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AN ANALYSIS OF FUNCTIONAL ANTAGONISM AND SYNERGISM

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1 A method is described for deriving null equations for functional antagonism and synergism. These null equations relate together the concentrations of agonist required to produce equivalent states of a cell or tissue in the presence and in the absence of a functional interactant

2 In one particular case the null equation leads to conclusions which are very similar to those reached by an earlier author who did not use the null method. However, the null equations give a clearer insight into the quantitative aspects of functional interaction.

3 It is concluded that the use of functional antagonism to estimate affinity constants and relative intrinsic efficacies of agonists has serious limitations. On the other hand, it may be possible to use the null equations, or similar principles, to test the validity of postulated mechanisms and sites of action of functional interactants.

Introduction

Functional antagonism and synergism can both be regarded as forms of functional interaction. The latter is said to occur when the response of a cell to a drug acting on one type of receptor can be modified by a second drug acting on a different receptor. The first quantitative model of functional interaction was proposed by Ariens, Van Rossum & Simonis (1956) who assumed that any one agonist can produce a maximal reponse only when it occupies all of its receptors. Van den Brink (1973a,b) showed that some experimental observations do not fit this model but can be explained if allowance is made for the possible existence of spare receptors, a receptor reserve, or spare stimulus capacity. Each of these terms implies that not all receptors need be occupied by an agonist to produce a maximal effect (Stephenson 1956).

When dose-response curves are used to study the properties of agonists and antagonists acting on only one type of receptor, the uncertainty that arises from lack of knowledge about the relation between response and number of receptors occupied by agonist has led to the use of the 'null method' to obtain information from such data (for a general review of this topic see Mackay, 1977). The null equations relate together those concentrations of agonist(s) required to produce equal responses from a cell or tissue. These equations should be independent of the response level and involve no assumption about the presence or absence of spare stimulus capacity.

As already mentioned, the revised model of functional interaction proposed by Van den Brink (1973a) was able to account for the variety of results obtained experimentally but the qualitative and semiquantitative conclusions were based on specific numerical examples. In the present paper the null method is applied to an essentially similar model of functional interaction. The resulting null equations allow general conclusions to be drawn concerning the kind of information which can be derived from such experiments.

Theory

Derivation of null equations for functional interaction

General assumptions The basic model is summarised in Figure 1. The primary stimulus ($S_{I\alpha}$ or $S_{II\alpha}$) generated by each receptor system produces a chain of stimuli which combine at some stage to produce a stimulus S_N which determines the state of the cell. In order to deal quantitatively with this model the following assumptions are made:

(a) Each stimulus, e.g. S_{x+1}, in a chain of stimuli is related to the preceding stimulus, S_x, by the equation

$$1/S_{x+1} = a_x + b_x/S_x \qquad 1$$

where a_x and b_x are 'step' constants. If a_x is zero then S_{x+1} is proportional to S_x otherwise S_{x+1} can approach a maximum. For a chain of sequential stimuli obeying equation 1, a similar equation should hold between the initial and final stimuli of the chain so that

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$$1/S_\Omega = a + b/S_\alpha \qquad 2$$

where a and b are chain constants which depend on the values of a_x and b_x for each step in the chain. The symbols α and Ω are used to indicate the beginning and end of each chain and do not imply anything about the number of steps in either chain.

(b) A definite relation exists between S_N, $S_{I\Omega}$ and $S_{II\Omega}$. Equivalent states of the cell or tissue are obtained under two sets of experimental conditions, denoted by the subscripts 1 and 2, when

$$S_{N1} = S_{N2} \qquad 3$$

The above statement defines the term 'equivalent state'. However, in practice S_N is not likely to be measured directly. Instead it may be assumed that equal values of S_N produce equal values of some measured property of the cell or tissue. The converse assumption that equal values of the measured property correspond to equal values of S_N may also be true but involves an implicit assumption that all other factors which may modify the measured property, such as X and Y in Figure 1, are also equal when values of the measured property are equal. With these assumptions, the length of a piece of tissue, for example, might be taken as a measure of its state. The 'state' of the tissue should be distinguished clearly from the 'response' which is usually taken to mean the change in state.

(c) The experimental data are assumed to consist of two (or more) concentration-state curves each measured for the agonist A_I in the presence of a different concentration of the second agonist A_{II}. It should therefore be possible to compare those concentrations of A_I which produce equivalent states in the presence of different concentrations of A_{II}.

(d) The primary stimulus produced by agonist A, acting on receptors type R, is

$$S_A = f_A R/(1 + 1/K_A[A]), \qquad 4$$

at equilibrium, where f_A is the intrinsic efficacy of the agonist, K_A is its affinity constant for the receptors, R is the total concentration (in arbitrary units) of receptors of type R and [A] is the molar concentration of the agonist. Equation 4 is the usual definition of the pharmacological stimulus on the basis of the occupation theory of drug action.

Type I interaction In this case S_N is assumed to be some function of $(S_{I\Omega} + S_{II\Omega})$ so that equal values of the former correspond to equal values of the latter. If two concentration-state curves are measured for the agonist A_I in the presence of concentrations $[A_{II}]_1$ and $[A_{II}]_2$ of the second agonist respectively then the requirement for equivalent states is

$$(S_{I\Omega})_1 + (S_{II\Omega})_1 = (S_{I\Omega})_2 + (S_{II\Omega})_2 \qquad 5$$

However the values of $(S_{II\Omega})_1$ and $(S_{II\Omega})_2$ depend on $[A_{II}]_1$ and $[A_{II}]_2$ respectively and are constant for the two curves. Equation 5 may therefore be rearranged to give

$$(S_{I\Omega})_2 - (S_{I\Omega})_1 = (S_{II\Omega})_1 - (S_{II\Omega})_2 = \Delta S_{II\Omega} \qquad 6$$

where $\Delta S_{II\Omega}$ is a constant for the particular pair of curves being compared. From equations 2 and 6 it follows that

$$1/\{a_I + b_I/(S_{I\alpha})_2\} - 1/\{a_I + b_I/(S_{I\alpha})_1\} = \Delta S_{II\Omega} \qquad 7$$

where a_I and b_I are constants characteristic of the chain of stimuli initiated by the agonist A_I up to the convergence step which determines S_N. Equation 7 relates together the primary stimuli required to produce equivalent states. Using the appropriate forms of equation 4 to substitute for $(S_{I\alpha})_1$ and $(S_{I\alpha})_2$ in equation 7 and rearranging gives the null equation for type I functional interaction,

$$[A]_2/[A]_1 = \alpha + \beta[A]_2 + \gamma/[A]_1 \qquad 8$$

where $[A]_1$ and $[A]_2$ are the concentrations of A_I which produce equivalent states of the cell or tissue in the presence of the concentrations $[A_{II}]_1$ and $[A_{II}]_2$ of the second agonist. The quantities α, β, and γ are given by the equations

$$\alpha = (1 + V)/(1 - V); \quad \beta = U/(1 - V) \quad \text{and } \gamma = W/(1 - V) \qquad 9a,b,c$$

where

$$U = \Delta S_{II\Omega}\{f_A K_A R_I/b_I\}\{a_I + b_I/f_A R_I\}^2 \qquad 10a$$

$$V = \Delta S_{II\Omega}\{a_I + b_I/f_A R_I\} \qquad 10b$$

and

$$W = \Delta S_{II\Omega}\{b_I/f_A K_A R_I\} \qquad 10c$$

In this case α, β, and γ would not be expected to be independent since it can be shown that

$$(\alpha - 1)^2 = 4\beta\gamma \text{ or } V^2 = U.W \qquad 11a,b$$

Type II interaction In this type of functional interaction A_{II} is assumed to change only the values of the chain constants a_I and b_I, by an indirect mechanism. There is no longer a convergence point for chains of stimuli from the two interactants so the chain S_α to S_Ω is not clearly defined by Figure 1. Instead the stimulus S_N becomes the stimulus S_{x+1} where a_x and b_x are the last step constants modified by the action of A_{II}. The condition for equivalent states is then

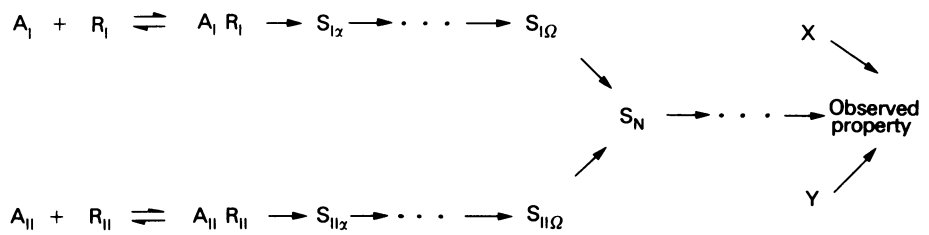

Figure 1 Agonists A_I and A_{II} act on different types of receptors R_I and R_{II} to produce sequential stimuli $S_{I\alpha}$ to $S_{I\Omega}$ and $S_{II\alpha}$ to $S_{II\Omega}$. $S_{I\Omega}$ and $S_{II\Omega}$ control the magnitude of S_N which in turn determines the magnitude of the observed property. X and Y are factors other than S_N which could also modify the magnitude of the observed property.

$$(S_{x+1})_1 = (S_{x+1})_2 \qquad 12$$

where the subscripts 1 and 2 indicate that the quantities refer to different experimental conditions. Combining equation 2 with equation 12 gives

$$1/\{a_{I1} + b_{I1}/(S_{I\alpha})_1\} = 1/\{a_{I2} + b_{I2}/(S_{I\alpha})_2\} \qquad 13$$

where a_{II} and b_{II} are the values of the appropriate chain constants in the presence of the concentration $[A_{II}]_1$ of functional interactant and a_{I2} and b_{I2} are their values in the presence of the concentration $[A_{II}]_2$. Using equation 4 to substitute for $(S_{I\alpha})_1$ and $(S_{I\alpha})_2$ in equation 13 and rearranging leads to the general null equation for type II functional interaction. The equation has the same form as equation 8 but with γ equal to zero and

$$\alpha = b_{I2}/b_{I1} \qquad 14a$$

$$\beta = K_A\{f_A R_I(a_{I2} - a_{I1})/b_{I1} + (b_{I2}/b_{I1} - 1)\} \qquad 14b$$

Type IIA interaction If $a_{I1} = a_{I2}$ then equations 14a and 14b reduce to

$$\alpha = b_{I2}/b_{I1} \text{ and } \beta = K_A\{b_{I2}/b_{I1} - 1\} \qquad 15a,b$$

It follows that in this special case

$$K_A = \beta/(\alpha - 1) \qquad 16$$

Type IIB interaction If $b_{I1} = b_{I2}$ then equations 14a and 14b reduce to

$$\alpha = 1.0 \text{ and } \beta = K_A\{f_A R_I(a_{I2} - a_{I1})/b_I\} \qquad 17a,b$$

Type III interaction Although, for reasons which will become apparent in the discussion, the most common type of interaction is probably type I it is possible that the two chains of stimuli, $S_{I\alpha}$ to $S_{I\Omega}$ and $S_{II\alpha}$ to $S_{II\Omega}$ might interact in a non-additive way. For example instead of S_N being determined by ($S_{I\Omega}$ + $S_{II\Omega}$), as was postulated for type I interaction, it might be determined by the ratio $S_{I\Omega}/S_{II\Omega}$. Applying the same principles used in the earlier sections, the null equation in this case can be shown to have the same form as equation 8 but with γ equal to zero,

$$\alpha = (S_{II\Omega})_2/(S_{II\Omega})_1 \qquad 18a$$

and $$\beta = (\alpha - 1)K_A\{a_I f_A R_I/b_I + 1\} \qquad 18b$$

Since for an experiment on a single piece of tissue a_I, b_I, R_I, f_A and K_A would all be expected to be constant the values of α and β from such data would be expected to be such that $\beta/(\alpha - 1)$ would be constant.

Qualitative predictions of the model

The most general form of the null equation is equation 8 which describes Type I interaction and requires three adjustable constants α, β, and γ. In all other cases γ is zero. Type III interaction is almost indistinguishable from type IIA, in terms of state-concentration curves, unless the actual value of K_A is known. The predicted effects of the various types of functional interaction on the position and shape of log concentration-state curves will therefore be illustrated using only the three cases corresponding to interactions of types I, IIA and IIB. In order to calculate the theoretical curves a log concentration-state curve was drawn arbitrarily and this was used in each case as the control curve. Numerical values were then assigned to a_I, b_I, K_A and $f_A R_I$. These values were chosen so that there would be spare stimulus capacity when the agonist acts on its receptors. Appropriate values of $\Delta S_{I\Omega}$ or of a_{I2} or b_{I2} were chosen to illustrate the effects of changing these quantities. At each state level the value of $[A]_1$ is known from the control curve. The value of $[A]_2$ required to produce the same state was calculated from

$$[A]_2 = \{\alpha[A]_1 + \gamma\}/\{1 - \beta[A]_1\}$$

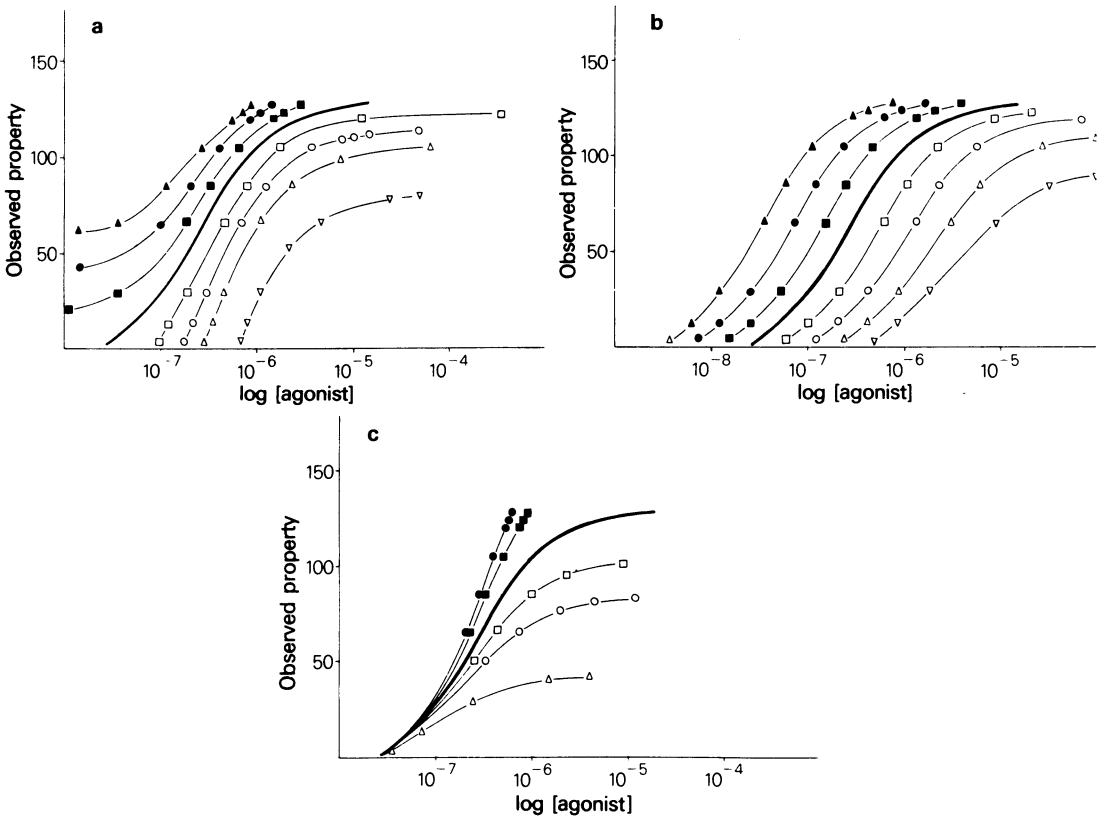

Figure 2 Predicted effects of various types of functional interaction on the shape and position of state-concentration curves. In each case the thick curve represents the initial control curve and the initial values of a_I, b_I, K_A and $f_A R_I$ are 1.0, 1.0, 10^5 and 20 respectively. (a) Type I interaction. The values of $\Delta S_{II\Omega}$ for the curves are −0.3 (▲), −0.2 (●), −0.1 (■), 0.1 (□), 0.2 (○), 0.3 (△) and 0.5 (▽). (b) Type IIA interaction. The values of b for the curves are 0.125 (▲), 0.25 (●), 0.50 (■), 2.0 (□), 4.0 (○), 8.0 (△) and 16.0 (▽). (c) Type IIB interaction. The values of a for the curves are 0.25 (●), 0.50 (■), 1.5 (□), 2.0 (○) and 4.0 (△).

which is an alternative form of equation 8, the values of α, β and γ being estimated from the appropriate equations. The results of these calculations are summarised in Figures 2a, 2b and 2c for type I, type IIA (or III) and type IIB respectively. It will be seen from Figure 2a that type I interaction is predicted to produce complex changes in the shapes and positions of the curves. A distinct characteristic of this type of interaction is the initial steepening of the curves obtained in the presence of low concentrations of functional antagonist. By contrast type IIA interaction, which involves only changes in b_I, produces mainly parallel displacement of the log concentration-state curve with a reduction of the maximal state at high concentrations of functional antagonist (Figure 2b). As already mentioned a very similar pattern would be expected for type III interaction. This pattern also clearly resembles that which would be expected from repeated treatment of a tissue with a selective irreversible antagonist. If only a_I is changed (type IIB interaction) there is no parallel displacment of the curves but a progressive reduction of the maximum (Figure 2c).

One other qualitative point remains, namely the explanation of the ability of some functional interactants to displace log concentration-state curves only to a limited extent (Van den Brink, 1973a, Figures 5 and 6). In terms of the null equations the curves cease to move when higher concentrations of A_{II} fail to produce any further change in $S_{II\Omega}$ (Figure 2a), b_I (or $S_{II\Omega}$) (Figure 2b) or a_I (Figure 2c). A_{II} would then be producing its maximal effect as a functional interactant, which might correspond to saturation of the receptors R_{II} for example.

Obviously the various types of functional interaction (except types IIA and III) would be expected to produce curve patterns which are clearly different. Type I interaction leads to patterns of curves which

Table 1 Theoretical criteria for the classification of types of functional interaction

Type of interaction	*Are these parameters significantly different from zero?* $(\alpha-1)$	β	γ	*Is there a general relationship between* α, β *and* γ?	*Can the affinity constant* K_A *be estimated from* α, β and γ?
I	Yes	Yes	Yes	$2\beta/(\alpha-1) = (\alpha-1)/2\gamma$ for any one piece of tissue	Only if the agonist A has a very low intrinsic efficacy
II (general)	Yes	Yes	No	No	No
IIA	Yes	Yes	No	$\beta/(\alpha-1)$ is constant	Yes, $K_A = \beta/(\alpha-1)$
IIB	No	Yes	No	No	No
III	Yes	Yes	No	$\beta/(\alpha-1)$ is constant, for any one piece of tissue	Only if the agonist A has a very low intrinsic efficacy

are very similar to those presented by Van den Brink (1973a, b).

Quantitative predictions

In order to test or use equation 8 two concentration-state curves for an agonist need to be determined each in the presence of a different concentration of the functional interactant. One of these concentrations may be zero. The concentrations $[A]_1$ and $[A]_2$, which produce the same state of the cell or tissue, are read off at a series of state levels within the range common to both curves. The values of α, β and γ can then be estimated for each pair of curves from the multiple linear regression of $[A]_2/[A]_1$ on $[A]_2$ and $1/[A]_1$. It should be possible to distinguish between some of the types of interaction discussed earlier from the magnitudes of α, β and γ and their variation with $[A_{II}]$, provided that one of these mechanisms is dominant. The theoretical criteria for distinguishing between the different types of interaction are summarised in Table 1. According to the theoretical equations α, β and γ depend on constants, some characteristic of the interactants and others of the tissue. The three types of interaction which offer the best prospects of obtaining useful information are types I, IIA and III.

Affinity constants and related quantities

Type I interaction In this case the value of K_A cannot be estimated from α, β and γ but another quantity given the symbol $K_A{}^F$ can be obtained from

$$K_A{}^F = V/W = (\alpha - 1)/2\gamma \qquad 19a$$

or

$$K_A{}^F = U/V = 2\beta/(\alpha - 1) \qquad 19b$$

or

$$K_A{}^F = (U/W)^{1/2} = (\beta/\gamma)^{1/2} \qquad 19c$$

These estimates of $K_A{}^F$ are not independent since according to the model

$$V^2 = UW \text{ or } (\alpha - 1)^2 = 4\beta\gamma.$$

One estimate of $K_A{}^F$ may be more reliable than another since the errors in α, β and γ may differ greatly. (The use of multiple linear regression to estimate these quantities and their standard errors is open to criticism on statistical grounds but this problem will not be discussed here). From equations 10a, b and c and equations 19a, b and c

$$K_A{}^F = K_A[a_I f_A R_I/b_I + 1] \qquad 20$$

$K_A{}^F$ will be called the functional affinity constant since it is an apparent affinity constant which, in the absence of information about the values of a_I, b_I, f_A and R_I, provides an estimate of the maximal value of K_A. Another way of interpreting $K_A{}^F$ is to note that $f_A R_I$ is the maximal value of the primary stimulus $S_{I\alpha}$ which agonist A can produce and that b_I/a_I is the magnitude of $S_{I\alpha}$ required to produce a value of $S_{I\Omega}$ equal to half $S_{I\Omega max}$ (see Figure 3). The functional affinity constant $K_A{}^F$ reduces to K_A only if $f_A R_I << b_I/a_I$. This requires agonist A to have an intrinsic efficacy so low that it acts as a weak partial agonist in the chain of stimuli from $S_{I\alpha}$ to $S_{I\Omega}$. It is interesting to note that this condition does not depend on the magnitude of $\Delta S_{II\Omega}$ nor on any limitation on the transfer of S_N to produce the final measured property. If the agonist A behaves as a partial agonist in terms of the final measured property, while another agonist acting on the same receptors under the same experimental conditions can produce a higher maximal value of the measured property then the condition $f_A R_I << b_I/a_I$ may be at least approximately satisfied.

On the basis of this theoretical analysis the con-

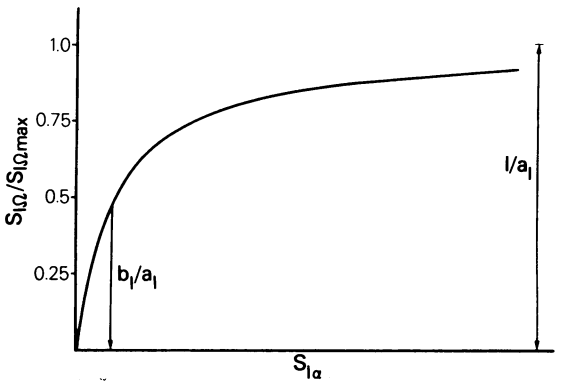

Figure 3 A graphical illustration of the assumed relation between $S_{I\alpha}$ and $S_{I\Omega}$. b_I/a_I is the value of $S_{I\alpha}$ required to produce one half of the maximal value of $S_{I\Omega}$.

centration-state curves obtained in the presence of a functional interactant should be interrelated according to equation 8. However at sufficiently high concentrations of a functional antagonist the term $\gamma/[A]_I$ may be numerically swamped by α and $\beta[A]_2$. If γ is not detectably different from zero, equation 8 can be rearranged to

$$1/[A]_1 = \alpha/[A]_2 + \beta \qquad 21$$

which has the same form as the null equation for irreversible antagonism. However, inability to measure γ accurately does not affect the meanings of α and β. Equations 19b and 21 can therefore be combined to give

$$\text{intercept}/(\text{slope} - 1) = \beta/(\alpha - 1) = 0.5\,K_A{}^F \qquad 22$$

where 'intercept' and 'slope' refer to the best straight line fitted to a plot of $1/[A]_1$ against $1/[A]_2$. The important point is that whereas for experiments involving a selective irreversible antagonist the quantity intercept/(slope − 1) gives an estimate of K_A this is not true for a type I functional antagonist. In the latter case intercept/(slope − 1) approximates to $0.5\,K_A{}^F$ but this in turn approximates to $0.5\,K_A$ only for an agonist of very low intrinsic efficacy.

Type II interaction In this case γ is zero and equation 8 can be rearranged to give equation 21. However in the general case α and β are given by equations 14a and 14b and so K_A cannot be estimated from α and β.

Type IIA interaction For this case too, equation 21 is valid and from equation 16

$$\text{intercept}/(\text{slope} - 1) = \beta/(\alpha - 1) = K_A \qquad 23$$

where 'intercept' and 'slope' have the same meanings as for equation 22.

Type IIB interaction As in the case of the general type II interaction the value of K_A cannot be estimated from α and β.

Type III interaction Combining equations 18b and 20 gives

$$\text{intercept}/(\text{slope} - 1) = \beta/(\alpha - 1) = K_A{}^F \qquad 24$$

Such interactions therefore provide estimates of a functional affinity constant which again reduces to K_A only if the agonist A has a suitably low intrinsic efficacy.

Relative intrinsic efficacies and maximal curve displacements

The relation between these quantities can be deduced from the null equations derived earlier. Suppose that two agonists C and D act on receptors type R_{II} and interact with another agonist A_I by a type I mechanism. The interactants C and D acting on the same piece of tissue produce only a limited shift of the concentration-state curves produced by agonist A_I. The magnitudes of these maximal displacements depend on the maximal stimuli which C and D can produce namely $S_{II\Omega MC}$ and $S_{II\Omega MD}$. If for each functional interactant the curve for A_I which approximates to maximal displacement is compared with that obtained in the absence of interactant then fitting equation 8 to the data will give maximal values, α_M, β_M and γ_M from which maximal values, U_M, V_M and W_M can be estimated using alternative forms of equations 9a, b and c. Equations 10a, b and c then give

$$S_{II\Omega MC}/S_{II\Omega MD} = U_{MC}/U_{MD} = V_{MC}/V_{MD} = W_{MC}/W_{MD} \qquad 25$$

The maximal primary stimuli produced by drugs C and D are respectively $f_C R_{II}$ and $f_D R_{II}$ (see equation 4). Using equation 2 to relate $S_{II\Omega}$ and $S_{II\alpha}$ it follows that

$$S_{II\Omega MC}/S_{II\Omega MD} = f_C[f_D R_{II} + b_{II}/a_{II}]/f_D[f_C R_{II} + b_{II}/a_{II}] \qquad 26a$$

which reduces to f_C/f_D only if both $f_C R_{II}$ and $f_D R_{II}$ are much less than b_{II}/a_{II}. If this condition is not satisfied then the maximal curve displacements provide only qualitative information about the ratio of the intrinsic efficacies f_C/f_D.

The same method can be applied to type III interaction for which

$\alpha_{MC}/\alpha_{MD} = S_{II\Omega MC}/S_{II\Omega MD}$, (see equation 18a).

It follows that α_{MC}/α_{MD} is also related to f_C/f_D but gives a valid estimate of this ratio for type III interaction only if $f_C R_{II}$ and $f_D R_{II}$ are much less than b_{II}/a_{II}.

Discussion

Type I functional interaction is essentially similar to that suggested by Ariens *et al.* (1956a) and subsequently extended by Van den Brink (1973a, b). The latter showed that the results of many experimental studies on functional interaction could be explained by this mechanism if the possibility of spare stimulus capacity is taken into account. Other conclusions were that functional synergism and antagonism can be described in terms of this one model and that functional antagonism may be used to distinguish between full agonists which act on the same receptors but have different amounts of spare stimulus capacity. The null equation for type I interaction leads to the same conclusions.

The null equation also shows that a type I functional antagonist may be used to estimate a functional affinity constant K_A^F for an agonist but if the latter has a high intrinsic efficacy then K_A^F may be considerably greater than K_A. Of the various mechanisms of functional interaction considered here, only type IIA leads to null equations which allow K_A to be estimated from concentration-state curves. This conclusion is of interest since, although the work of Van den Brink seems to indicate that many cases of functional interaction belong to type I, Buckner & Saini (1975) have used the null equation for irreversible antagonism (analogous to equations 21 and 23) to estimate 'affinity constants' of agonists from dose-response curves measured in the presence of functional antagonists. They obtained good agreement (within a factor of two) between the affinity constant of soterenol estimated in this way and values obtained by other methods. Since soterenol behaved as a partial agonist this agreement would be expected whether the functional antagonism was type I (equations 19b and 20), type IIA (equation 23) or type III (equations 24 and 20). The extension of this method to estimate affinity constants of agonists with higher intrinsic efficacies, as suggested by Buckner & Saini (1975), may produce erroneously high estimates of K_A if the functional antagonism is not type IIA.

More recently the technique suggested by Buckner & Saini (1975) has been used by Broadley & Nicholson (1979) who reached the surprising conclusion that several drugs, normally thought of as partial agonists at β-adrenoceptors, had intrinsic efficacies greater than that of isoprenaline in producing positive chronotropic responses on guinea-pig isolated atria. If the functional antagonism is not type IIA then K_A may be overestimated and this overestimation would be expected to be greater for those agonists with higher intrinsic efficacies. In order to calculate the ratio of the intrinsic efficacies of two agonists A and B acting on the same receptors it is necessary to know, at least implicitly, either their potency ratio or the more general quantity $\psi_{AB} = f_A K_A/f_B K_B$, (see Mackay 1966), and the ratio of their affinity constants. If the latter, K_A/K_B, is overestimated then the intrinsic efficacy ratio, f_A/f_B, will be underestimated. Such an effect may at least partly explain the surprisingly low values obtained by Broadley & Nicholson (1979) for the intrinsic efficacy of isoprenaline relative to salbutamol and other β-agonists.

The main aim in deriving null equations for functional interaction was to see what information might be obtained from such data. The previous discussion indicates some of the problems which can arise in the absence of an adequate model. However, the null equations derived here are based on several assumptions and may have their own limitations. For example, it has been assumed that the reciprocal of each stimulus in a chain of stimuli is linearly related to the reciprocal of the preceding stimulus (see equation 1). Although this assumption seems reasonable it is arbitrary. It is also assumed that functional interactants belong to one or other of the 'pure' types. In fact null equations can also be derived for 'mixed' interactions such as types (I + II) or type (II + III) but such complications do not seem to be justified at the present time. A third important assumption is that each chain of stimuli is strictly linear, with no loops which return to the chain between $S_{I\alpha}$ and the measured property. Lastly, the primary stimulus has been defined according to the occupation theory of drug action. This last assumption seems unlikely to limit the applicability of the null equations for functional interaction since in most situations occupation theory leads to null equations which are of the same general form as those derived on the basis of other models of drug-receptor interaction (Thron, 1973; Colquhoun, 1973; Mackay, 1977). Since the derivation of the null equations for functional interaction did not require any assumption about the exact form of $S_{II\Omega}$ or about the mechanism by which a_I or b_I might be changed, these equations would be expected to apply even if the functional interactant A_{II} does not act via receptors in the usual sense. If any of the assumptions mentioned above are seriously in error then the true state of affairs is likely to be more complicated than the model analysed here. Such complications are therefore unlikely to invalidate the criticisms made earlier concerning the use of functional antagonism to estimate values of affinity constants or relative intrinsic efficacies.

Provided that the null equations for functional

interaction do provide an adequate description of such systems then they might be used to: (1) summarise experimental data in terms of α, β and γ; (2) estimate affinity constants of partial agonists, though other methods are also available for this purpose; (3) classify functional interactants; (4) test postulated sites and mechanisms of functional interaction. The first three uses listed above need no further comment but the fourth may not be immediately obvious. For a functional interactant acting by a type I or type III mechanism the magnitude of K_A^F should depend on a_I and b_I which in turn are characteristic of the series of steps from $S_{I\alpha}$ to $S_{I\Omega}$. If another interactant acts at a different point between $S_{I\alpha}$ and the measured property then the values of a_I and b_I, and therefore the value of K_A^F for the same agonist acting on the same piece of tissue, would be expected to be different. Such experiments should provide information about the relative positions of the sites of action of these interactants.

Although the emphasis here has been on functional interaction the preceding discussion raises questions about the possible application of this model to other problems such as comparison of the drug sensitivities of different kinds of tissue and comparison of the sensitivities of a chosen kind of tissue under different conditions. These topics will not be discussed further for the moment except to point out the similarity between Figures 2b and 2c of the problems discussed by Kalsner (1974).

It will only be possible to judge the practical usefulness of the null equations for functional interaction when they have been tested on a variety of biological systems. However, studies on the interaction between isoprenaline and muscarinic agonists on guinea-pig atria, described in the following paper, indicate that the interaction is type I and that the data are in reasonable agreement with the model. Preliminary experiments on the use of the model to test the validity of postulated sites of functional interaction have also given encouraging results.

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