CLASSIC ARTICLE

An operational model of pharmacological agonism: the effect of E/[A] curve shape on agonist dissociation constant estimation

JW Black, P Leff[1]* NP Shankley with an Appendix by J. Wood*

Rayne Institute, King's College Hospital, Denmark Hill, London and Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS*

1 An operational model of pharmacological agonism has been analysed to predict the behaviour of rectangular hyperbolic and non-hyperbolic agonist-concentration effect, E/[A], curves with variation in receptor concentration, [R$_o$].

2 Irreversible antagonism is predicted to cause E/[A] curve gradient changes in non-hyperbolic cases but not in hyperbolic cases; in both cases estimation of agonist dissociation constants (K_As) is theoretically valid.

3 5-Hydroxytryptamine (5-HT) produced 'steep' E/[A] curves in contracting the rabbit isolated aorta preparation. Irreversible antagonism by phenoxybenzamine (Pbz) produced a flattened E/[A] curve, consistent with theoretical predictions.

4 Fitting 5-HT E/[A] curves in the presence and absence of Pbz to the model provided an estimate of K_A for 5-HT which was not significantly different from the estimate obtained using Furchgott's null method.

5 The operational model of agonism appears to account qualitatively and quantitatively for the effects of [R$_o$] changes on hyperbolic and non-hyperbolic E/[A] curves. Under conditions where irreversible antagonism may be used to estimate K_As, fitting the operational model directly to E/[A] data represents a valid, economical and analytically simple alternative to the conventional null method.

Introduction

In a previous paper, Black & Leff (1983) undertook an analysis of pharmacological agonism, aiming to develop an operational model which explicitly described agonist-concentration effect, E/[A], curves and their behaviour under various experimental conditions. The approach to the problem began with the assumption that the initial event in the generation of a pharmacological effect by an agonist, hormone or drug, is a simple bimolecular interaction with a receptor obeying the Law of Mass Action. An attempt was made to define the 'transducer function' linking agonist occupied receptors, AR, to pharmacological effect, E, which would account for experimentally observed E/[A] curves. For rectangular hyperbolic E/[A] curves this transducer function is necessarily hyperbolic (Paton

& Rothschild, 1965; Black & Leff, 1983). For E/[A] curves which deviate from hyperbolicity an alternative transducer function is necessary and alogistic-type function was sufficient to account for such cases.

Here, the theoretical analysis is extended to predict the behaviour of non-hyperbolic E/[A] curves, particularly in circumstances where total receptor concentration, [R$_o$], is varied. [R$_o$] variation may occur physiologically between different tissues for the same agonist-receptor combination and [R$_o$] variation is introduced using irreversible antagonists to allow estimation of agonist dissociation constants (K_As) by the method of Furchgott (1966). In the latter circumstances particularly, theoretical expectation of E/[A] curve shape behaviour with variable [R$_o$] is relevant in order to establish conditions under which estimation of K_As are valid. In a recent paper (McPherson *et al.*, 1983) the absence of such a framework for expectation

[1]Author for correspondence

led to the assertion that 'to calculate accurate K_A values it is essential that the pre- and post-inactivation concentration-response curves [in the method of Furchgott (1966)] be rectangular hyperbolas', a claim which contravenes the principle of the null methods (for reviews see Colquhoun, 1973; Jenkinson, 1979).

In this paper we use the operational model of agonism (Black & Leff, 1983) to demonstrate that K_A estimation using irreversible receptor antagonism should be valid, in principle, regardless of the shape of the E/[A] curve so long as only one receptor-transducer system is involved and that the agonist-receptor interaction is a simple, non-cooperative, one. Both assumptions are implicit in Furchgott's method.

The validity of the operational model as a basis for analysis is assessed by comparing its theoretical predictions with experimental data obtained using 5-hydroxytryptamine (5-HT) as agonist and phenoxybenzamine (Pbz) as irreversible antagonist. In the rabbit isolated thoracic aorta preparation, 5-HT produces E/[A] curves which are 'steep' compared to rectangular hyperbolae. Therefore this system offered the opportunity to challenge the ability of the operational model to account quantitatively for nonhyperbolic E/[A] curves and, in addition, to allow the assertion of McPherson *et al.*, to be examined experimentally. In analysing the interaction between 5-HT and Pbz we compare the K_A value estimated using Furchgott's method with that obtained by fitting the operational model directly to E/[A] curve data.

Theory: The operational model of agonism

Hyperbolic El/A] curves

In deriving the operational model of agonism (Black & Leff, 1983) it was assumed that the initial event in the production of a pharmacological effect by an agonist, A, is a simple bimolecular interaction between the agonist and a receptor obeying the law of mass action. Therefore, at equilibrium, the concentration of occupied receptors, [AR], is given by

$$[AR] = \frac{[R_o][A]}{K_A + [A]} \tag{1}$$

in which $[R_o]$ is the total receptor concentration and K_A is the dissociation constant for AR. Equation (1) describes a rectangular hyperbolic, [AR]/[A], function. For E/[A] curves which are rectangular hyperbolic we proved that the relation between pharmacological effect, E, and [AR] must also be rectangular hyperbolic, that is

$$E = \frac{E_m[AR]}{K_E + [AR]} \tag{2}$$

in which K_E is the value of [AR] for half the maximum possible effect, E_m. This 'transducer function' could also be linear, which is the limiting case of the rectangular hyperbola with $K_E \gg [AR]$, but in order to account for receptor reserve the relation is necessarily saturable.

The resulting E/[A] relation, found by substitution of (1) into (2), is:

$$E = \frac{E_m \tau[A]}{K_A + (1 + \tau)[A]} \tag{3}$$

τ, which we called the 'transducer ratio', defines the operational efficacy, for an agonist. This can be appreciated by considering the location, $[A_{50}]$, and asymptote, α, parameters of the E/[A] function:

$$[A_{50}] = K_A/(1 + \tau) \tag{5}$$

$$\alpha = E_m \tau/(1 + \tau) \tag{6}$$

When τ is large, α is approximately E_m and $[A_{50}]$ is much less than K_A, meaning full agonism. When τ is small, α is less than E_m and $[A_{50}]$ is approximately equal to K_A, meaning partial agonism or, for very small τ, competitive antagonism.

Non-hyperbolic E/[A] curves

For non-rectangular hyperbolic E/[A] curves, if receptor occupancy is still assumed to be non-cooperative, and to obey equation (1), then an alternative transducer function to equation (2) is required. In our previous analysis we chose a transducer function of the logistic form

$$E = \frac{E_m[AR]^m}{K_E^n + [AR]^n} \tag{7}$$

Combination of equations (1) and (7) retains the definition and meaning of the transducer ratio, τ, and leads to an E/[A] function of the form

$$E = \frac{E_m \tau^n[A]^n}{(K_A + [A])^n + \tau^n[A]^n} \tag{8}$$

The location and asymptote parameters of this function are:

$$[A_{50}] = K_A\left/\left((2 + \tau^n)^{1/n} - 1\right)\right. \tag{9}$$

$$\alpha = E_m \tau^n/(1 + \tau^n) \tag{10}$$

While a flexible transducer function is required to account for non-hyperbolic E/[A] curves, the particular function used, equation (7), cannot be

regarded as necessary. In the present study, the sufficiency of this choice of function is all that can be examined.

Figure 1 illustrates the model in the three-dimensional display adopted previously (Black & Leff, 1983).

E/[A] curve gradients

The model predictions for EI[A] curve gradients are conveniently made by finding the derivative of equation (8) at the mid-point of the curve. Pharmacological E/[A] curves are usually displayed in semi-logarithmic (base 10) mode, so equation (8) must be differentiated with respect to lg[A], then [A$_{50}$], as defined by equation (9), is substituted for [A]. Also, in order to making meaningful comparisons of curves with different asymptotes, the gradient must be 'normalized' by dividing the derivative by $E_m \tau^n/(1 + \tau^n)$ (equation 10). The resulting definition of the mid-point gradient is

$$G = \frac{0.576n(2+\tau^n)\left((2+\tau^n)^{1/n}-1\right)}{(2+\tau^n)^{1/n}(1+\tau^n)} \qquad (11)$$

Note that when n = 1, corresponding to a rectangular hyperbolic E/[A] curve, the gradient G has the value 0.576 which is the mid-point gradient for normalized semi-logarithmic rectangular hyperbolae. This value is constant regardless of the value of τ.

When n is not equal to 1 equation (11) does not simplify and G is dependent on τ. When τ is very large, that is for substantial receptor reserves, $G \rightarrow 0.576n$ which is the expected value of a logistic function of the form,
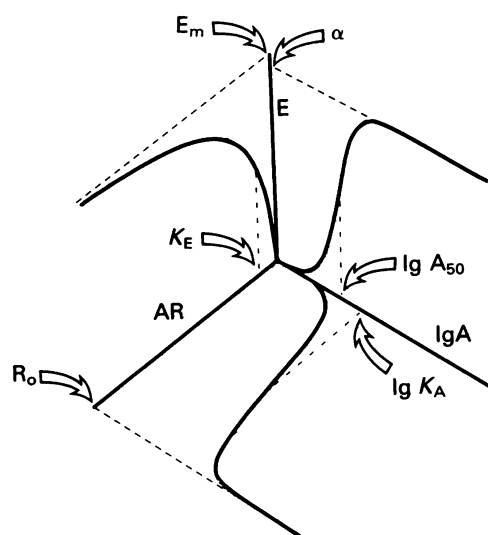

Figure 1 The operational model of pharmacological agonism: the model was simulated with n = 1 and [R$_o$]/K$_E$ = 10. So α = 0.91 E$_m$ and [A$_{50}$] =0.09 K_A.

$$E = \frac{\alpha[A]^n}{[A_{50}]^n + [A]^n} \qquad (12)$$

This reflects the fact that for large τ the transducer function (equation (7)) determines the form of the E/[A] relation. This is because occupancy, [AR], is proportional to [A] in the range that [AR] is saturating the E/[AR] function (Paton & Rothschild, 1965).

When τ is very small, $G \rightarrow 0.576n$ $(2(1 - 1/2^{1/n}))$. In cases when n > 1, G decreases with decreasing τ; in cases when n < 1, G increases with decreasing τ. The dependence of G on τ and n is illustrated in Figure 2 which shows computer simulated E/[A] curves and accompanying mid-point gradient values.

The important point from this analysis is that the gradients of the non-hyperbolic E/[A] curves are predicted by the model to vary with changes in the receptor reserve τ, whereas the gradient of hyperbolic E/[A] curves remain fixed and independent of τ. Therefore, in experiments employing irreversible receptor antagonism the variation in τ produced by [R$_o$] reduction is expected to be accompanied by E/[A] curve gradient changes if the initial, control, curve is non-hyperbolic.

K_A estimation by [R$_o$] reduction

Model equations (9) and (10) establish the predicted behaviour of the location and asymptote curve parameters with variations in τ. The relationship between [A$_{50}$] and K_A as τ is reduced is of particular relevance to the estimation of K_A by irreversible antagonism. Equation (9) predicts that as [R$_o$], and therefore τ, is reduced [A$_{50}$] approaches a limiting value of $K_A/(2^{1/n} - 1)$. Under this condition the asymptote α is reduced towards zero and A behaves as a progressively less efficacious partial agonist. When n = 1, the limiting value of [A$_{50}$] is K_A and therefore K_A can be estimated 'by eye' by extrapolating the trend in the [A$_{50}$] values. However, when n is not equal to 1 the extrapolated [A$_{50}$] value no longer corresponds to K_A. For example, when n = 2, the [A$_{50}$] approaches a limiting value of $K_A/0.41$ which therefore, is an underestimate of agonist affinity. This case, which implies positive cooperativity at the transducer level, was considered by Furchgott (1966). Conversely, when n is less than 1, the limiting value of [A$_{50}$] is an overestimate of agonist affinity. Figure 2 exemplifies these predictions.

Importantly, regardless of n, and therefore regardless of the gradients of the E/[A] curves, the method of Furchgott (1966) to estimate K_A can be shown to be valid. This is also shown in Figure 2, where double reciprocal plots of equi-effective agonist concentrations have been constructed from
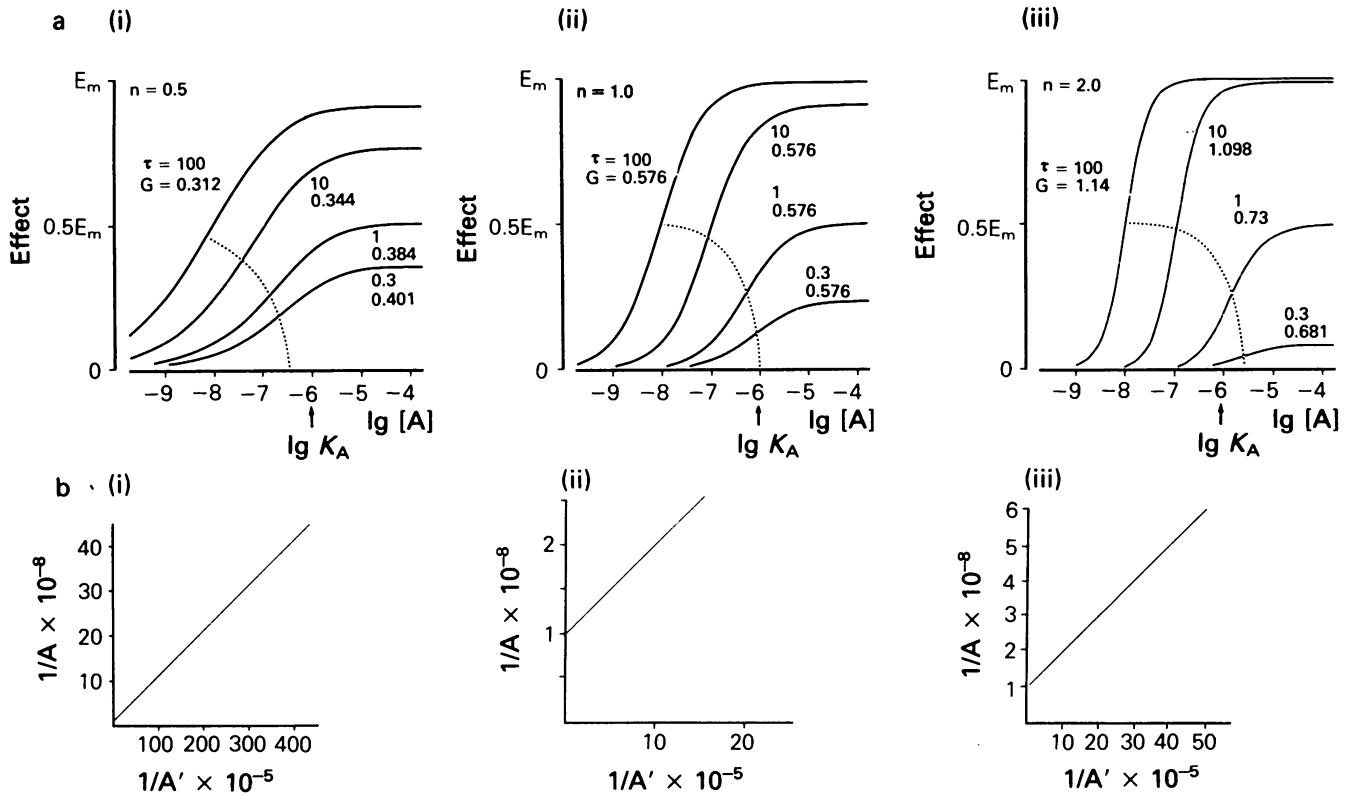
Figure 2 E/[A] curve simulations for varying n and t: the operational model was simulated using equation (8) with K_A fixed at 10^{-6}. In a(i), (ii) and (iii), n is set at 0.5, 1.0 and 2.0 respectively. In each panel t is varied as shown. Mid-point gradients, calculated from equation (11), are given by each curve together with the corresponding τ value. The dotted lines drawn through the curves indicate the trend in p[A$_{50}$]s as $\tau \rightarrow 0$. In panels b (i), (ii) and (iii), double reciprocal plots are constructed, according to the method of Furchgott (1966). Equi-effective agonist concentrations [A] and [A]' were interpolated from curves at $\tau = 100$ and $\tau = 1$ respectively. Interpolations were made in the effect range 0.3 to 0.999 of the maximum of the curve at $\tau = 1$. The three graphs are actually identical although the range of [A] and [A]' values differ due to the different relative gradients of the paired E/[A] curves in each case. The value of (slope − 1)/intercept was 10^{-6} in each instance corresponding to the true K_A for the simulated curves.

the simulated curves according to Furchgott's method. In each instance a control curve corresponding to $\tau = 100$ and a postantagonism curve corresponding to $\tau = 1$ were used. Straight lines are evident in all three cases corresponding to the same true K_A.

Methods

Rabbit thoracic aorta
Male NZW rabbits (2.0–2.5 kg) were killed by intravenous injection of pentobarbitone sodium (60 mg kg^{-1}). Isolated thoracic aortic ring segments were prepared according to the method of Stollak & Furchgott (1983) and suspended in 20 ml organ baths, thermostatically controlled at 37°C, containing modified Krebs solution, pH 7.4, of the following (mM) composition: NaCl 118.41, NaHCO$_3$ 25.00, KCl 4.75, KH$_2$PO$_4$ 1.19, MgSO$_4$ 1.19, glucose 11.10, CaCl$_2$ 2.50, aerated continuously with 95:5; O$_2$:CO$_2$.

Tissue responses were measured as increases in isometric force using Grass FT03C strain gauges and displayed on Bryans 2000 single-channel flat-bed, recorders.

Experimental protocols
The protocol adopted for these experiments is shown in Figure 3.

The second force application was made to reinstate a total load of 3 g. Tissues achieved steady tension before each drug intervention. All washes (indicated by W in the protocol) were by exchange of bath contents for fresh Krebs solution. Benextramine tetrahydrochloride (BHC) was included in order to avoid possible confounding of 5-HT responses due to direct or indirect α-adrenoreceptor stimulation by the agonist (Innes, 1962; Fozard & Mwaluko, 1976; Apperley et al., 1976; Marin et al., 1981). The sighting dose of 10 μM 5-HT was used only to establish tissue viability. Two cumulative 5-HT curves were constructed in each tissue accord-
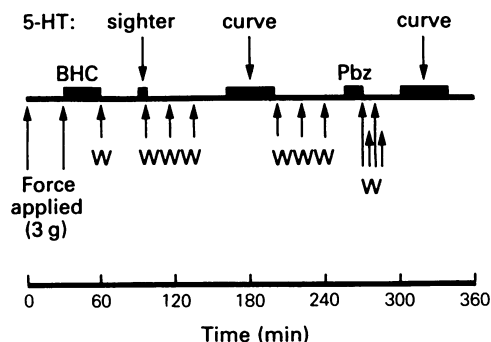
Figure 3 Experimental protocol. BHC = benextramine hydrochloride; Pbz = phenoxybenzamine.

ing to Van Rossum (1963) using $0.5\log_{10}M$ increments. 5-HT responses after Pbz treatment were measured as fractions of the maximum of the corresponding control curve.

Paired curve analysis

Each aorta provided six ring segments, three of which were used for Pbz treatment and the other three to provide replicate 5-HT curves. For both control and Pbz treatment groups a total of nine pairs of curves were obtained, the control pairs providing information on time-dependent differences between first and second curves.

Drugs and solutions

Drugs used were: pentobarbitone sodium (Sagatal, May and Baker), 5-HT creatinine sulphate complex (Sigma), benextramine tetrahydrochloride monohydrate (BHC) (Aldrich Chemical Company), phenoxybenzamine (Pbz) (Dibenyline, SK & F). 5-HT and BHC solutions were aqueous. Pbz was prepared in acidic solution which, at the concentrations used in the experiment, produced no alteration in the Krebs solution pH. 5-HT and Pbz solutions were freshly prepared and BHC solutions prepared from a frozen 1 mM stock. The maximum volume of drug solution administered to the organ bath was 300 μl.

Data analysis

All the following fitting procedures were unweighted iterative least squares minimization computer programmes. They were locally written with the exception of BMDP Module AR (BMDP Statistical Software, 1981) which was the programme used to fit data to the model of agonism.

Pragmatic curve-fitting

Individual sets of 5-HT E/[A] curve data were fitted to a logistic function of the form

$$E = \frac{a[A]^p}{b^p + [A]^p} \quad (13)$$

in which a, b and p are the asymptote, location ($[A_{50}]$) and slope parameters respectively. The location parameters were actually estimated as logarithms by making the subsitution $b = 10^{lgb}$.

Modelfitting

Pairs of control and Pbz-treatment 5-HT curves were fitted to model equation (8) providing direct estimates of E_m, n and K_A, and two estimates of τ for each curve in a pair. Goodness-of-fit was assessed by examining fitted and experimental data points for systematic deviations. A more comprehensive goodness-of-fit analysis will be the subject of a subsequent paper.

Estimation of K_A by Furchgott's method

Equi-effective 5-HT concentrations were interpolated from logistic curves fitted to each pair of control and Pbz treatment 5-HT curves. Five interpolations were made for each pair using the upper part of the Pbz treatment curve as recommended by Thron (1970). K_A values were estimated by fitting the interpolated values, [A] and [A]′ (the equi-effective concentrations of 5-HT before and after Pbz treatment respectively) to the equation

$$A = \frac{M[A]'}{K + [A]'} \quad (14)$$

in which $M = K_A(1 - y_1)/y_1$ and $K = K_A/y_1$ where y_1 is the fraction of receptors irreversibly occluded. K_A is estimated as $K - M$ (see Parker & Waud, 1971).

Results

5-Hydroxytryptamine E/[A] curves

5-HT produced sustained, concentration-dependent, contractions of the aortic rings. This was the case for both curves in control and Pbz-treatment pairs. Typical tracings are reproduced in Figure 4. Analysis of the paired curves was initially performed by logistic curve fitting using equation (13) and the estimated asymptote, location and slope parameters were compared by paired *t* test.

Analysis of paired control 5-HT curves indicated a significant fractional increase in asymptote between first and second curves of 0.094 ($P < 0.001$, $n = 9$). This factor was used to correct 5-HT responses after Pbz treatment. No significant slope parameter changes were observed ($P = 0.402$, $n = 9$) but a small but significant ($P = 0.044$, $n = 9$) left shift in the

location parameter was noted. This increment in $\log_{10}$ units was only 0.05 and was considered to be too small to be meaningful since it was of the same order as the fitting error on individual curve location parameters.

The results of logistic fitting of paired 5-HT and corrected Pbz-treatment curves are shown in Table 1.

Analysis showed that there was significant differences between their slope parameters ($P < 0.001$, $n = 9$). The control 5-HT curves were steeper than the Pbz treatment curves in all cases, although both groups were steeper than rectangular hyperbolae. Pbz treatment reduced asymptotes significantly ($P < 0.001$, $n = 9$) as expected and also produced significant right shift ($P < 0.001$, $n = 9$) of the location parameters.

Model-fitting of E/[A] curves

Each pair of control and corrected Pbz-treatment curves were fitted to the operational model of agonism (equation 8). A typical set of data with the fitted lines superimposed is shown in Figure 5.
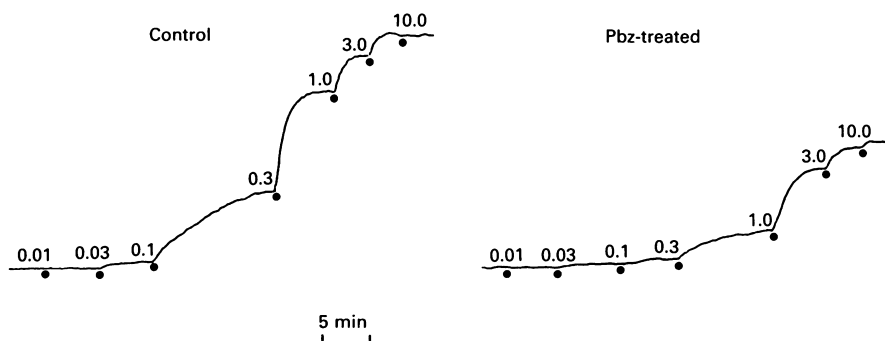
Figure 5 Model-fitting of E/[A] curve data: the diagram shows the results of model-fitting 5-hydroxytryptamine (5-HT) data for a typical control/phenoxybenzamine-treatment pair, using equation (8). Single values of E_m, K_A and n were estimated and two values of τ Correspondending to the pre- and post-inactivation curves respectively.

Figure 4 Experimental 5-hydroxytryptamine (5-HT) E/[A] curve tracings: a representative pair of control and phenoxybenzamine (Pbz)-treatment curves are shown. Applied concentrations of 5-HT are given in μM units.

Table 1 Logistic fitting of paired control and phenoxybenzamine (Pbz)-treatment 5-hydroxytryptamine E/[A] curves

Treatment	Asymptote								
Control	1	1	1	1	1	1	1	1	1
Pbz	0.24	0.18	0.24	0.49	0.36	0.46	0.15	0.14	0.11
Mid-point gradient									
Control	0.89	0.97	0.97	1.07	1.23	1.09	1.02	1.00	1.09
Pbz	0.77	0.86	0.85	0.98	0.95	0.98	0.85	0.88	0.87
$P[A_{50}]$									
Control	6.89	6.79	6.87	7.11	7.05	7.07	7.02	7.00	6.98
Pbz	6.43	6.44	6.45	6.56	6.50	6.47	6.52	6.53	6.42

Curves in each pair were fitted to equation (13) expressing responses as fractions of control curve maxima. The slope parameter, n, has been converted to the mid-point gradient by multiplying by 0.576 and location parameters are given as $p[A_{50}]$s
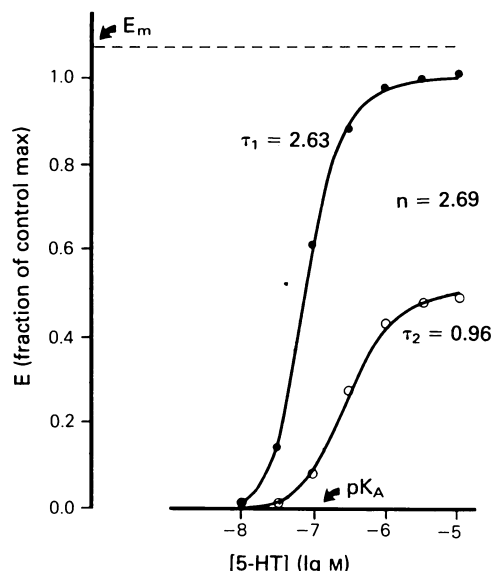
Comparisons of observed data points and corresponding fitted values revealed systematic deviations for the Pbz-treatment curves. However, even the largest residual difference between observed and fitted points represented only 5% of the amplitude of the corresponding curve and generally the residuals were much smaller. Figure 5 gives an indication of the extent of departure of model-fitted lines from the data points.

For each pair of curves estimates were made of E_m, n, K_A and two τ values, τ_1 and τ_2, corresponding to the control and Pbz-treatment curves respectively. The ratio between τ_2 and τ_1 for each pair reflects the reduction in $[R_o]$ due to Pbz-treatment. In addition to the slope parameter, n, estimated for each pair the mid-point gradients G_1 and G_2 of each curve in a pair were calculated from model equation (11). Table 2 summarizes the parameter estimates. Comparison of G_1 and G_2 illustrates the flattening effect of irreversible antagonism on the steep 5-HT E/[A] curves, seen previously by logistic curve fitting.

Estimated E_m values were invariably greater than the asymptote of the control 5-HT curve, the latter always being set at unity. This indicates that 5-HT was not behaving as a full agonist but was on average generating only 85% of the maximum possible response.

Comparison of the control τ values, τ_1, with the ratio τ_2/τ_1, implied that although the initial operational efficacy of 5-HT varied between tissues, presumably due to $[R_o]$ differences, the effect of Pbz in each tissue was similar, reducing $[R_o]$ and, therefore T, by almost the same fraction in each case. Agonist affinity estimates, expressed as pK_As, were evidently consistent across the nine preparations with an average value of 6.95.

Estimation of agonist affinity by Furchgott's method

Analysis of paired control Pbz treatment curves by the method of Furchgott, gave the results shown in Table 3. Agonist affinities as pK_As are given for each pair and also the estimated fraction of receptors remaining after Pbz treatment. The average pK_A value estimated by this method was 6.81.

Table 2 Operational model fitting of paired control and phenoxybenzamine (Pbz)-treatment 5-hydroxytryptamine E/[A] curves

E_m	1.23	1.38	1.25	1.07	1.08	1.05	1.20	1.21	1.10
n	2.87	3.35	3.01	2.69	4.09	2.84	3.32	3.12	2.87
G_1	0.94	0.91	0.94	1.06	1.22	1.12	1.00	0.98	1.07
G_2	0.73	0.74	0.74	0.77	0.79	0.77	0.74	0.73	0.72
τ_1	1.71	1.36	1.61	2.63	1.87	2.74	1.67	1.69	2.38
τ_2	0.60	0.58	0.63	0.96	0.85	0.93	0.57	0.53	0.49
τ_2/τ_1	0.35	0.43	0.39	0.37	0.45	0.34	0.34	0.31	0.21
pK_A	6.90	7.01	6.95	6.86	7.08	6.81	7.11	7.06	6.81

Curves in each pair were fitted to model equation (8). For each pair a value of E_m, n and K_A was computed and two values of t corresponding to the control and Pbz-treatment curve. Mid-point gradients were calculated according to model equation (11). Agonist affinities are given as the negative logarithm (base 10) of the K_A in each case. Curve parameters estimates are given in the same order, left to right, as for Table 1.

Table 3 Analysis of paired control and phenoxybenzamine (Pbz)-treatment 5-hydroxytryptamine (5-HT) E/[A] data by Furchgott's method

pK_A	6.68	6.76	6.92	6.80	6.92	6.74	6.84	6.85	6.79
$1 - y_1$	0.23	0.28	0.58	0.34	0.37	0.31	0.20	0.21	0.18

Paired data were analysed according to the method described in Analytical procedures. Interpolations between equieffective 5-HT concentrations on the control and Pbz-treatment curves were made at the 0.3, 0.6, 0.9, 0.99 and 0.999 fractional levels of the Pbz-treatment curve asymptote (Thron, 1970). Affinity estimates are given as the negative logarithm (base 10) of the K_A in each case and the fractional receptor concentration remaining after irreversible antagonism $(1 - y_1)$ was calculated using the definitions in equation (14). Results are given in the same order, left to right, as in Tables 1 and 2.

Comparison of parameter estimation by model-fitting and Furchgott's method

The average pK_A values estimated by operational model fitting and Furchgott's method were evidently similar, the difference being only 0.14 log_{10} units.

There appears to be no obvious way of comparing these two values statistically due to the different approaches used in their estimation and in particular the somewhat arbitrariness of certain steps involved in the Furchgott method of calculation. The similarity between the two estimates seems to make complex statistical analysis inappropriate.

The ratio τ_2/τ_1 provided by operational modelfitting is equivalent to $(1 - y_1)$ in Furchgott's method, both corresponding to the fractional concentration of receptors remaining after irreversible antagonism. The average value of τ_2/τ_1 was 0.35 compared with an average value of $1 - y_1$ of 0.30. Once again a similarity between the two methods of estimation is evident.

Discussion

In this paper we have extended our previous operational analysis of pharmacological agonism in order to make quantitative predictions about nonrectangular hyperbolic E/[A] curves, particularly under conditions where receptor concentration, $[R_o]$, is varied. In order to test the utility of the operational model in this respect, we have examined an agonist-receptor combination which clearly demonstrates 'non-hyperbolic' agonism.

Pragmatic, logistic, curve fitting of 5-HT E/[A] data in the rabbit aortic ring preparation showed that the curves were steeper than rectangular hyperbolae. It was also evident that, after irreversible antagonism, the curves became inherently flattened, an effect which is emphatically not merely due to depression of the asymptote of the curves. Logistic slope parameters and mid-point gradients have been calculated for selfnormalized E/[A] curves in order to avoid such confusion. This result is entirely consistent with model predictions when a 'steep' transducer E/[AR] function operates, and fitting the E/[A] curve data to the model showed that the model quantitatively accommodates this flattening effect.

By simple inspection of observed and model-fitted curves and examination of residuals for each of the nine replicate experiments we conclude that the operational model accounts quantitatively and qualititatively for the data presented here.

The other criterion by which the model can be judged quantitatively is in its ability to determine accurately the agonist dissociation constant, K_A, and also the fractional reduction in receptor concentration produced by irreversible antagonism. To this end the data were analysed by the method of Furchgott (1966) following the optimizing recommendations of Thron (1970) and Parker & Waud (1971). This null approach, in principle, should provide the most assumption-free, pragmatic, estimate of K_A and, in addition, fractional receptor reduction. Comparison of the estimates of these two quantities by Furchgott's method and by model-fitting, showed negligible differences. Therefore, on the basis of the present data, we conclude that the operational model, despite containing an explicit assumption regarding the transducer function (an assumption which the null method of Furchgott explicitly avoids), can be used to provide a reliable estimate of the dissociation constant by a direct model-fitting approach.

For the purposes of data analysis such a modelfitting approach has a number of attractions. In Furchgott's method a number of choices have to be made regarding data to be analysed and the means by which to analyse them. Equi-effective agonist concentrations in the presence and absence of irreversible antagonism 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_A estimation (Thron, 1970). The optimal way of analysing data by the Furchgott method appears to be that described by McPherson *et al.* (1983) in which both pre- and post-inactivation E/[A] curves are fitted by a logistic function. However, the validity of using the logistic function may be questioned as it can be proven (see Appendix) that there is no single definable transducer function which can translate a set of rectangular hyperbolic occupancy functions with varying $[R_o]$s into a corresponding set of logistic E/[A] relations. As the transducer function, is in principle, fixed for a particular system (assuming that the irreversible antagonist does not affect it), the corollary is that the resulting E/[A] function cannot be logistic. It is to be emphasized that the E/[A] curve as defined by the operational model (equation (8)) is not of the logistic form (see equation (12) for contrast). Having calculated equi-effective agonist concentrations, by whatever means, the choice must then be made regarding which graphical method to use to relate them in order to estimate K_A (Parker & Waud, 1971). At this stage questions arise as to whether regression is an appropriate means of analysis as both sets of concentration data are estimated with error (Thron, 1970).

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. Therefore, from the point of view of analytical simplicity, this approach appears to be favourable.

While the operational model accounts for the present data, its general acceptability depends on its application to a variety of agonist-receptor combinations. Essentially, the feature of the model under test is the transducer function, as it is conventional in the analysis of pharmacological agonism (Stephenson, 1956; Mackay, 1966; Furchgott, 1966; Barlow et al., 1967), to avoid explicit assumptions about the form of the E/[AR] relation. Several important points should be made about the nature of this relation. Firstly, for rectangular hyperbolic E/[A] curves a rectangular hyperbolic transducer function can be deduced (Black & Leff, 1983). Therefore, in these cases the operational model (with n = 1, see equation (4)) must, in principle, serve as a quantitative basis for analysis. When E/[A] curves are non-rectangular hyperbolic, if hyperbolic occupancy is still assumed, it can be inferred that the non-hyperbolicity must reside at the transducer level. While the precise choice of logistic function at this level is not necessary it can be concluded to be sufficient from the present study. We have also performed similar analyses of experimental data in the literature (not shown). Model-fitting of non-hyperbolic E/[A] data from Van Rossum (1966) and Besse & Furchgott (1976), involving irreversible antagonism at histamine (H_1) and α-adrenoceptors respectively, produced the same estimates of K_A as those obtained by the null method.

In the case of E/[A] curves, which have steeper midpoint gradients than rectangular hyperbolae, accepting that the steepness resides in the transducer function, means that a logistic transducer relation with n > 1 provides at least a qualitative general guide to expectations, even if our quantitative analyses were not to hold in general. Under these circumstances, irreversible antagonism is expected to produce a progressive reduction of E/[A] curve mid-point gradients, as shown in Figure 2. This result should hold generally and indeed was a feature of the data presented here.

McPherson et al. (1983), in their analysis of β-adrenoceptor irreversible antagonism by Ro 03-7894, assume that the pre- and post-inactivation curves should have the same gradient even although they are inherently steep. Having shown by computer simulation that Furchgott's method does not hold in these circumstances, they deduced that the irreversible antagonism method is not applicable for non-hyperbolic E/[A] curves. Our analysis implies that gradients for pre- and post-inactivation curves should only remain unchanged by $[R_o]$ variation in the case when the control, pre-inactivation curve, is rectangular hyperbolic. As shown in Figure 2, Furchgott's method is valid regardless of the gradients of the control E/[A] curve so long as the non-hyperbolicity of the curve resides at the transducer level, an assumption which is explicit in the operational model and implicit in Furchgott's method.

The preceding argument illustrates the utility of the explicit description of agonism that the operational model provides. In this instance the model provides the basis for predicting E/[A] curve gradient changes with varying $[R_o]$, thereby clarifying the conditions under which K_A estimation is valid. If, in a particular experimental system, the pre- and post-inactivation E/[A] curves were actually observed to be steep and of the same gradient, then the model predictions serve to alert the experimenter to an inconsistency which may reflect expression of other pharmacological properties in the agonist or in the irreversible antagonist. The estimation of K_A by Furchgott's method would indeed be invalid as McPherson et al. (1983) point out, but the result should be associated with a particular system rather than with a general principle that the method is invalid other than for hyperbolic curves. Indeed, since their initial study using Ro 03-7894, the same workers have questioned the classification of this compound as an irreversible, β-adrenoceptor antagonist (Krstew et al., 1984).

Another of the model predictions borne out in the present experimental analysis is that, for steep E/[A] curves, the location parameter $[A_{50}]$, approaches a concentration value which is higher than the K_A as $[R_o]$ is reduced; the extrapolated value of the $[A_{50}]$ underestimates agonist affinity. In fact, for the 5-HT E/[A] curves the average control $[A_{50}]$ (6.98) was very similar to the pK_A estimated (6.95) (see Table 1 and Figure 5). The curves obtained after Pbz treatment were located on average at 6.48. This result is important as it is intuitively tempting when inspecting 'partial agonist' E/[A] curves to approximate them to occupancy curves and, therefore, to approximate their location parameters to affinity constants. Rough K_A estimates by such 'eye-fitting' evidently can be substantially inaccurate.

In conclusion, the operational model, in addition to providing a general qualitative framework for the understanding of pharmacological agonism, appears to succeed quantitatively in accounting for the effects of irreversible antagonists on non-rectangular hyperbolic E/[A] curves. The results of our analysis suggest that the model may be fitted directly to E/[A] curve data to obtain agonist disso-

ciation constants and that this model-fitting approach is a valid, efficient and economical way to treat such data.

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Appendix

In this appendix it is shown that a set of rectangular hyperbolic occupancy [AR]/[A] curves, with the same location, K_A, but with different asymptotes, [R_o] cannot be transformed into an equivalent set of logistic E/[A] curves using a single, fixed, transducer function.

The occupancy relation is of the form

$$[AR] = \frac{[R_o][A]}{K_A + [A]} \tag{i}$$

A logistic E/[A] curve can be represented as follows:

$$E = \frac{a[A]^p}{b^p + [A]^p} \tag{ii}$$

[R_o], K_A, a, b and p are as defined in the text.

If (i) and (ii) hold, then the transducer function, E/[AR], can be found by rearranging (i) to define [A] in terms of [AR] and substituting into (ii). This gives

$$E = \frac{a[AR]^p}{K^p\left([R_0] - [AR]^p + [AR]^p\right)} \tag{iii}$$

in which $K = b/K_A$.

Equation (iii) describes the form of the transducer function. When p = 1, that is, for rectangular hyperbolic E/[A] curves, (iii) simplifies to

$$E = \frac{a[AR]}{K[R_o] + (1 - K)[AR]} \tag{iv}$$

In this case, a and K can be redefined in order to make the E/[AR] relation independent of [R_o]. Defining a = Em[R_o]/([R_o] + K_E) and $K = K_E/([R_o] + K_E)$ allows (iv) to be rewritten

$$E = \frac{E_m [AR]}{K_E + [AR]} \tag{v}$$

This is the familiar hyperbolic transducer function (see text), which is clearly independent of [R_o]. When p ≠ 1, the parameters in equation (iii) cannot be redefined in such a way as to make the transducer function independent of [R_o]. Consequently in this case, [R_o] necessarily determines the form of the transducer function; for different values of [R_o] a different transducer function pertains.

Therefore, if a set of logistic E/[A] curves is assumed, each individual member of the equivalent set of occupancy curves requires a different transducer function.

An alternative, more general, way of approaching the problem is to make use of the fundamental relation between equi-effective agonist concentrations which is the basis for Furchgott's null method (1966). This relation can be written as

$$\frac{1}{[A]} = \alpha + \frac{\beta}{[A]'} \tag{vi}$$

that is, a straight line in which [A] and [A]' are, respectively, the equi-effective agonist concentrations in the absence and presence of irreversible antagonist. The problem is to identify E/[A] functions which preserve their form when [R_o] is varied, that is, when equation (vi) applies. Considered in terms of reciprocal concentrations, we require a function f so that E = f (1/[A]) is of the same form as E = f (α + β/[A]'). Writing the logistic E/[A] relation (equation (ii)) in reciprocal form

$$\frac{1}{E} = c + \frac{d}{[A]^p} \tag{vii}$$

when 1/[A] is substituted by (α + β/[A]')

$$\frac{1}{E} = c + d\left(\alpha + \beta/[A]'\right)^p \tag{viii}$$

By inspection, equation (viii) is of a different form from equation (vii) when p ≠ 1; (viii) is no longer the reciprocal form of a logistic. When p = 1, that is, for rectangular hyperbolic E/[A] curves, both (vii) and (viii) are of the same form.

Therefore, it is proven that a set of logistic E/[A] curves cannot be generated as a result of [R_o] variation, assuming that the occupancy relation is rectangular hyperbolic and that the transducer is fixed and independent of [R_o].

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