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Pharmacological analysis of β-adrenoceptor-mediated agonism in the guinea-pig, isolated, right atrium

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1 The recently developed, operational model of pharmacological agonism defines the efficacy of agonists by $\tau = [R_o]/K_E$, where $[R_o]$ is the total functional concentration of receptors and K_E is the concentration of agonist-occupied receptors for half-maximal effect. Theoretically, variations in $[R_o]$ and K_E affect τ and in turn, E/[A] curve profiles similarly.

2 Using the β-adrenoceptor mediated chronotropic responses of the guinea-pig isolated right atrial preparation we have investigated the consequences of experimental $[R_o]$ and K_E variation.

3 Bromoacetylalprenolol menthane (M–75) produced displacements of isoprenaline and dichloroisoprenaline E/[A] curves consistent with $[R_o]$ reduction. Cholera toxin produced displacements consistent with decreases in K_E.

4. The operational model provides a simple conceptual framework for the prediction and interpretation of changes in E/[A] curve profile resulting from experimental interventions at the post-receptor (K_E) level as well as at the receptor ($[R_o]$) level.

Introduction

Recently, Black & Leff (1983) developed a simple, operational, model of pharmacological agonism. By assuming that the Law of Mass Action applied at the agonist-receptor binding stage, it was deduced that a saturable relationship was necessary between occupied receptors (AR) and pharmacological effect (E). The necessary inclusion of this saturable relationship not only accounted for phenomena such as partial agonism and receptor reserve but also led to a conceptually simple definition of the operational efficacy of an agonist in a system. In the model, operational efficacy is determined by the ratio between the total, functional, receptor concentration, $[R_o]$, and a parameter, K_E, which defines the value of [AR] for half-maximal effect. This ratio was defined as τ, for which the term 'transducer ratio' was introduced in order to emphasise its meaning in the model.

For any specified agonist-receptor interaction, variations in either $[R_o]$ or K_E should produce identical changes in concentration-effect, E/[A], curves. Therefore, the general applicability of the operational model of agonism would be seriously challenged if separate experimental variations in $[R_o]$ or K_E did not produce congruent effects. In this paper we have examined the experimental consequences of deliberately trying to vary $[R_o]$ and K_E independently.

Agonist effects were measured in guinea-pig, isolated, right atrial preparations. In these preparations, isoprenaline and its analogue, dichloroisoprenaline, behaved as full and partial agonists respectively. Bromoacetylalprenolol menthane (M–75) has been classified as an irreversible antagonist of the β-receptors subserving these agonist responses (Baker & Pitha, 1982; Pitha *et al.*, 1982). Therefore, controlled exposure of the preparations to this receptor reagent has been assumed to produce selective variations in $[R_o]$. Cholera toxin has been shown, in functionally-reduced biochemical systems, to enhance β-receptor mediated adenylate cyclase activity (Cassell & Selinger, 1977). In functionally intact physiological systems, cholera toxin would be expected to increase the efficiency with which receptor occupation is transduced into pharmacological effect. Therefore, we have assumed that exposure of the tissues to this 'transducer reagent' will have the effect of producing K_E changes in model terms.

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Methods

Guinea-pig isolated right atrial preparation

Chronotropic effects were studied in isolated, spontaneously-beating, right atria from male guinea-pigs (Dunkin-Hartley, 375–475g), which were suspended in 20 ml glass organ baths in Krebs-Henseleit buffer (composition, mM: Na^+ 143, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128, $H_2PO_4^-$ 2.2, HCO_3^- 24.9, SO_4^{2-} 1.2, dextrose 10) at 37°C (± 0.3°C) and gassed with 95% O_2, 5% CO_2 (for details see Angus & Black, 1980). Isometric transducer outputs were processed by a ratemeter (ADG Instruments) which gave a direct readout of rate (beats min^{-1}) continuously displayed on a potentiometric recorder (Bryans 28000). Tissues were subjected to 0.5g resting tension and washed at approximately 15 min intervals during an initial 60 min stabilization period. Krebs-Henseleit solution was routinely prepared containing 10^{-4}M ascorbate to reduce the oxidation of catecholamines. This concentration produced no intrinsic chronotropic effect.

Experimental protocols

Preliminary experiments indicated that cholera toxin (0.3–5.0 $\mu g\,ml^{-1}$) produced a concentration-dependent tachycardia. The response to all concentrations attained a plateau after approximately 160 min. Accordingly, cholera toxin was incubated for 180 min. Additions of the irreversible β-receptor antagonist, bromoacetylalprenolol menthane (M–75), were made 60 min before the end of this incubation period at which time the preparations were washed twice. Two further washes were made at 10 min intervals and full E/[A] curves, using the β-receptor agonists isoprenaline and dichloroisoprenaline, were obtained in a cumulative manner (Van Rossum & Van den Brink, 1963) at increments of 0.5 log units. Each preparation was used only once. The final volume of drug solutions added to the bath was not greater than 500 μl, i.e. 2.5% of the bath volume.

Six preparations were used simultaneously and drug treatments were randomized across organ baths and days.

Statistical methods

Agonist responses were measured from the resting rate immediately before the E/[A] curve whether or not cholera toxin was present in the pre-incubation period. The responses were expressed as a percentage of the maximum change observed with isoprenaline.

Logistic curves were fitted to individual E/[A] curves by means of an iterative, least squares, programme which computed location ($\log[A_{50}]$), upper asymptote (α) and midpoint slope (n) parameters. Asymptote and slopes were tested by one-way analysis of variance comparing computed parameter values between and within-drug treatment groups.

For display purposes the individual computed parameters estimated for each treatment group were expressed as means and a single logistic curve generated using these values.

Theory

The operational model for pharmacological agonism

The operational analysis of agonism (Black & Leff, 1983) began with the assumption that the initial event in the interaction of an agonist with a tissue is chemical binding to a receptor obeying the Law of Mass Action, so that,

$$[AR] = \frac{[R_o][A]}{K_A + [A]} \qquad \text{(i)}$$

where [A] represents the concentration of free agonist, $[R_o]$ the total concentration of receptors and K_A the dissociation constant for the agonist-receptor complex, AR.

For rectangular hyperbolic E/[A] curves it could be deduced that the relation between E and [AR] is necessarily linear or rectangular hyperbolic. In order to account for the phenomena of a receptor reserve and inter- and intra-tissue differences in agonist expression, the hyperbolic choice is necessary, that is a relationship of the form

$$E = \frac{E_m[AR]}{K_E + [AR]} \qquad \text{(ii)}$$

where E_m is the maximum effect that can be generated in the system and K_E is the value of [AR] for half E_m. The E/[A] relationship is found by substituting equation (i) into equation (ii) giving

$$E = \frac{E_m[R_o][A]}{K_A K_E + (K_E + [R_o])[A]} \qquad \text{(iii)}$$

which appears to be a necessary and sufficient description of hyperbolic E/[A] curves.

Dividing equation (iii) throughout by K_E leads to

$$E = \frac{E_m([R_o]/K_E)[A]}{K_A + (1 + [R_o]/K_E)[A]} \qquad \text{(iv)}$$

which shows that hyperbolic E/[A] curves are completely characterized by three parameters, K_A, E_m and the ratio $[R_o]/K_E$ which we define as τ, the transducer ratio. Making this definition in equation (iii) leads to

$$\frac{E}{E_m} = \frac{\tau[A]}{K_A + (1 + \tau)[A]} \qquad \text{(v)}$$

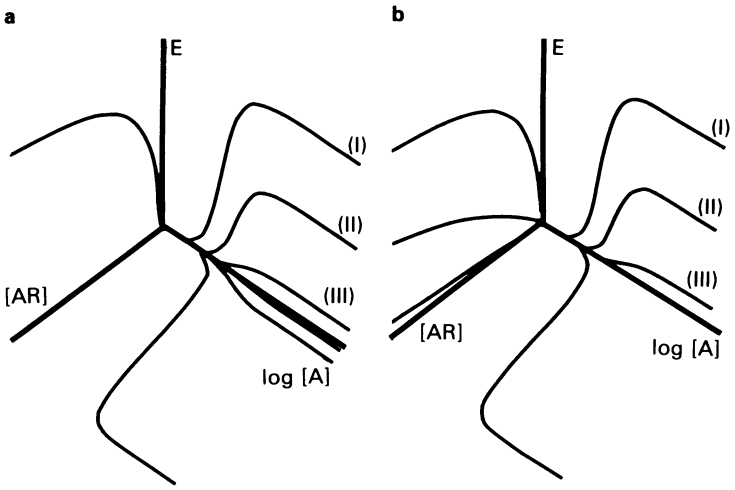

Figure 1 Three dimensional display of the operational model of agonism. The diagram illustrates simulations of the model using equation (iii) (see Methods) for a fixed K_A, with varying $[R_o]$ at fixed K_E (a), and varying K_E at fixed $[R_o]$ (b). In both cases the three associated values of τ (= $[R_o]/K_E$) were 10, 1 and 0.1 giving, therefore, the same family of E/[A] curves (see equation (v) Methods). In (a): curve (I) $[R_o] = 10$, (II) $[R_o] = 1$, (III) $[R_o] = 0.1$ and $K_E = 1$ for all curves. In (b): curve (I) $K_E = 1$, (II) $K_E = 10$, (III) $K_E = 100$ and $[R_o] = 10$ for all curves.

Large values of τ are associated with full agonism; low values of τ are associated with partial agonism or, in the extreme, competitive antagonism. This can be appreciated by considering the following model definitions of the location ($[A_{50}]$) and asymptote (α) parameters of the E/[A] curve:

$$\alpha = \frac{E_m \tau}{1 + \tau}$$

$$[A_{50}] = \frac{K_A}{1 + \tau}$$

For example, when $\tau = 99$, α is $0.99\,E_m$ and $[A_{50}]$ is $0.01\,K_A$; the agonist acts as a full agonist whose potency is much higher than its affinity. When τ is in the region of unity α is only a fraction of E_m and $[A_{50}]$ approaches K_A.

Figure 1 illustrates the model and, in particular, shows how changes in either $[R_o]$ or K_E, with the other fixed, affects the location and asymptote of the E/[A] curves. The same family of curves can be generated in each case because the same τ value can be produced by different absolute values of $[R_o]$ and K_E.

Similar arguments can be developed for non-rectangular hyperbolic E/[A] curves (Black & Leff, 1983) but these will not be given here because, as will be shown, the experimental curves in the present study are indistinguishable from rectangular hyperbolae.

Compounds

Isoprenaline hydrochloride (Sigma), dichloroisoprenaline hydrochloride (Aldrich), cholera enterotoxin (Wellcome Research Laboratories). Bromoacetylalprenololmenthane (M−75) was a generous gift from Dr J. Pitha of the Gerontology Research Center, Baltimore City Hospitals, Baltimore, Maryland 21224, USA.

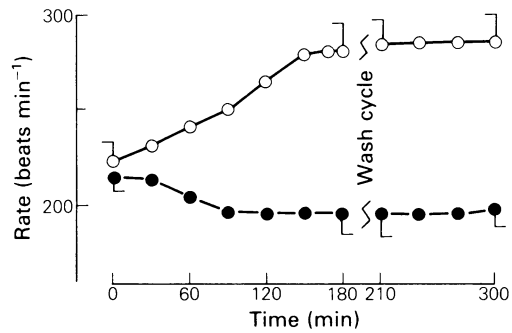

Figure 2 The effect of cholera toxin on basal rate of beating of the guinea-pig atria. The atria were incubated in cholera toxin (1 μg ml^{-1}) for 3 h followed by a 30 min wash cycle and a further 90 min incubation, as detailed in the text. The data from each of 5 replicate experiments in the absence (●) and presence (○) of cholera toxin are expressed as mean beats min^{-1} with vertical lines showing s.e.

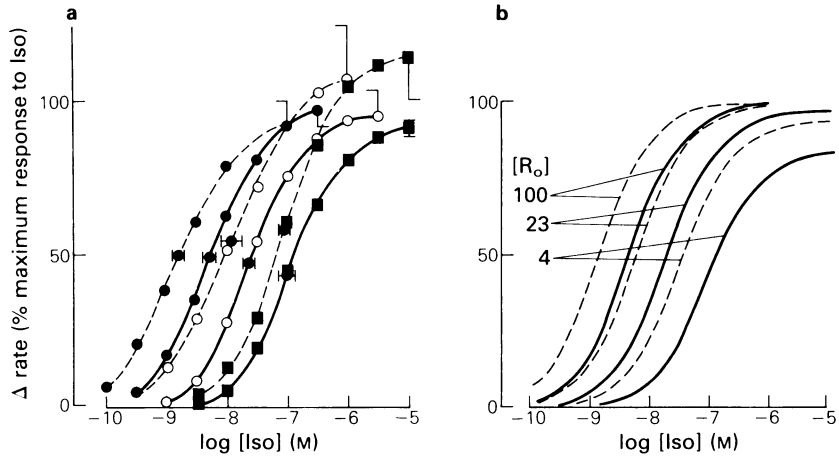

Figure 3 Effect of cholera toxin pre-incubation and β-receptor irreversible antagonism on isoprenaline E/[A] curves. (a) Experimental data. Average, computer-fitted, emilogarithmic E/[A] curves for isoprenaline following incubation with 0 (●), 10^{-6} M (○) and 10^{-5} M (■) M−75 in the absence (—) and presence (....) of cholera toxin 1 μg ml^{-1}. Each line represents the average of 5 or 6 replicate curves expressed as a percentage of the mean isoprenaline control curve maximum increase in rate. Standard errors are shown (vertical lines) for the upper asymptote and log [A_{50}] parameter estimates of each curve. (b) Simulation. The experimental data illustrated (a) were simulated using equation (iii) (see Methods). (—) $K_E = 0.82$, (....) $K_E = 0.27$ and $K_A = 5 \times 10^{-7}$ M.

Results

The intrinsic effect of cholera toxin

Cholera toxin (1 μg ml^{-1}) produced a significant increase ($P < 0.05$) in atrial rate reaching a plateau after approximately 160 min (Figure 2). The elevated rate did not alter significantly after washing the preparations for 30 min, and was observed to remain unchanged for a further 90 min. Therefore, a steady-state response with cholera toxin, which could be maintained for the time required to obtain a cumulative E/[A] curve, has been assumed.

The effect of pre-incubation with cholera toxin on isoprenaline and β-receptor irreversible antagonism by M−75

Figure 3a illustrates average, computer-fitted, semilogarithmic E/[A] curves for isoprenaline following incubation with increasing concentrations of M−75 (0, 10^{-6} M and 10^{-5} M) in the absence and presence of cholera toxin (1 μg ml^{-1}).

Both with and without cholera toxin pretreatment, M−75 produced a significant concentration-dependent parallel displacement of the isoprenaline E/[A] curves over approximately 1.5 log units. Pre-incubation with cholera toxin gave a leftward displacement of the curves at each concentration of M−75. The significant change in log [A_{50}] values, without alterations in upper asymptotes, was consistent with theoretical expectations for a full agonist when τ, but not E_m, is altered.

The effect of pre-incubation with cholera toxin on dichloroisoprenaline and β-receptor irreversible antagonism by M−75

Figure 4a illustrates average, computer-fitted, semilogarithmic dichloroisoprenaline E/[A] curves following incubation with increasing concentrations of M−75 (3×10^{-7} M and 10^{-6} M) in the absence and presence of cholera toxin (1 μg ml^{-1}). With the exception of the 10^{-6} M M−75 treatment group in the absence of cholera toxin, best-fit logistic curves were obtained for each replicate experimental curve and mean estimates of the log[A_{50}], midpoint slope and upper asymptote could be calculated. Due to the small responses, and the absence of response in one out of five preparations, it proved impossible to fit the data obtained after pretreatment with 10^{-6} M M−75. The upper asymptote value of this treatment group was therefore obtained by expressing the individual maximal responses as a mean.

Pre-incubation with M−75 gave a significant progressive decrease in the upper asymptotes of both the control and cholera toxin pretreated dichloroisoprenaline curves. Midpoint slopes were unaffected and the curves were progressively subject to a dextral, shift over approximately 0.5 log units. However, as occurred with isoprenaline curves, cholera toxin gave a leftward displacement of the

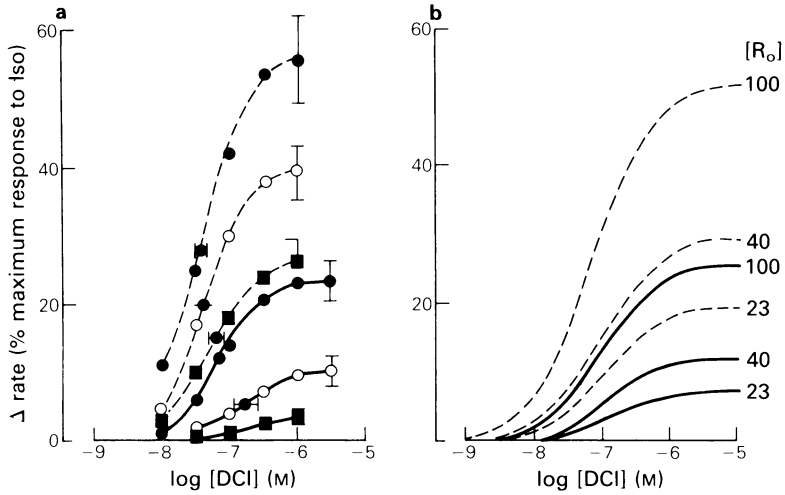

Figure 4 Effect of cholera toxin pre-incubation on β-receptor irreversible antagonism of the dose-response curves to dichloroisoprenaline (DCI). (a) Experimental data. Average, computer-fitted, semilogarithmic E/[A] curves for DCI following incubation with O (●), 3×10^{-7} M (○) and 10^{-6} M (■) M−75 in the absence (—) and presence (....) of cholera toxin 1 μg ml^{-1}. Each line represents the average of 5 or 6 replicate curves expressed as a percentage of the mean isoprenaline (Iso) control curve maximum increase in rate. Standard errors are shown (vertical lines) for the upper asymptote and log [A_{50}] parameter estimates for each curve. (b) Simulation. The experimental data illustrated in (a) were simulated using equation (iii) (see Methods). (—) $K_E = 280$, (....) $K_E = 93$ and $K_A = 1.35 \times 10^{-7}$ M.

curves from the control location at each concentration of M−75.

Cholera toxin pretreatment significantly increased the intrinsic activity, α in the model, of dichloroisoprenaline in this tissue. The upper asymptotes measured in the presence of 0, 3×10^{-7} M and 10^{-6} M M−75 were all significantly greater ($P < 0.001$) than the corresponding asymptotes in the absence of cholera toxin.

Computer simulations of the agonism model

Use of the model required that E/[A] curves were rectangular hyperbolic. In fact the majority of experimental curves were insignificantly different from rectangular hyperbolae as judged by their computer estimated midpoint slopes (0.576 for a normalized rectangular hyperbola in semilogarithmic, base 10, space).

In order to generate theoretical curves simulating the effects of M−75 and cholera toxin on the responses to isoprenaline and dichloroisoprenaline, [R_o] was assigned an arbitrary starting value of 100. K_A values were fixed for each agonist as shown in Figures 3 and 4 and initial K_E values were selected which accounted for the control isoprenaline and dichlorisoprenaline E/[A] curves: 0.82 and 280, respectively. Therefore, for isoprenaline the control operational efficacy, that is [R_o]/K_E (= τ) was 122 and for dichloroisoprenaline τ was 0.36.

The effects of M−75 were simulated by reducing [R_o] from its initial value with K_E fixed for each agonist, and the effects of cholera toxin were simulated by decreasing the initial K_E for each agonist with [R_o] fixed.

Figures 3b and 4b show model simulations of [R_o] reduction for isoprenaline and dichloroisoprenaline curves, respectively. With K_E set at control values or at reduced values to account for the effect of cholera toxin the same range of [R_o] values accounted for the essentially parallel rightward shift of the isoprenaline curves and depression of the dichloroisoprenaline curves under the influence of M−75 in the absence and presence of cholera toxin. For this choice of parameter values, the model predicted depression of the isoprenaline curve at 10^{-5} M M−75. Such a small change would not be detectable in the data due to experimental variation.

The effect of cholera toxin itself on each agonist E/[A] curve could be simulated by decreasing the 'control' K_E value in each case by a factor of 3, as shown by Figures 3 and 4 for isoprenaline and dichloroisoprenaline, respectively. In general, this factor accounted for the isoprenaline and dichloroisoprenaline data shown in Figures 3 and 4, although, in particular cases, ideal fits to the data were not obtained. However, even

in these cases, the curve parameters for the simulated curves fell into the 95% confidence bands of their observed counterparts.

Discussion

In the present study we have demonstrated that pretreatment with cholera toxin significantly increases the potency ($\log[A_{50}]$) of a full agonist and the intrinsic activity (α) as well as the potency of a partial agonist. These effects were reversed by irreversible β-adrenoceptor blockade in a concentration-dependent manner. Cholera toxin appears, therefore, to be capable of increasing the operational efficacy of agonists, a result which is consistent with, although not proof of, a decrease of K_E in the theoretical model (Black & Leff, 1983). In principle, cholera toxin could be causing an increase in $[R_o]$, since, from the definition $\tau = [R_o]/K_E$, $[R_o]$ increases would be indistinguishable from K_E decreases.

However, cholera toxin (10^{-8} M incubation for 3 h) has been shown not to affect the specific binding of [^{3}H]-alprenolol, in terms of maximal binding as well as affinity, to turkey erythrocyte ghosts (Rudolph *et al.*, 1977). Therefore, the only alternative, according to the model definition of efficacy, is that K_E is being reduced by cholera toxin treatment. This can be rationalized by considering the reported mechanism of action of cholera toxin on β-receptor linked adenylate cyclase.

β-Adrenoceptor agonists activate adenylate cyclase to produce cyclic AMP by a mechanism believed to involve the following steps (Cassel & Selinger, 1977): agonist-receptor (AR) formation; AR interaction with a regulatory nucleotide-binding component (N) to produce the ternary complex ARN; and then activation by ARN of the catalytic unit (C), a process requiring CTPase hydrolysis of GTP. The rate of production of cyclic AMP depends, therefore, on the steady-state concentration of active ARNC complex. Inhibition of the GTPase termination step results in a larger steady-state concentration of ARNC and therefore a higher cyclic AMP production rate. This sequence of events is represented in essence by a receptor-transducer form of the general model of agonism (see Black & Leff, 1983). Cholera toxin has been demonstrated to activate adenylate cyclase by catalyzing the adenosine diphosphate ribosylation of the GTP-binding regulatory component of the enzyme complex (Cassel & Pfeuffer, 1978). The consequences of this covalent modification include inhibition of GTPase activity, prolonged life time of the ARNC complex and, ultimately, more effective generation of biological response per concentration unit of AR. In terms of the agonism model therefore a decrease in K_E, that is, an increase in the efficiency of transduction of AR into E can be expected. In general, the experimental interactions made in this study affected the isoprenaline and dichloroisoprenaline E/[A] curves in a manner consistent with τ changes in the model. Although, in particular cases, fitted lines deviated from average data points (see Figure 4a and b) they still fell within the confidence bands of the data. Therefore, the model was justifiably used without modification as a basis for interpreting the data.

The dependence of the transducer ratio, τ, on $[R_o]$ was demonstrated by decreasing this quantity with the irreversible β-receptor antagonist, M−75. The essentially parallel rightward shift of isoprenaline E/[A] curves elicited by M−75 indicates that a considerable 'receptor reserve' exists for this agonist. The τ value, which effectively scales the size of the reserve was about 122. In fact, a slight depression of the isoprenaline curve asymptote at 10 μM M−75 is predicted by the model (Figure 3b), and, although this feature was not observed in the data, the simulated curve was insignificantly different from its experimental counterpart (Figure 3a). In contrast to isoprenaline, the asymptote (α) of the dichloroisoprenaline E/[A] curve was sensitive to decreases in $[R_o]$ (Figure 4a and b), a result expected in the case of a partial agonist in the system. M−75, at concentrations above 10^{-5} M, was found to depress basal rates. Thus it was not possible to reduce $[R_o]$ sufficiently to obtain depressed isoprenaline curves and so the estimation of a K_A for isoprenaline was precluded. Therefore, in simulating the data, we used an estimate of the K_A for isoprenaline obtained by De Lean *et al.* (1980) for the displacement of bound [^{3}H]-dihydroalprenolol in turkey erythrocyte membranes. The K_A for dichloroisoprenaline used in simulating the data was also a realistic estimate similar to that measured by the above workers. Therefore, the effects of M−75 on isoprenaline and dichloroisoprenaline agonism were quantitatively in accord with $[R_o]$ reduction in the model. Importantly, the same changes of $[R_o]$ in the model accounted for the effects of M−75 on both the isoprenaline and the dichloroisoprenaline curve profiles. Cholera toxin produced an essentially parallel leftward shift of isoprenaline E/[A] curves, indicating that τ was increased by cholera toxin with no change in E_m. A 3 fold increase in τ accounted for this potentiation. The same factor accounted for the changes in dichloroisoprenaline E/[A] curves produced by cholera toxin, which, as dicholoroisoprenaline behaved as a partial agonist, involved an increase in asymptote as well as a leftward shift.

In considering τ, $[R_o]$ and K_E so far we have not differentiated between the tissue-dependent and agonist-dependent factors contributing to operational efficacy. Although $[R_o]$ is clearly a tissue-dependent quantity, K_E contains both agonist and tissue information. Cholera toxin appears to alter K_E by the same factor for both agonists in the present study, exposing

the essentially tissue-dependent GTPase contribution to efficacy. However, the relative K_E values for isoprenaline and dichloroisoprenaline at each condition employed must reflect a difference in their intrinsic efficacies since all the tissue-dependent information cancels out. The estimated ratio for the present data was approximately 340:1, isoprenaline:dichloroisoprenaline. This ratio probably reflects the relative values of the dissociation constants for the ternary, ARN, complexes formed by these two compounds. The reciprocal of the ARN dissociation constant reflects the affinity of a particular AR complex for the N subunit and is therefore the likely candidate for 'intrinsic efficacy' in such systems (Black & Leff, 1983). Interestingly, assuming a 'ternary complex' model in their analysis of β-receptor agonist binding to turkey erythrocyte ghosts, De Lean *et al.* (1980) calculated dissociation constants for the ternary complexes formed by isoprenaline and dichloroisoprenaline of 5.56×10^{-13} M and 10^{-10} M respectively, giving a ratio of 180:1.

The definition of efficacy, τ, in the operational model of agonism conveniently separates the contributions of the receptor, ($[R_o]$) and the transducer system (K_E) to the pharmacological effects produced by particular agonist-receptor combinations. Therefore, the model provides a simple conceptual framework for predicting and interpreting the effects of experimental interventions at the receptor and transducer level. The consistency between theoretical prediction and experimental data in the present study suggests the applicability of the model as both a qualitative and quantitative means for analysing agonism.

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