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Demonstration of α_{1s}-adrenoceptors after exposure of the rat anococcygeus to benextramine

Jill Coates & D.F. Weetman

Department of Pharmacology, Sunderland Polytechnic, Wharncliffe Street, Sunderland, SR2 3SD

1 Sgd 101/75 (4(2-imidazoline-amino)-2-methylindazol-chlorhydrate) and noradrenaline exerted similar, full agonist effects on the α-adrenoceptors of the rat anococcygeus.

2 Benextramine (30 μM for 30 min, followed by 20 washes over 30 min) abolished contractions of the anococcygeus to Sgd 101/75, but only reduced those to noradrenaline.

3 Sgd 101/75 (400 μM) did not significantly reduce contractions of the benextramine-treated (30 μM) anococcygeus to noradrenaline.

4 It is concluded that benextramine can replace phenoxybenzamine as the alkylating agent in the demonstration of α_{1s}-adrenoceptors in the rat anococcygeus.

Introduction

Sgd 101/75 (4(2-imidazoline-amino)-2-methylindazol-chlorhydrate) can stimulate adrenoceptors on smooth muscle in two distinct ways. It is a partial agonist with weak antagonist activity for α_1-adrenoceptors, when compared with noradrenaline, on the isolated taenia of guinea-pig caecum (Ismail, Jahn & Weetman, 1981; Ismail, 1981). On the rat anococcygeus, Sgd 101/75 is a full agonist relative to noradrenaline with no antagonist action (Ismail, 1981). These results could not be explained on the basis of different efficacies of the two drugs for a single α-adrenoceptor (noradrenaline high and Sgd 101/75 low) and different receptor reserves on the preparations (it would necessitate that on the taenia they were low and high on the anococcygeus). It was concluded that two distinct α-adrenoceptors (α_1 and α_{1s}) existed on the rat anococcygeus (Coates, Jahn & Weetman, 1982). The critical evidence in favour of this interpretation was that when the rat anococcygeus was made insensitive to Sgd 101/75 by phenoxybenzamine pretreatment, Sgd 101/75 failed to antagonize contractions of the smooth muscle to noradrenaline, thus precluding an action of the two drugs on a single receptor.

One criticism of these experiments was that phenoxybenzamine was used before the interaction (or lack of interaction) between Sgd 101/75 and noradrenaline could be determined. Phenoxybenzamine can exert several different actions on peripheral tissues (see below) which might have affected the result of these experiments. Although a very low concentration of phenoxybenzamine was used in the demonstration of the existence of α_{1s}-adrenoceptors, it was thought worthwhile to see if another alkylating agent, benextramine, could reveal the new receptor. Benextramine (BHC, N,N'-*bis* (*o*-methoxybenzylamino-*n*-hexyl) cystamine) causes an irreversible blockade of α_1-adrenoceptors, yet unlike phenoxybenzamine does not inhibit noradrenaline uptake into adrenergic nerves, nor antagonize muscarinic, 5-HT or histamine H_1-receptors (Melchiorre, Yong, Benfey & Belleau, 1978; Furchgott, 1980; Melchiorre, 1981).

Methods

Anococcygeus muscles (Gillespie, 1972) were dissected from male Sprague-Dawley rats (230–270 g) and suspended in isolated organ baths (10 ml) which contained McEwen's solution (McEwen, 1956) at 37° ± 1°C gassed with 95% O_2 and 5% CO_2. The initial tension on the preparations was 0.5 g and an equilibration period of 30 min was allowed before drugs were added, during which time preparations were washed 5–10 times with McEwen's solution. The tension was measured by a Grass FT 03C force-displacement transducer and recorded on a Grass 79D polygraph. Noradrenaline or Sgd 101/75 was administered cumulatively at 2 min intervals by the method of van Rossum (1963) until a maximum response was obtained (i.e. when two or more increases in concentration failed to augment the tension.) When each of a pair of anococcygeus muscles

Table 1 The effect of benextramine on the response of the rat anococcygeus to Sgd 101/75 and noradrenaline

Drug	*Characteristic*	*Benextramine treatment* None	3 μM	P	30 μM	P
Sgd 101/75	Maximum response (g)	6.49 ± 0.50 (29)	3.33 ± 1.06 (4)	<0.02	response abolished	
	EC_{30} (nM)	51.2 ± 23.6 (29)	1070 ± 450 (4)	<0.05	response abolished	
Noradrenaline	Maximum response (g)	6.61 ± 0.49 (29)	6.06 ± 0.78 (4)	>0.05	3.63 ± 0.55 (5)	<0.005
	EC_{30} (nM)	164 ± 15.5 (29)	280 ± 80 (4)	>0.05	820 ± 150 (5)	<0.001

Mean values ± s.e.mean are given; *n* in parentheses.
Preparations were incubated with benextramine for 30 min, and then washed 20 times over the next 30 min, before the addition of the stimulant drug.

was incubated with benextramine (3 or 30 μM for 30 min, followed by 20 washes over 30 min), it was before a contractile drug was administered. Then the control anococcygeus received noradrenaline (cumulatively from 6 nM to the concentration that produced a maximal response), whereas the contralateral preparation first received Sgd 101/75 (cumulatively up to 400 μM, the benextramine pretreatment preventing a contractile effect) and then noradrenaline. From such paired preparations, it was possible to estimate the interactions between Sgd 101/75 and noradrenaline by comparing the maximal response and sensitivity (EC_{30} value; see below).

Individual concentration-response curves for agonists were plotted and EC_{30} values (i.e. concentration of drug producing a tension equivalent to 30% of the control maximum to noradrenaline, viz 1.98 g) and the maximum response were measured. Values in the text refer to the mean ± s.e. mean of *n* such determinations. Differences in means were determined by Student's *t* test (Snedecor & Cochran, 1967), and a probability level of <0.05 was considered significant.

Drugs

Sgd 101/75 (Siegfried AG), (−)-noradrenaline bitartrate (Sigma) benextramine tetrahydrochloride monohydrate (Aldrich) were used.

Drugs were dissolved in distilled water. Solutions of noradrenaline contained approximately 50 μg ml^{-1} ascorbic acid (B.D.H.).

The composition of the McEwen's solution was as follows (mM): NaCl 130, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 25, NaH_2PO_4 1.2 glucose 11.1 and sucrose 13.2.

Concentrations in the text are expressed in molarities.

Results

Effects of benextramine on concentrations of the anococcygeus to Sgd 101/75 and noradrenaline

Benextramine pretreatment (0.3–300 nM) failed to antagonize the contractile effects of either noradrenaline or Sgd 101/75 ($n = 8$). After benextramine (3 μM), neither the maximum contraction nor the sensitivity to noradrenaline were significantly depressed; however, the maximum contraction and the sensitivity to Sgd 101/75 were decreased (Table 1). Increase in the benextramine concentration (to 30 μM) abolished the response to Sgd 101/75 (up to 400 μM), and reduced that to noradrenaline (Table 1).

Table 2 Interaction between Sgd 101/75 and noradrenaline in five pairs of benextramine-treated rat anococcygeus muscles

	Noradrenaline alone	*Noradrenaline in the presence of Sgd 101/75* 400 μM	P
Maximum response (g)	3.63 ± 0.55	3.70 ± 0.71	>0.05
EC_{30} (nM)	820 ± 150	2060 ± 900	>0.05

Values are mean ± s.e.mean.
The sensitivity of the anococcygeus to noradrenaline was assessed by estimating EC_{30} values (i.e. concentration producing a tension equivalent to 30% of the control maximum to noradrenaline, viz. 1.98g)
The maximum response to the rat anococcygeus to noradrenaline in the presence of 400 μM Sgd 101/75 was 102% of its control, and the sensitivity was reduced only 2.5 times.

Interaction between Sgd 101/75 and noradrenaline in benextramine-pretreated anococcygeal preparations

Pairs of anococcygeus muscles were initially incubated with benextramine (30 μM). Sgd 101/75 (400 μM) was added to one anococcygeus muscle and no contraction resulted, so noradrenaline was added cumulatively to determine its effects in the presence of Sgd 101/75. The contralateral anococcygeus was treated identically, except that Sgd 101/75 was not present. The effect of noradrenaline were compared on five such pairs of preparations. There was no interference by Sgd 101/75 in the response to noradrenaline in that a very high concentration of Sgd 101/75 (400 μM i.e. 7800 times its EC_{30} value in untreated preparations) failed to depress either the maximum response of the anococcygeus to noradrenaline (102% of the paired controls) or the sensitivity (dose-ratio 2.5) significantly, thus excluding the possibility that both drugs act on a single receptor (Table 2).

Discussion

Sgd 101/75 and noradrenaline contract the anococcygeus muscle by activating different receptors (Coates *et al.*, 1982), although the effects of both drugs could be blocked by prazosin, a selective antagonist for α_1-adrenoceptors (Doxey, Smith & Walker, 1977; Cambridge, Davey & Massingham, 1977; Drew, 1982). The rat anococcygeus is similarly sensitive to Sgd 101/75 and noradrenaline, but contractions to the former can be prevented by incubating preparations with concentrations of phenoxybenzamine below those normally required to inactivate α_1-adrenoceptors (see Coates *et al.*, 1982). When Sgd 101/75 is added to such preparations in 3000 times the concentration that causes 50% maximal contraction of a normal preparation, no contraction results, and the response of the tissue to noradrenaline is unaffected. These results should be contrasted with those of Kenakin (1981), who showed that the partial agonist for α_1-adrenoceptors, dobutamine, exerted an antagonistic action on the anococcygeus. When the contractile effects of dobutamine were abolished by partial alkylation of the adrenoceptors with benextramine, dobutamine reduced the sensitivity of the anococcygeus to noradrenaline (10 μM gave a dose-ration of about 100). This is the result that would be predicted for a partial agonist. However, because no antagonism of noradrenaline by Sgd 101/75 could be demonstrated by Coates *et al.* (1982), it was concluded that the two drugs activated different receptors, which were designated the α_{1s}-adrenoceptor (specifically stimulated by Sgd 101/75) and the α_1-adrenoceptor (responsible for 70% of the maximum response of noradrenaline, the other 30% being due to an action on the α_{1s}-adrenoceptor).

A possible weakness in this argument lies in the use of phenoxybenzamine, a drug known to interact with a variety of receptors and to interfere with noradrenaline uptake into tissues. Although this possibility was thought to be unlikely, considering the very low concentration of phenoxybenzamine used (see Coates *et al.*, 1982), it has now been shown that benextramine can replace phenoxybenzamine without affecting the pattern of results obtained. Benextramine has a narrow range of pharmacological activity relative to phenoxybenzamine (see Introduction), so its effectiveness provides further support for the conclusion that two distinct α_1-adrenoceptors exist in the anococcygeus, both subserving contraction of the smooth muscle.

The adrenoceptors in the anococcygeus were not particularly sensitive to benextramine; 30 μM had to be used to abolish the response to Sgd 101/75. This finding contrasts with the very low concentrations of phenoxybenzamine (0.3nM) previously used in this type of experiment (Coates *et al.*, 1982; Coates & Weetman, 1983). Benextramine also exhibited a low sensitivity in antagonizing the contractile effects of noradrenaline, a result that contrasts with that obtained by Kenakin (1981). In his experiments, cocaine and EDTA were included in the Ringer solution to prevent the loss of noradrenaline from the biophase. However, even in the presence of desmethylimipramine (0.35 μM) the effectiveness of benextramine against noradrenaline was not increased (Coates & Weetman, unpublished results). The difference in the activity of benextramine may lie in the differences in the strains of rats used.

The heterogeneity of the α_1-adrenoceptor in the anococcygeus has clear implications in the interpretation of results obtained from experiments in which ligands are bound to isolated membrane preparations. Affinity of drugs for the α_1-adrenoceptor is frequently measured by the inhibition of specific binding of prazosin to membrane preparations. As prazosin does not discriminate between α_1- and α_{1s}-adrenoceptors, it cannot be certain which receptor is involved in such experiments. With membranes isolated from rat brain, Sgd 101/75 was of very low potency in displacing [^{3}H]-prazosin binding (65 μM Sgd 101/75 caused 50% inhibition of specific binding: Mathy, Thoolen, Timmermans & van Zwieten, unpublished results), suggesting that α_{1s}-adrenoceptors were not involved in the binding. The same situation may not pertain with membranes from other tissues.

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