Brit. J. Pharmacol. (1966), **26**, 9–16.

A GENERAL ANALYSIS OF THE RECEPTOR–DRUG INTERACTION

BY

D. MACKAY

From the Department of Pharmacology, University of Leeds

(*Received January* 5, 1965)

The concept of receptors, first introduced by Langley (1878, 1905), has become extremely important in pharmacology. The law of mass action can be applied to the drug-receptor interaction and this approach was first employed by Clark (1937). He also assumed that the response of a tissue is proportional to the fraction of the receptors occupied by an agonist, although he realized that this assumption might not be valid.

It was later observed that the maximal responses produced by some agonists are less than those produced by others. In order to explain these results Ariens (1954) introduced the term " intrinsic activity," while Stephenson (1956) introduced the term " efficacy." Both terms arise from the idea that the drug-receptor complexes of all agonists are not equally effective in producing a response. In his earlier work Ariens retained Clark's assumption that the response of a tissue is directly proportional to the fraction of the receptors occupied by the agonist. Stephenson, however, abandoned this idea and assumed instead that in order to produce a maximal response a fully active agonist need occupy only a very small fraction of the receptors. Subsequently, van Rossum & Ariens (1962) modified the definition of intrinsic activity so as to allow for the possible existence of an excess of receptors.

Two important points in Stephenson's modification of receptor theory were his clear distinction between the pharmacological stimulus and the response, and his use of what may be called the " null method." This method, which had previously been applied to studies of drug antagonism (Clark & Raventos, 1937; Gaddum, 1937; Schild, 1947, 1954), depends on the assumption that any given stimulus applied to a tissue always produces the same response.

The aim of this paper is to introduce a general method for the analysis of drug-receptor interactions, which eliminates all assumptions about the relationship between stimulus and response by suitable application of the null method. The interaction of the receptor with the agonist is assumed to be characterized by two fundamental parameters which are called the affinity constant and the intrinsic efficacy. The latter term, which is conceptually the same as intrinsic activity and efficacy, was suggested by Furchgott (April 1965, unpublished), and is used here to define the fundamental drug parameter, as distinct from any experimental estimate of this parameter.

THEORY

It is assumed that receptors are present in or on the cells of a tissue, and that these receptors interact with various drugs to produce measurable responses. Some time after a drug has come into contact with the receptors an equilibrium or steady-state concentration of drug-receptor complexes is produced. The response results from the pharmacological stimulus which is defined as the product of the number of receptors occupied by the drug and its intrinsic efficacy. It is assumed that a given stimulus always produces the same response. These assumptions will now be applied to analyse the responses of single cells.

The bimolecular reaction of the drug A with the receptor R may be written as:

$$A+R \rightleftharpoons RA$$

The total number of receptors on the cell, or on the responsive region of the cell, may be written as R_T. The fraction of those receptors occupied by the drug A, under steady state conditions, is then:

$$y_A = \frac{1}{1+1/K_A(A)} \tag{1}$$

where K_A is the affinity constant of the receptor for drug A, and the curved brackets indicate molar concentrations. An analogous equation applies for any other drug B. The stimuli produced by drugs A and B separately are written as s_A and s_B where:

$$s_A = f_A y_A R_T \tag{2a}$$

and

$$s_B = f_B y_B R_T \tag{2b}$$

f_A and f_B are the intrinsic efficacies of the drugs A and B respectively.

Suppose that reproducible dose/response curves have been obtained for the actions of each drug on the cell. Any particular value of the response, r, will be produced by definite concentrations of the drugs A and B, written as $(A)_r$ and $(B)_r$. These concentrations can be read directly from the dose/response curves, and correspond to definite values of y_A and y_B, written as $[y_A]_r$ and $[y_B]_r$, which in turn correspond to definite stimuli $[s_A]_r$ and $[s_B]_r$ respectively. However, since these stimuli produce the same response r, it follows from the null hypothesis that they must be equal. Then, from equations (2a) and (2b):

$$f_A[y_A]_r = f_B[y_B]_r$$

or

$$\frac{\beta_{AB}}{[y_B]_r} = \frac{1}{[y_A]_r}$$

where β_{AB} is the ratio of the intrinsic efficacy of drug A to that of drug B. By use of equation (1), this may be rewritten as:

$$\beta_{AB}\{1+1/K_B(B)_r\} = \{1+1/K_A(A)_r\}$$

which on re-arrangement gives:

$$1/(A)_r = \{K_A/K_B\}\beta_{AB}/(B)_r + K_A\{\beta_{AB}-1\} \tag{3}$$

Equation (3) indicates that, if the reciprocal of $(A)_r$ is plotted against that of $(B)_r$, then a straight line should be obtained of slope Ψ_{AB} and intercept I_{AB} where:

$$\Psi_{AB} = \{K_A/K_B\}\beta_{AB} \tag{4}$$

and

$$I_{AB} = K_A\{\beta_{AB}-1\} \tag{5}$$

Ψ_{AB} and I_{AB} are experimentally determinable constants which are related to the funda-

mental drug-receptor parameters K_A, K_B and β_{AB}. If I_{AB} is positive then β_{AB} must be greater than one, so that f_A is greater than f_B. It is therefore possible to arrange a number of agonists in a series with decreasing intrinsic efficacies, by comparing the values of I_{AB} for different pairs of drugs.

Since for any pair of agonists there are only two independent equations (4 and 5), but three unknown parameters (K_A, K_B and β_{AB}), these parameters cannot be estimated by comparison of simple dose/response curves. However, even if the fundamental parameters are not determined, the method of analysis described here can provide useful information when applied to more complicated drug-receptor systems.

If two drugs compete reversibly for the same receptors then the equation:

$$(A)_r' = (A)_r \{ 1+K_B(B)' [\beta_{AB}-1]/\beta_{AB} \} - K_B(B)'/K_A\beta_{AB} \qquad (6)$$

can be derived by the same method as was used to derive (3). $(A)_r'$ is the concentration of drug which, in the presence of a concentration $(B)'$ of drug B, produces the same response as does a concentration $(A)_r$ of drug A in the absence of drug B. K_A, K_B and β_{AB} have the same significance as before. The required values of $(A)_r$ and $(A)_r'$ can be read from appropriate dose/response curves. It follows from (6) that a plot of $(A)_r'$ against $(A)_r$, at a constant value of B', should give a straight line. The slope L and intercept N, of this line, should be related to Ψ_{AB} and I_{AB} (see equations (4) and (5)) by the equations:

$$L = 1+I_{AB}(B)'/\Psi_{AB} \qquad (7)$$

$$\text{and} \quad N = -(B)'/\Psi_{AB} \qquad (8)$$

Equations (7) and (8) therefore provide a direct test of whether or not two agonists A and B compete for the same receptor. If compound A is an agonist and B is a competitive reversible antagonist then β_{AB} becomes infinitely large and equation (6) reduces to:

$$1/(A)_r = \{ 1+K_B(B)' \} . 1/(A)_r'$$

This is merely another form of equations derived previously for competitive antagonism (Clark & Raventos, 1937; Arunlakshana & Schild, 1959) by direct application of the null hypothesis.

A more general equation for reversible drug antagonism, which can be derived by direct application of the null method, is:

$$1/(A)_r = \{ 1+K_B(B)' \} . 1/(A)_r' + \{ K_AK_{AB}(B)' \} \qquad (9)$$

where K_A and K_B have their usual meaning and K_{AB} is the affinity constant of the agonist-receptor complex for the antagonist. In deriving the above equation it was assumed that equilibrium was attained between the receptors and drugs A and B during the measurement of the response. Under such conditions equation (9) can be used to estimate values of K_B and of $\{ K_AK_{AB} \}$. However, if the antagonist is displaced only slowly from the receptors then the responses may be measured under non-equilibrium conditions. In such cases a positive intercept will be obtained on plotting $1/(A)_r$ against $1/(A)_r'$, even if the antagonist is really of the competitive type.

The equations so far derived apply only to comparisons of graded dose/response curves measured on single cells. However, these equations can also be applied to multicellular tissues. Suppose that a piece of tissue contains n cells and that concentrations of drugs A and B are found which produce equal effects on the tissue. The total observed effect, E,

will be some function of the individual cell responses. This may be written as:

$$E = \sum_{i=1}^{n} r_i - \Phi\{\xi\} \tag{10}$$

where r_i is the individual response of the ith cell and the summation is over all n cells. $\Phi\{\xi\}$ is a function which depends on the type and magnitude of the effect observed, and on the structure of the tissue. Provided that $\Phi\{\xi\}$ is a continuous increasing function of E then any given value of E will correspond to a definite value of $\sum_{i=1}^{n} r_i$.

Suppose that the concentration $(A)_E$ of drug A which produces a total effect E, produces responses r_1 from cell 1, r_2 from cell 2, and so on. Provided that the values of Ψ_{AB} and I_{AB} are the same for every cell of the tissue, then equation (3), which may be written in the form:

$$1/(A)_r = \Psi_{AB} \,.\, 1/(B)_r + I_{AB}$$

will be valid for each cell. When $(A)_E$ produces response r_1 from cell 1, then a concentration $(B)_E$ of drug B will produce the same response, so that the above equation becomes:

$$1/(A)_E = \Psi_{AB} \,.\, 1/(B)_E + I_{AB} \tag{11}$$

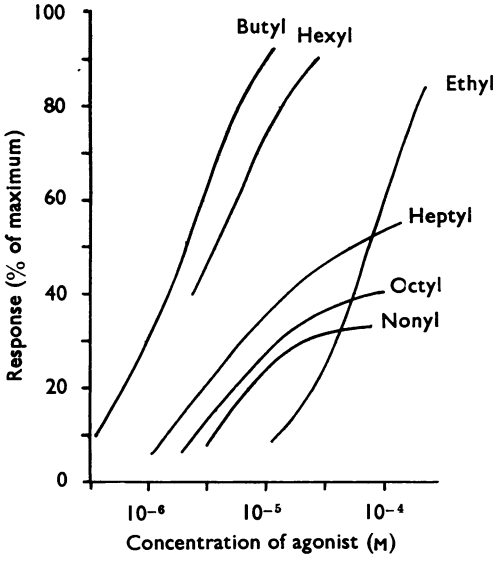
The same equation is valid for cell 2, giving response r_2, and so on. Therefore $(A)_E$ and $(B)_E$ produce equal responses from any individual cell, and so produce the same values of $\sum_{i=1}^{n} r_i$, and hence equal values of E for the whole tissue. Since equation (11) holds for each cell of the tissue it is also valid for the whole tissue. It follows that this equation can be used to obtain values of Ψ_{AB} and I_{AB} from dose/response curves measured on a multicellular tissue. If the values of these experimental constants vary from one cell to another then the values obtained from multicellular tissues will be complicated mean values. The application to multicellular tissues of the other equations derived here for single cells can be justified in a similar way.

Equations analogous to (10) can also be set out for two different pieces of tissue containing n_1 and n_2 cells respectively. The response of each tissue can then be expressed as its " fractional response," which is the fraction of its maximal response. Dose/" fractional response " curves obtained with different pieces of tissue can then be compared in the same way as dose/response curves obtained on a single piece of tissue. The results so obtained would not be expected to be as accurate as those obtained by the latter method, but they might be acceptable, provided that: (1) the same recording system is used; (2) the tissues are in the same metabolic state, so that the same average stimulus/response relationship applies to the cells of the two pieces of tissue; and (3) either $\Phi\{\xi\}$ is small compared with $\sum_{i=1}^{n} r_i$, or $\Phi\{\xi\}/\sum_{i=1}^{n} r_i$ is the same for the two pieces of tissue. Direct comparison of dose/response curves measured on different pieces of tissue are therefore likely to give accurate values of Ψ_{AB} and I_{AB} only in very carefully controlled experiments.

DISCUSSION

In order to carry out a preliminary test of these ideas, use has been made of dose/response curves, shown in Fig. 1, which were published by Stephenson (1956). Values of Ψ_{AB} and

of I_{AB}, for several alkyltrimethylammonium compounds, were calculated from his results and are given in Table 1. In every case but the last, good straight lines were obtained when $1/(A)_r$ was plotted against $1/(B)_r$. Some typical examples are shown in Fig. 2. Since it is unlikely that all of Stephenson's results were obtained with a single piece of guinea-pig ileum, these calculations of the experimental constants, Ψ_{AB} and I_{AB}, probably involve the additional assumptions which were discussed in the last paragraph of the preceding section. Nevertheless, agreement between theory and experiment is satisfactory and suggests that these drug-receptor interactions are bimolecular.



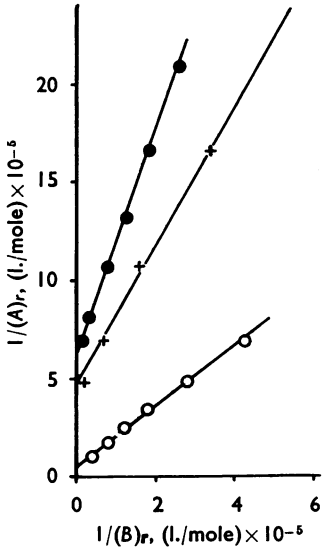


Fig. 1. Log dose/response curves for some alkyltrimethylammonium ions on guinea-pig ileum. The results are those of Stephenson (1956) and only the smoothed curves have been plotted. The nature of the alkyl group is indicated on each curve.

Fig. 2. $1/(A)_r$ is plotted against $1/(B)_r$, where $(A)_r$ and $(B)_r$ are those molar concentrations of agonists A and B which produce the same responses when they act on the same tissue. In the above examples A is butyltrimethylammonium and B is either octyltrimethylammonium (●), heptyltrimethylammonium (+) or hexyltrimethylammonium (o). The test object was guinea-pig ileum in Tyrode solution at 37° C. The results are those of Stephenson (1956).

TABLE 1

VALUES OF Ψ'_{AB} AND I_{AB} FOR SEVERAL ALKYLTRIMETHYLAMMONIUM COMPOUNDS

The values of the constants Ψ'_{AB} and I_{AB} are respectively the slopes and intercepts of lines obtained by plotting $1/(A)_r$ versus $1/(B)_r$ for given pairs of agonists A and B acting on the same tissue. $(A)_r$ and $(B)_r$ are the concentrations of drugs A and B required to produce a given response r. These values of $(A)_r$ and $(B)_r$ are from the results in Fig. 1, which were taken from the paper by Stephenson (1956). These results were obtained using guinea-pig ileum in Tyrode solution at 37° C. All of the drugs are alkyltrimethylammonium compounds and are therefore characterized by the name of the alkyl group

Alkyl group of alkyltrimethylammonium compound			
A	B	Ψ'_{AB}	I_{AB} (l./mole)× 10^{-5}
Butyl	Ethyl	37·4	0·103
Butyl	Hexyl	1·51	0·59
Butyl	Heptyl	3·30	4·89
Butyl	Octyl	5·55	6·22
Butyl	Nonyl	7·45	6·83

TABLE 2

CALCULATION OF FUNDAMENTAL PARAMETERS

β_{bn} is the ratio of the intrinsic efficacy of butyltrimethylammonium to that of nonyltrimethylammonium. Values of β_{bn} were chosen arbitrarily and for each such value the corresponding values of the affinity constant K_x and the relative intrinsic efficacy β_{xn} of each compound x, were calculated from the values of Ψ_{AB} and I_{AB} given in Table 1. The values of K_x are in (l./mole)$\times 10^{-5}$ and β_{xn} is the ratio of the intrinsic efficacy of compound x to that of nonyltrimethylammonium. Values of the parameters measured by Stephenson's (1956) method are shown in the last column

Alkyl group (x) of alkyltrimethyl-ammonium compound	$\beta_{bn}=2{\cdot}0$		$\beta_{bn}=5{\cdot}0$		$\beta_{bn}=11{\cdot}0$		$\beta_{bn}=25{\cdot}3$		$\beta_{bn}\to\infty$		Results from Stephenson (1956)	
	K_x	β_{xn}	K_x	β_{xn}	K_x	β_{xn}	K_x	β_{xn}	K_x	β_{xn}	K_x	Relative efficacy
Ethyl	0·20	1·9	0·05	4·7	0·02	9·6	0·01	18·7	0·01	18·7	0·006	31
Butyl	7·3	2·0	1·7	5·0	0·69	11·0	0·28	25	0·29	25·3	0·04	200
Hexyl	5·3	1·8	1·5	3·7	0·85	5·9	0·58	8·1	0·58	8·4	0·19	21
Heptyl	3·7	1·2	2·0	1·3	1·7	1·4	1·57	1·4	1·57	1·4	0·41	2·2
Octyl	2·6	1·0	1·4	1·1	1·3	1·1	1·18	1·1	1·18	1·1	0·63	1·4
Nonyl	1·9	1·0	1·2	1·0	1·0	1·0	0·96	1·0	0·95	1·0	1·10	1·00

In general for N agonists acting on a tissue, an examination of the signs of the experimental constants I_{AB} allows the drugs to be placed in order of decreasing intrinsic efficacies. For such a series, A, B, C . . . N, there will be $(2N-1)$ unknown parameters, but only $(2N-2)$ independent values of the experimental constants. The fundamental parameters therefore cannot be estimated from such data alone, but if one parameter is arbitrarily defined then all the corresponding values of the other parameters can be calculated. The results given in Table 2 were obtained in this way. Each set of parameters given in the table corresponds to an arbitrarily chosen value of β_{bn}, which is the ratio of the intrinsic efficacy of butyltrimethylammonium to that of nonyltrimethylammonium. These calculations show that the experimental dose/response curves can be described by an infinitely large number of sets of values of the fundamental parameters. Each such set corresponds to a different stimulus/response curve, which can be calculated. This curve depends on the arbitrarily chosen value of β_{bn}. It is worth noting that these conclusions are valid whether the values of Ψ_{AB} and I_{AB} given in Table 1 are real results or hypothetical values.

It will be seen from Table 2 that the order of the intrinsic efficacies of the agonists is independent of the chosen value of β_{bn} but this is not true for the affinity constants. The special case when β_{bn} is assumed to be infinitely large would be expected to give values of the parameters which should be in reasonable agreement with those obtained by the method of Stephenson (1956) (see Table 2). This assumption is, of course, not necessarily correct.

In order to obtain accurate values of the fundamental parameters, one or other of the following conditions must be met: (1) the assumption that β_{AN} is infinitely large must be justified; (2) a value for at least one of the fundamental parameters must be obtained by an independent method; or (3) another independent equation relating the fundamental parameters must be found.

The most promising methods for determining these parameters seem to be those based on the use of specific irreversible antagonists. Two such methods, which are based on the type of analysis outlined here, will be discussed elsewhere.

SUMMARY

1. A general method for the analysis of drug-receptor interactions, which involves a minimum of assumptions, is presented.

2. The method is first developed for dose/response curves obtained from a single cell, and is then extended to dose/response curves from a multicellular tissue.

3. The two fundamental parameters of a drug-receptor interaction have been termed the affinity constant and the intrinsic efficacy. The latter is conceptually the same as intrinsic activity or efficacy.

4. It is shown that experimental constants, Ψ_{AB} and I_{AB}, which are related to the fundamental parameters, can be obtained from the dose/response curves of any two agonists A and B acting separately on the same tissue. No assumption is made about the form of the relationship between stimulus and response, but a steady response is assumed at equilibrium. These experimental constants should depend only on the drugs, the tissue, and the experimental conditions.

5. The fundamental parameters cannot be calculated directly from the experimental constants. Nevertheless, the order of the intrinsic efficacies of a series of agonists can be obtained, in principle, from the signs of the constants I_{AB}.

6. If two drugs are applied simultaneously to the tissue then this method of analysis provides a direct test of whether or not they compete for the same receptors. If one of the drugs is an agonist and the other is an antagonist then the affinity constant of the antagonist can be calculated.

7. This type of analysis therefore provides a uniform, general method for the study of drug-receptor interactions.

I wish to thank Dr S. G. Williams and Professor D. R. Wood of this department, and Dr J. M. van Rossum of the University of Nijmegen, for useful comments.

REFERENCES

ARIENS, E. J. (1954). Affinity and intrinsic activity in the theory of competitive inhibition. Part 1. *Arch. int. Pharmacodyn.*, **99**, 32–49.

ARUNLAKSHANA, O. & SCHILD, H. O. (1959). Some quantitative uses of drug antagonists. *Brit. J. Pharmacol.*, **14**, 48–58.

CLARK, A. J. (1937). In HEFFTER, *Hbk. exp. Pharmak., Ergangungswerk*, **4**, pp. 63–64. Berlin: Springer.

CLARK, A. J. & RAVENTOS, J. (1937). The antagonism of acetylcholine and of quaternary ammonium salts. *Quart. J. exp. Physiol.*, **26**, 375–392.

GADDUM, J. H. (1937). The quantitative effects of antagonistic drugs. *J. Physiol. (Lond.)*, **89**, 7–8*P*.

LANGLEY, J. N. (1878). On the physiology of the salivary secretion, part 2: on the mutual antagonism of atropin and pilocarpin having a special reference to their relation in the submaxillary gland of the cat. *J. Physiol. (Lond.)*, **1**, 339–3t9.

LANGLEY, J. N. (1905). On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. *J. Physiol. (Lond.)*, **33**, 374–413.

SCHILD, H. O. (1947). pA, a new scale for the measurement of drug antagonism. *Brit. J. Pharmacol.*, **2**, 189–206.

SCHILD, H. O. (1954). Non-competitive drug antagonism. *J. Physiol. (Lond.)*, **124**, 33–34*P*.

STEPHENSON, R. P. (1956). A modification of receptor theory. *Brit. J. Pharmacol.*, **11**, 379–393.

VAN ROSSUM, J. M. & ARIENS, E. J. (1962). Receptor-reserve and threshold-phenomena. *Arch. int. pharmacodyn.*, **136**, 385–413.