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Quantification of the actions of agonists that simultaneously act on a particular type of receptor and have separate functional interactant properties

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1 Null equations have been derived which allow quantification of the agonist properties of a compound that is able to modify the state of a tissue simultaneously by interacting with a particular type of receptor and by other means.

2 Parameters can be estimated which separately characterize the agonist properties of the compound and its functional interactant effect.

3 The null equations have been tested in a model system by using a mixture of papaverine (5 μM) and hexyltrimethylammonium bromide (30 μM) to mimic an agonist which also has functional antagonist properties.

4 The values obtained for the various parameters measured directly and indirectly are in good general agreement, confirming the validity of the model.

Introduction

In an earlier paper (Hughes & Mackay, 1985) a technique was described which enabled the estimation of affinity constants of antagonists which have both competitive and functional antagonist properties. This technique was applied to analyse the action of (+)-meptazinol on the opioid receptors of the electrically stimulated isolated vas deferens of the mouse and ileum of the guinea-pig (Goodall *et al.*, 1985). Although (+)-meptazinol seemed to have no direct agonist action on the mouse vas deferens, more elaborate experiments suggested the existence of a subthreshold agonist action. In the present paper the method of analysis developed previously for drugs that act simultaneously as competitive antagonists and as functional interactants has been extended to drugs that have both agonist and functional interactant properties.

Theoretical basis of methods of analysis

It is assumed that two agonists A and B are available which interact with the population of receptors being studied and that a purely competitive antagonist I can also interact with these receptors. Agonist B however may have other properties which may synergise with or act against its agonist action. In extreme cases the agonist action of B might be obliterated. The agonist properties of B are best summarised in terms of the receptor-dependent quantities ψ_{AB} and I_{AB} (Mackay, 1966) while its functional-interactant properties can be described in terms of the quantities α, β and γ (Mackay, 1981). These various quantities can be estimated by comparing appropriate $\log_{10}$ concentration-tissue state curves as discussed below.

Method (a) Direct comparison of agonists A and B

The appropriate null equation for direct comparison of the separate concentration-tissue state curves of two agonists which act on the same receptors (Mackay, 1966) is

$$\frac{1}{[A]} = \frac{1}{[B]}\ \psi_{AB} + I_{AB} \qquad (1)$$

where [A] and [B] are equi-effective molar concentrations of A and B acting on the same piece of isolated tissue,

$$\psi_{ZB} = f_A K_A / f_B K_B$$
$$\text{and } I_{AB} = K_A(f_A/f_B - 1).$$

The symbols K and f denote affinity constants and intrinsic efficacies of the agonist indicated by the subscript. If A and B are full agonists producing

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parallel curves then ψ_{AB} is the potency of agonist A relative to agonist B.

However, direct application of these equations to concentration-tissue state curves of agonist A and agonist B involves the implicit assumption that the two agonists act through the same receptors and have no other effects on the tissue.

Method (b) Indirect comparison of agonists A and B by studying the effect of a fixed concentration of agonist B on the concentration-tissue state curve of agonist A

The appropriate null equation in this case (Mackay, 1966, Kenakin & Black, 1978) is

$$[A]' = [A]\,L + N \qquad (2a)$$

where [A]′ and [A] are molar concentrations of A which are equi-effective in the presence and absence respectively of a fixed concentration of B,

$$L = 1 + \frac{I_{AB}[B]}{\psi_{AB}} \text{ and } N = \frac{-[B]}{\psi_{AB}} \qquad (2b)$$

Note that in order to be physically meaningful N must be <0. The values of ψ_{AB} and I_{AB} can be estimated from L and N since

$$\psi_{AB} = \frac{-[B]}{N} \text{ and } I_{AB} = \frac{(L-1)\psi_{AB}}{[B]} \qquad (2c)$$

Equation (2a) also involves the assumption that the agonists only modify the state of the tissue by interacting with the receptors which are being studied.

It should be noted that the definitions of I_{AB} and of L given here are correct but differ from those in the previous paper (Goodall *et al.*, 1985) due to a transcription error which was noticed only recently.

Method (c) The action of agonist B which results from an effect on the receptor under study can be separated from its effect as a functional interactant if its action on the receptors is modified by adding a fixed concentration of the pure competitive antagonist I while at the same time keeping constant the concentration, and therefore the functional interactant effect, of B

Using the same method and assumptions as those used to derive equation 2a (Mackay, 1966) a null equation can be derived which relates the molar concentrations $[A]_2$ and $[A]_3$, of agonist A, which produce the same state of the tissue respectively in the presence of a fixed concentration of agonist B and in the presence of the same fixed concentration of B together with a fixed concentration of the competitive antagonist I. The appropriate null equation in this case can be shown to be

$$[A]_3 = [A]_2\,P + Q \qquad (3a)$$

$$\text{where } P = \frac{\psi_{AB}(1 + K_I[I]) + [B]\,I_{AB}}{\psi_{AB} + [B]\,I_{AB}} \qquad (3b)$$

$$\text{and } Q = \frac{[B]\,K_I[I]}{\psi_{AB} + [B]\,I_{AB}} \qquad (3c)$$

Solving these equations for ψ_{AB} and I_{AB},

$$\psi_{AB} = [B](P-1)/Q \text{ and } I_{AB} = (1 + K_I[I] - P)/Q \qquad (3d)$$

$$\text{Alternatively } L = K_I[I]/(P-1)$$
$$\text{and } N = -Q/(P-1) \qquad (3e)$$

Since in this case agonist B is present at the same concentration during the estimation of both concentration-tissue state curves for agonist A this equation would be expected to be valid whether or not B has functional interactant properties.

Method (d) This allows an estimate to be made of the functional interactant properties of drug B by comparing appropriate curves

If drug B had only functional interactant properties then the curve for agonist A alone, say curve 1, would be modified to curve x by the presence of a fixed concentration of B (see Figure 3). The curve codes are used to subscript [A] in order to indicate the curve from which the equieffective agonist concentration has been read. The null equation relating these two curves would be then be of the form

$$\frac{[A]_x}{[A]_1} = \alpha_x + \beta_x[A]_x + \frac{\gamma_x}{[A]_1} \qquad (4a)$$

where α_x, β_x and γ_x are adjustable constants whose values depend on the agonist A, the concentration and properties of the functional interactant B, and the properties of the tissue (Mackay, 1981).

The effect of the purely agonist properties of B on curve x would be to produce curve 2 where

$$[A]_2 = [A]_x\,L + N \qquad (4b)$$

Eliminating $[A]_x$ from equations (4a) and (4b) gives the null equation which relates the curve for agonist A alone to that obtained for agonist A in the presence of a fixed concentration of agonist B when the latter has both agonist and functional interactive properties, namely

$$\frac{[A]_2}{[A]_1} = \alpha_{21} + \beta_{21}[A]_2 + \frac{\gamma_{21}}{[A]_1} \qquad (5a)$$

$$\text{where } \alpha_{21} = \alpha_x L - \beta_x N$$
$$\beta_{21} = \beta_x$$
$$\text{and } \gamma_{21} = \gamma_x L + N$$

Rearranging these equations

$$\alpha_x = (\alpha_{21} + \beta_{21} N)/L \quad (5b)$$

$$\beta_x = \beta_{21} \quad (5c)$$

$$\text{and} \quad \gamma_x = (\gamma_{21} - N)/L \quad (5d)$$

If dependable values can be obtained for ψ_{AB} and I_{AB}, or for L and N, then values of α_x, β_x and γ_x can be estimated from the above equations. One method which might give such values of ψ_{AB} and I_{AB}, uncontaminated by functional interaction effects, is method (c).

Experimental methods

Experiments were carried out on jejunum taken from male rats (150 to 220 g). The length of the tissue was recorded isotonically (1 g resting tension) and this was taken as a measure of the state of the tissue in arbitrary units. The tissue was bathed in Krebs solution (mM: NaCl 118, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.1, $NaHCO_3$ 25, $CaCl_2$ 2.5 and glucose 11), gassed with 5% CO_2 in O_2, at 35°C. Carbachol was used as the reference agonist A and atropine as the purely competitive antagonist I. In some experiments hexyltrimethylammonium (hexyl-TMA) alone was used as agonist B while in other experiments hexyl-TMA and papaverine were present together, though added at different times, to mimic an agonist B with mixed actions. All concentration-tissue state curves were determined by the cumulative method. In those experiments in which a fixed concentration of hexyl-TMA was present during the estimation of the concentration-tissue state curve for carbachol, hexyl-TMA was allowed to act for 1 min to produce a new steady state before starting administration of carbachol. When papaverine and/or atropine were present during the estimation of concentration-tissue state curves they were added 10 min before beginning the cumulative additions of carbachol. For each experiment smooth curves were drawn by eye through the experimentally-determined points and equi-effective concentrations of agonist read from these curves were substituted into the appropriate null equations to obtain values of the various parameters.

Drugs used

Atropine sulphate (Sigma), carbachol chloride (B.D.H.) and papaverine hydrochloride (Sigma) were all commercial products. Hexyltrimethylammonium bromide (hexyl-TMA) was prepared in this department by Dr J. Wheeler.

Results

The following series of experiments were carried out.

Series 1

Concentration-tissue state curves were estimated for carbachol alone, for hexyl-TMA alone and finally for carbachol in the presence of 30 μM hexyl-TMA. The values of ψ_{AB} and I_{AB} were estimated from the appropriate curves using analytical methods (a) and (b). The results of these calculations are presented in Table 1. A typical set of curves obtained from one experiment of this series is shown in Figure 1.

Table 1 Direct and indirect estimates of ψ_{AB} and I_{AB}, obtained using various methods, for carbachol (A) and hexyltrimethylammonium (B) on rat isolated jejunum

Series	*Analysis*	ψ_{AB}	$I_{AB} \times 10^{-6}$ M	*Comments*
1	method (a)	117.8 ± 29.2 (11)	4.38 ± 0.44 (11)	Papaverine was absent during experiments in series 1 and 2
1	method (b)	110.1 ± 13.6 (11)	4.50 ± 0.50 (11)	
2	method (b)	144.7 ± 19.7 (9)	8.32 ± 1.74 (9)	
2	method (c)	60.6 ± 11.5 (9)	7.45 ± 3.06 (9)	
3	method (c)	138.9 ± 54.4 (6)	5.90 ± 1.63 (6)	Papaverine (5 μM) was present during experiments in series 3 and 4
4	method (b)	127.8 ± 22.7 (8)	10.5 ± 1.50 (8)	
4	method (c)	60.5 ± 10.5 (8)	0.62 ± 0.30 (8)	

Values of ψ_{AB} and I_{AB} are mean values ± s.e.mean with the number of results in parentheses.
The concentrations of hexyltrimethylammonium and of atropine used where appropriate in these experiments were 30 μM and 10 nM respectively.

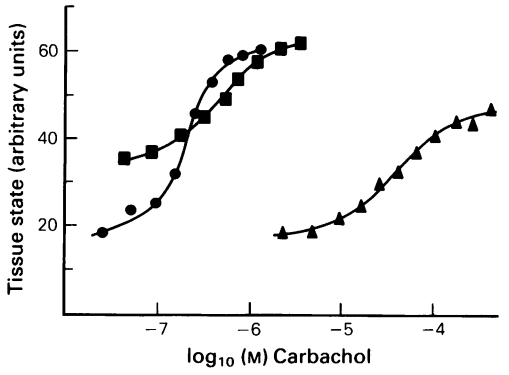

Figure 1 Log_{10} concentration-tissue state curves determined on rat isolated jejunum for carbachol alone (●), hexyltrimethylammonium alone (▲) and for carbachol together with a fixed concentration of hexyltrimethylammonium (30 μM) (■).

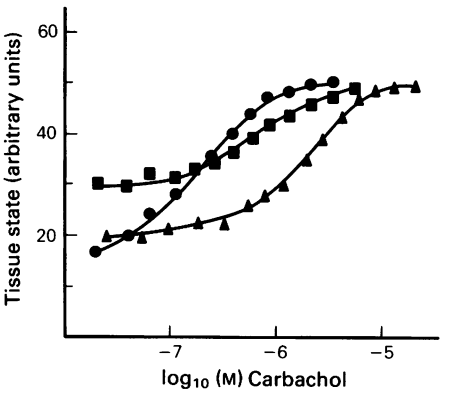

Figure 2 Log_{10} concentration-tissue state curves determined on rat isolated jejunum, for carbachol alone (●), in the presence of hexyltrimethylammonium (30 μM) ■) and in the presence of both hexyltrimethylammonium (30 μM) and atropine (10 nM) together (▲).

Series 2

Concentration-tissue state curves were estimated for carbachol alone, for carbachol in the presence of hexyl-TMA (30 μM) and finally for carbachol in the presence of both hexyl-TMA (30 μM) and of atropine (10 nM). The values of ψ_{AB} and I_{AB} were again estimated by applying the appropriate null equations (methods (b) and (c)) to equi-effective concentrations of carbachol read from the smooth curves. The values obtained are presented in Table 1 and curves obtained in a typical experiment illustrated in Figure 2.

Series 3

Concentration-tissue state curves were obtained for carbachol, for carbachol in the presence of papaverine (5 μM) and hexyl-TMA (30 μM), and finally for carbachol in the presence of papaverine (5 μM), hexyl-TMA (30 μM) and atropine (10 nM). Values for ψ_{AB} and I_{AB} obtained from these curves using method (c) are presented in Table 1.

Series 4

Concentration-tissue state curves were obtained for carbachol alone (curve 1), for carbachol in the presence of papaverine (5 μM) (curve x), for carbachol in the presence of papaverine (5 μM) and hexyl-TMA (30 μM) (curve 2) and finally for carbachol in the presence of papaverine (5 μM), hexyl-TMA (30 μM) and atropine (10 nM) (curve 3). A typical set of curves is shown in Figure 3. Values of ψ_{AB} and I_{AB} were calculated by applying methods (b) and (c) to curves x, 2 and 3. These estimates, obtained from curves measured in the presence of papaverine, are also shown in Table 1. Direct estimates of α_x, β_x and γ_x were obtained by comparing curve x with curve 1. However since carbachol concentrations equi-effective in the absence and presence of papaverine generally fitted equation 4a equally well with β_x set equal to zero as with nonzero values, this quantity has been set equal to zero routinely. This is in accord with the finding that papaverine (5 μM) lowers the carbachol curve and moves it to the right with no effect on the maximal response (see Figure 3). Comparison of curve 1 with curve 2 gave estimates of α_{21} and γ_{21} (β_{21} again being taken as zero) for the combined effect of functional antagonist and muscarinic agonist (method (d), equation 5a). Indirect estimates of α_x and γ_x were obtained

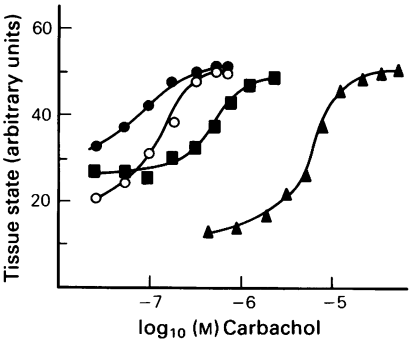

Figure 3 Log_{10} concentration-tissue state curves determined on rat isolated jejunum for carbachol alone (curve 1, ●), with papaverine (5 μM) (curve x, ○), with papaverine (5 μM) and hexyltrimethylammonium (30μM) (curve 2, ■) and finally with papaverine (5 μM), hexyltrimethylammonium (30 μM) and atropine (10 nM) (curve 3, ▲).

from α_{21} and γ_{21} using three alternative sets of mean values of ψ_{AB} and I_{AB} taken from Table 1. These values of α_x and γ_x estimated directly and indirectly are summarised in Table 2.

Discussion

Examination of the results presented in Table 1 shows that the values of ψ_{AB} and I_{AB} obtained by direct comparison of the individual curves obtained for carbachol and hexyl-TMA (method (a)) are not appreciably different from those obtained indirectly by comparing curves obtained for carbachol alone and for carbachol in the presence of a fixed concentration of hexyl-TMA (method (b)). This conclusion applies whether method (b) is used to analyse appropriate curves obtained in the absence of papaverine or to analyse such curves obtained in the presence of a constant background concentration of papaverine. On the other hand method (c), which requires the comparison of curves obtained to agonist A first in the presence of a fixed concentration of agonist B and then in the presence of this same concentration of B and a fixed concentration of a pure competitive antagonist I, produced a mean value of ψ_{AB} which in two cases (series 2 and 4) was appreciably lower but in another case (series 3) was very similar to those obtained by methods (a) and (b). However, the standard error of ψ_{AB} from series 3 was exceptionally high and there must be some suspicion that method (c) tends to give somewhat lower values of ψ_{AB} in these experiments. The values of I_{AB} obtained using method (c) were very similar for series 2 and 3 but were markedly lower for series 4. The duration of the individual experiments in series 4 was longer than in the other series since an extra concentration-tissue state curve had to be obtained. It is possible that the last curve in such experiments was being measured under conditions where the reproducibility of the curves was deteriorating. This conclusion tends to gain support from the fact that such low values of I_{AB} were not obtained when method (c) was applied to the shorter experiments of series 3 (with papaverine) nor in series 2 (without papaverine). Related evidence in favour of this interpretation will be discussed in the next section.

The results of experiments in series 4 have been used to test the ability of method (d) to separate the agonist-receptor properties of drug B from any functional interactant properties which it might have. For this purpose papaverine was used as a functional antagonist of carbachol, while the same concentration of papaverine together with a fixed concentration of hexyl-TMA was used to imitate the action of an agonist with functional antagonist properties. Comparison of curve 1 with curve 2 (see Figure 3) produced values of α_{21} and γ_{21} which contain both agonist-receptor and functional antagonist components. According to the analysis set out under method (d) these should be separable if dependable values of ψ_{AB} and I_{AB} are available. In the case of an agonist with additional functional interactant properties the only

Table 2 Comparison of direct and indirect estimates of α_x and γ_x obtained on rat isolated jejunum using different estimates of ψ_{AB} and I_{AB}

	Curve x cf curve 1, yielding direct estimates (series 4)		*Curve 2 cf curve 1, yielding indirect estimates, assuming values for ψ_{AB} and I_{AB} of* $\psi_{AB} = 60.5$, $I_{AB} = 0.62 \times 10^6\ M^{-1}$		$\psi_{AB} = 60.6$, $I_{AB} = 7.45 \times 10^6\ M^{-1}$		$\psi_{AB} = 127.8$, $I_{AB} = 10.5 \times 10^6\ M^{-1}$	
Experiment number	α_x	$\gamma_x \times 10^8\ M^{-1}$	α_x	$\gamma_x \times 10^8\ M^{-1}$	α_x	$\gamma_x \times 10^8\ M^{-1}$	α_x	$\gamma_x \times 10^8\ M^{-1}$
1	0.93	10.1	2.37	31.0	0.66	8.7	0.90	4.2
2	1.23	13.8	2.53	32.2	0.71	9.0	0.96	4.7
3	1.44	3.2	2.74	35.0	0.76	9.7	1.04	5.7
4	1.68	0.9	4.44	17.5	1.24	4.9	1.68	−0.9
5	1.19	7.9	5.00	45.8	1.40	12.8	1.89	9.8
6	1.32	8.3	3.46	44.5	0.96	12.4	1.31	9.3
7	1.75	5.1	4.35	33.4	1.21	9.3	1.64	5.1
8	1.60	2.7	6.40	26.7	1.78	7.4	2.42	2.5
Mean value ± s.e.mean	1.39 ± 0.10	6.50 ± 1.52	3.91 ± 0.49	33.3 ± 3.2	1.09 ± 0.14	9.28 ± 0.90	1.48 ± 0.19	5.05 ± 1.22

The curves used were for carbachol, Curve 1: alone; Curve x: with papaverine (5 μM); Curve 2: with papaverine (5 μM) and hexyltrimethylammonium bromide (30 μM).

method likely to be dependable is method (c). Table 2 shows values of α_x and γ_x calculated assuming the following sets of values for ψ_{AB} and I_{AB}:

Set 1 $\psi_{AB} = 60.5$, $I_{AB} = 0.62 \times 10^6 M^{-1}$ (method (c), series 4)

Set 2 $\psi_{AB} = 60.6$, $I_{AB} = 7.45 \times 10^6 M^{-1}$ (method (c), series 2)

Set 3 $\psi_{AB} = 127.8$, $I_{AB} = 10.5 \times 10^6 M^{-1}$ (method (b), series 4)

Comparing these values of α_x and γ_x estimated indirectly with the values obtained directly (Table 2) it will be seen that Set 1 produces poor agreement, Set 2 produces quite good agreement and Set 3 very good agreement. That this last set should produce such good agreement is not surprising since the mean values of ψ_{AB} and of I_{AB} used in this particular case were derived from the same set of curves in series 4. However, the fact that the values of ψ_{AB} and I_{AB} in Set 2 produce quite good agreement between direct and indirect estimates of α_x and γ_x shows that the main reason for the poor agreement obtained with Set 1 is the exceptionally low value for I_{AB}. This supports the idea that in the experiments of series 4 a discrepancy exists between the last curve and the earlier curves. Possible reasons for the abnormally low value obtained for I_{AB} in these experiments have already been discussed.

It is concluded that the equations presented here can be used to analyse concentration-tissue state curves to obtain estimates of the parameters ψ_{AB} and I_{AB} which can be used to characterize receptors. In particular, by comparing the concentration-tissue state curve obtained for pure agonist A in the presence of a fixed concentration of another agonist B with that obtained for A in the presence of that same fixed concentration of B together with a fixed concentration of a pure competitive antagonist I, it is possible to obtain estimates of these parameters even if drug B also has functional interactant properties.

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