_B, or with one value of r, from

$$K_{est}=\frac{x_B}{r-1} \tag{25}$$

which, from (24), gives K_B.

The relation between x_A and x'_A, for all cooperative models of the class defined by equation (5), is given by (21). An ideal competitive antagonist, that had no other effect than to exclude agonist, would have equal affinities for both R and T states ($M_B=1$) so that it would not disturb the R⇌T equilibrium. When $M_B=1$ is put into (21), we obtain

$$\frac{x'_A}{x_A}\equiv r=1+\frac{x_B}{K_{BT}} \tag{26}$$

which is just the same as the Schild equation (24), and shows that, as for partial agonists, we obtain (for example, from equation 25) the equilibrium constant of the antagonist (B) for the T conformation, K_{BT}.

The cooperative models include the possibility that the antagonist will not merely fail to open channels, but it may actually close whatever channels are open in the resting state, namely, we could have $M_B>1$. From equation (21) we obtain, in general

$$\frac{x'_A}{x_A}=r=\frac{x_B}{K_{BT}}\left[\left(\frac{1-M_A/M_B}{1-M_A}\right)+\left(\frac{1-1/M_B}{1-M_A}\right)\frac{K_{AR}}{x_A}\right]+1 \tag{27}$$

where x_A is the concentration of agonist that produces, when given alone, the standard response level at which the dose ratios are measured. The appearance of this quantity on the right-hand side shows that the shift of the response–log concentration curve will not be exactly parallel when the antagonist prefers the shut (T) conformation ($M_B>1$). Correspondingly,

the estimate of the equilibrium constant from (25) is, in general,

$$K_{est} = K_{BT}\left[\frac{(1-M_A)}{(1-M_A/M_B)+(1-1/M_B)\,K_{AR}/x_A}\right] \tag{28}$$

which is only exactly K_{BT} when $M_B = 1$.

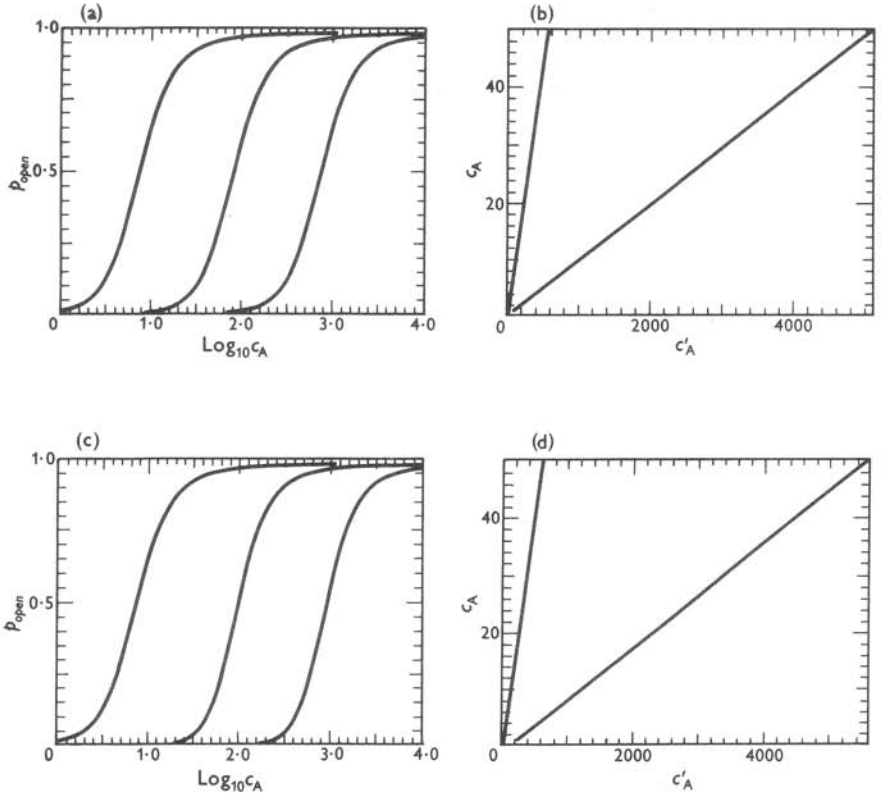


FIG. 9. Theoretical predictions for antagonists. MWC model, $n=4$, $L=1000$, so $p_{open}(0)=0{\cdot}001$. Agonist (A) has $M_A=0{\cdot}067$, so $p_{open}(\infty)=0{\cdot}98$. (a), Parallel shift of effect–log concentration curves by an antagonist (B) with $M_B=1{\cdot}0$, in concentrations producing dose ratios of 11 and 101 ($c_{BR}=10$ and 100). The dose ratio method gives K_{BT} exactly (equation 26). (b), Plot of c_A against c'_A corresponding to Fig. 9a. Both lines are straight, and go through the origin showing that the dose ratio $r=x'_A/x_A$ is a constant, independent of x_A, i.e. that the shift is parallel in Fig. 9a. (c), Shift of effect–log concentration curves by an antagonist with preferential affinity for the shut conformation, $M_B=100$. The shift is not quite parallel. Although the nonparallelism does not look striking, the equilibrium constant estimated from the dose ratio at the bottom end of the curves is $0{\cdot}67\,K_{BT}$ whereas measuring the dose ratio at the top gives $0{\cdot}88\,K_{BT}$ (from equation 28). (d), Plot of c_A against c'_A corresponding to Fig. 9c. The fact that the lines do not go exactly through the origin reflects the nonparallelism in Fig. 9c, that is, the dependence of r on x_A. The slope of either of these lines, if concentration rather than normalized concentration were used, would give the equilibrium constant as $K_{BT}(1-M_A)/(1-M_A/M_B)=0{\cdot}93\,K_{BT}$.

However, equation (27) *does* predict that the plot of log $(r-1)$ against log x_B, illustrated in Figs. 8 and 9, will still be linear with unit slope even if $M_B > 1$, provided that all dose ratios are measured at the same response level, so that x_A is constant. In fact, the degree of nonparallelism predicted when $M_B > 1$ is small, as illustrated in Fig. 9. This is true especially, as equations (27) and (28) show, if $x_A \gg K_{AR}$, which is the case for many plausible parameter values (see Fig. 9, for example). Experimentally the straight dose-ratio plots with unit slope, shown in Fig. 8b, are much better documented than *precise* parallelism.

It is interesting that even if $M_B > 1$ then, from equation (21), the plot of x_A against x'_A should still be exactly straight, and the slope of this plot should give, using equation (20), the result

$$K_{est} = K_{BT}\left(\frac{1-M_A}{1-M_A/M_B}\right)$$

just as for a partial agonist. This result is the same as equation (22) and shows that this method will give K_{BT} as long as a sufficiently powerful agonist is used, (that is, $M_A \ll M_B$). It is illustrated in Fig. 9 that, although the x_A versus x'_A plot should always be straight, it only goes exactly through the origin when $M_B = 1$, so only in this case is $r = x'_A/x_A$ a constant. Equation (21) and Figs. 7 and 9 show that when $M_B < 1$ (partial agonist) the line passes above the origin, and when $M_B > 1$ (antagonist) it passes below the origin.

VI. Comparison of full agonists with partial agonists

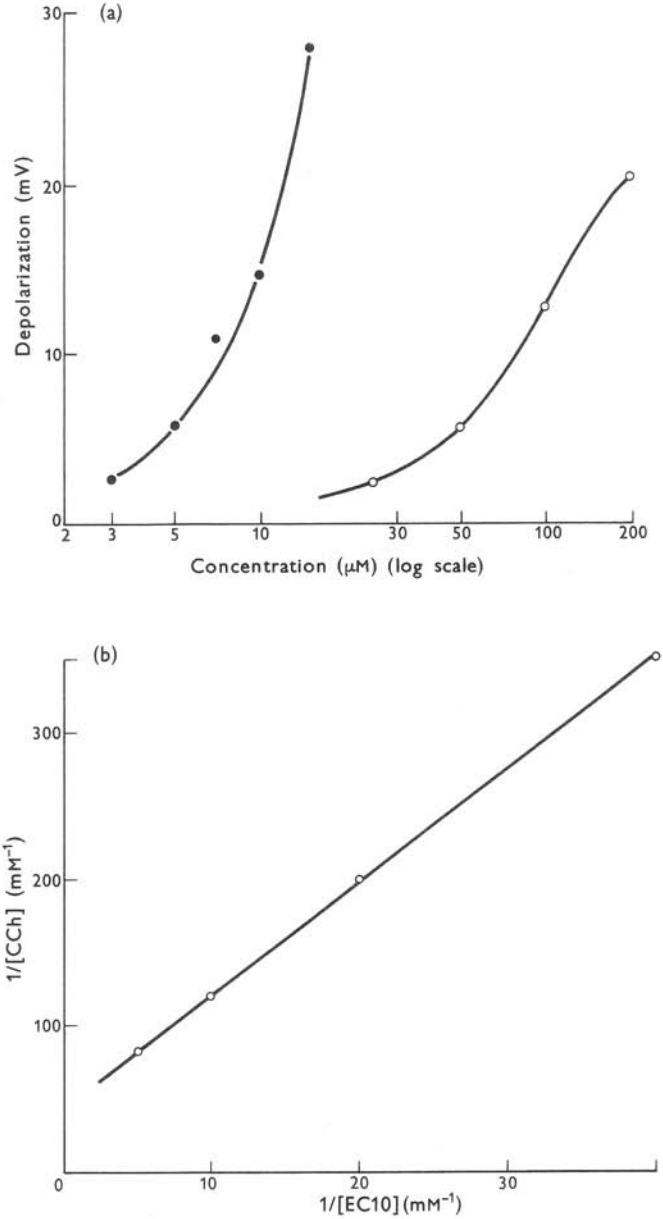
The equilibrium constants, and relative efficacies, of partial agonists can also be estimated by comparing the doses of full agonist (x_A) and partial agonist (x_B) that produce the same response when each drug is given on its own (Barlow, Scott & Stephenson, 1967; Waud, 1969). Fig. 10 shows typical experimental results. In this case the classical theory predicts that a double reciprocal plot of $1/x_A$ against $1/x_B$ will be straight and, as shown in Fig. 10, this is observed. The equilibrium constant can be estimated as

$$K_{est} = \frac{slope}{intercept} \tag{29}$$

from this plot, and on the classical theory, this gives $K/(1-e_B/e_A)$, exactly as for the interaction method. And, again exactly as before, all the cooperative models of the form of equation (5) predict linear plots (see Fig. 10), and the quantity estimated by (29) is $K_{BT}(1-M_A)/(1-M_A/M_B)$, as in equation (22).

It should be stressed that double reciprocal plots are shown here only because they have been widely used by other authors. Such plots are usually a poor way of estimating parameters, especially if the experimental

results are variable (see, for example, Colquhoun, 1971). The properties of estimates made by means of the usual unweighted double reciprocal plot are not known in detail for the present sort of experiment, but past experience in simpler situations certainly suggests that properly weighted fitting, as described by Parker & Waud (1971), should be used.



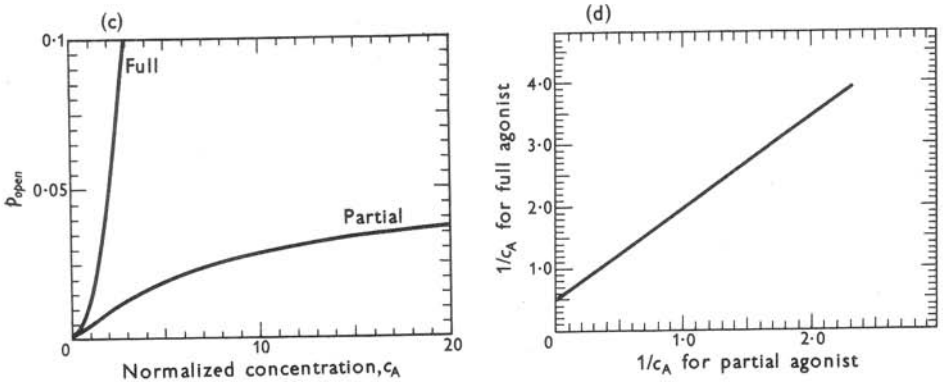


FIG. 10. Comparison method for partial agonists. (a), Concentration–effect curves for the depolarization produced at the frog neuromuscular junction by carbachol (CCh), (●); and decamethylene-*bis*(ethyldimethylammonium) (EC10), (○). (Rang, unpublished.) (b), Double reciprocal plot of equieffective concentrations of CCh and EC10, determined in the experiment shown in Fig. 10a. (Rang, unpublished.) (c), MWC model $n=4$, $L=1000$. Effect–concentration curves for two agonists given separately. Full agonist (A) has $M_A=0\cdot067$, so $p_{open}(\infty)=0\cdot98$. Partial agonist (B) has $M_B=0\cdot371$, so $p_{open}(\infty)=0\cdot05$. (d), Double reciprocal plot of equieffective concentrations, $1/c_A$ against $1/c_B$, corresponding to Fig. 10c. This gives an estimated equilibrium constant of $K_{BT}(1-M_A)/(1-M_A/M_B)=1\cdot14\ K_{BT}$, from equation (29).

VII. The use of irreversible antagonists to determine the affinities and relative efficacies of agonists

This method, used by Waud (1963), Furchgott (1966), Mackay (1966), and Stephenson (1966), is the only available method for investigation of powerful agonists. Unfortunately, experiments done by this method are not performed under equilibrium conditions and are not described by equations of the form of equation (5). No such simple conclusions as those in earlier sections seem possible at the moment. However, the discussion below certainly shows no obvious inconsistency, either qualitative or quantitative, between the experimental observations and cooperative models.

Classical model

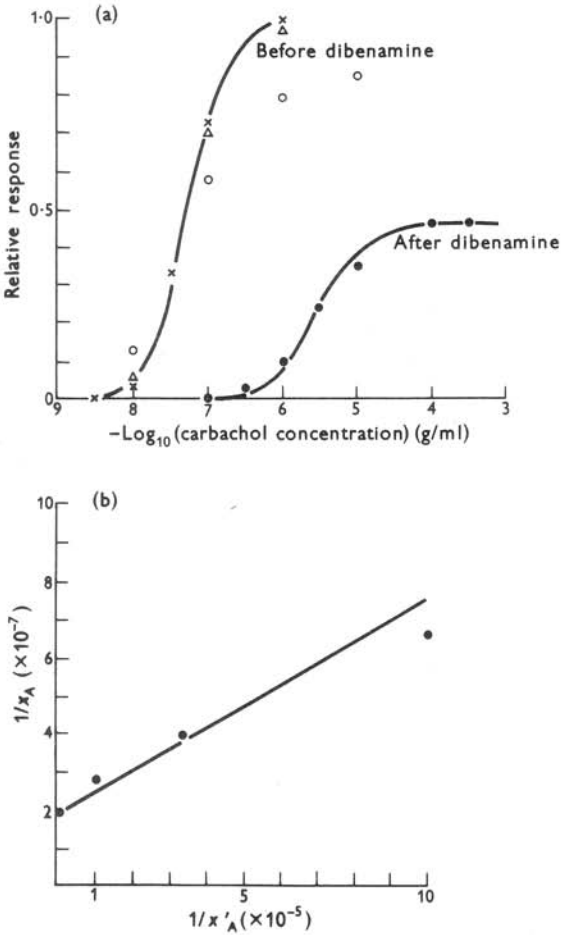
The concentrations of agonist that produce the same response before (x_A) and after (x'_A) exposure to an irreversible antagonist are measured. The classical model predicts that a double reciprocal plot of $1/x_A$ agonist $1/x'_A$ will be straight. From this plot

$$K_{est}=\frac{slope\ -1}{intercept} \tag{30}$$

provides an estimate of the equilibrium constant, K_A, for the agonist, and $1/slope$ = fraction of receptors not blocked. Experimental results are shown in Fig. 11.

Independent model

The simplest assumption is that the irreversible antagonist will permanently inactivate a certain fraction of channels, leaving the rest unchanged. This would happen, for example, if it occupied one or more of the n receptor sites associated with each channel. If the fraction of *sites* not occupied by the irreversible antagonist is p_{OB} then the fraction of open channels is equation (9) multiplied by p_{OB}^n (which is the fraction of channels remaining functional). This model does not predict linear double reciprocal plots in general, but numerical calculations show that in many cases the deviation is small. In particular, when $x_A \gg K_{AR}$ over the range of measurement, the plot will be straight. This condition is met when L is large enough, values of L in the guessed range, above $1000^{1/n}$ (see p. 163) usually giving reasonable



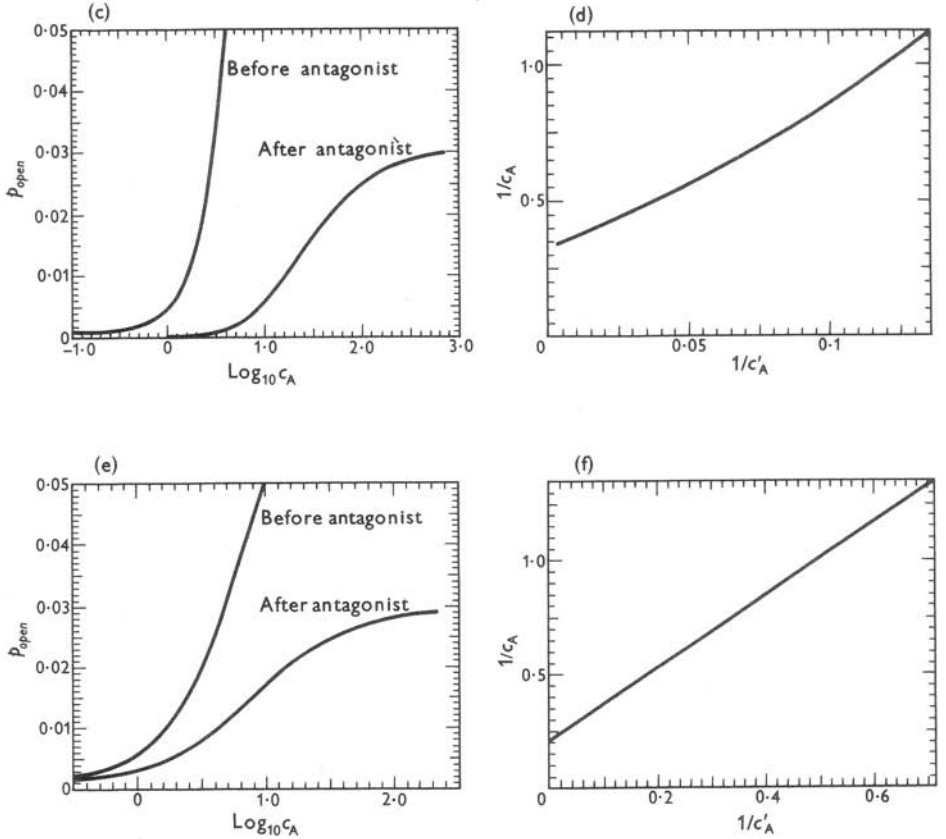


FIG. 11. Irreversible antagonist method. (a), Response of rabbit stomach smooth muscle to carbachol before and after dibenamine treatment. (Redrawn from Furchgott & Bursztyn (1967).) (b), Double reciprocal plot of equieffective concentrations of carbachol before and after dibenamine, $1/x_A$ against $1/x'_A$, from Fig. 11a. (c), Independent model $n=4$, $L=5\cdot6$, for a full agonist with $M_A=0\cdot00136$; that is, $p_{open}(\infty)=0\cdot98$. Fraction of binding sites not irreversibly blocked, $p_{OB}=1\cdot0$, $0\cdot422$. (d), Double reciprocal plot corresponding to Fig. 11c. This plot gives an estimate of the equilibrium constant for the agonist of $9\cdot7\ K_{AR}$, compared with $K_{AT}=730\ K_{AR}$ and $K_{AR}(1+L)/(1+LM)=6\cdot6\ K_{AR}$ (see equation (31)). $1/slope=0\cdot24$ compared with $p_{OB}=0\cdot42$. In this example equation (31) is only a rough approximation. (e), MWC model (equation (32)), $n=4$, $L=1000$, for a partial agonist with $M_A=0\cdot308$, that is, $p_{open}(\infty)=0\cdot1$. Fraction of binding sites not irreversibly blocked, $p_{OB}=1\cdot0$, $0\cdot614$. (f), Double reciprocal plot corresponding to Fig. 11e. This gives an estimated equilibrium constant of $0\cdot92\ K_{AT}$, close to the equilibrium constant for the shut conformation. The reciprocal of the slope is $0\cdot62$, close to p_{OB}.

lines, as shown in Fig. 11. When this condition holds, equation (30) estimates approximately

$$K_R\left(\frac{1+L}{1+LM_A}\right) \tag{31}$$

For partial agonists this quantity is close to K_T (that is, K_R/M), which is roughly consistent with experimental results as discussed in Section VIII. This happens mostly because for quite a large range of partial agonists, the value of K_T cannot be grossly different from the value of LK_R, that is, LM is of the order of unity. For more potent agonists, equation (31) suggests that the method will provide an approximate estimate of LK_R, rather than K_T (cf. p. 159).

Monod–Wyman–Changeux model

There is no good experimental basis for predicting the effect of an irreversible inhibitor. One assumption would be to suppose that a certain fraction of the oligomers was completely blocked, the remainder being normal. Most of the calculated examples give rather nonlinear double reciprocal plots in this case. Another plausible approach is to suppose that the antagonist is randomly bound to individual sites where it has no other effect but to exclude the agonist, so that a tetramer, for example, would be effectively converted to a mixture of tetramers, trimers, dimers, monomers and completely blocked tetramers. In this case

$$p_R = \sum_{i=0}^{n} \frac{\binom{n}{i} p_B^i \, p_{OB}^{n-i}}{1 + L\left(\frac{1+\sum Mc}{1+\sum c}\right)^{n-i}} \qquad (32)$$

where $p_{OB} = 1 - p_B$ is the fraction of binding *sites* not occupied by the irreversible inhibitor. Again, this does not, in general, give straight double reciprocal plots. However, if the conditions are such that the agonist concentrations, c_A', used in the presence of the antagonist, would open only a small fraction of channels even in the *absence* of antagonist, then the plots will be approximately straight, as in Fig. 11. This condition must be true for almost all partial agonists (for which even $p_{open}(\infty) \ll 1$), and numerical calculations suggest that it may well be true for many full agonists too, but ignorance of realistic M values for full agonists prevents more precise conclusions. When this approximation is valid, equation (30) gives quite a close approximation to K_T, and $1/slope$ estimates p_{OB}, as with the classical model. An example is shown in Fig. 11.

Furchgott's method

Furchgott & Bursztyn (1967) used an irreversible inhibitor to reduce the response of a partial agonist to near zero, while leaving a measurable response to a full agonist. The partial agonist then behaved like an antagonist, producing a parallel shift to the right of the log concentration–response curve for the full agonist. The equilibrium constant for the partial agonist was calculated from the shift (that is, the dose ratio) in the conventional way,

by means of the Schild equation (equations 24 and 25). Some experimental results obtained by this method are shown in Table 1. This method is simple to analyse because the receptor blockade by the irreversible antagonist is the same before and after the addition of the partial agonist, so the equations discussed earlier in this section conform with equation (5). Consequently, the dose ratio produced by the partial agonist would be expected to have the same form as before, that given in equation (27), where A stands for the full and B for the partial agonist. Clearly, the lines before and after B will become parallel when enough irreversible inhibitor has been bound to make $x_A \gg (1-1/M_B)$ over the range of measurement, so that r is independent of x_A. And in this case equation (28) shows that this method should estimate $K_{BT}(1-M_A)/(1-M_A/M_B)$, exactly like the methods of Sections V and VI.

VIII. Conclusions concerning the interpretation of experiments

Three facts that must be considered when thinking of alternatives to the classical model are (1) when the classical theory predicts a straight line, an approximately straight line has usually been observed; (2) several authors (Furchgott & Bursztyn, 1967; Waud, 1969; Parker, 1972; Rang, unpublished) have found that several methods for determining the equilibrium constants for partial agonists all give approximately (within a factor of 2 or so) the same result (some of these results are shown in Table 1); and (3) in the case of, for example, atropine (Paton & Rang, 1965) and tetrodotoxin (Colquhoun & Ritchie, 1972a; Colquhoun, Henderson & Ritchie, 1972), the affinity measured by binding to intact cells agrees with that found by various indirect null methods.

If any of the wide class of two-state models discussed in Section III were a

TABLE 1. *Estimates of equilibrium constants for cholinergic agonists*

Drug	Method	K	
MeN^+Et_3 (frog *rectus*)	Interaction (Me_4N^+)	4·07 mM	Barlow, Scott & Stephenson (1967)
	Comparison (Me_4N^+)	2·75 mM	
Pilocarpine (rabbit stomach)	Irrev. (DBN)	1·42 μM	Furchgott & Bursztyn (1967)
	CCh antag. (DBN)	1·28 μM	
Heptyl-TMA (guinea-pig ileum)	Comparison (CCh)	71 μM	Waud (1969)
	Irrev. (DBN)	105 μM	
	CCh antag. (DBN)	32 μM	
Decamethonium (frog neuromuscular junction)	Interaction (CCh)	54 μM	Rang (unpublished)
	Comparison (CCh)	48·7 μM	
	Irrev. (DNM)	39·4 μM	

Methods: *Interaction*, interaction with the stated full agonist (Section V). *Comparison*, comparison with the stated full agonist (Section VI). *Irrev.*, use of the stated irreversible antagonist (Section VII). *CCh antag.*, Furchgott's method with the stated irreversible antagonist (Section VII). CCh, Carbachol; DBN, dibenamine; DNM, dinaphthyldecamethonium mustard.

good approximation to the true explanation for the observed cooperativity, it would suggest the following conclusions.

The agreement with classical theory is expected to be very good for antagonists. And good agreement is expected between the equilibrium constants of antagonists found by binding and by the Schild method. Both should give approximations to K_T (see p. 153 and Section V). The observed linearity and unit slope of the Schild plot (Fig. 8), is, however, predicted even when the shift of response–log concentration curves is not exactly parallel, and if this happens a plot of x_A against x'_A would be more appropriate, as discussed in Section V. This would give $K_{BT}(1-M_A)/(1-M_A/M_B)$, which is close to K_{BT} if the full agonist (A) is potent ($M_A \ll 1$) *or* if the antagonist (B) has a similar affinity for both open and shut conformation (M_B near 1).

For partial agonists (B), both interaction (Section V) and comparison (Section VI) with a full agonist (A) estimate the same quantity, $K_{BT}(1-M_A)/(1-M_A/M_B)$, and the same quantity would be expected using Furchgott's method (Section VII) also. This too should be close to K_T, though the term $(1-M_A)/(1-M_A/M_B)$* may not be completely negligible. For the example in Fig. 10 the error would be about 14%. The estimate of K_T is biased by the same factor using all three methods (as in the classical case), so agreement between the methods does not indicate lack of bias.

The methods using irreversible antagonists are more difficult (except for Furchgott's method). The considerations in Section VII make it very probable that the equilibrium constant estimated by the use of irreversible antagonists would also be about K_T for *partial* agonists. This, and the predicted approximate linearity of the plots, shown in Fig. 11, certainly explain the experimental agreement between this method and the others, shown in Table 1. However, for powerful agonists the discussion in Section VII makes it unlikely that this method estimates K_T. Unfortunately, in this case, no other method is known to check the values obtained. In Section VII it is suggested that the quantity estimated for a very potent agonist is about LK_{AR} for the independent model.

I am grateful to Professor H. P. Rang for many discussions on the topics discussed in this article.

Note added in proof

Thron (1973, *Mol. Pharmac.*, **9**, 1) has recently published a comparison of the classical and MWC models which independently arrives at some of the results discussed in this paper. The main difference lies in the treatment of irreversible antagonists.

In contrast to Thron's result, which is similar to equation (31) above, the results in Section VII suggest that, for the MWC model, the irreversible

* If $1/M-1$ were taken as an analogue of efficacy (pp. 156, 157, 169), this factor would be the same as that occurring in the classical theory, $1/(1-e_B/e_A)$, (pp. 167, 173).

antagonist method should give K_T approximately for a *partial* agonist, as should other methods, thus explaining the experimental agreement between methods for partial agonists: but for agonists of high efficacy the present treatment suggests that there is at present not enough knowledge to interpret with any certainty the irreversible antagonist method in terms of cooperative models.

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DISCUSSION

Worcel (Paris)

The Langmuir equation in the case of a reaction order greater than one predicts a sigmoid rather than a hyperbolic saturation curve. Although this interpretation demands integral values for the Hill coefficient, would you invariably exclude it as an explanation of sigmoid concentration–effect curves?

Colquhoun (Yale)

The reaction *order* (as opposed to molecularity) is essentially an empirical quantity, so it merely describes the observations and does not explain them. In order to find any sort of explanation one must postulate some sort of physical mechanism and see how its predictions agree with experiment. There does not seem to be any strong evidence that Hill slopes are usually integers, and even when they are, the observation would be compatible with a number of different mechanisms. One such mechanism would be the sort first postulated by A. V. Hill for haemoglobin, which is implied in your question. But this has not turned out to be physically correct in other systems such as haemoglobin, and it is not very plausible physically because it implies an infinite interaction energy between subunits.