Br. J. Pharmac. (1985), **85**, 271–275

Quantification of the characteristics of antagonists exhibiting both competitive antagonism and functional interaction

I.E. Hughes & D. Mackay[1]

Department of Pharmacology, Worsley Medical and Dental Building, University of Leeds, Leeds-2, LS2 9JT, Yorkshire.

1 Null equations have been derived which, when applied to $\log_{10}$ concentration-tissue state curves for an agonist determined in the presence and absence of a competitive antagonist which also exhibits functional interaction, allow quantitation of the characteristics of the competitive and functional interactant effects.

2 Both the affinity constant of the antagonist for its receptors and numerical values characterizing the functional interaction can be obtained.

3 The null equations have been tested in a model system by using a mixture of papaverine hydrochloride (either 5 or 20 μM) and methyl atropine bromide (10 nM) to mimic a competitive antagonist which also shows functional interaction.

4 Agreement between values derived directly and indirectly from the model is good and validates the use of the null equations.

Introduction

Antagonists tend to be classified according to their main pharmacological effects but it is not uncommon for antagonists to have more than one action. Such mixed actions may result in Schild plots which are non-linear or whose slopes differ from unity. Estimation of affinity constants by methods which assume competitive action is invalid in these circumstances and quantitation of antagonistic action presents a problem. This is especially acute in studies of structure-activity relationship where drugs may have a variety of actions and an estimate of the effectiveness of interaction of the drug with a particular receptor type may be required so that structures which optimise interaction with that receptor may be selected.

The present paper discusses the derivation and use of null equations which, when applied to $\log_{10}$ concentration-tissue state curves, can be used to separate the competitive and 'non-competitive' (i.e. functional antagonistic or synergistic) activities of such mixed antagonists.

Derivation of null equations

The simplest approach to the problem is to consider what would be expected to happen to the $\log_{10}$ concentration-tissue state curve for an agonist A if it is determined in the presence of a constant concentration of a pure functional interactant X (antagonist or synergist) and then in the presence of the same concentration of X together with a fixed concentration of a purely competitive antagonist I_1. The expected changes are shown in Figure 1.

The general null equation which summarises the relation between curve 1 and curve × (Mackay, 1981) is

$$\frac{[A]_x}{[A]_1} = \alpha_x + \beta_x[A]_x + \frac{\gamma_x}{[A]_1} \qquad (1)$$

Where $[A]_x$ and $[A]_1$ are equieffective molar concentrations of agonist A read from curve × and curve 1 respectively. The quantities α_x, β_x and γ_x are adjustable curve fitting constants. Since the presence of the pure functional interactant X merely changes the state of the tissue the addition of a fixed concentration, $[I_1]$, of the competitive antagonist I_1, in the presence of the previous concentration of X, would be expected to produce curve 2, to the right of curve × and parallel to it. The appropriate null equation which relates curve 2 to curve × is the well-known equation for competitive antagonism,

[1] Author for correspondence.

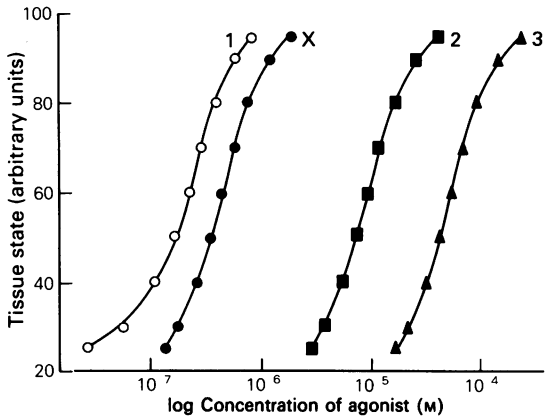

Figure 1 Log_{10}agonist molar concentration-tissue state curves obtained by calculation from the null equations assuming the following values: α_x 1.3, β_x 0.05×10^7, γ_x 1.0×10^{-7}, K_{I1} $2.0 \times 10^9 M^{-1}$, $[I_1] = 1.0 \times 10^{-8} M$, K_{I2} $1.0 \times 10^9 M^{-1}$, $[I_2] = 1 \times 10^{-7} M$. These have been chosen to mimic more closely the curves obtained from rat jejunum. Curve 1 is for the agonist alone, curve × for agonist + functional interactant X, curve 2 for agonist + functional interactant X + competitive antagonist I_1, curve 3 for agonist + functional interactant + competitive antagonist I_1 + competitive antagonist I_2.

$$\frac{[A]_2}{[A]_1} = 1 + K_{I1}[I_1] \qquad (2)$$

where K_{I1} is the affinity constant of the antagonist-receptor complex.

Eliminating $[A]_x$ between equations 1 and 2 gives the null equation which relates curve 2 to curve 1, namely

$$\frac{[A]_2}{[A]_1} = \alpha_{21} + \beta_{21}[A]_2 + \frac{\gamma_{21}}{[A]_1} \qquad (3a)$$

where $[A]_2$ and $[A_1]$ are equieffective concentrations of agonist A read from curve 2 and curve 1 respectively,

$$\begin{aligned} \alpha_{21} &= \alpha_x\{1 + K_{I1}[I_1]\} \\ \beta_{21} &= \beta_x \\ \text{and } \gamma_{21} &= \gamma_x\{1 + K_{I1}[I_1]\} \end{aligned} \qquad (3b)$$

The mixture of functional interactant and pure competitive antagonist imitates the properties of a mixed antagonist but in a real situation the 'components' of the mixture could not be separated and only curves 1 and 2 would be obtained experimentally.

Comparison of these curves would not allow calculation of K_{I1}, α_x, β_x and γ_x which are the quantities of interest. However, this difficulty can be overcome if another antagonist I_2 is available which is a pure competitive antagonist for the same receptors as those for which I_1 and A compete. Curve 3 of figure 1 represents a $\log_{10}$[A]-tissue state curve for the agonist A estimated in the presence of the previously used concentrations of the functional interactant X and of the competitive antagonist I_1 together with a fixed concentration of I_2. The null equation which relates curve 3 to curve 2 can readily be shown to be

$$\frac{[A]_3}{[A]_2} = 1 + \{K_{I2}[I_2]/(1 + K_{I1}[I_1])\} \qquad (4)$$

where $[A]_2$ and $[A]_3$ are equieffective concentrations of agonist A read from curves 2 and 3 respectively and K_{I1} and K_{I2} are the affinity constants for the antagonist-receptor complexes of antagonists I_1 and I_2 used at concentrations $[I_1]$ and $[I_2]$ respectively.

Since K_{I2} for the purely competitive antagonist I_2 can be estimated by the standard methods for pA_2 determinations, it follows that the value of K_{I1} can be calculated from the parallel displacement of curve 3 relative to curve 2. Equation 4 should be valid whether or not I_1 is a mixed antagonist. Use of equation 4 should therefore provide information about the value of K_{I1} in all circumstances. If the functional interactant properties of a mixed antagonist are also of interest then the values of α_{21}, β_{21}, and γ_{21} can be obtained by applying equation 3a to the $\log_{10}$[A]-tissue state curves obtained in the absence and presence of the mixed antagonist. The values for α_x, β_x and γ_x can then be calculated from α_{21}, β_{21} and γ_{21} knowing the values of K_{I1} and $[I_1]$ (see equations 3b). Possible interpretation of the values of α_x, β_x and γ_x have been discussed elsewhere (Mackay, 1981).

Methods

Experiments were carried out on jejunum taken from male rats (150 to 220 g) and on ileum taken from male guinea-pigs (200 to 350 g). The length of the tissue was recorded isotonically and this was taken as a measure of the 'state' of the tissue in arbitary units. In the case of the rat jejunum the tissue was bathed in Krebs solution (NaCl 118, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.1, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11 mM), gassed with 5% CO_2 in O_2, at 35°C. For guinea-pig ileum the physiological solution was Tyrode solution (NaCl 137, KCl 2.7, $MgSO_4$ 1, NaH_2PO_4 0.3, $NaHCO_3$ 12, $CaCl_2$ 1.8 and glucose 5.5 mM), gassed with O_2, at 32°C. For both tissues the resting tension was approximately 1 g. Only in the case of the rat jejunum were the $\log_{10}$ concentration-tissue state curves determined using the cumulative technique and with both tissues the antagonists were added to the

physiological saline and allowed to remain in contact with the tissue for at least 20 min before measurements were made. For each experiment smoothed curves were drawn by eye through the experimentally-determined points and equi-effective concentrations of agonist were read from the smoothed curves. These equi-effective concentrations were then substituted into the appropriate null equations to obtain values of drug-receptor affinity constants and other parameters.

Drugs used

Atropine methyl bromide (Sigma), atropine sulphate (Sigma), carbachol chloride (BDH) and papaverine hydrochloride (Sigma) were used.

Results

Experiments were carried out on rat isolated jejunum using carbachol as the agonist and atropine as the antagonist, papaverine (5 μM) being present in both cases. Comparison of the $\log_{10}$[carbachol]-tissue state curves gave a value of $(0.99 \pm 0.32)\,\text{nM}^{-1}$ ($n = 4$) as the affinity constant of atropine for the muscarinic receptors of this tissue.

A series of experiments was then carried out on rat jejunum and on guinea-pig ileum. In each case the $\log_{10}$[carbachol]-tissue state curves were determined for the agonist alone (curve 1), in the presence of papaverine (curve ×), in the presence of papaverine and methyl atropine (curve 2) and finally in the presence of papaverine, methyl atropine and atropine (curve 3). Typical examples of results obtained in such experiments are shown in Figures 2 and 3.

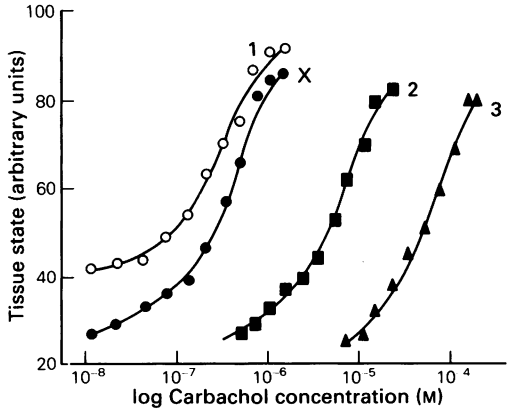

Figure 2 $\log_{10}$[carbachol]-tissue state curves obtained on rat isolated jejunum. The state of the tissue was recorded isotonically and expressed in arbitary units. Curve 1, carbachol alone (○); curve ×, carbachol + papaverine (5 μM, ●); curve 2, carbachol + papaverine + methyl atropine (10 nM, ■); curve 3, carbachol + papaverine + methyl atropine + atropine (100 nM, ▲). From such data the values given in Table 1 were obtained.

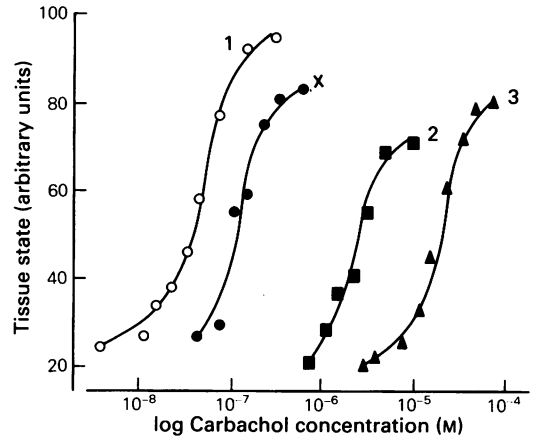

Figure 3 $\log_{10}$[carbachol]-tissue state curves obtained on guinea-pig isolated ileum. The state of the tissue was recorded isotonically and expressed in arbitary units. Curve 1, carbachol alone (○). Curve ×, carbachol + papaverine (20 μM; ●). Curve 2, carbachol + papaverine + methyl atropine (10 nM; ■). Curve 3, carbachol + papaverine + methyl atropine + atropine (100 nM; ▲). From such data the values given in Table 2 were obtained.

Comparison of curve × with curve 1 gave *direct* estimates of α_x, β_x and γ_x (see equation 1). Comparison of curve 2 and curve × provided a *direct* estimation of K_{I1}, the affinity constant of methyl atropine for the muscarinic receptors (equation 2). By comparing curve 3 with curve 2 and assuming a value for K_{I2} (i.e. $1\,\text{nM}^{-1}$ for atropine) an *indirect* estimate was made of K_{I1} using equation 4. Finally, by comparing curve 2 with curve 1 values were obtained for α_{21}, β_{21} and γ_{21} (see equation 3a). These were used to obtain *indirect* estimates of α_x, β_x and γ_x by using equations 3b together with the indirectly estimated value of K_{I1}. The results of such calculations are shown in Tables 1 and 2 for experiments carried out on rat jejunum and guinea-pig ileum respectively.

Discussion

Examination of Figures 1, 2 and 3 shows that papaverine and combinations of papaverine with competitive antagonists produce shifts in the $\log_{10}$[concentration]-tissue state curve which are

Table 1 Comparison of direct and indirect estimates of K_{I1}, α_x, β_x and γ_x obtained on rat isolated jejunum

	Direct estimates				*Indirect estimates*			
		Curve × cf. curve 1		*Curve 2 cf. curve x*		*Curve 2 cf. curve 1*		*Curve 3 cf. curve 2*
Experiment number	α_x	$\beta_x \times 10^{-7}$M	$\gamma_x \times 10^{7}$M^{-1}	K_{I1}(nM^{-1})	α_x	$\beta_x \times 10^{-7}$M	$\gamma_x \times 10^{7}$M^{-1}	K_{I1}(nM^{-1})
1	1.80	0.02	0.26	1.10	1.85	0.04	0.67	1.10
2	1.46	−0.04	1.30	1.20	1.77	0.03	2.10	0.95
3	0.52	0.08	1.30	1.60	0.60	0.11	0.97	3.10
4	1.11	0.26	0.17	1.00	2.20	0.04	0.45	0.46
5	2.34	−0.08	0.44	0.37	2.54	0.10	1.20	0.39

The curves used were for carbachol, Curve 1: alone; Curve ×: + papaverine (5×10^{-6}M); Curve 2: + papaverine (5×10^{-6}M) + methyl atropine, I_1, (10^{-8}M); Curve 3: + papaverine (5×10^{-6}M) + methyl atropine (10^{-8}M) + atropine (10^{-7}M).

qualitatively as expected. In the model tested here the actions of papaverine and of methyl atropine can be analysed separately to provide *direct* estimates of α_x, β_x, γ_x and K_{I1}. However, normally, the mixed effect mimicked by papaverine and methyl atropine would be produced by a single compound with mixed actions and curve × (see Figures 1 and 2) would not be obtainable experimentally. In such a case curves 1, 2 and 3 can be analysed using equations 4, 3a and 3b to obtain *indirect* estimates of K_{I1}, α_x, β_x and γ_x.

Comparison of the direct and indirect estimates of K_{I1} (see Tables 1 and 2), shows quite good agreement, though the variability of the indirect estimates is greater. This increased variability is not surprising in view of the more complex and prolonged nature of the experiments required to obtain indirect estimates of K_{I1}. Comparison of direct estimates of α_x, β_x and γ_x for each experiment with the indirect estimates also shows generally good agreement allowing for normal variation and changes in tissue sensitivity (see Tables 1 and 2).

The agreement between the direct and indirect estimates for K_{I1} supports the use of equation 4 to obtain values of this quantity for antagonists with mixed competitive and functional properties. However, as already mentioned, the method should also be applicable if I_1 is a pure competitive antagonist. In this special case the value of K_{I1} obtained by applying equation 4 should be in agreement with that estimated by applying equation 2 to what should then be a parallel shift of the $\log_{10}$concentration-tissue state curve produced by I_1 acting alone. In these special circumstances the technique described above would be closely related to that suggested by Paton & Rang (1965) for testing whether two antagonists compete for the same receptors, though the emphasis in this present paper is on the estimation of K_{I1} knowing K_{I2}.

In conclusion, the null equations presented here have been shown to be capable of giving satisfactory quantitative information about both the competitive and functional interactive properties of substances which have both types of action.

Table 2 Comparison of direct and indirect estimates of K_{I1}, α_x, β_x and γ_x obtained on guinea-pig isolated ileum

	Direct estimates				*Indirect estimates*			
		Curve × cf. curve 1		*Curve 2 cf. curve x*		*Curve 2 cf. curve 1*		*Curve 3 cf. curve 2*
Experiment number	α_x	$\beta_x \times 10^{-7}$M	$\gamma_x \times 10^{7}$M^{-1}	K_{I1}(nM^{-1})	α_x	$\beta_x \times 10^{-7}$M	$\gamma_x \times 10^{7}$M^{-1}	K_{I1}(nM^{-1})
1	1.14	1.41	5.85	1.40	1.38	1.01	4.30	1.13
2	2.63	1.36	0.25	1.70	2.74	1.23	0.14	2.40
3	1.52	0.39	0.44	1.16	1.39	1.09	0.70	0.51

The curves used were for carbachol, Curve 1: alone; Curve ×: + papaverine (2×10^{-5}M); Curve 2: + papaverine (2×10^{-5}M) + methyl atropine, I_1, (10^{-8}M); Curve 3: + papaverine (2×10^{-5}M) + methyl atropine (10^{-8}M) + atropine (10^{-7}M).

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(*Received November 26, 1984.*
Accepted December 23, 1984.)