Br. J. Pharmac. (1984), **81**, 131–141

The relative contribution of affinity and efficacy to agonist activity: organ selectivity of noradrenaline and oxymetazoline with reference to the classification of drug receptors

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1 Oxymetazoline demonstrated a pronounced organ selectivity, when compared to noradrenaline, by being a potent full agonist in rat anococcygeus muscle and a partial agonist in rat vas deferens.

2 Responses of rat anococcygeus muscles to oxymetazoline were relatively more sensitive to antagonism by phenoxybenzamine (Pbz) an alkylating α-adrenoceptor antagonist. Therefore, although oxymetazoline was more potent than noradrenaline in this tissue, after Pbz (0.3 μM for 10 min), the responses to oxymetazoline were completely inhibited while those to noradrenaline were only partially inhibited.

3 Schild analysis with phentolamine, corynanthine, prazosin and yohimbine indicated no α-adrenoceptor heterogeneity within the rat anococcygeus muscle or between this tissue and rat vas deferens. Measurement of agonist K_d values and Schild analysis of oxymetazoline antagonism of responses to noradrenaline (after alkylation) confirmed the homogeneity of α-adrenoceptors with respect to these two agonists.

4 The above profiles of activity would be predicted if oxymetazoline had a higher affinity but lower efficacy than noradrenaline. Experimentally this was confirmed when it was found that oxymetazoline had 5 times the affinity but 0.2 to 0.3 times the efficacy of noradrenaline.

5 These results serve as a caveat to the use of selective receptor desensitization and/or selective receptor alkylation (or protection from alkylation) as means of differentiating drug receptors.

6 Theoretical modelling and these experimental results indicate that high affinity/low efficacy agonists are much more sensitive to receptor coupling. The implications for therapeutic selectivity could be important in that high affinity/low efficacy agonists theoretically have a much greater potential for organ selectivity.

Introduction

An agonist produces responses by virtue of two drug related properties: affinity and efficacy. The affinity determines the extent of binding of agonist to the receptor at any given concentration and is defined as the reciprocal of the equilibrium dissociation constant (K_d) of the drug for the receptor. The efficacy (Stephenson, 1956) determines the magnitude of stimulus produced by the agonist for any given fraction of receptor occupancy. Receptor theory predicts that very different patterns of responses would be obtained in a range of organs for two drugs which have different ratios of efficacies and affinities. These different patterns of responses have direct relevance to the method of receptor classification by alteration of receptor number either by selective desensitization or alkylation (or selective protection from alkylation). Theoretically, if two drugs are both full agonists in a given tissue, the responses to the drug with the higher efficacy will be more resistant to alkylation and/or desensitization than those to the drug with the lower efficacy. Also, when comparing the responses of two such agonists in two tissues with different stimulus-response characteristics, their relative order of agonist activity can reverse.

Recently, a study by Ruffolo & Waddell (1982) showed oxymetazoline to possess a higher affinity but

lower efficacy than noradrenaline for α-adrenoceptors in rabbit aortae. This is a provocative finding since it suggests a way to test some of these predictions of receptor theory for high affinity/ low efficacy drugs. The effects of receptor coupling described in this paper have direct relevance to experiments aimed at drug receptor classification specifically as a caveat to ascribing these effects to receptor sub-types.

Methods

Rat anococcygeus muscle

Rats (male Sprague-Dawley, 200–250 g) were killed by cervical dislocation and both anococcygeal muscles were removed and placed in Krebs-Henseleit solution of the following composition (mM): Na^+ 151, K^+ 3.4, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.4, HCO^-_3 25.0, SO^{2-}_4 1.2, $H_2PO_4^-$ 1.0 and (+)-glucose 5.5. The muscles were mounted in heated (37°C) 17 ml organ baths (filled with pre-warmed Krebs-Henseleit solution and gassed with 95% O_2 plus 5% CO_2) on a perspex holder and tied, under 0.5 g resting tension, to a Grass FT.03 isometric transducer. Tissues were equilibrated for 60 min (solution changed every 10 min) before commencement of experimental procedures. During this period and thereafter, desmethylimipramine (DMI) (0.3 μM), corticosterone (30 μM) and propranolol (0.3 μM) were added to the medium to inhibit neuronal and extra-neuronal uptake of noradrenaline and possible β-adrenoceptor effects, respectively.

Rat vasa deferentia

Vasa deferentia were removed from rats (male Sprague-Dawley, 250–350 g; killed by cervical dislocation) and placed in aerated (95% O_2 plus 5% CO_2) Krebs-Henseleit solution. Tissues were trimmed of fat and desheathed, and then mounted in heated (37°C) 17 ml organ baths containing gassed Krebs-Henseleit solution. Vasa deferentia were tied to perspex holders and Grass FT.03 isometric transducers (under 0.5 g resting tension). Muscle tension was recorded in grams on a Beckman R511A dynograph recorder. Tissues were washed (bath changed every 10 minutes) for a further two 30 min periods before the start of experimental procedures. During this period, tissues were exposed to noradrenaline (1 μM and 10 μM) every 20 min for stabilization of α-adrenoceptor responses (Kenakin, 1980). After the first hour of washing, DMI (0.3 μM), corticosterone (30 μM) and propranolol (0.3 μM) were included in the bathing medium to inhibit neuronal and extra-neuronal uptake of noradrenaline and possible activation of β-adrenoceptors. Stable responses to noradrenaline (1 μM and 10 μM) were obtained after two hours.

Concentration-response curves

Cumulative concentration-response curves (van Rossum, 1963) were obtained in rat anococcygeus muscles at 0.5 log unit multiples of concentration added to the organ baths in < 0.1 ml volumes. In vasa deferentia, concentration-response curves were determined by measurement of peak responses to bolus injections of single concentrations of drugs (< 0.1 ml volumes); tissues were washed for three min before further addition of agonist. After determination of concentration-response curves to either noradrenaline or oxymetazoline (order was randomized), tissues were washed for at least 60 min before a second concentration-response curve was obtained. Separate experiments showed no effects of desensitization and/or reversal of antagonism by phenoxybenzamine (Pbz) with this protocol.

Schild regressions

After determination of control concentration-response curves, tissues were equilibrated with a competitive antagonist for 40 min. Concentration-response curves then were obtained in the presence of the antagonist and the procedure repeated with a higher (either 3x or 10x) concentration of antagonist. Equi-active dose-ratios (DR) of agonist (concentration of agonist producing 50% maximal response, EC_{50}, in the presence of antagonist ÷ EC_{50} in the absence of antagonist) were utilized in the Schild equation (Arunlakshana & Schild, 1959):

$$\log (DR\text{-}1) = n \log [B] - \log K_B \qquad (1)$$

where [B] is the molar concentration of antagonist and K_B the equilibrium dissociation constant of the antagonist for the receptor, in the form of a linear regression of log (DR − 1) upon log [B]. If the regression was linear and had a slope of unity ($n = 1$), the intercept [value of the abscissa when log (DR − 1) = 0] was considered to be an estimate of the pK_B ($-\log K_B$). In tissues with elevated baseline tension in the presence of partial agonist (oxymetazoline in rat vasa deferentia), the DR was measured from the control EC_{50} to the half maximal response to noradrenaline in the presence of oxymetazoline (van Rossum, 1963; Kenakin & Black, 1978; Kenakin & Beek, 1980).

Furchgott (1966). Thus, after determination of a control concentration-response curve, tissues were equilibrated with Pbz (0.1 μM or 0.3 μM for 10 min) and then washed for 2 h. For the first hour of washing, sodium thiosulphate (100 μM) was included in the medium to prevent further alkylation of α-adrenoceptors by newly formed aziridinium ion from residual Pbz (Nickerson & Goodman, 1947; Kenakin & Cook, 1976). After 2 h of washing, stable concentration-response curves were obtained which were depressed and shifted to the right of the control. Equi-active concentrations of agonist before [A] and after [A'] alkylation were utilized in the following equation (Furchgott, 1966):

$$\frac{1}{[A]} = \frac{1}{[A']}\frac{1}{q} - \frac{1}{K_d}\frac{(1-q)}{q}$$

where q is the fraction of α-adrenoceptors not alkylated. A linear regression of 1/[A] upon 1/[A'] yielded an estimate of K_d from the equation

$$K_d = \frac{(\text{slope} - 1)}{\text{intercept.}}$$

Relative efficacy

The relative efficacy of oxymetazoline and noradrenaline was measured by the method of Furchgott & Bursztyn (1967). Briefly, the responses to both agonists were expressed as functions of the fractional receptor occupancy (y), which was calculated by the Langmuir absorption isotherm with the experimentally estimated K_d. In terms of receptor theory, the response was considered to be a single valued function of stimulus (S) (Stephenson, 1956) which, in turn, was the product of the fractional receptor occupancy and efficacy (S = e y). Thus, assuming that equal responses emanate from equal stimuli, the ratio of equal responses of the concentration-occupancy curves reflected relative efficacy; i.e., response (oxymetazoline; OXY) = response (noradrenaline; NA) = $S_{OXY} = S_{NA} = e_{OXY}\, y_{OXY} = e_{NA}\, y_{NA}$ thus

$$\frac{y_{NA}}{y_{OXY}} = \frac{e_{OXY}}{e_{NA}}$$

Therefore relative efficacy was calculated as the antilog of the distance between the occupancy response curves along the log (occupancy) scale.

Drugs

Drugs used were (−)-noradrenaline bitartrate, corynanthine HCl, propranolol HCl, corticosterone, yohimbine HCl (all from Sigma Chemical Co., St. Louis, Missouri); cocaine HCl (Mallinckrodt Chemical Works, St. Louis, MO) and oxymetazoline (E. Merck, Darmstadt, Germany). I wish to thank Pfizer, Inc. (New York, NY) for prazosin, Ciba-Geigy (Summit, NJ) for phentolamine HCl and Smith, Kline and French Laboratories for phenoxybenzamine HCl. All drugs were prepared freshly in ascorbate (100 μM) and kept on ice during the experiments. Pbz was prepared immediately before use for each experiment.

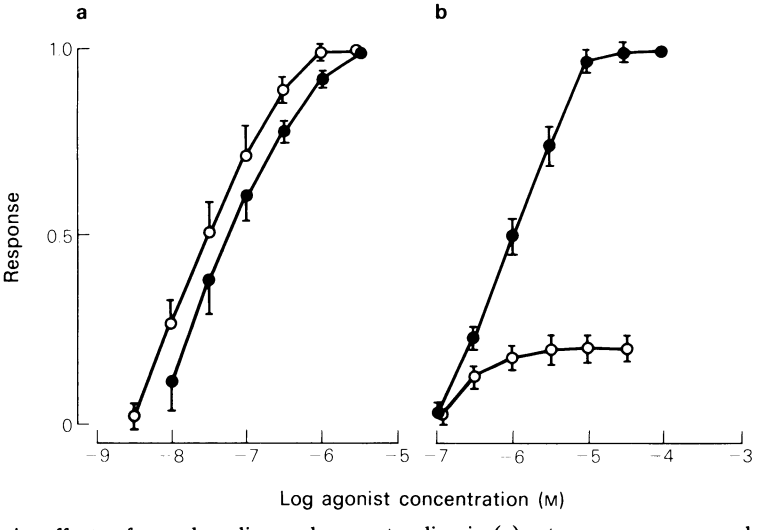

Figure 1 Agonist effects of noradrenaline and oxymetazoline in (a) rat anococcygeus muscle and (b) rat vas deferens. The response is expressed as a fraction of the maximal response to noradrenaline. Responses to noradrenaline (●, $n = 6$) and oxymetazoline (○, $n = 6$) for each tissue. The vertical bars represent s.e.mean.

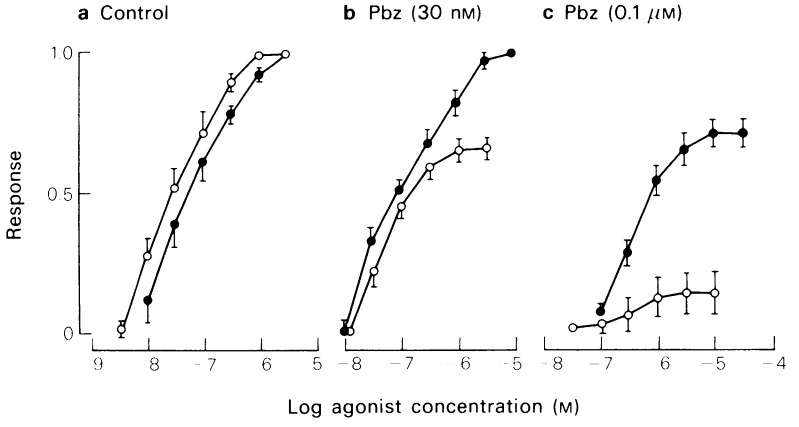

Figure 2 Effect of partial alkylation of the α-adrenoceptor population on the responses of rat anococcygeus muscles to agonists. Responses are expressed as fractions of the control maximal response to noradrenaline. Responses to noradrenaline (●, $n = 6$) and oxymetazoline (○, $n = 6$) in (a) control tissues, and (b and c) after treatment with phenoxybenzamine (Pbz) (30 nM and 0.1 μM respectively for 10 min). The vertical bars represent s.e.mean.

Results

Agonist activity

Both noradrenaline and oxymetazoline were agonists in the rat anococcygeus muscle and vas deferens. However, while both were full agonists in the rat anococcygeus muscle and oxymetazoline was more potent than noradrenaline, oxymetazoline was a much weaker agonist in vasa deferentia, producing only partial agonist activity (oxymetazoline maximal response 19 ± 4% of that to noradrenaline—see Figure 1).

Partial alkylation of the α-adrenoceptor populations of rat anococcygeus muscles, by controlled exposure to Pbz, was initiated in an attempt to delineate the relevance of receptor reserve to the different profile of activity obtained for these two agonists in these tissues. Accordingly, separate samples of rat anococcygeus muscles were equilibrated with Pbz (30 nM for 10 min and 0.1 μM for 10 min) and then washed for 2 h (bath changed every 10 min) to remove non-alkylated Pbz. The first hour of washing included sodium thiosulphate (100 μM) to prevent further cyclization of Pbz to aziridinium ion and subsequent alkylation of receptors (see Methods). Under these conditions stable concentration-response curves to the agonists were obtained after 2 h of washing as judged by the fact that no further change in responses were observed after 2–3/4 h of washing. The effects of partial alkylation of the α-adrenoceptor population in these tissues are shown in Figure 2. While the concentration-response curves to both noradrenaline and oxymetazoline shifted to the right with more extreme treatment with Pbz, the maximal response to oxymetazoline was disproportionately more depressed than that of noradrenaline by this treatment. The relative resistance of noradrenaline responses to alkylation of α-adrenoceptors was shown by the fact that in tissues with 58% of the receptors alkylated ($q = 0.42$), oxymetazoline pro-

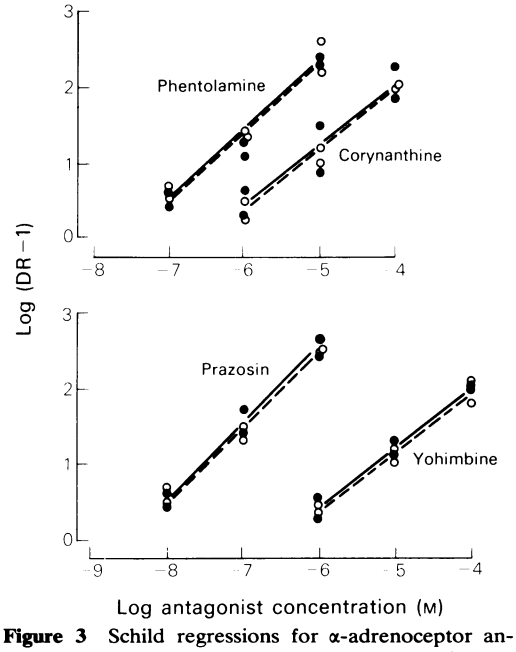

Figure 3 Schild regressions for α-adrenoceptor antagonists, phentolamine, corynanthine, prazosin and yohimbine, as antagonists of responses to noradrenaline (●—●) and oxymetazoline (○---○) in the rat anococcygeus muscle.

Table 1 Schild regressions in rat anococcygeus muscles

Antagonist	*Agonist*					
	Noradrenaline (NA)			*Oxymetazoline*		
	n	*slope*	pA_2	n	*slope*	pA_2
Prazosin	6	1.0 (0.9–1.1)	8.5[a] (8.3–8.8)	6	1.0 (0.9–1.1)	8.6 (8.4–8.7)
Phentolamine	6	0.9 (0.8–1.0)	7.6[b] (7.4–7.9)	6	0.9 (0.8–1.0)	7.5 (7.3–7.7)
corynanthine	6	0.8 (0.7–0.9)	6.5[c] (6.2–7.0)	6	0.8 (0.7–0.9)	6.4 (6.2–6.6)
Yohimbine	6	0.8 (0.7–0.9)	6.6[d] (6.4–6.75)	6	0.8 (0.7–0.9)	6.5 (6.4–6.7)

[a]8.2 (NA; Doxey, Smith & Walker, 1977), 8.5 (graphical estimation; phenylephrine; Docherty & Starke, 1981).
[b]7.7 (NA; Doxey *et al.*, 1977).
[c]6.65 (NA; Doxey *et al.*, 1977).
[d]6.4 (NA; Doxey *et al.*, 1977), 6.49 (NA; Doxey, Roach & Vindee, 1982).
Values in parentheses represent 95% confidence limits of the estimates.

duced 57% of the control maximal response while in tissues with 91% of the receptors alkylated ($q = 0.09$), noradrenaline produced 75% maximal response.

Receptor analyses by antagonists

The antagonism of responses of rat anococcygeus muscles to both oxymetazoline and noradrenaline was quantified by Schild analysis. The salient features of the Schild regressions (shown in Figure 3) are given in Table 1. The regressions for prazosin, phentolamine, corynanthine and yohimbine were not significantly different for antagonism of responses to oxymetazoline or noradrenaline with respect to slope or elevation of regression lines (analysis of covariance, Snedecor & Cochrane, 1967). Thus, the data with these four antagonists indicate that the two agonists activate a common drug receptor in this tissue.

Table 2 Schild regressions in rat vasa deferentia

Antagonist	n	*Agonist (Noradrenaline)* *slope*	pA_2
Prazosin	6	1.1 (1.0–1.2)	8.6[a] (8.4–8.7)
Phentolamine	6	1.1 (1.0–1.3)	7.5[b] (7.3–7.7)
Corynanthine	6	1.0 (0.9–1.1)	6.5[c] (6.4–6.7)
Yohimbine	6	1.0 (0.8–1.1)	6.5[d] (6.3–6.8)

[a]8.76 (amidephrine; Michel & Whiting, 1981).
[b]7.9 (phenylephrine; Ruffolo & Patil, 1979).
[c]6.84 (amidephrine; Michel & Whiting, 1981).
[d]6.25 (amidephrine; Michel & Whiting, 1981); 5.99 (phenylephrine; Ruffolo & Patil, 1979); 6.1 (noradrenaline; Mottram & Kapur, 1975).
Values in parentheses represent 95% confidence limits of the estimate.

The antagonism of responses of rat vasa deferentia to noradrenaline by these same four antagonists was also quantified with Schild regressions; and the data describing these regressions are given in Table 2. Insufficient agonist activity was observed with oxymetazoline in this tissue to allow accurate estimation of pK_B values with this agonist. The pK_B values in rat vasa deferentia (measured with noradrenaline) were not significantly different from those in rat anococcygeus muscles indicating no evidence to suggest that the α-adrenoceptors were different in these two tissues with respect to the affinity of prazosin, phentolamine, corynthanine and yohimbine.

Receptor analyses by agonists

The equilibrium dissociation constants (K_d) of both noradrenaline and oxymetazoline were estimated in rat anococcygeus muscles by the method of Furchgott (1966). For both agonists there was no significant correlation between the estimate of K_d and the fraction of receptors alkylated by Pbz (noradrenaline, t value for correlation 1.0, d.f. = 2, NS; oxymetazoline, $t = 2.1$, d.f. = 3, NS).

An independent method was utilized to estimate the K_d for oxymetazoline. By partial alkylation of the α-adrenoceptor population in the rat anococcygeus muscle with Pbz, the agonist responses to oxymetazoline could be abolished in tissues which still contracted appreciably to noradrenaline. Under these circumstances oxymetazoline was utilized as a competitive antagonist of noradrenaline and an estimate of the equilibrium dissociation constant for

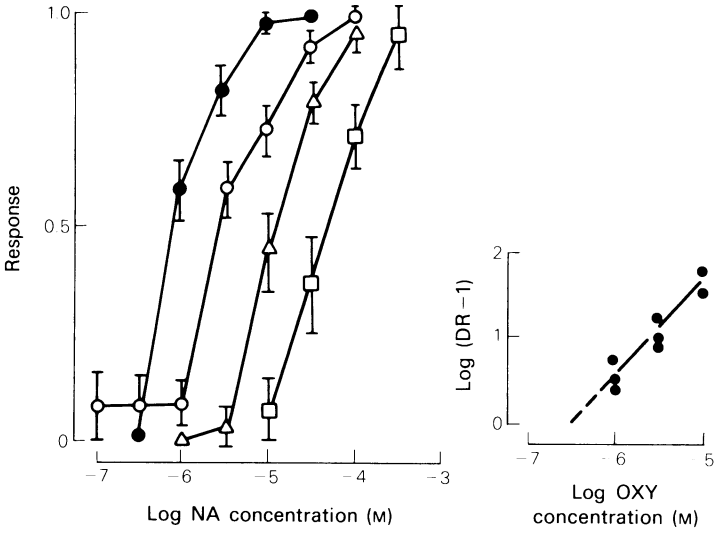

Figure 4 Antagonism of responses of phenoxybenzamine (Pbz)-treated rat anococcygeus muscles to noradrenaline (NA) by oxymetazoline (OXY). Responses are expressed as fractions of the control maximal response to noradrenaline: inset; as for Figure 3. Concentration-response curves to noradrenaline in the absence (●, $n = 6$) and presence of oxymetazoline 1 μM (○, $n = 3$), 3 μM (△, $n = 3$) and 10 μM (□, $n = 2$). The vertical bars represent s.e.mean or range when $n < 3$. Inset: Schild regression of antagonism of responses to noradrenaline by oxymetazoline.

oxymetazoline was obtained from a Schild regression (Furchgott & Bursztyn, 1967) (Figure 4). Accordingly, rat anococcygeus muscles were equilibrated with Pbz (0.3 μM for 10 min) and then washed for 2 h (bath changed every 10 min, sodium thiosulphate, 100 μM, present for the first hour of washing). Under these circumstances a stable concentration-response curve to noradrenaline was obtained which was shifted to the right of non-Pbz-treated curves and did not change appreciably with respect to location along the concentration axis or maximal response for a further 2 h. Oxymetazoline was a simple competitive antagonist of responses to noradrenaline with a pK_B not significantly different from the pK_d estimated by the method of Furchgott (1966) (see Table 3).

The agonist responses of rat vasa deferentia to

Table 3 The pK_d and efficiacy in isolated tissues for noradrenaline (NA) and oxymetazoline (OXY)

	Rat anococcygeus		*Rat vas deferens*	
A. *Affinity* (pK_d)				
Method	*NA*	*OXY*	*NA*	*OXY*
Alkylation	5.9[a] (5.6–6.4) ($n = 4$)	6.7[b] (6.5–6.9) ($n = 5$)		
Schild		6.5[c] (6.3–6.8) ($n = 8$)		6.5[d] (6.2–6.9) ($n = 9$)
B. *Efficacy* (±s.e.mean)				
$\frac{\varepsilon_{NA}}{\varepsilon_{OXY}}$	3.5 ± 0.4[e] ($n = 14$)		4.0 ± 0.6 ($n = 5$)	

[a]5.93 (Ruffolo & Waddell, 1982)
[b]6.8 (Ruffolo & Waddell, 1982)
[c]slope = 1.0 (0.9–1.2).
[d]slope = 1.1 (0.9–1.3).
[e]1.74 (Ruffolo & Waddell, 1982)
Values in parentheses indicate 95% confidence limits of the estimate.

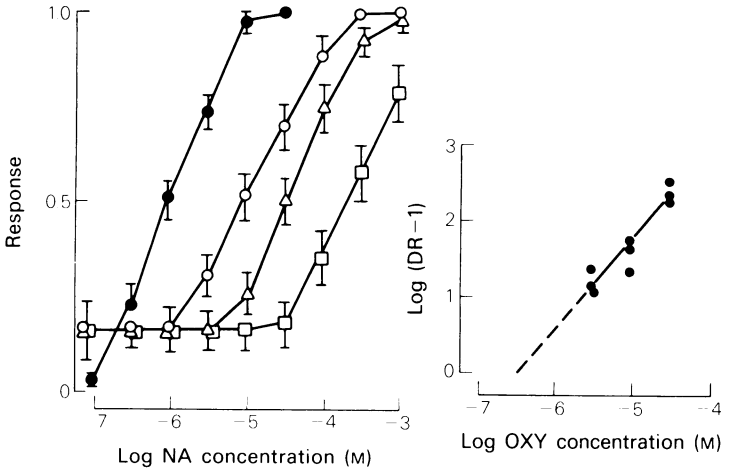

Figure 5 Antagonism of responses of rat vasa deferentia to noradrenaline (NA) by oxymetazoline (OXY). Ordinates as for Figure 4. Concentration-response curves in the absence (●, $n = 6$) and presence of oxymetazoline 3 μM (○, $n = 3$), 10 μM (△, $n = 3$) and 30 μM (□, $n = 3$). The vertical bars represent s.e.mean. Inset: Schild regression for antagonism of responses to noradrenaline by oxymetazoline.

oxymetazoline were sufficiently weak to enable Schild analysis of oxymetazoline antagonism of responses to noradrenaline in this tissue. Oxymetazoline produced dextral displacement of concentration-response curves to noradrenaline and the resulting dose ratios were utilized in the Schild equation (Figure 5) to yield an estimate of the equilibrium dissociation constant of oxymetazoline (Table 3). This pK_B estimate was not significantly different from the pK_B or pK_d estimated for oxymetazoline by two separate methods in rat anococcygeus muscles.

Relative efficacy

The relative efficacy of noradrenaline and oxymetazoline was estimated by the method of Furchgott & Bursztyn (1967) in the rat anococcygeus muscle and vas deferens. The relative efficacies of noradrenaline and oxymetazoline (given in Table 3) indicated no selective efficacy for either agonist in the rat anococcygeus muscle or vas deferens.

Effects of varying affinity/efficacy ratios

The preceding data were consistent with the view that noradrenaline and oxymetazoline activate identical receptors in both the rat anococcygeus muscle and vas deferens. Therefore, in an attempt to simulate the selective profile of agonism observed experimentally with these drugs, equations from receptor theory were utilized to model responses. Thus, stimulus (S) (Stephenson, 1956) was defined as (Furchgott, 1966):

$$S = \frac{\varepsilon[R_t]}{1 + K_d/[A]} \quad (3)$$

where ε was intrinsic efficacy (Furchgott, 1966), $[R_t]$ the total concentration of receptors, K_d the equilibrium dissociation constant of the agonist for the receptor and [A] the molar concentration of agonist. The tissue response (R) was assumed to be a nonlinear function of the stimulus

$$R = \frac{S}{(S + \beta)}$$

where β was a fitting parameter. It should be noted that the magnitude of β and, in fact, the nature of the function used to calculate response are not critical to the predictions of these calculations and serve only to introduce (and manipulate the magnitude of) the concept of a nonlinear relationship between receptor activation and tissue response. Changes in β-allow manipulation of the effective receptor reserve. The experimental estimates of K_d (Table 3) were utilized in equation 4, while the values of β for the anococcygeus muscle and ε for noradrenaline were chosen arbitrarily to simulate the data shown in Figure 2. The value for ε (oxymetazoline) was defined by the experimental estimate of the ratio of the relative efficacies of noradrenaline and oxymetazoline (Table 3). The theoretical concentration-response curves generated with these parameters are shown in Figure 6 where it can be seen that the agonist with the higher affinity, but lower efficacy (broken line) is slightly more potent than the agonist with the lower affinity, but higher efficacy (solid line) when the receptor concentration $[R_t]$ is set to a value of unity. However,

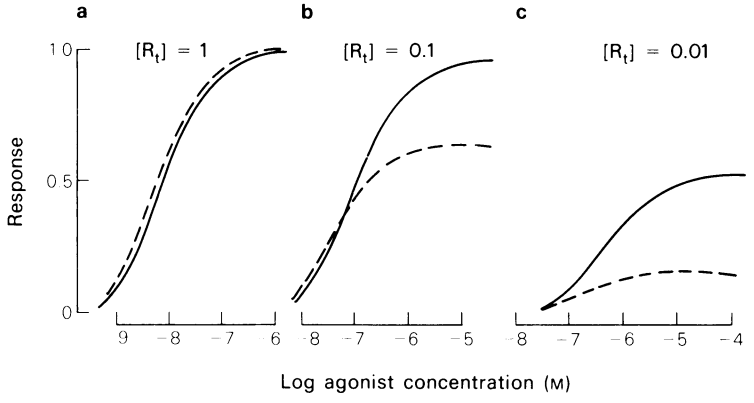

Figure 6 Theoretical concentration-response curves modelling responses of rat anococcygeus muscle to noradrenaline and oxymetazoline. Responses are expressed as a fraction of the maximum response calculated from theoretical equations (see text). Responses to oxymetazoline (---, high affinity/low efficacy, $\beta = 0.1$, $K_d = 0.2\ \mu M$, $\varepsilon = 2$) and noradrenaline (——, high efficacy/low affinity, $\beta = 0.1$, $K_d = 1\ \mu M$, $\varepsilon = 10$) with varying concentrations of receptors ([R$_t$] = 1, 0.1 and 0.01).

note that as the number of receptors is reduced ([R$_t$] = 0.1 and 0.01), the maximal response to the agonist with the lower intrinsic efficacy (oxymetazoline) is disproportionately more depressed than that to the agonist with the higher intrinsic efficacy (noradrenaline). This effect can occur in the same tissue when the number of receptors are reduced (as, for example, by alkylation, Figure 2) or in two different tissues with identical receptors but different efficiencies of coupling between stimulus and response (for example, compare theoretical curves for [R$_t$] = 1 and 0.01 to Figure 1). Another example was calculated to illustrate this further. In Figure 7, the theoretical concentration-response curves are shown for two agonists with even greater disparities between K_d and ε than noradrenaline and oxymetazoline. Thus, one agonist was defined as having 1,000 times the affinity, but only 0.03 times the efficacy of the other. Under these circumstances, the agonist with the lower intrinsic efficacy is 30 times more potent than the agonist with the higher intrinsic efficacy in a tissue with a large effective receptor reserve ([R$_t$] = 1) but demonstrates no agonist response in a tissue with a very low effective receptor reserve ([R$_t$] = 0.001) which nevertheless is quite responsive to the agonist with the higher efficacy.

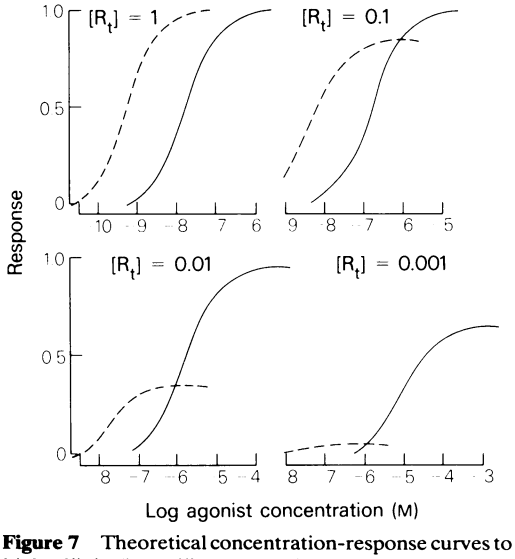

Figure 7 Theoretical concentration-response curves to high affinity/low efficacy and high efficacy/low affinity agonists in a range of tissues varying in receptor number. Ordinates as for Figure 6. Responses to a high affinity/low efficacy (---, $\beta = 0.05$, $K_d = 30\ nM$, $\varepsilon = 3$) and high efficacy/low affinity (——, $\beta = 0.05$, $K_d = 30\ \mu M$, $\varepsilon = 100$) agonist with varying numbers of receptors ([R$_t$] = 1, 0.1, 0.01 and 0.001).

Discussion

Although noradrenaline and oxymetazoline showed an opposite selectivity in rat anococcygeus muscles and vasa deferentia, classical pharmacological procedures showed that these agonists activate indistinguishable α-adrenoceptors in these tissues. This apparent paradox is compounded by the fact that the responses to oxymetazoline are much more sensitive to receptor alkylation than are those to noradrenaline. Before discussion of the relevance of this finding it is worth considering the evidence for receptor homogeneity in these two tissues with respect to these two agonists. To explain the differential agonists profiles of noradrenaline and oxymetazoline in terms of receptor differences, the α-adrenoceptors in

the rat anococcygeus muscle would have to be different from those in the vas deferens and oxymetazoline to be a more selective agonist of the anococcygeal α-adrenoceptors. To explain the differential sensitivity to Pbz (Figure 2), however, requires a further postulate of heterogeneous receptors within the rat anococcygeus muscle which would propose that oxymetazoline and noradrenaline stimulate two different α-adrenoceptors in this tissue, one being more susceptible to alkylation by Pbz.

It is unlikely that there is a heterogeneous population of α-adrenoceptors within the rat anococcygeus muscle, with respect to noradrenaline, oxymetazoline and Pbz for two reasons. Firstly, the pK_B estimates for phentolamine, corynanthine, prazosin and yohimbine, a series of antagonists with differing potencies for α_1- and α_2-adrenoceptors, were identical whether they were measured in experiments with noradrenaline or oxymetazoline as the agonist. In terms of the fundamental theory of Schild analysis this indicates that both agonists activate a common receptor with respect to antagonist binding (Schild, 1947; Arunlakshana & Schild, 1959) and argues against separate noradrenaline and oxymetazoline receptors. In the latter case, it might be expected that the antagonists would have had different potencies for each receptor and, therefore, that the pK_B estimates would have differed with the different agonists. However, it should be pointed out that the heterogeneity in the pK_B values is in direct proportion to the selectivity of the antagonist for the two receptor types (Kenakin, 1982). Thus failure to observe differences in the pK_B values for the four α-adrenoceptor antagonists is not proof that heterogeneity in the receptors is not present. Rather, it provides no evidence to support the concept of separate receptors for these two agonists.A stronger argument against receptor heterogeneity in the rat anococcygeus muscle for these two agonists is furnished by the K_d data for oxymetazoline.

Experiments utilizing the method of Furchgott (1966) yielded the equilibrium dissociation constant of oxymetazoline for the α-adrenoceptors which mediate the generation of responses to this agonist. After partial alkylation by Pbz, to a point where oxymetazoline produced no response, and thus served to antagonize the responses to noradrenaline (Figure 5), Schild analysis indicated an identical affinity constant. These data provided no evidence to support the hypothesis that separate receptors, with differential sensitivity to Pbz, mediate the responses to oxymetazoline and noradrenaline in this tissue. In the latter case, a different affinity constant for oxymetazoline might have been predicted after alkylation by Pbz if this treatment selectively eliminated oxymetazoline receptors.

The quantitative data also do not support a hypothesis proposing different α-adrenoceptors in the anococcygeus muscle and vas deferens. Firstly, the pK_B estimates for antagonism of noradrenaline responses in these two tissues by phentolamine, corynanthine, prazosin and yohimbine were the same, indicating no receptor heterogeneity with respect to the interaction of these antagonists with noradrenaline. Also, the affinity constants for oxymetazoline measured by two independent methods in the rat anococcygeus muscle were not significantly different from the estimate, by Schild analysis, in the rat vas deferens.

The differential sensitivity of the agonist effects of these two drugs theoretically can result from their relative values of affinity and intrinsic efficacy. Thus, an agonist with a high affinity but low efficacy may be a full agonist in a tissue with a highly efficient stimulus-response mechanism, but since efficacy is the prime determinant of agonist activity, the low efficacy of the same drug may be insufficient to produce any agonist response in a tissue with an inefficient stimulus-response mechanism. This idea must be considered as a caveat to receptor classification by (a) selective agonists, (b) selective desensitization, and (c) selective receptor protection. In the absence of evidence that the receptors in the rat anococcygeus muscle and vas deferens are homogeneous, the fact that oxymetazoline was a more powerful agonist than noradrenaline in one tissue and much less so in another would suggest a receptor difference. Also, the results in Figure 2 show that irreversible removal of a portion of the receptor pool (by, in this case, alkylation) as would be produced by desensitization of the tissue, would have a greater effect on the responses to oxymetazoline. This is because noradrenaline probably requires a smaller proportion of the receptor pool to generate responses than does oxymetazoline. Thus, selective receptor protection from alkylation would show differences between noradrenaline and oxymetazoline which could be ascribed to receptor differences. The same theoretical objection to the use of this method has been raised for the use of full and partial agonists in conjunction with receptor alkylation (Waud, 1968). The results in this paper show that the effects of differential receptor reserve extend to full agonists if there is a substantial difference in intrinsic efficacy.

A corollary to this hypothesis provides a caveat to comparing the potencies of agonists with differing ratios of efficacy and affinity. In tissues with large effective receptor reserves, where two drugs of different efficacy are full agonists, their potency ratios reflect receptor parameters and are constant. However, in tissues when one or both agonists do not produce the maximal tissue response, the potency ratio of the two agonists can change unpredictably (as seen in the rat anococcygeus muscle and vas defe-

rens) because of differences in relative affinities and efficacy. This phenomenon has been described by Furchgott (1972) (see Figure 1).

There are other examples of high affinity/low efficacy agonists. Dobutamine has 25 times the affinity but 1/40 times the efficacy of noradrenaline for α-adrenoceptors (Kenakin, 1981). Also, a series of agonists related to clonidine have been described which have higher affinity but lower efficacy than noradrenaline for α-adrenoceptors (Ruffolo, Waddell & Yaden, 1980). Finally, impromidine, a recently described histamine H_2-agonist indicates a profile of high affinity/low efficacy agonist, being a full agonist and more potent than histamine in guinea-pig right atria (Durant, Duncan, Ganellin, Parsons, Blakemore & Rasmussen, 1978) and a partial agonist (but still more potent) when compared to histamine in mouse isolated stomach (Parsons & Sykes, 1980).

Theoretically, the potential for organ selectivity of high affinity/low efficacy agonists is greater than that of high efficacy agonists. This is because the responses to high affinity/low efficacy agonists are more sensitive to the efficiency of receptor coupling than are high efficacy agonists. An example of the selectivity which can be attained is found in the variety of responses to prenalterol, a β-adrenoceptor agonist of low efficacy. Prenalterol is nearly a full agonist in guinea-pig trachea and right atria from guinea-pigs treated with thyroxine, a partial agonist in cat, rat and guinea-pig left atria and a complete antagonist in guinea-pig digitorum longus muscle and canine coronary artery (Kenakin & Beek, 1980; 1982). Also, the organ selectivity of morphine, being a partial agonist in guinea-pig ileum and an antagonist in rat vas deferens, has been described as being a function of differing receptor numbers in these two preparations (Henderson, Robinson & Sim, 1982).

The experiments described in this paper show how different ratios of K_d and ε can produce agonist selectivity which very much resembles receptor selectivity. However, no receptor mediated selectivity was found, and classical receptor theory predicts that none is required to explain the organ selective effects.

I wish to thank Mrs Debbie Beek for excellent technical assistance in these experiments.

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(Received June 22, 1983.
Revised August 31, 1983.)