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Review Article

The mathematics of drug-receptor interactions

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MOST pharmacological observations support the hypothesis that drugs produce their effects by interacting in a specific way with some component of the living cell. This component, which is likely to be either an enzyme or a site on a cell membrane, is called the receptor. Substances which act on receptors may be classified as agonists, which produce an observable response from a tissue, or as antagonists, which do not themselves produce an observable response but prevent the response to agonists. The concept of specific receptors is supported mainly by the ability of some antagonists to block selectively the response of tissues to certain agonists.

EXPERIMENTAL EVIDENCE

Any detailed conclusions about drug-receptor interactions must rest on information derived from studies of the kinetics of drug action or of dose-response curves. The kinetics of drug action, however, when measured on isolated tissues, are likely to depend on the rate of diffusion of the drug to the receptors, on the rate of reaction of the drug with the receptors, and on the rate of response of the cells to the drug-receptor reaction. These problems, which were discussed fully by Clark (1933a), may arise even when responses are measured on single cells. Analyses of drug-receptor interactions therefore tend to be based on dose-response curves measured under equilibrium conditions, so that complicated kinetic factors are eliminated. The responses obtained are then assumed to correspond to an equilibrium, or steady state, occupation of the receptors to which the law of mass action may be applied.

1. APPLICATION OF THE LAW OF MASS ACTION TO DRUG-RECEPTOR INTERACTIONS

If the drug is given the symbol A and the specific receptor with which it interacts the symbol R, then the reaction of the drug with the receptor may be written as

$$R + A \rightleftharpoons RA$$

The reaction is usually assumed to be bimolecular. Then by the law of mass action, the affinity constant, K_A, of the drug for the receptor is given by the equation

$$K_A = \frac{\{RA\}}{\{R\}\,(A)} \qquad .. \quad .. \quad .. \quad (1)$$

where (A) is the equilibrium molar concentration of the drug in the region about the receptors. The braces around the symbols R and RA

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indicate that the concentrations of receptor and of drug-receptor complex are in arbitrary units. Since the arbitrary units cancel, K_A is in litres/mole.

The total concentration of receptors in or on a cell is $\{R\}_T$ where

$$\{R\}_T = \{R\} + \{RA\} \quad .. \quad .. \quad .. \quad (2)$$

Eliminating $\{R\}$ from equation (2), by use of equation (1), and re-arranging, gives

$$\{R\}_T = \{RA\}\left[1 + \frac{1}{K_A(A)}\right]$$

Then the fraction of the receptors occupied by the drug A, at equilibrium, is

$$y_A = \frac{\{RA\}}{\{R\}_T} = \frac{1}{\left[1 + \frac{1}{K_A(A)}\right]} \quad .. \quad .. \quad (3)$$

The fraction of the receptors occupied at any given value of (A), therefore, depends only on K_A, and y_A tends to unity when the concentration of A is made sufficiently high.

It can be shown, in the same way, that when two drugs A and B compete for the same receptor R, then the fraction of the receptors occupied by drug A, at equilibrium, is

$$y_A = \frac{\{RA\}}{\{R\}_T} = \frac{1}{1 + \frac{1}{K_A(A)} + \frac{K_B(B)}{K_A(A)}} \quad .. \quad .. \quad (4)$$

If, on the other hand, the drug B is a non-competitive antagonist then

$$y_A = \frac{1}{1 + \frac{1}{K_A(A)} + \frac{K_B(B)}{K_A(A)} + K_{AB}(B)} \quad .. \quad .. \quad (5)$$

where K_{AB} is the affinity constant of the agonist-receptor complex for the antagonist and K_A and K_B have their usual significance [see equation (1)]. If B is a non-competitive antagonist in the strictest sense then K_B and K_{AB} are equal.

2. THE RELATIONSHIP BETWEEN RECEPTOR-OCCUPATION AND THE OBSERVED RESPONSE

This problem may be considered both qualitatively and quantitatively.

Qualitative considerations. According to Clark's ideas, occupation of a receptor by an agonist causes a change in some property of the cell, and this change persists as long as the agonist occupies the receptor. This hypothesis is known as "occupation theory". Other possibilities have been suggested (Paton, 1961; Mackay, 1963), but fortunately, from a mathematical point of view, occupation theory and the alternative ideas all lead to the conclusion that the response, under equilibrium or steady-state conditions, is likely to be some function of the concentration of agonist-receptor complex.

Quantitative considerations. It must be emphasised that the relation between the fraction of receptors occupied by the agonist and the observed

response is not known. Indeed, it is interesting to consider the various types of response which might be measured. In the case of muscle contraction the response might be the isometric tension produced, or the change in length of the tissue under isotonic conditions. Alternatively, changes in the electrical properties or membrane permeabilities of the cells might be used as a measure of the response. It seems very unlikely that these various types of response would all be related in the same way to the fraction of receptors occupied by the agonist.

3. CLARK'S QUANTITATIVE TREATMENT OF RECEPTOR THEORY

The first quantitative treatment of receptor theory was that of Clark (1933c). He applied the law of mass action to the drug-receptor interaction and also assumed that the response of a tissue is directly proportional to the fraction of receptors occupied by the agonist, although he clearly realised (Clark, 1933a,b) that this assumption might not be valid. Clark's assumption may be written as

$$r_A = ky_A \quad .. \quad .. \quad .. \quad .. \quad (6)$$

where y_A is the fraction of receptors occupied and is given by equation (3), and r_A is the response to the agonist A. The constant k applies to all agonists interacting with these specific receptors. At sufficiently high concentrations all the receptors are occupied and y_A is then equal to unity. Under such conditions equation (6) becomes

$$r_A = k = r_{max}$$

where r_{max} is the maximal response of the tissue and is the same for all agonists.

Hence, on the basis of Clark's assumptions, the fraction of receptors occupied should be related to the response by the equation

$$y_A = \frac{r_A}{r_{max}}$$

When $r_A = \frac{1}{2} r_{max}$, then $y_A = \frac{1}{2}$

$$= \frac{1}{1 + \frac{1}{K_A(A)_{50}}}, \text{ from equation (3),}$$

where $(A)_{50}$ is the concentration of agonist which produces 50% of the maximal response. It follows that

$$K_A = \frac{1}{(A)_{50}} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

and so, on the basis of Clark's assumptions, the affinity constant of an agonist for its receptor may be estimated from the dose-response curve.

Gaddum (1937) extended Clark's theory to account for the effects of specific antagonists. These compounds were assumed to adsorb onto the receptors without producing the changes necessary for a response. In this way antagonists could prevent the formation of agonist-receptor complexes. Gaddum therefore suggested that equation (6) might also

apply to the action of an agonist in the presence of a competitive antagonist, the value of y_A being given by equation (4), where B would then be the competitive antagonist.

4. APPLICATION OF THE NULL METHOD TO STUDIES OF DRUG ANTAGONISM

A basic weakness of Clark's quantitative treatment of receptor theory was the assumption of direct proportionality between the response and the fraction of receptors occupied by the agonist.

However, both Clark and Gaddum sometimes compared the concentrations of an agonist required to produce a selected response from a tissue before and after it had been treated with an antagonist. Clark & Raventos (1937) suggested that "an alternative method of estimating antagonistic power is to determine the concentration of B (the antagonist) which alters by a selected proportion (e.g. tenfold) the concentration of A (the agonist) needed to produce a selected effect." This suggestion contains the basis of the null method which was applied by Schild (1947) to the study of drug antagonism.

Suppose that a given value of the response, r, is produced by a concentration $(A)_1$ of the agonist acting alone. The response is some function of the fraction of receptors occupied by the agonist, but this function is not necessarily a linear one. The fraction of receptors occupied, when the concentration of A is $(A)_1$, is

$$y_{A_1} = \frac{1}{1 + \frac{1}{K_A(A)_1}} \quad \text{[see equation (3)]} \quad .. \qquad (8a)$$

When a competitive antagonist is also present it reduces the concentration of agonist-receptor complex produced by $(A)_1$ by competing for the receptors. This in turn reduces the response produced by $(A)_1$, but this antagonism can be counteracted by increasing the concentration of the agonist from $(A)_1$ to some higher value $(A)_2$. The value of y_A, when the concentration of agonist $(A)_2$ acts on the tissue in the presence of a concentration (B) of antagonist, is

$$y_{A_2} = \frac{1}{1 + \frac{1}{K_A(A)_2} + \frac{K_B(B)}{K_A(A)_2}} \quad \text{[see equation (4)]} \quad .. \qquad (8b)$$

If the response is determined by the fraction of receptors occupied by the agonist then equal values of y_A should produce equal responses, and vice versa. This conclusion does not depend on the form of the relationship between y_A and the response, since only equal responses are compared. Suppose that a value of $(A)_2$ is chosen and (B) is adjusted until the response to $(A)_2$, in the presence of (B), is equal to the response to $(A)_1$ alone. Then

$$y_{A_1} = y_{A_2}$$

and so, from equations (8a) and (8b),

$$1 + \frac{1}{K_A(A)_1} = 1 + \frac{1}{K_A(A)_2} + \frac{K_A(B)}{K_A(A)_2}$$

It follows that

$$\frac{1}{(A)_1} = \frac{1}{(A)_2}[1 + K_B(B)] \quad .. \quad .. \quad (9)$$

and if $(A)_2 = x\,(A)_1$

then $$(B)_x = \frac{[x-1]}{K_B} \quad .. \quad .. \quad .. \quad .. \quad (10)$$

where $(B)_x$ is the corresponding concentration of the antagonist. Schild defined the pA_x as

$$pA_x = -\log_{10}(B)_x$$
$$= \log_{10}K_B - \log_{10}[x-1], \text{ from equation (10)} \quad .. \quad .. \quad (11a)$$

From equation (11a), when x is equal to 2,

$$pA_2 = \log_{10}K_B, \quad .. \quad .. \quad .. \quad (11b)$$

and when x is equal to 10

$$pA_{10} = \log_{10}K_B - \log_{10}9$$
$$= pA_2 - \log_{10}9, \text{ from equation (11b).}$$

Therefore $$pA_2 - pA_{10} = \log_{10}9.$$

If this relationship between pA_2 and pA_{10} is found to be valid then the antagonist is probably acting competitively and K_B can be calculated from equation (11b).

However, a more general test for competitive antagonism can be applied by using equation (11a) (Arunlakshana & Schild, 1959). This equation can be rearranged to give

$$\log_{10}[x-1] = \log_{10}K_B - pA_x \quad .. \quad .. \quad (12)$$

Various values of x can be chosen and the corresponding values of pA_x can be found experimentally. Then, for a competitive antagonist, a plot of $\log_{10}[x-1]$ against pA_x should give a straight line with an intercept equal to $\log_{10}K_B$.

When applied to competitive antagonists the pA_x method should give correct values of the affinity constants of the antagonist for the receptor, since this method involves no assumptions about the form of the relationship between the response and the fraction of receptors occupied by the antagonist.

5. INTRINSIC ACTIVITY

According to Clark's quantitative treatment of receptor theory, drugs which act on any particular type of receptor should be either agonists or antagonists. In sufficiently high concentrations all agonists should be able to produce a maximal response from a tissue.

However, it was later observed (Raventos, 1937; Ariëns, 1954) that the maximal responses produced by some agonists were less than those produced by others. Agonists which produce the maximal response of the tissue may be called full agonists, while those which produce maximal responses which are less than the maximal response of the tissue may be

called partial agonists. In order to account for such findings, Ariëns (1954) introduced the term *intrinsic activity* and described it as "a substance-constant determining the effect per unit of pharmacon-receptor complex." In other words, it was suggested that a complex of a receptor with one agonist might differ from the complex with another agonist, in its ability to contribute to a response. In order to obtain values of the intrinsic activity, Ariëns retained Clark's assumption that the response is directly proportional to the fraction of receptors occupied by the agonist. The constant k in equation (6) was therefore considered to vary from one agonist to another.

According to Ariëns' assumptions the response to any agonist A is r_A where

$$r_A = k_A y_A \quad .. \quad .. \quad .. \quad .. \quad (13)$$

The term k_A is the intrinsic activity of the agonist and y_A is the fraction of receptors occupied. The value of y_A is given by equation (3), and tends to a maximum of unity when the concentration of agonist is made sufficiently high. Then, from equation (13), the maximum response to the agonist A is

$$[r_A]_{max} = k_A \quad .. \quad .. \quad .. \quad .. \quad (14)$$

and the intrinsic activity of an agonist is proportional to the maximum response which it can produce. Then for two agonists A and B, the ratio of their intrinsic activities is

$$\frac{k_B}{k_A} = \frac{[r_B]_{max}}{[r_A]_{max}} \quad .. \quad .. \quad .. \quad .. \quad (15)$$

Suppose that the maximum response which can be elicited from the tissue is r_{max}. Then all agonists which produce this response will be observed to have the same intrinsic activity, which may be set equal to unity. If $[r_A]_{max}$ is equal to r_{max} then $k_A = 1$, and equation (15) becomes

$$k_B = \frac{[r_B]_{max}}{r_{max}} \quad .. \quad .. \quad .. \quad .. \quad (16)$$

If the simplifying assumptions made by Ariëns are correct, then the (relative) intrinsic activity of a partial agonist can be obtained by comparing maximum responses (see Fig. 1).

From equations (13) and (14)

$$\frac{r_A}{[r_A]_{max}} = y_A$$

Suppose that the concentration of agonist, or partial agonist, which produces a response equal to one half of $[r_A]_{max}$, is written as $(A)_{50}$. Then the corresponding fraction of receptors occupied is

$$y_A = \frac{r_A}{[r_A]_{max}} = \tfrac{1}{2}$$

$$= \frac{1}{1 + \dfrac{1}{K_A(A)_{50}}} \text{ [from equation (3)]}$$

It follows that

$$[1 + \frac{1}{K_A(A)_{50}}] = 2$$

and so

$$K_A = \frac{1}{(A)_{50}} \quad .. \quad .. \quad .. \quad .. \quad (17)$$

The simplifying assumptions made by Ariëns lead to the conclusion that the affinity constant of an agonist or partial agonist for its receptor can be calculated directly from the simple dose-response curve, since $(A)_{50}$ is readily determined (see Fig. 1). Clearly equation (17) is similar to

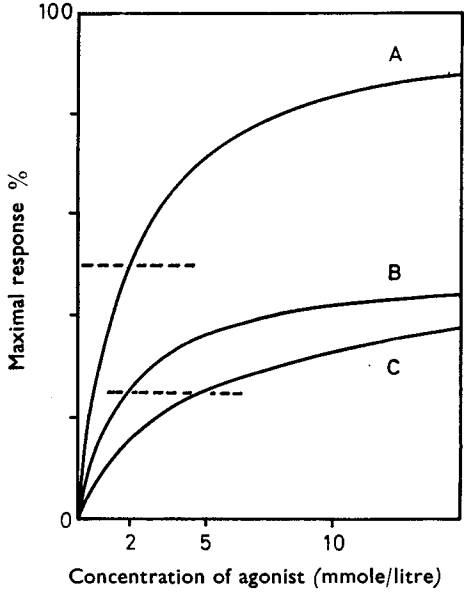

FIG. 1. The determination of affinity constants and intrinsic activities of agonists, on the basis of Ariëns' simplifying assumptions. Compound A has an intrinsic activity of unity and an affinity constant of 0·5 litre/mmole. Compounds B and C have intrinsic activities of 0·5. The affinity constants of compounds B and C are respectively 0·5 litre/mmole and 0·2 litre/mmole. The values of the affinity constants are equal to the reciprocal of the concentrations of the drugs which produce a response equal to one half of the maximal response which the drug can elicit from the tissue.

equation (7) except that $(A)_{50}$ now represents the concentration of drug which produces a response equal to one half of the maximal response which the drug can elicit from the tissue.

It may be noted that Ariëns' treatment separates agonists into two groups which are full agonists and partial agonists. However, as with Clark's treatment, the intrinsic activities of all fully active agonists are the same, since such compounds are assumed to occupy all the receptors when producing the maximal response of the tissue. If some fully active agonists could produce maximal responses from the tissue without occupying all the receptors, the intrinsic activities of these compounds

would appear to be the same, although actually different. Attempts have been made to differentiate between fully active agonists by using irreversible antagonists (van Rossum & Ariëns, 1962).

6. EFFICACY

The term efficacy, introduced by Stephenson (1956), is conceptually the same as the intrinsic activity. However, Stephenson did not retain Clark's assumption that the response is directly proportional to the fraction of receptors occupied by the agonist. Instead he assumed that some full agonists may produce a maximum response from a tissue when only a very small fraction of the receptors are occupied. If this assumption is valid then various full agonists may elicit the maximum response of which the tissue is capable, by occupying different fractions of the total number of available receptors.

Stephenson defined a quantity called the *stimulus* and distinguished clearly between this stimulus and the response. The stimulus is defined by the equation

$$s = ey \quad .. \quad .. \quad .. \quad .. \quad (18)$$

where e is a constant termed the efficacy, and y is the fraction of the receptors which the drug occupies when it produces the response corresponding to this stimulus. For any particular agonist, the stimulus is proportional to y, and y is given by equation (3). The response r is regarded as being a definite function of the stimulus, so that a given stimulus always produces the same response. It follows that any particular response might be produced by a very large number of values of e and y, provided that the product of e and y has the appropriate constant value.

By using the null method and by making certain simplifying assumptions, Stephenson was able to calculate the efficacies of partial agonists. The stimulus produced by an agonist A is

$$s_A = e_A y_A$$

$$= e_A \frac{K_A(A)}{1 + K_A(A)} \text{ [from equation (3)]} \quad .. \quad .. \quad (19a)$$

If the agonist A produces a maximal response from the tissue when y_A is very much less than unity, which corresponds to the case when $K_A(A)$ is also very much less than unity (see equation 3), then equation (19a) reduces to

$$s_A = e_A K_A(A) \quad .. \quad .. \quad .. \quad (19b)$$

and this equation is then approximately valid for all values of (A) which produce responses between zero and the maximal response. In the case of a partial agonist P, which cannot produce the maximal response of the tissue, the stimulus is

$$s_P = e_P y_P \quad .. \quad .. \quad .. \quad .. \quad (20a)$$

where

$$y_P = \frac{K_P(P)}{1 + K_P(P)} \text{ [from equation (3)]} \quad .. \quad .. \quad (20b)$$

Suppose that a concentration $(A)_1$ of agonist, and a concentration (P) of partial agonist each produce the same response r_1, when applied separately to the same piece of tissue. Then provided that the stimulus-response relationship is constant, these concentrations of drugs also produce equal stimuli, so that from equations (19b) and (20a),

$$e_A K_A (A)_1 = e_P y_P \quad .. \quad .. \quad .. \quad (21)$$

Suppose also that a concentration $(A)_2$ of agonist produces a response r_2 which can also be produced by concentrations $(A)_3$ of agonist and (P) of partial agonist acting together. Then the corresponding stimuli are equal, so that

$$e_A K_A (A)_2 = e_A K_A (A)_3 [1 - y_P] + e_P y_P$$

[It was assumed at this stage that y_A is negligible compared with y_P.] Then substituting equation (21) into the above equation,

$$e_A K_A (A)_2 = e_A K_A (A)_3 [1 - y_P] + e_A K_A (A)_1$$

It follows that

$$(A)_2 = (A)_3 [1 - y_P] + (A)_1$$

Then

$$y_P = \frac{(A)_3 - (A)_2 + (A)_1}{(A)_3} \quad .. \quad .. \quad .. \quad (22)$$

and so the value of y_P, which corresponds to (P), can be calculated from the experimentally observed values of $(A)_1$, $(A)_2$ and $(A)_3$. The value of K_P is then estimated from the equation

$$K_P = \frac{y_P}{1 - y_P} \cdot \frac{1}{(P)} \quad .. \quad .. \quad .. \quad (23)$$

which is obtained by rearrangement of equation (20b).

Let the efficacy of a partial agonist which can produce a maximal response equal to one half of the maximal response of the tissue, be set equal to unity. When such a partial agonist elicits its maximal response then all of the receptors are occupied, and y is equal to unity. The stimulus which corresponds to this response is then

$$s = ey = 1.$$

Then for any other drug acting on the same receptors,

$$e = \frac{s}{y} = \frac{1}{y_{50}} \quad .. \quad .. \quad .. \quad .. \quad (24)$$

where y_{50} is the fraction of the receptors occupied by the drug when it produces unit stimulus, which in turn produces a response equal to 50% of the maximal response of the tissue. In order to calculate y_{50} directly from equation (3), it is necessary to know both the affinity constant of the agonist for the receptor and the concentration of the agonist which produces 50% of the maximal response of the tissue. The latter is readily obtained from the dose-response curve.

Stephenson was able to produce a solution to this problem by obtaining values of the affinity constants of partial agonists using equation (23).

Since the group of compounds which he studied comprised a homologous series, he was able to estimate the affinity constants of full agonists for the receptors by extrapolating the values which he obtained for those members of the series which were partial agonists. In this way he obtained estimates of the efficacies of both agonists and partial agonists, from equation (24).

7. THE STIMULUS-RESPONSE RELATIONSHIP

In applying the null method the aim is to eliminate assumptions about the relationship between stimulus and response. This is only partly achieved in the method described above, because the form of the stimulus-response relationship is partly determined by Stephenson's simplifying assumptions.

The concentration of any agonist A which produces the response r can be read from its dose-response curve and if K_A is known then y_A can be calculated from equation (3). If the value of e_A is also available, then the stimulus which produces the response r can be obtained from equation (18), or from the related equation (19a). This calculation can be repeated for several values of r and so the stimulus-response relationship can be plotted. The type of stimulus-response curve obtained by Stephenson is shown in Fig. 2. Its validity depends on the validity of the estimated values of e and K.

8. COMPARISON OF INTRINSIC ACTIVITY AND EFFICACY

Although intrinsic activity and efficacy are conceptually the same they are quantitatively different, because of the different assumptions made in their calculation. What is even more important, from the point of view of structure-activity relationships, is the fact that these two approaches give different values of the affinity constants of agonists for the receptors. If the response is proportional to the fraction of receptors occupied by the agonist (as was assumed initially by Ariëns) then it follows that the response would be proportional to the stimulus. The differences in the mathematical treatments of Ariëns and Stephenson can therefore be summarised as differences in the assumed forms of the stimulus-response relationships, as shown in Fig. 2. According to Stephenson, an agonist which produces a maximal response equal to 50% of the maximal response of the tissue then produces unit stimulus and has an efficacy of unity. Since the assumed stimulus-response relationships are very similar below unit stimulus (see Fig. 2) it follows that an efficacy of 1·0 or less corresponds to an intrinsic activity approximately equal to one half of the efficacy. However, for compounds with intrinsic activities greater than 0·5, the discrepancy between the intrinsic activity (calculated in its simplest form) and the efficacy increases rapidly and is large for compounds which produce the maximal response of the tissue.

The assumption that the response is directly proportional to the fraction of receptors occupied by the agonist automatically means that all agonists producing the maximum response of the tissue should have the same intrinsic activity. On the other hand, Stephenson's assumptions would

allow drugs with different efficacies to produce the maximum response of the tissue, by occupying different fractions of receptors. The fraction of receptors occupied could be increased still further by increasing the concentration of agonist until all the receptors were saturated, but, because of the shape of the stimulus-response curve suggested by Stephenson (see Fig. 2), the increased stimulus would produce very little change in the response.

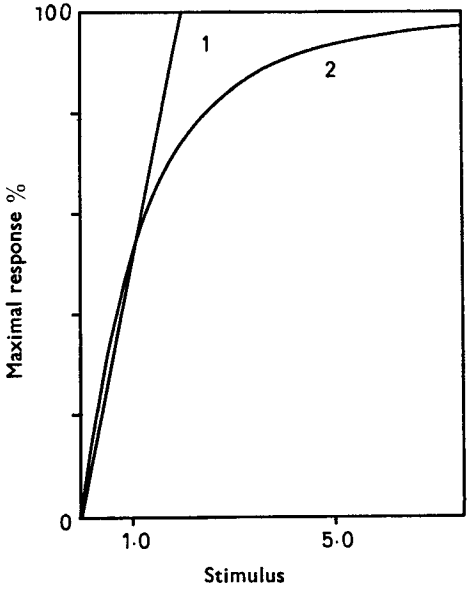

FIG. 2. The stimulus-response relationship. 1. As assumed by Ariëns. 2. As derived by Stephenson (1956) on the basis of his simplifying assumptions.

9. FURCHGOTT'S METHOD FOR THE DETERMINATION OF THE AFFINITY CONSTANTS OF AGONISTS FOR RECEPTORS

According to the ideas of Ariëns and Stephenson, agonist-receptor interactions are characterised by two parameters, the affinity constant and the intrinsic activity or efficacy. Furchgott (1965) suggested that the hybrid term *intrinsic efficacy* might be used for the second parameter. Since the terms intrinsic activity and efficacy are associated with certain assumptions which are not necessarily correct, or which may be correct only in certain cases, it seems advisable to use this hybrid term to describe the parameter itself, as distinct from any experimental estimate of the parameter.

If the affinity constants of agonists for their receptors could be calculated by some dependable method, relative values of their intrinsic efficacies could be obtained from a knowledge of equi-effective concentrations. The symbol f will be used here to denote intrinsic efficacy, although Furchgott uses the symbol ϵ. Suppose that concentrations (A) of drug A and

(B) of drug B, each acting alone, produce the same response r. Then the respective stimuli are s_A and s_B, where

$$s_A = f_A y_A \{R\}_T \quad .. \quad .. \quad .. \quad (25)$$

and

$$s_B = f_B y_B \{R\}_T \quad .. \quad .. \quad .. \quad (26)$$

(It may be noted that the stimulus as used in this and in subsequent sections differs slightly from Stephenson's earlier definition. The stimulus is now re-defined as the product of the intrinsic efficacy and the concentration of drug-receptor complex.) Since the stimuli s_A and s_B produce the same response they are assumed to be equal, so that equations (25) and (26) give

$$\frac{f_A}{f_B} = \frac{y_B}{y_A} \quad .. \quad .. \quad .. \quad .. \quad (27)$$

If K_A, K_B, (A) and (B) are known, then the values of y_A and y_B can be calculated from equation (3) and so the ratio of the intrinsic efficacies of the drugs A and B can be estimated from equation (27).

Furchgott (1965) suggested a method for calculating the affinity constants of agonists, based on the use of irreversible antagonists. These antagonists are assumed to react with the receptors in such a way as to inactivate them for a period of time which is long compared with the duration of the experiment. This is conducted in the following manner. First the log dose-response curve of an agonist A is determined for a piece of tissue. The tissue is then incubated with an irreversible antagonist for a period of time. Excess antagonist is then washed out of the tissue, and the log dose-response curve is re-determined for the treated tissue. The irreversible antagonist will have blocked some of the receptors, so that the log dose-response curve will be altered. Treatment with the irreversible antagonist is then repeated, log dose-response curves being determined after each such treatment. The type of results obtained is shown in Figs 3a and 3b.

These observations can be explained by the form of stimulus-response relationship suggested by Stephenson (1956). At very high concentrations of agonist, y_A tends to unity. The maximum stimulus which the agonist can produce is therefore $[s_A]_{max}$ where

$$[s_A]_{max} = f_A\{R\}_T \text{ [from equation (25)]} \quad .. \quad .. \quad (28)$$

As the value of $\{R\}_T$ decreases, due to reaction of receptors with the irreversible antagonists, so the value of $[s_A]_{max}$ decreases. Ultimately it reaches a value such that it can no longer produce the maximal response of the tissue. The value of $\{R_T\}$ at which this occurs depends on the value of f_A, since it is the stimulus $[s_A]_{max}$ which then determines the response. The greater the value of f_A the smaller must be $\{R\}_T$ and hence the longer must be the time of incubation of the tissue with the irreversible antagonist before the maximum response is reduced. It follows that with an agonist of high intrinsic efficacy, repeated treatment of the tissue with an irreversible antagonist causes the log dose-response curve to be shifted almost parallel to itself, before producing a reduction in the maximal response. On the other hand, if A is a partial agonist then the

maximal response is reduced immediately by treatment with an irreversible antagonist (see Figs. 3a and 3b).

Suppose that the stimulus response relationship can be written in the form

$$s = ar + br^2 + cr^3 + \ldots, \qquad \ldots \qquad \ldots \quad (29a)$$

where s is the stimulus, r is the response and a, b, c, . . . are constants.

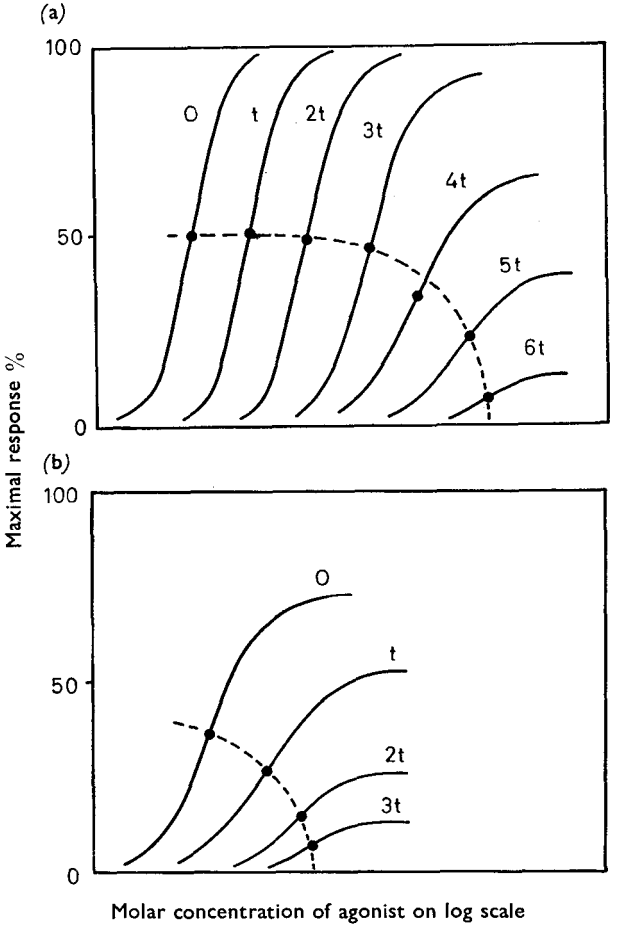

FIG. 3. Dose-response curves measured on a tissue which has been repeatedly treated with an irreversible antagonist. The standard time of incubation with the irreversible antagonist is taken as t. The total times of incubation, which apply to each dose-response curve, are indicated by the multiples of t. The results in Fig. 3a suggest that this agonist produces a maximal response when it occupies only a fraction of the total number of available receptors. The results in Fig. 3b suggest that in the case of a partial agonist a maximal response is produced only when all of the receptors are occupied. In both figures the filled circles indicate $(A)_{50}$ values. The dotted curve indicates the method of obtaining limiting values of $(A)_{50}$, at very small maximal responses.

Equation (29a) could be fitted to a large number of possible stimulus-response relationships. Provided that the constant a is not equal to zero then the limiting form of equation (29a), at very small values of r, is

$$s = ar \quad .. \quad .. \quad .. \quad .. \quad (29b)$$

This indicates that at sufficiently small values of the response, its value is directly proportional to the stimulus. If the entire dose-response curve of the agonist falls within the range of r for which equation (29b) applies, then Ariëns' approximations are valid [see section 8, Fig. 2, and equation (17)] and the affinity constant of the agonist for its receptor can be calculated from the corresponding value of $(A)_{50}$ by use of equation (17). As the irreversible antagonist inactivates the receptors it reduces the maximum stimulus which the agonist A can produce, and hence reduces the maximal response until finally equation (29b) may become valid. The value of $(A)_{50}$ to be used in the calculation of K_A is the limiting value as $[r_A]_{max}$ tends to zero, and is obtained by extrapolation as shown in Figs 3a and 3b. It is assumed that the irreversible antagonist merely inactivates the receptors and does not alter the stimulus-response relationship.

10. A GENERAL METHOD FOR THE ANALYSIS OF DRUG-RECEPTOR INTERACTIONS

(i) *Application to Reversible Interactions*

As already pointed out, the main difficulty in analysing the interactions of drugs with receptors is the lack of knowledge of the stimulus-response relationship. This difficulty was overcome, in the case of drug antagonism, by applying the null method. Mackay (1965a,b) suggested that a simple mathematical transformation should be applied to dose-response curves to obtain useful information about the interaction of receptors with antagonists, partial agonists, and full agonists.

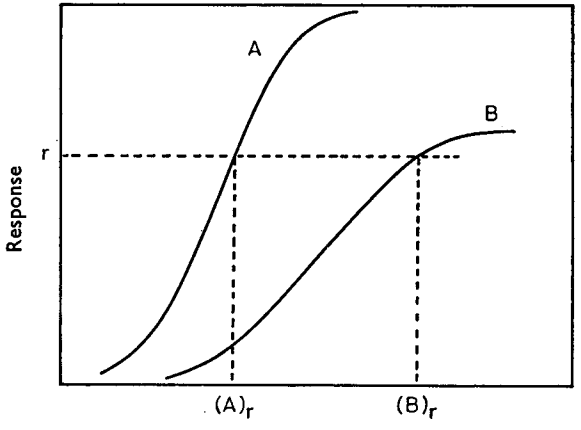

FIG. 4. Comparison of the dose-response curves of two agonists, A and B, measured on the same cell or tissue. $(A)_r$ and $(B)_r$ are the concentrations of the agonists which produce the chosen response r.

Suppose that the log dose-response curves shown in Fig. 4 have been obtained for two agonists A and B acting on the same cell or tissue. If a response r is chosen, then the corresponding pharmacological stimulus produced by the agonist A is given by equation (25). The stimulus s_A is then that producing the response r, and may therefore be written as $[s_A]_r$. The term y_A in equation (25) then also has a definite value, written as $[y_A]_r$, which in turn corresponds to a definite value of the concentration of agonist A, written as $(A)_r$. Thus equation (25) takes the general form

$$[s_A]_r = f_A[y_A]_r\{R\}_T \quad .. \quad .. \quad .. \quad (30)$$

which applies to any chosen response r. Similarly for the agonist B acting on the same cell or tissue,

$$[s_B]_r = f_B[y_B]_r\{R\}_T \quad .. \quad .. \quad .. \quad (31)$$

From equation (3),

$$[y_A]_r = \frac{1}{1 + \dfrac{1}{K_A(A)_r}} \quad .. \quad .. \quad .. \quad (32)$$

and

$$[y_B]_r = \frac{1}{1 + \dfrac{1}{K_B(B)_r}} \quad .. \quad .. \quad .. \quad (33)$$

If $(A)_r$ and $(B)_r$ are the concentrations of agonists A and B which produce the same response r, then they also produce equal stimuli. This is an application of the null method, and involves the assumption that the stimulus-response relationship has remained unchanged during the determination of the dose-response curves. Then from equations (30) and (31)

$$\beta_{AB} = \frac{f_A}{f_B} = \frac{[y_B]_r}{[y_A]_r} \quad .. \quad .. \quad .. \quad (34)$$

where β_{AB} is the ratio of the intrinsic efficacy of drug A to that of drug B. Substituting equations (32) and (33) into equation (34) and rearranging gives

$$\frac{1}{(A)_r} = \frac{K_A}{K_B}\beta_{AB}\frac{1}{(B)_r} + K_A[\beta_{AB} - 1] \quad .. \quad .. \quad (35)$$

This equation indicates that if $1/(A)_r$ is plotted against $1/(B)_r$ then a straight line should be obtained of slope ψ_{AB} and intercept I_{AB}

$$\text{where } \psi_{AB} = K_A\beta_{AB}/K_B \quad .. \quad .. \quad .. \quad (36)$$

$$\text{and } I_{AB} = K_A[\beta_{AB} - 1] \quad .. \quad .. \quad (37)$$

The values of $(A)_r$ and $(B)_r$ which produce the response r, are simply read from the log dose-response curves, as shown in Fig. 4. $1/(A)_r$ is then plotted against $1/(B)_r$ for a series of chosen values of r. This method therefore makes maximum use of the information available in the log dose-response curves.

If I_{AB} is positive then β_{AB} must be greater than one and so f_A must be greater than f_B. The sign of I_{AB} can therefore be used to decide which of the agonists A and B has the greater intrinsic efficacy. The experimental constants ψ_{AB} and I_{AB} are related to the fundamental parameters K_A, K_B and β_{AB}. (Only relative values of the intrinsic efficacies can be determined.) However, there are only two equations, (36) and (37), containing these three unknowns. The individual parameters of the agonist-receptor interactions therefore cannot be determined from these equations. In the more general case of N agonists, all of which act on the same type of receptor, comparison of the log dose-response curves gives $(N - 1)$ independent values of ψ and $(N - 1)$ independent values of I. The order of the intrinsic efficacies can be determined from the signs of the values of I. The N agonists will have N unknown values of K and $(N-1)$ unknown values of β. There will therefore be $(2N - 2)$ independent equations with $(2N - 1)$ unknown parameters. It follows that even if all the experimental constants can be accurately determined, the values of the fundamental parameters cannot be obtained from such data alone. In fact, an infinitely large number of sets of fundamental parameters can be made to fit any given group of dose-response curves (Mackay, 1965b). The fundamental parameters of the series of agonists could however, be estimated from the values of ψ and I, provided that *one* of the following conditions is valid.

(1) The ratio of the intrinsic efficacy of one of the agonists to that of another (partial) agonist, is very much greater than unity. The values of β, obtained on the basis of this assumption, are then similar to Stephenson's efficacies (Mackay, 1965b).

(2) The affinity constant of one of the agonists is known.

(3) Another independent equation is available which relates the fundamental parameters.

Ideally, the values of the experimental constants ψ_{AB} and I_{AB} should be obtained from the log dose-response curves of the agonists, measured on the same piece of tissue with the same recording system. The values of the experimental constants should not depend on the method of recording the response (since the null method is employed) provided that K_A, K_B and β_{AB} do not themselves depend on the recording method.

The method of analysis described above can also be applied to other drug-receptor systems. If the log dose-response curve of an agonist, acting on a cell or tissue, is determined first in the absence and then in the presence of a competitive antagonist, it can be shown that the appropriate equation for the comparison of the dose-response curves is

$$\frac{1}{(A)_r} = \frac{1}{(A)'_r}[1 + K_B(B)] \quad .. \quad .. \quad .. \quad (38)$$

where $(A)_r$ is the concentration of the agonist which produces the response r in the absence of the antagonist, and $(A)'$ is the concentration which produces the response r in the presence of a constant concentration (B) of the competitive antagonist. [If this equation is compared

with equation (9) it will be seen that they are equivalent.] A plot of $1/(A)_r$ against $1/(A)'_r$ should give a straight line of slope $[1 + K_B(B)]$ passing through the origin, and so K_B can be estimated from the slope.

It can also be shown, by application of this method, that if B is a non-competitive antagonist then the appropriate equation, assuming bimolecular drug-receptor interactions, is

$$\frac{1}{(A)_r} = \frac{1}{(A)'_r}[1 + K_B(B)] + K_A K_B(B) \quad .. \quad .. \quad (39)$$

Once again, a plot of $1/(A)_r$ against $1/(A)'_r$ should give a straight line. In this case the slope is $[1 + K_B(B)]$ and the intercept is $[K_A K_B(B)]$. The values of K_B and of K_A can therefore be calculated. The term *non-competitive* is used here in the enzymic sense. The antagonist is supposed to be adsorbed close to the adsorption site for the agonist, without interfering with the adsorption of the agonist. However, the presence of the adsorbed non-competitive antagonist is assumed to block the stimulus which normally results from the agonist-receptor interaction.

Mackay (1965d) also suggested a method for the determination of affinity constants and relative intrinsic efficacies of agonists, based on the kinetics of action of specific irreversible antagonists. However, this method has not been used, since it is more difficult to apply and theoretically less satisfactory than Stephenson's new method which is described in the next section.

(ii) *Application to Irreversible Antagonism*

Stephenson (1965) pointed out that dose-response curves obtained before and after treatment of a tissue with a specific irreversible antagonist, can be compared as described in the previous section, so as to obtain the affinity constant of an agonist for its receptors.

Suppose that the log dose-response curve of an agonist A, acting on a single cell or tissue, is determined, and that the tissue is then incubated with an irreversible antagonist B. The incubation may be continued until the maximum response which the agonist can produce on the treated tissue, after washing out the excess antagonist, is definitely reduced. The log dose-response curve is then re-determined on the treated tissue. Let the concentration of receptors, before treatment of the tissue with the antagonist, be $\{R\}_T$, and the concentration after treatment $\{R\}'_T$. Then

$$\{R\}'_T = \{R\}_T[1 - y_B] \quad .. \quad .. \quad .. \quad (40)$$

where y_B is the fraction of the receptors inactivated by the irreversible antagonist. If $(A)_r$ is the concentration of agonist which produces the response r from the untreated tissue then the corresponding stimulus is

$$[s_A]_r = f_A[y_A]_r\{R\}_T$$
$$= \frac{f_A\{R\}_T}{1 + \dfrac{1}{K_A(A)_r}} \quad \text{[from equation (3)]}$$

If $(A)'_r$ is the concentration of agonist which produces the same response r from the treated tissue then the corresponding stimulus is written as

$$[s_A]'_r = f_A[y_A]'_r\{R\}'_T$$
$$= \frac{f_A\{R\}_T[1 - y_B]}{1 + \dfrac{1}{K_A(A)'_r}} \text{ [from equations (3) and (40)].}$$

Provided that the stimulus-response relationship has not been altered by the action of the irreversible antagonist then the response r will have been produced by equal stimuli, so that

$$[s_A]_r = [s_A]'_r.$$

It follows that

$$\frac{1}{1 + \dfrac{1}{K_A(A)_r}} = \frac{[1 - y_B]}{1 + \dfrac{1}{K_A(A)'_r}}$$

Rearrangement of this equation gives

$$\frac{1}{(A)_r} = \frac{1}{[1 - y_B]} \cdot \frac{1}{(A)'_r} + K_A \frac{y_B}{[1 - y_B]} \quad .. \quad .. \quad (41)$$

Therefore a plot of $1/(A)_r$ against $1/(A)'_r$ gives a straight line. In this case the slope is $1/[1 - y]_B$ and the intercept is $K_A y_B/[1 - y_B]$. Then K_A can be calculated from the equation,

$$K_A = \frac{\text{intercept}}{[\text{slope} - 1]} \quad .. \quad .. \quad .. \quad (42)$$

This equation can also be applied to dose-response curves obtained in the presence of pseudo-irreversible and non-competitive antagonists, as discussed in detail elsewhere (Mackay, 1965c). Equations (41) and (42) have been derived on the assumption that the dose-response curves to be compared are those measured before and after treatment of the tissue with an irreversible antagonist. However, these equations apply equally well to comparisons of two dose-response curves obtained after two different periods of incubation of the tissue with the irreversible antagonist.

The validity of the equations derived in section 10 depends almost entirely on the assumption that the stimulus-response relationship does not change while the dose-response curves are being determined. This is a basic assumption of the null method. If this assumption is valid then the application of equations (41) and (42) may give good estimates of the affinity constants of agonists for their receptors. If the value of the affinity constant of each agonist is determined in this way, together with the equi-effective concentrations, then the relative intrinsic efficacies of the agonists can be estimated from equation (27) (see section 9).

However, it was pointed out in section 10 (i) that if accurate experimental estimates of ψ_{AB} and I_{AB} are available for a series of agonists then knowledge of the value of one of the affinity constants would enable all the other fundamental parameters to be calculated. Therefore, if all of the values of the affinity constants are determined by use of equations

(41) and (42), and the value of ψ and I are also determined, there will be a surplus of experimental data and cross-checks become possible. Thus, the value of β_{AB} can be estimated from equation (36), if ψ_{AB}, K_A and K_B are known. A theoretical value of I_{AB} can then be calculated from equation (37), and this can be compared with the observed values. Any serious discrepancy between the calculated and observed values of I_{AB} would throw doubt on the estimated values of the fundamental parameters, since the null method is more likely to be valid in the case of equation (35), than in the case of equation (41).

It must also be emphasised that the equations derived in section 10 ought to be taken as applying to graded responses measured on single cells, since there are the responding units. In certain circumstances these equations can also be applied to multicellular tissues (Mackay, 1965b).

EXPERIMENTAL CONSIDERATIONS

The equations discussed in this review should strictly be applied only to measurements made on tissues which contain cells capable of producing graded responses. In applying these equations it is also assumed that the response which is measured is produced by the cell on which the drug-receptor interaction occurs and that the response is a result of the interaction of the drug with only one type of receptor.

The values of the concentrations of drugs which have to be inserted in the various equations are strictly the concentrations close to the receptors at equilibrium. However, these concentrations are usually assumed to be the same as those present in the bathing solution before it was applied to the tissue. This approximation is satisfactory only if the receptors are on the surface of the cells and if the amount of drug adsorbed by the tissue is not sufficient to produce any significant change in the concentration of the drug in the solution. This can usually be ensured, if necessary, by using a small piece of tissue and a large volume of bathing solution. Similar problems arise if the drug is metabolised or absorbed by the tissue, but these are not so readily solved. In some instances it may be possible to block the metabolism or uptake of the drug.

In section 10 it has been stated that the dose-response curves which are to be compared should be measured on a single piece of tissue, or on a single cell. This is because the validity of the equations derived in that section usually depends on the constancy of the stimulus-response relationship (and sometimes also of $\{R\}_T$) during the determination of the dose-response curves. However, *all* the dose-response curves of a group of agonists cannot be measured on a single piece of tissue, and in any case the stimulus-response relationship may vary with time. Such practical difficulties can be overcome to a large extent by carrying out experiments in such a way that one agonist is repeatedly used as a reference compound.

General discussion and conclusion

It has already been emphasised that in analysing drug-receptor interactions no assumptions should be made about the form of the relationship

between the response and the fraction of receptors occupied by the agonist. This means that those methods of analysis discussed in sections 4 and 10, which are based only on the null method and the law of mass action, are the most likely to give dependable values for the fundamental parameters of drug-receptor interactions. However, such results must also be considered cautiously since these methods depend implicitly on the validity of two basic assumptions. The first assumption is that when drugs interact with receptors to produce an equilibrium or steady state concentration of drug-receptor complex then this corresponds to a steady response. The second assumption is that a definite stimulus-response relationship exists for any given piece of tissue. The exact validity of these assumptions can be questioned.

Experimentally, steady responses are seldom seen. Instead, the response to an agonist usually reaches a maximum and subsequently declines, sometimes to zero, even though the agonist is still present. Such observations were partly responsible for the introduction of alternative forms of receptor theory, such as the *rate theory* proposed by Paton (1961) and the *flux-carrier hypothesis* suggested by Mackay (1963). All of the mathematical treatments discussed here have been derived on the basis of *occupation theory*. The equations derived in sections 4 and 10 could also be applied to the alternative forms of receptor theory, provided that the appropriate responses are measured from the response-time curves. These appropriate responses are respectively the steady-state plateau response in the case of rate theory, and the maximal response in the case of the flux-carrier hypothesis.

Although the alternative forms of receptor theory require investigation and evaluation, there seems to be no strong reason for discarding occupation theory at the present time. The fact that the variation of a response with time does not follow the pattern predicted by occupation theory, does not necessarily mean that the response is not due to simple occupation of the receptors. The complicated forms of the response-time curves may be due to secondary effects. In such circumstances the methods of analysis discussed here can be applied, provided that the maximum response corresponds to equilibrium occupation of the receptors.

It seems likely that any increased permeability of the cell membrane would cause a greater influx of sodium ions. The ionic composition of the intracellular fluid is maintained by one or more "pump" systems. The properties of the sodium pump and of its adenosine-triphosphatase, have recently been reviewed by Skou (1965). An increased influx of sodium ions would be expected to stimulate the sodium pump and so tend to annul the effect of any increased permeability. The initial change in membrane permeability might be brought about by the interaction of an agonist with its receptors, and the sodium pump would provide a negative feedback. Many interesting pharmacological phenomena, which cannot be explained on the basis of simple occupation theory alone, could be explained by such a "feedback" model. If such a time-dependent feedback process does in fact occur, then the idea of a definite stimulus-response relationship can be only an approximation to the truth. The

feedback model is still highly speculative, but such biochemical considerations are likely to be of some importance, regardless of whether an agonist produces its primary action by simply occupying the receptors, or by the mechanisms suggested by the rate theory or the flux-carrier hypothesis.

It may therefore be concluded that, of the various methods which have been considered for analysing drug-receptor interactions, those which depend solely on the application of the null method and on the law of mass action, are probably the best available at the present time. Studies of response-time relationships may lead to new concepts which in turn may require further modification of receptor theory.

Summary

The law of mass action can be applied to the interaction of a drug with a receptor, but the relationship between the response and the fraction of receptors, y_A, occupied by the agonist, is not known. In fact, it seems unlikely that the relationship between the response and y_A would be the same for all the different types of response which might be measured.

The first quantitative treatment of receptor theory, set out by Clark (1933c), separated drugs into two groups which were the agonists and the antagonists. In the case of drug antagonism, techniques were developed (Gaddum, 1937; Clark & Raventos, 1937; Schild, 1947) which enabled the affinity constants of competitive antagonists to be estimated without making any assumptions about the relationship between y_A and the response. This technique was based on the comparison of equal responses and was therefore called a null method.

The discovery of partial agonists led to the introduction of the terms *intrinsic activity* (Ariëns, 1954) and *efficacy* (Stephenson, 1956), which are conceptually the same but which are quantitatively different, especially in the case of agonists which elicit the maximal response of the tissue. The terms intrinsic activity and efficacy both imply that the complexes between the receptor and various agonists may differ in their ability to contribute to a response. Whereas Ariëns assumed that all of the receptors have to be occupied in order to produce a maximal response from the tissue, Stephenson assumed that some agonists can produce maximal responses when they occupy only a small fraction of the receptors. Stephenson also drew a clear-cut distinction between receptor occupation and the response, by introducing the term stimulus.

According to the ideas of Ariëns and of Stephenson, any agonist-receptor interaction can be characterised by two parameters, the affinity constant and the intrinsic activity or efficacy. Furchgott (1965) introduced the hybrid term *intrinsic efficacy* for the latter parameter. His method for the determination of the affinity constants of agonists depends on the use of irreversible antagonists. The assumption made by Furchgott, in order to estimate these affinity constants, is that when the maximal response of the tissue to a fully active agonist is made vanishingly small then the stimulus is proportional to the response. This assumption is in some way less restrictive than Stephenson's earlier assumptions. When

the affinity constants of the agonists for the receptors have been obtained, then their relative intrinsic efficacies can be estimated from their equieffective concentrations, assuming the null method to be valid.

Mackay (1965a,b) introduced a mathematical transformation of dose-response curves, which allowed the null method to be applied to the analysis of the interactions of receptors with competitive and non-competitive antagonists, partial agonists, and fully active agonists. He showed that in the case of partial agonists and fully active agonists, experimental constants could be obtained by comparing simple dose-response curves measured on a single piece of tissue. These experimental constants are related to the fundamental parameters of the agonist-receptor interactions, but can be broken down into the constituent parameters only under certain circumstances.

Stephenson (1965) pointed out that the same basic principles could be applied to obtain the affinity constants of agonists for their receptors, by comparing the dose-response curves of the agonist measured on a piece of tissue before and after it had been treated with an irreversible antagonist.

It is obvious that the most dependable techniques for analysing drug-receptor interactions are those which involve the smallest number of doubtful assumptions. The methods discussed in sections 4 and 10 are therefore recommended, since they depend only on the validity of the null method and the law of mass action. Application of these methods, assuming that the maximal response corresponds to equilibrium occupation of the receptors, has so far given satisfactory results. Nevertheless, the response-time relationship requires more detailed investigation and such studies may lead to further modification of receptor theory.

References

Ariëns, E. J. (1954). *Archs int. Pharmacodyn. Thér.*, **99**, 32–49.

Arunlakshana, O. & Schild, H. O. (1959). *Br. J. Pharmac. Chemother.*, **14**, 48–58.

Clark, A. J. (1933a). *The Mode of Action of Drugs on Cells*, p. 61. London: Arnold.

Clark, A. J. (1933b). *Ibid.*, p. 133.

Clark, A. J. (1933c). *Ibid.*, p. 147.

Clark, A. J. & Raventos, J. (1937). *Q. Jl exp. Physiol.*, **26**, 375–392.

Furchgott, R. F. (1965). At the Symposium on "The Interaction of Drugs with Receptors" held at the Chelsea College of Science and Technology, April, 1965.

Gaddum, J. H. (1937). *J. Physiol., Lond.*, **89**, 7P–8P.

Mackay, D. (1963). *Nature, Lond.*, **197**, 1171–1173.

Mackay, D. (1965a). *Br. J. Pharmac. Chemother.*, **24**, 302.

Mackay, D. (1965b). *Ibid.*, **26**, 9–16.

Mackay, D. (1965c). *Advances in Drug Research*, Vol. 3. In the press.

Mackay, D. (1965d). At the Symposium on the "Interaction of Drugs with Receptors," held at the Chelsea College of Science and Technology, April, 1965.

Paton, W. D. M. (1961). *Proc. R. Soc., Ser. B*, **154**, 21–69.

Raventos, J. (1937). *Q. Jl exp. Physiol.*, **26**, 361–374.

Rossum, J. M. van & Ariëns, E. J. (1962). *Archs int. Pharmacodyn. Thér.*, **136**, 385–413.

Schild, H. O. (1947). *Br. J. Pharmac. Chemother.*, **2**, 189–206.

Skou, J. C. (1965). *Physiol. Rev.*, **45**, 596–617.

Stephenson, R. P. (1956). *Br. J. Pharmac. Chemother.*, **11**, 379–393.

Stephenson, R. P. (1965). At the Symposium on the "Interaction of Drugs with Receptors" held at the Chelsea College of Science and Technology, April, 1965.