Estimation of partial agonist affinity by interaction with a full agonist: a direct operational model-fitting approach

P. Leff, ¹I.G. Dougall & D. Harper

Department of Pharmacology, Fisons plc, Bakewell Road, Loughborough, Leicestershire, LE11 0RH

1 The operational model of agonism (Black & Leff, 1983) has been extended to describe the interaction between a partial agonist and a full agonist at the same receptor. The derived equation explicitly describes the interaction and allows the affinity (and efficacy) of the partial agonist to be estimated by direct fitting of raw experimental agonist concentration-effect ($E/[A]$) curve data.

2 The model was used to analyse experimental $E/[A]$ curve data generated for the interaction between pilocarpine (partial agonist) and carbachol (full agonist) at the M_3 -muscarinic receptor mediating contraction of the guinea-pig isolated trachea. Pilocarpine affinity estimates obtained by operational model-fitting were compared with those obtained by use of the null method (Stephenson, 1956). These analyses demonstrated that the two methods gave comparable results (mean pK_B estimates were 5.79 and 5.86 for the operational model and null method respectively).

3 When multiple concentrations of partial agonist are used, simultaneous operational model-fitting of all the $E/[A]$ curve data allows the competitive nature of the interaction to be studied.

4 We conclude that operational model-fitting is a valid and analytically simple alternative to the conventional null method of analysing full/partial agonist interactions.

Keywords: Guinea-pig trachea; muscarinic receptor; partial agonist; affinity; efficacy; operational model; null method

Introduction

The commonly used pharmacological approaches to estimating agonist affinities and efficacies are the receptor inactivation method (Furchgott, 1966) which allows quantification of either full or partial agonists and the comparative method (Barlow *et al.*, 1967) which allows quantification of partial agonists. A less frequently used means of estimating these drug-receptor parameters for a partial agonist is to study its interaction with a full agonist (Stephenson, 1956). This method has the advantage of allowing confirmation, in a single tissue, that the effects of the full and partial agonist are mediated by the same receptor. The comparative method makes this assumption but does not test it. Conventionally, estimation of affinity and efficacy by the interaction method (as well as by the other methods) utilises the null equation approach, as originally described by Stephenson (1956) and later advanced by Marano & Kaumann (1976). An alternative approach which has been successfully applied to the receptor inactivation and comparative methods, is to analyse data directly by operational model-fitting (Black *et al.*, 1985; Leff *et al.*, 1990). In this paper we show how this approach can be extended to the interaction method. Initially, we advance the operational model to describe interactions between a partial agonist and a full agonist at the same receptor. Then, we explain how the derived equation is applied in practice to estimate affinity and efficacy of the partial agonist. This analysis is illustrated with experimental $E/[A]$ curve data generated for the interaction between pilocarpine (partial agonist) and carbachol (full agonist) at the M_3 -muscarinic receptor mediating contraction of the guinea-pig isolated trachea. Finally, the results of operational model-fitting analysis are compared with those using the null method. A preliminary account of this work was presented to the British Pharmacological Society (Dougall *et al.*, 1993).

Methods

Guinea-pig isolated trachea

Male albino Dunkin-Hartley guinea-pigs (400–550 g) were killed by cervical dislocation and the whole trachea removed.

After removing the adherent connective tissue, the trachea was cut into five segments, each three cartilage bands wide and then suspended in 10 ml organ baths containing Krebs solution of the following composition (mM): NaCl 117.56, KCl 5.36, NaH_2PO_4 1.15, $MgSO_4$ 1.18, glucose 11.10, $NaHCO_3$ 25.00 and $CaCl_2$ 2.55. This was maintained at 37°C and continually gassed with 5% CO_2 in oxygen. Indomethacin (2.8 μM), hexamethonium (300 μM) and propranolol (1 μM) were added to the Krebs solution: indomethacin to prevent development of smooth muscle tone due to the synthesis of cyclo-oxygenase products, hexamethonium to block any nicotinic actions of the muscarinic agonists used and propranolol to prevent any possible interference of catecholamines released by stimulation of ganglionic M_1 -muscarinic receptors. The tracheal rings were suspended between two tungsten wire hooks, one attached to an Ormed Beam isometric force transducer and the other to a stationary support in the organ bath. Changes in isometric force were recorded on 3-channel Advance Bryans flat bed recorders.

Experimental protocols

General At the beginning of each experiment a force of 1.0 g was applied to the tissues. Subsequently each tissue was exposed to the irreversible antagonist phenoxybenzamine (Pbz) (1 μM) for a period of 10 min. This procedure was employed because preliminary experiments indicated that in untreated tissues pilocarpine produced a maximum response that was on average approximately 70% of that achieved with carbachol (data not shown); that is, the intrinsic activity of pilocarpine was 0.7. The decrease in functional muscarinic receptor reserve that resulted from Pbz treatment allowed well-defined carbachol $E/[A]$ curves in the presence of pilocarpine to be constructed. At the end of the incubation period the tissues were washed four times at 5 min intervals to remove the Pbz. The force of 1.0 g was reinstated at this time point and 30 min later tissues were exposed to 10 μM carbachol, to assess tissue viability. All subsequent contractile agonist concentration-effect, $E/[A]$, curves were constructed by cumulative additions of carbachol at 0.5 $\log_{10}$ unit increments. In all experiments a paired curve design was used with a period of 60 min elapsing between the first and

¹ Author for correspondence.

second curves. Responses were recorded as percentage maximum of the first curve.

Analysis of carbachol by the receptor inactivation method In each tissue a carbachol E/[A] curve was constructed. After washing, tissues were incubated with Pbz (10 μ M) or vehicle (ethanol) for 10 min. Following removal of the irreversible antagonist by four changes of the organ bath Krebs solution at 5 min intervals, a second carbachol E/[A] curve was obtained.

Pilocarpine affinity estimation by the interaction method In each tissue a carbachol E/[A] curve was constructed. Tissues were subsequently exposed to pilocarpine (10 μ M, 30 μ M or 100 μ M). When the responses had stabilized a second carbachol E/[A] curve was constructed on top of the response to pilocarpine.

Drugs

The following drugs were used: carbachol chloride (Sigma Chemical Company), pilocarpine hydrochloride (Sigma Chemical Company), phenoxybenzamine hydrochloride (Research Biochemicals Inc.), indomethacin (Sigma Chemical Company), hexamethonium bromide (Sigma Chemical Company) and ($\pm$)-propranolol hydrochloride (Sigma Chemical Company).

Indomethacin was dissolved in 10% w/v Na₂CO₃ and Pbz was dissolved in ethanol. Pbz solutions were made up just prior to use. All other drugs were dissolved in distilled water.

Data analysis

Logistic curve fitting In control experiments designed to determine any time-dependent changes in tissue sensitivity, and in the null analysis (see below), individual E/[A] curves were fitted to a logistic of the form:

$$E = \beta + \frac{\alpha[A]^p}{[A_{50}]^p + [A]^p} \quad (1)$$

where β is the basal effect level and α , $[A_{50}]$ and p are the asymptote, location and slope parameters respectively. In the present study, A refers to the full agonist carbachol and β is the effect level produced by the partial agonist pilocarpine.

Analyses of full/partial agonist interactions

Operational model-fitting By extending the operational model of agonism (Black & Leff, 1983; see equation (3) below) to describe the interaction between a partial agonist and a full agonist it can be shown (see Appendix) that the mathematical relationship between pharmacological effect (E) and the concentrations of a full agonist ([A]) and a partial agonist ([B]) is described by the following equation:

$$E = \frac{E_m([A]K_B + \tau_B[B][A_{50}])^n}{[A_{50}]^n (K_B + [B])^n + ([A]K_B + \tau_B[B][A_{50}])^n} \quad (2)$$

in which E_m is the maximum possible effect; n determines the steepness of the occupancy-effect relation; K_B is the dissociation constant of the partial agonist; τ_B is the efficacy of the partial agonist; $[A_{50}]$ is the midpoint location of the control full agonist E/[A] curve.

The analysis involves simultaneously fitting the E/[A] curve data for the full agonist obtained in the absence ($[B] = 0$) and presence of the partial agonist to equation 2. This allows the parameters of interest, namely K_B and τ_B to be estimated and also provides estimates of, E_m , n and $[A_{50}]$. In practice, K_B , τ_B and $[A_{50}]$ are estimated as logarithms. Figure 1 illustrates the fitting procedure for typical experimental E/[A] curve data obtained in a single tissue employing one concentration of partial agonist.

Figure 1 Operational model-fitting of full/partial agonist interaction data. Carbachol E/[A] curves were obtained in the absence (○) and presence of 30 μ M pilocarpine (●). The symbols represent the data from a single tissue. The lines drawn through the data are the results of operational model-fitting. For this particular tissue the estimated model parameters were as follows: $E_m = 101.84$; $n = 0.90$; $\tau_B = 1.17$ ($\log \tau_B = 0.07$); $pK_B = 5.49$; $p[A_{50}]$ (control) = 6.47.

Null method analyses The null method was carried out by fitting logistics to each pair of curves (control and in the presence of 10 μ M, 30 μ M or 100 μ M pilocarpine) using equation (1). Equi-effective carbachol concentrations were then interpolated at the 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 fractional levels of the second curve asymptote. Linear regressions of these interpolated values, [A] and [A'] (the equi-effective concentrations of carbachol in the absence and presence of pilocarpine) were performed and K_B was estimated as $[\text{pilocarpine}] \times \text{slope}/(1 - \text{slope})$.

Testing for simple competition The operational model-fitting procedure also allows data from tissues in which different concentrations of partial agonist are used to be fitted simultaneously, thus providing an estimate of a model parameter, m , which is analogous to the slope parameter of a Schild plot. That is, the model tests if the interaction between the full and partial agonist is consistent with simple competition. This fitting procedure involves raising the concentration of partial agonist to the power m , each time it appears in equation 2 (i.e. $[B]^m$ replaces $[B]$) and then simultaneously fitting the control curves and the curves in the presence of the different concentrations of partial agonist to this modified equation. This analysis was carried out for each of the six experiments.

Estimation of the efficacy of the reference full agonist In order to apply the above methods to the analysis of partial agonists it was necessary to ensure that the reference agonist was truly full under the experimental conditions employed. To estimate the efficacy (and affinity) of the full agonist carbachol, experimental E/[A] curve data were fitted using the operational model of agonism (Black & Leff, 1983; Black *et al.*, 1985):

$$E = \frac{E_m \tau^q [A]^n}{(K_A + [A])^n + \tau^q [A]^n} \quad (3)$$

in which E_m and n are as defined above and τ and K_A are the efficacy and dissociation constant respectively of the full agonist, A. This analysis allows τ values before and after Pbz treatment to be estimated as well as K_A , E_m and n .

Experimental design

A single experiment consisted of a carbachol control pair, a carbachol analysis by the inactivation method and three pilocarpine analyses by the interaction method. This pro-

cedure was repeated with tissues from six different animals. All the data obtained in these experiments were analysed as paired curves.

All data fitting procedures were carried out using the statistical package BMDP and a Vax 11/780 mainframe computer. Differences were assessed by Student's *t* test and considered significant at the level of $P < 0.05$.

Results

Carbachol efficacy estimation by the receptor inactivation method

E/[A] curves for carbachol were obtained before and after Pbz-treatment ($10 \mu\text{M}$ for 10 min). Control experiments showed that paired carbachol E/[A] curves were not significantly different in terms of slope (p). In contrast, small but significant differences in asymptote (α) and location ($p[A_{50}]$) occurred between first and second curves, the second curves being depressed and right-shifted (see Figure 3a). These time-dependent changes were not corrected for (see Discussion). The mean logistic parameters ($\pm$ s.e.; $n = 6$) of curves were as follows: 1st curve: $\alpha = 99.40 (\pm 0.35)$; $p = 1.15 (\pm 0.12)$; $p[A_{50}] = 6.63 (\pm 0.09)$. 2nd curve: $\alpha = 93.25 (\pm 0.82)$; $p = 1.21 (\pm 0.11)$; $p[A_{50}] = 6.40 (\pm 0.10)$.

Figure 2 illustrates typical results obtained in a single Pbz-treated tissue. The lines drawn through the data are the results of operational model-fitting. The substantial rightward shift and depression of the carbachol E/[A] curve produced by Pbz treatment indicated that the agonist has a high efficacy in this tissue. Analysis of 6 experiments gave an average estimate of efficacy (τ) of 64.6 ($\log \tau = 1.81 (\pm 0.05)$) and an estimate of affinity (pK_A) of 4.78 (± 0.09). The high efficacy confirmed that carbachol was indeed a full agonist in this system.

Analysis of full/partial agonist interactions

Operational model-fitting Figure 3 illustrates the results of a typical interaction experiment. The control data in panel (a) illustrates the small changes in location and asymptote of the second carbachol E/[A] curve. The lines drawn through the data in panels (b), (c) and (d) are the results of fitting the paired E/[A] curve data from each individual tissue to equation (2) and illustrate the goodness-of-fit of the data to the model. The affinity (pK_B) estimates obtained in all six experiments are given in Table 1. The average pK_B value was 5.79 ± 0.05 (mean $\pm$ s.e.; $n = 18$).

Figure 2 Effect of phenoxybenzamine (Pbz) treatment on carbachol E/[A] curves. Carbachol E/[A] curves were obtained before (○) and following 10 min exposure to $10 \mu\text{M}$ (●) Pbz. The symbols represent the data from a single tissue. The lines drawn through the data are the results of operational model-fitting. For this particular tissue the estimated model parameters were as follows: $E_m = 100.72$; $n = 1.07$; τ_1 (control) = 72.4 ($\log \tau_1 = 1.86$); τ_2 ($10 \mu\text{M}$ Pbz) = 0.76 ($\log \tau_2 = -0.12$); $pK_A = 4.73$.

Figure 3 Operational-model fitting analyses of pilocarpine by the interaction method. The data shown are from a single experiment (experiment 5). In all panels (○) represents the carbachol E/[A] curve data in the absence of pilocarpine. In (a) (●) represents the 2nd control carbachol curve. In (b), (c) and (d) (▲), (■), and (▼) illustrate the carbachol E/[A] curve data in the presence of $10 \mu\text{M}$, $30 \mu\text{M}$ and $100 \mu\text{M}$ pilocarpine respectively. The lines drawn through the data are the results of logistic fitting (a) and operational model-fitting (b, c and d). The model parameter estimates were as follows: panel (b) $E_m = 96.72$; $n = 1.30$; $\tau_B = 1.26$ ($\log \tau_B = 0.10$); $pK_B = 5.66$; $p[A_{50}]$ (control) = 6.51; panel (c) $E_m = 99.59$; $n = 1.14$; $\tau_B = 1.26$ ($\log \tau_B = 0.10$); $pK_B = 5.69$; $p[A_{50}]$ (control) = 6.51; panel (d) $E_m = 95.82$; $n = 1.47$; $\tau_B = 1.34$ ($\log \tau_B = 0.13$); $pK_B = 5.79$; $p[A_{50}]$ (control) = 6.50.

Table 1 Comparison of pilocarpine affinity (pK_B) estimates obtained by operational model-fitting and the null method

Pilocarpine concentration	Method	pK_B Estimate							Mean ($\pm$ s.e.)
10 μ M	Operational	5.55	5.70	5.96	5.75	5.66	5.55		5.70 $\pm$ 0.06
	Null	5.63	5.79	6.03	5.79	5.72	5.63		5.77 $\pm$ 0.06
	Difference	0.08	0.09	0.07	0.04	0.06	0.08		0.07 $\pm$ 0.01
30 μ M	Operational	5.48	5.91	5.79	5.92	5.69	5.49		5.71 $\pm$ 0.08
	Null	5.52	5.97	5.91	6.00	5.79	5.55		5.79 $\pm$ 0.09
	Difference	0.04	0.06	0.12	0.08	0.10	0.06		0.08 $\pm$ 0.01
100 μ M	Operational	5.91	6.06	5.99	6.17	5.79	5.86		5.96 $\pm$ 0.06
	Null	5.96	6.11	6.06	6.21	5.89	5.93		6.03 $\pm$ 0.05
	Difference	0.05	0.05	0.07	0.04	0.10	0.07		0.06 $\pm$ 0.01

All figures in a given column are from the same experiment; the first column refers to experiment 1, the last to experiment 6.

Figure 4 Plots of equi-effective concentrations of carbachol in the absence ($[A]$) and presence ($[A']$) of pilocarpine. The regression analyses were performed on the same data set as shown in Figure 3. Panels (a), (b) and (c) show plots for 10 μ M, 30 μ M and 100 μ M pilocarpine respectively. K_B was estimated as $[\text{pilocarpine}] \times \text{slope}/(1-\text{slope})$.

Table 2 Testing for competitive behaviour: results of simultaneously fitting experimental carbachol $E/[A]$ curve data obtained in the presence of different concentrations of pilocarpine to the operational model

Experiment No.	pK_B	Slope (m)
1	8.19	1.58
2	8.22	1.52
3	5.85	0.99
4	9.11	1.70
5	6.05	1.07
6	6.77	1.25
Mean ($\pm$ s.e.)	7.37 $\pm$ 0.54	1.35 $\pm$ 0.12

Figure 5 Correlation diagrams of pK_B estimates and pilocarpine concentration; (a) illustrates the correlation for pK_B estimates obtained by operational model fitting; (b) illustrates the correlation obtained for the null method. A significant correlation was seen in both cases.

Null method analyses The $E/[A]$ curve data shown in Figure 3 were used to produce the plots of $[A]$ against $[A']$ that are shown in Figure 4. The complete set of pK_B estimates obtained from such analyses are shown in Table 1 with the corresponding estimates obtained by operational model-fitting. The average pK_B value was 5.86 ± 0.05 (mean $\pm$ s.e.; $n = 18$). The affinities obtained by the two methods were significantly different, the null method giving a small but consistently higher estimate (mean difference = 0.07 log units; $n = 18$).

Testing for simple competition

The results of simultaneously fitting all the interaction $E/[A]$ curve data from each experiment to the modified version of equation 2 are shown in Table 2. The mean slope parameter (m) estimate was 1.35 ± 0.12 ($\pm$ s.e.; $n = 6$). This value was significantly greater than unity indicating that the interaction was not consistent with simple competitive behaviour. This lack of adherence to simple competition is also evident when the individual pK_B estimates (Table 1) obtained by both the operational model and null method are plotted as a function of the pilocarpine concentration (Figures 5a and b respectively). There is a significant correlation in both plots; for simple competitive behaviour the estimated pK_B values should be independent of the pilocarpine concentration. These plots also show that it is the data obtained with the highest concentration of pilocarpine ($100 \mu\text{M}$) that are responsible for the observed deviation from competitive behaviour; the pK_B estimates obtained using $10 \mu\text{M}$ and $30 \mu\text{M}$ are not significantly different (see Table 1).

Discussion

Previous publications (Black *et al.*, 1985; Leff *et al.*, 1990) have shown how the operational model of agonism is used to estimate agonist affinities and efficacies when the receptor inactivation method (Furchgott, 1966) and the comparative method (Barlow *et al.*, 1967) are employed.

The aim of this study was to extend the operational model to describe the interaction between a partial agonist and a full agonist. This method is important in pharmacology as the displacement of the control full agonist $E/[A]$ curve by the partial agonist confirms that the two agonists interact with the same receptor type. The interaction method thus confirms mechanism and establishes that the partial agonist has genuine low efficacy. In contrast, the comparative method, which is also used to analyse partial agonists makes the assumption that the full and partial agonist interact with the same receptor type but does not test it.

The derived equation (equation 2) was used to analyse experimental $E/[A]$ curve data generated for the interaction between pilocarpine (partial agonist) and carbachol (full agonist) at the M_3 -receptor mediating contraction of the guinea-pig isolated trachea. The goodness-of-fit of the model-fitted curves to the experimental data (see Figures 1 and 3) suggest that the operational model accounts quantitatively and qualitatively for the data presented here. The other criterion by which the model can be judged quantitatively is in its ability to determine accurately the partial agonist dissociation constant, K_B . To this end the data were analysed by the method of Stephenson (1956). This null approach, in principle should provide the most assumption-free pragmatic estimate of K_B . Comparison of the estimates obtained by the two methods showed small but significant differences, the null method producing a higher affinity estimate than the operational model (mean difference = 0.07 log units; see Table 1). It is likely that part of this difference can be explained by the different fitting procedures employed by the two methods since analysis of simulated data resulted in a higher affinity estimate by the null method (mean difference = 0.03 log units; $n = 6$, data not shown) than by the

operational model. Whatever the reason for the small differences observed, the similarity of the results obtained by the two methods leads us to conclude that the model can be used to provide reliable estimates of the dissociation constant of a partial agonist.

For the purposes of data analysis such a model-fitting approach has several advantages. In the null method a number of choices have to be made regarding data to be analysed and the means by which to analyse them. Equi-effective concentrations of full agonist in the absence and presence of the partial agonist are interpolated between curves, either drawn by eye or produced by logistic fitting. In neither case do the interpolated concentrations necessarily correspond to 'real' data points and differences in the choice of interpolations introduces differences in precision of K_B estimation. Having calculated equi-effective agonist concentrations by whatever means they are typically analysed by linear regression, which may be inappropriate as both sets of concentration data are estimated with error. The direct-fitting approach using the operational model not only eliminates the need for all these considerations but also uses the raw $E/[A]$ curve data without transformation. Furthermore, this direct treatment of the data means they can be displayed along with the model-fitted lines on the scale on which the responses are recorded (in contrast to a plot of equi-effective concentrations for the null method). This facilitates assessment of goodness-of-fit.

We analysed the experimental data by both a single curve (using the second curve obtained in each tissue) analysis (results not shown) and a paired curve (using both curves from each tissue) analysis. The paired curve analyses were preferred despite the small right shift and depression observed in the second carbachol control curves (see Results and Figure 3a). The reasons for this preference were as follows. Firstly, this is analytically the simplest way to treat the data since it allows a single estimate of K_B (and τ_B) to be made in each tissue. Individual estimates obtained in different tissues can then be averaged. Such analysis also encompasses the effect of τ_B on K_B estimation in different tissues. Secondly, in the null method a single curve design would necessitate using the same control $E/[A]$ curve to analyse the effects of different concentrations of partial agonist. This would have the effect of overweighting the control data; in the case of the operational model simultaneous fitting of the data allows each curve to be given similar weight in the analysis.

When multiple concentrations of partial agonist are used, as in this study, the fitting procedure can be extended to allow the competitive nature of the interaction to be studied. An equivalent test of competition for the null method has been described (Kaumann & Marano, 1982) but involves plotting the slope of the regression analyses of individual null plots as a function of partial agonist concentration; that is $\log(1/\text{slope}-1)$ is plotted against $\log [B]$. Use of the operational model simply involves fitting all the data simultaneously to a modified version of equation (2) in which the term $[B]$ is raised to the power m , the order of the reaction of the partial agonist with its receptors. The model parameter m is thus analogous to the slope parameter of a Schild plot.

When the data were analysed in this way the estimated slope parameter (m) was found to be significantly greater than unity (1.35) (see Table 2) indicating that the interaction was not consistent with simple competition. This deviation from competitive behaviour was also evident when the individual pK_B estimates were plotted as a function of pilocarpine concentration (see Figure 5). Furthermore, it was evident from these plots that it was the data obtained with the highest concentration of pilocarpine ($100 \mu\text{M}$) that caused the deviation from competitive behaviour. The reason for this is unclear at present but will be the subject of future investigations. In conclusion, the results of this study suggest that the operational model may be fitted directly to $E/[A]$ curve data to estimate partial agonist dissociation constants

and that this model-fitting approach is a valid and analytically simple alternative to the conventional null method.

Appendix: Derivation of equation (2)

A two-agonist-one receptor model

Following the nomenclature of Furchgott (1966) the system under consideration may be represented by the scheme:

The full agonist, A and the partial agonist, B occupy receptors R to form agonist-receptor complexes AR and BR respectively. The affinities of the agonists for the receptors are defined by K_A and K_B , the agonist dissociation constants. The agonist-receptor complexes impart stimuli, S_A and S_B to the system that are proportional to the agonists respective intrinsic efficacies (ϵ_A and ϵ_B). The measured pharmacological effect (E) is some function of the total stimulus ($S_{\text{tot}} = S_A + S_B$). The derivation of equation (2) therefore necessitates finding E as a function of S_{tot} .

From the above:

$$\begin{aligned}
 S_{\text{tot}} &= S_A + S_B \\
 &= \epsilon_A[AR] + \epsilon_B[BR]
 \end{aligned} \quad (i)$$

The equilibrium concentrations or receptors occupied by A and B in the presence of each other are given by the following equations:

$$[AR] = \frac{[R_0][A]}{[A] + K_A(1 + [B]/K_B)} \quad (ii) \quad [BR] = \frac{[R_0][B]}{[B] + K_B(1 + [A]/K_A)} \quad (iii)$$

where $[R_0]$ represents the total functional receptor concentration in the tissue.

References

- BARLOW, R.B., SCOTT, N.C. & STEPHENSON, R.P. (1967). The affinity and efficacy of onium salts on frog rectus abdominis. *Br. J. Pharmacol.*, **31**, 188–196.
- BLACK, J.W. & LEFF, P. (1983). Operational models of pharmacological agonism. *Proc. R. Soc. B*, **220**, 141–162.
- BLACK, J.W., LEFF, P., SHANKLEY, N.P. & WOOD, J. (1985). An operational model of agonism: the effect of $E/[A]$ curve shape on agonist dissociation constant estimation. *Br. J. Pharmacol.*, **84**, 561–571.
- DOUGALL, I.G., HARPER, D. & LEFF, P. (1993). A direct operational model-fitting approach to the estimation of partial agonist affinity by the interaction method. *Br. J. Pharmacol.*, (in press).
- FURCHGOTT, R.F. (1966). The use of β -haloalkylamines in the differentiation of dissociation constants of receptor-agonist complexes. *Adv. Drug Res.*, **3**, 21–55.

Substituting (ii) and (iii) into (i) gives:

$$S_{\text{tot}} = \frac{\epsilon_A[A][R_0]K_B + \epsilon_B[B][R_0]K_A}{[A]K_B + K_AK_B + K_A[B]} \quad (iv)$$

Let

$$E = \frac{E_m(S_{\text{tot}})^n}{1 + (S_{\text{tot}})^n} \quad (v)$$

thus E is a logistic function of (S_{tot}), n accounts for non-hyperbolic agonist concentration-effect curves and when $S_{\text{tot}} = 1$, $E = 0.5 E_m$.

Inserting equation (iv) into equation (v) gives:

$$E = \frac{E_m(\epsilon_A[A][R_0]K_B + \epsilon_B[B][R_0]K_A)^n}{([A]K_B + K_AK_B + K_A[B])^n + (\epsilon_A[A][R_0]K_B + \epsilon_B[B][R_0]K_A)^n} \quad (vi)$$

The terms $\epsilon_A[R_0]$ and $\epsilon_B[R_0]$ are equivalent to the operational model parameters τ_A and τ_B therefore equation (vi) can be rewritten as:

$$E = \frac{E_m(\tau_A[A]K_B + \tau_B[B]K_A)^n}{([A]K_B + K_AK_B + K_A[B])^n + (\tau_A[A]K_B + \tau_B[B]K_A)^n} \quad (vii)$$

For a full agonist $K_A \gg [A]$ therefore equation (vii) reduces to:

$$E = \frac{E_m(\tau_A[A]K_B + \tau_B[B]K_A)^n}{K_A^n(K_B + [B])^n + (\tau_A[A]K_B + \tau_B[B]K_A)^n} \quad (viii)$$

Dividing throughout by τ_A^n and defining K_A/τ_A as $[A_{50}]$ gives:

$$E = \frac{E_m([A]K_B + \tau_B[B][A_{50}])^n}{[A_{50}]^n(K_B + [B])^n + ([A]K_B + \tau_B[B][A_{50}])^n} \quad (ix)$$

This model describes the interaction of a partial agonist and a full agonist in terms of 5 parameters, E_m the maximum possible effect; n the slope index of the common transducer function linking agonist occupancy to effect; K_B the dissociation constant of the partial agonist; τ_B the efficacy of the partial agonist and $[A_{50}]$ the midpoint location of the control full agonist $E/[A]$ curve.

- KAUMANN, A.J. & MARANO, M. (1982). On equilibrium dissociation constants for complexes of drug-receptor subtypes. *Naunyn-Schmied. Arch. Pharmacol.*, **318**, 192–201.
- LEFF, P., PRENTICE, D.J., GILES, H., MARTIN, G.R. & WOOD, J. (1990). Estimation of agonist affinity and efficacy by direct, operational model-fitting. *J. Pharmacol. Methods*, **23**, 225–237.
- MARANO, M. & KAUMANN, A.J. (1976). On the statistics of drug-receptor constants for partial agonists. *J. Pharmacol. Exp. Ther.*, **198**, 518–525.
- STEPHENSON, R.P. (1956). A modification of receptor theory. *Br. J. Pharmacol. Chemother.*, **11**, 379–393.

(Received February 8, 1993)

Revised May 5, 1993

Accepted May 10, 1993)