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Occurrence of α_{1s}-adrenoceptors in the mouse but not in the rabbit isolated anococcygeus preparations

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1 Both Sgd 101/75 (4(2-imidazoline-amino-2-methylindazol-chlorhydrate) and noradrenaline contracted anococcygeal preparations from the mouse and rabbit.

2 The maximal response to and sensitivity (EC_{30} values) of each drug on the mouse was similar, whereas in the rabbit, Sgd 101/75 was a partial agonist relative to noradrenaline (intrinsic activity 0.50) and the EC_{30} value was 14.5 times higher than for noradrenaline.

3 In both species, phenoxybenzamine (0.3 nM for 30 min followed by 20 washes over 30 min) reduced the effects of Sgd 101/75 preferentially. When noradrenaline was then added in the presence of a high concentration of Sgd 101/75 (400 μM), its maximum response and EC_{30} value were decreased in the rabbit, but not in the mouse.

4 When a range of concentrations of phenoxybenzamine (30 pM–3 μM for 30 min followed by 20 washes over 30 min) was tested, the maximum response of the rabbit anococcygeus to noradrenaline declined in a concentration-dependent manner. Similar experiments in the mouse resulted in two distinct sensitivities of the maximum response of noradrenaline to phenoxybenzamine (at 0.3 and 300 nM).

5 The results indicate that noradrenaline acts on two receptors to contract the mouse anococcygeus (α_1 and α_{1s}-adrenoceptors), but only one (α_1-adrenoceptor) in preparations derived from the rabbit.

Introduction

The rat anococcygeus preparation contracts in response to sympathomimetic agents. Recent evidence indicates that two adrenoceptors are activated in the tissue, although both resemble in some respects the α_1-adrenoceptor subtype (McGrath, 1982; Coates, Jahn & Weetman, 1982). Coates *et al.* (1982) designated one subtype as an α_{1s}-adrenoceptor on the basis that it could be activated specifically by Sgd 101/75 (4(2-imidazoline-amino)-2-methylindazol-chlorhydrate) and blocked by very low concentrations of phenoxybenzamine. Noradrenaline also activated the α_{1s}-adrenoceptor, about 30% of the maximal response being via this receptor. We now provide evidence for the existence of α_{1s}-adrenoceptors in the mouse but not in the rabbit anococcygeus.

Methods

Anococcygeal preparations were dissected from male albino mice (20–30 g) or New Zealand White rabbits (2–4 kg) by the methods of Gibson (1981) and Creed, Gillespie & McCaffery (1977). Whereas both muscles were used to make one preparation in the mouse, the muscles were divided in the rabbit to provide paired preparations.

Tissues were set up in isolated organ baths (10 ml) maintained at 37 ± 1°C and containing McEwen's solution (McEwen, 1956) of the following composition (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. The McEwen's solution was gassed with 95% O_2 and 5% CO_2. The tension in the muscles was measured with a Grass FT 03C force-displacement transducer and displayed on a Grass 79D polygraph. The initial tension on the muscles was 125 mg for the mouse and 0.5 g for the rabbit.

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), or in some cases up to 400 μM Sgd 101/75. Phenoxybenzamine was incubated with preparations for 30 min at the con-

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centrations indicated in the text and then washed from the bath over the following 30 min (20 changes in McEwen's solution).

In preparations from mice, only one concentration-response curve to a stimulant drug was obtained on each tissue, because repeated curves resulted in a depression of the maximum contraction. Although no such depression occurred in the rabbit anococcygeus, the same procedure was adopted, but in this species comparisons were made on pairs of preparations derived from one animal. In some phenoxybenzamine-treated preparations, Sgd 101/75 was administered first followed by noradrenaline without washing out the Sgd 101/75 (400 μM).

Individual concentration-response curves for agonists were plotted and the EC_{30} value (concentration producing a tension equivalent to 30% of the control maximum to noradrenaline) 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).

Drugs

Sgd 101/75 (Siegfried AG), (−)-noradrenaline bitartrate (Sigma) and phenoxybenzamine hydrochloride (S.K. & F.) were used.

Drugs were dissolved in distilled water. Solutions of noradrenaline contained approximately 50 μg/ml ascorbic acid (B.D.H.). Stock solutions of phenoxybenzamine were prepared by dissolving 50 mg in 0.5 ml absolute ethanol and were made up to 5 ml with distilled water and one drop of 1M HCl to remove turbidity. Subsequent dilutions were made in distilled water.

Results

Response of the anococcygeus to noradrenaline and Sgd 101/75

Both noradrenaline and Sgd 101/75 contracted mouse and rabbit anococcygeus preparations (Figure 1), although the relative sensitivity and maxima of

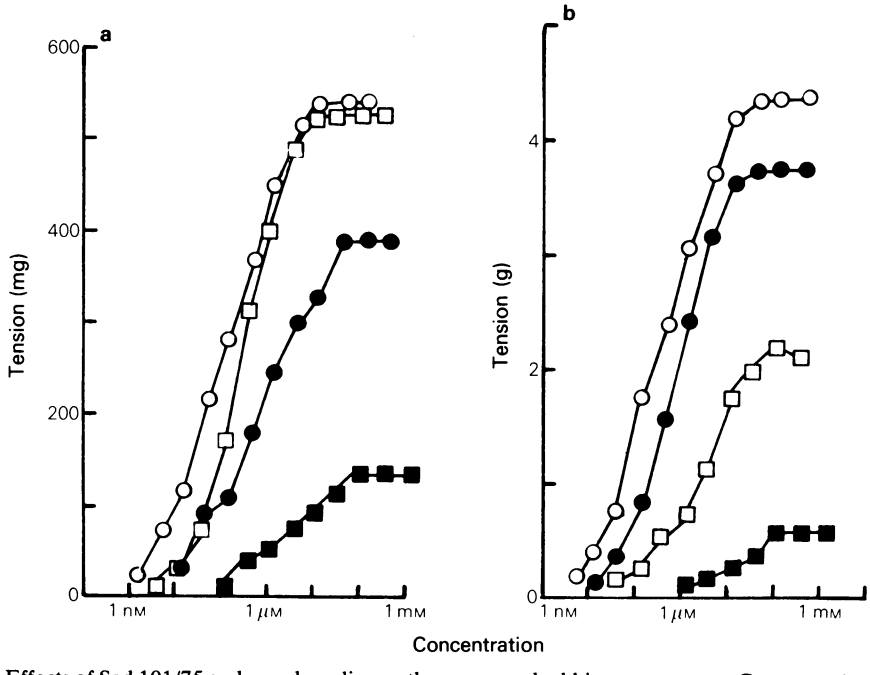

Figure 1 Effects of Sgd 101/75 and noradrenaline on the mouse and rabbit anococcygeus. Concentration-response curves for noradrenaline (○) and Sgd 101/75 (□) on mouse (a) and rabbit (b) untreated preparations. In a separate series of experiments, preparations were incubated with phenoxybenzamine (0.3 nM for 30 min followed by 20 washes over 30 min) before either noradrenaline (●) or Sgd 101/75 (■) was applied. Note that Sgd 101/75 is a full agonist in the mouse and a partial agonist in the rabbit anococcygeus, and that phenoxybenzamine exerts a greater antagonism against Sgd 101/75 in both preparations.

the drugs in the two species differed (sensitivity in the mouse, noradrenaline EC_{30} 64 ± 24 nM, $n = 8$; Sgd 101/75 EC_{30} 141 ± 61 nM, $n = 8$, $P > 0.05$: maximum response in the mouse, noradrenaline 541 ± 48 mg, $n = 8$; Sgd 101/75 522 ± 76mg, $n = 8$, $P > 0.05$: sensitivity in the rabbit, noradrenaline EC_{30} 147 ± 15nM, $n = 6$; Sgd 101/75 EC_{30} 2.13 ± 0.4μM, $n = 6$, $P < 0.001$: maximum response in the rabbit, noradrenaline 4.4 ± 0.48 g, $n = 6$; Sgd 101/75 2.21 ± 0.27 g, $n = 6$, $P < 0.005$). Thus in the mouse preparation, there was no significant difference between the maximal responses to noradrenaline and Sgd 101/75 whereas in the rabbit there was (intrinsic activity of Sgd 101/75 = 0.5). The sensitivity of the anococcygeus to Sgd 101/75 relative to noradrenaline also differed in the two species, being 2.12 times less in the mouse and 14.49 times less in the rabbit.

Sensitivity of the contractions to a low concentration of phenoxybenzamine

When the rat anococcygeus is treated with a low concentration of phenoxybenzamine (0.3 nM for 30 min followed by 20 washes over 30 min) it fails to contract to Sgd 101/75, whereas the effect of noradrenaline is only partially reduced (maximal response 30% less than in untreated tissues: Coates *et al.*, 1982). In this way, the low concentration of phenoxybenzamine could be used to reveal α_{1s}-adrenoceptor-mediated contractions.

Phenoxybenzamine (0.3 nM for 30 min followed by 20 washes over 30 min) reduced both the maximum effect (by 28%) and sensitivity (by 16.3 fold) of the mouse anococcygeus to noradrenaline (control maximum 541 ± 48 mg, $n = 8$; after phenoxybenzamine 390 ± 39 mg, $n = 5$, $P < 0.05$: control EC_{30} 64 ± 24 nM, $n = 8$; after phenoxybenzamine 1.04 ± 0.28 μM, $n = 5$, $P < 0.001$). In the mouse, phenoxybenzamine exerted a much greater effect against Sgd 101/75 than against noradrenaline (control maximum 522 ± 76 mg, $n = 8$; after phenoxybenzamine 131 ± 51 mg, $n = 5$, $P < 0.005$; control EC_{30} 141 ± 61 nM, $n = 8$; after phenoxybenzamine the sensitivity was reduced, but was not measurable because the maximum contraction was less than 30% of the control maximum for noradrenaline): see Figure 1a.

Phenoxybenzamine (0.3 nM for 30 min followed by 20 washes over 30 min) failed to reduce significantly the maximum response of the rabbit anococcygeus to noradrenaline, although the sensitivity was reduced (control maximum 4.4 ± 0.48 g, $n = 6$; after phenoxybenzamine 3.7 ± 0.58 g, $n = 6$, $P > 0.05$; control EC_{30} 147 ± 15 nM, $n = 6$; after phenoxybenzamine 750 ± 290 nM, $n = 6$, $P < 0.02$, Figure 1b). Contractions of the rabbit anococcygeus to Sgd 101/75 were reduced by phenoxybenzamine pretreatment (control maximum 2.21 ± 0.27 g, $n = 6$; after phenoxybenzamine 0.58 ± 0.17 g, $n = 6$, $P < 0.001$: sensitivity reduced but not measurable, Figure 1b). Thus in both species, the low concentration of phenoxybenzamine reduced the effects of Sgd 101/75 more than those of noradrenaline, and consequently this does not by itself constitute a useful test for the presence of α_{1s}-adrenoceptors in a preparation.

Interaction of Sgd 101/75 and noradrenaline in the phenoxybenzamine-treated anococcygeus

Although phenoxybenzamine did not differentiate between the effects of Sgd 101/75 on the two anococcygeal preparations, the subsequent addition of noradrenaline in the presence of a high concentration of Sgd 101/75 (400 μM) revealed two distinct types of interaction. In preparations from rabbits, contractions to noradrenaline in the presence of Sgd 101/75 exhibited a reduced maximum effect and were only achieved with higher concentrations of the catecholamine relative to paired control preparations (treated identically except that Sgd 101/75 was not present). Thus Sgd 101/75 antagonized noradrenaline (Table 1), a result consistent with the former's partial agonist effect (see Discussion). When a similar experiments were performed on the mouse anococcygeus, Sgd 101/75 (400 μM) failed to depress either the maximum response or the sensitivity to noradrenaline (noradrenaline in the presence of Sgd 101/75 413 ± 28 mg, $n = 5$; noradrenaline

Table 1 Interaction between Sgd 101/75 and noradrenaline in phenoxybenzamine-treated preparations

Species	*Excess Sgd 101/75* (400 μM Sgd 101/75 + control EC_{30} to Sgd 101/75)	*Maximum response to noradrenaline* (%) (in presence of 400 μM Sgd 101/75 relative to absence)	*Sensitivity to noradrenaline* (EC_{30} in presence of 400 μM Sgd 101/75 + EC_{30} in absence)
Rabbit	188	66 (< 0.005)	12.4 (< 0.001)
Mouse	2837	106 (> 0.05)	1.56 (> 0.05)
Rat	7143	99 (> 0.05)	1.84 (> 0.05)

A high concentration of a partial agonist should depress both the maximum effect and the sensitivity of a full agonist acting on the same receptor: this is the case in the rabbit, but not in the mouse or rat (rat data taken from Coates *et al.*, 1982). Statistical tests were performed on the values contained in the text, not the ratios.

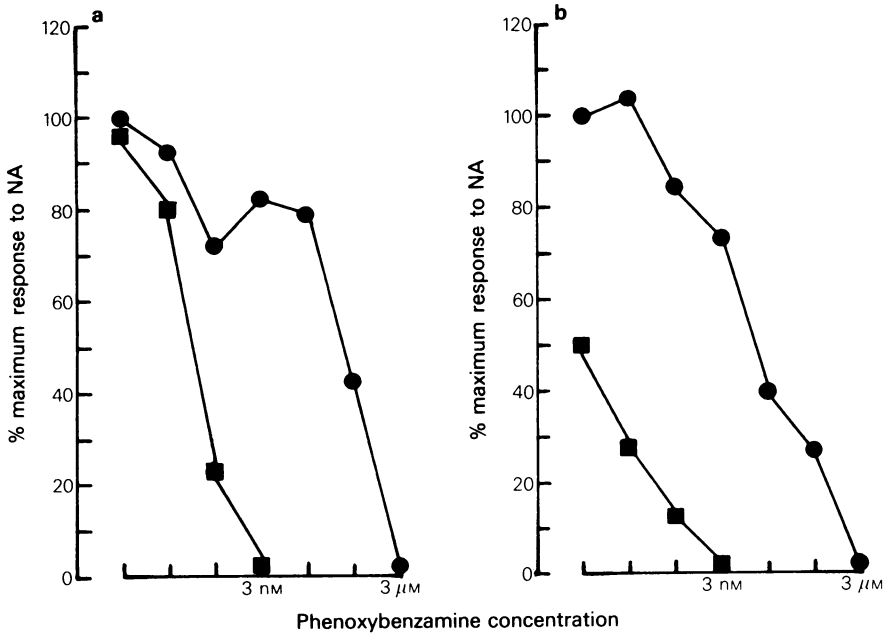

Figure 2 Antagonism of noradrenaline by phenoxybenzamine in the mouse and rabbit anococcygeus. Decline in the maximum response of the anococcygeus to noradrenaline (NA) (●) or Sgd 101/75 (■) due to 30 min incubation with phenoxybenzamine (followed by 20 washes in 30 min): the values are the mean of 4 experiments. Note that in the mouse there are two levels of sensitivity of the noradrenaline response to phenoxybenzamine, separated by a 100 fold increase in the concentration of the antagonist.

without Sgd 101/75 390 ± 39 mg, $n = 5$, $P > 0.05$: EC_{30}, noradrenaline in the presence of Sgd 101/75 1.62 ± 0.37 μM, $n = 5$; noradrenaline without Sgd 101/75 1.04 ± 0.28 μM, $n = 5$, $P > 0.05$: Table 1).

Effect of various concentrations of phenoxybenzamine on the maximum response to noradrenaline

When a series of mouse preparations was exposed to a range of concentrations of phenoxybenzamine (30 pM–30 μM for 30 min followed by 20 washes over 30 min), the maximum response to noradrenaline declined in three phases (Figure 2a). The first phase, which corresponded to the loss in response to Sgd 101/75, occurred with low concentrations of phenoxybenzamine (0.3 nM). Increasing the concentration of phenoxybenzamine failed to reduce the maximum response to noradrenaline further, until 300 nM phenoxybenzamine was used when a second level of inhibition was encountered. With similar experiments in rabbit tissues, the regression of decline in maximum response against concentration of phenoxybenzamine was monophasic (Figure 2b).

Discussion

Evidence is now presented describing the interaction of drugs with the adrenoceptors of rabbit and mouse anococcygeus. Although both preparations respond similarly to noradrenaline, distinct differences between them are seen when Sgd 101/75 is applied. This difference leads to the conclusion that more than one α_1-adrenoceptor must be involved in the response to noradrenaline in the mouse, but not in the rabbit. In the rat is was originally considered that sensitivity of the Sgd 101/75-induced contractions to a very low concentration of phenoxybenzamine represented a test for the second (i.e. α_{1s}-) adrenoceptor (Coates *et al.*, 1982). However, this can now be seen not to be a criterion for distinguishing between α_1-adrenoceptor subtypes, because the low concentration of phenoxybenzamine can inhibit contractions due to Sgd 101/75 by two mechanisms. In the rabbit, where Sgd 101/75 acts as a partial agonist, phenoxybenzamine blocks preferentially contractions to this drug by occluding receptors, revealing the reduced capacity of Sgd 101/75 to initiate a response. Thus a

reduction in the number of receptors available in the rabbit tissue, considered with the low efficacy of Sgd 101/75 relative to noradrenaline, causes the specific block. When the mouse anococcygeus is considered, phenoxybenzamine also causes a preferential reduction of the contractions to Sgd 101/75, but in this case, as shown below, the effect is due to antagonism of the α_{1s}-adrenoceptor. Clearly, phenoxybenzamine is not the ideal drug to differentiate between these dissimilar mechanisms, so the interaction between high concentrations of Sgd 101/75 and noradrenaline in phenoxybenzamine-treated preparations becomes critical in the development of the argument that there are two distinct α_1-adrenoceptor subtypes in the mouse anococcygeus.

In the rabbit anococcygeus, Sgd 101/75 acts as a partial agonist: the maximum effect was less than that of noradrenaline (intrinsic activity = 0.5), and the muscle in this species was not particularly sensitive to the imidazoline derivative (EC_{30} value is 14.5 times higher than for noradrenaline). After phenoxybenzamine (0.3 nM for 30 min followed by 20 washes over 30 min) the maximum response to Sgd 101/75 was reduced by 74%. It was then possible to contract preparations with noradrenaline in the continued presence of 400 μM Sgd 101/75. In this situation, the effect of noradrenaline was characterized by a significant reduction in maximum (34% less than in paired control preparations) and there was an increase in the noradrenaline EC_{30} value (12.4 times: see Table 1). This result is exactly that anticipated for a partial agonist, where the drug with the lower efficacy (Sgd 101/75) interferes with the action of a full agonist (noradrenaline) (Stephenson, 1956; Van Rossum & Ariëns, 1962: Thron, 1973).

A similar analysis conducted on the mouse anococcygeus preparation yielded quite different results. In untreated preparations, the maximum effects of noradrenaline and Sgd 101/75 were similar, and the EC_{30} value for Sgd 101/75 was only 2.2 times higher than that for noradrenaline. Phenoxybenzamine depressed the maximum response of Sgd 101/75 (by 75%) more than that of noradrenaline (28%), as in the rabbit. However, there was no evidence that a high concentration of Sgd 101/75 (400 μM) interfered with the capacity of noradrenaline to contract phenoxybenzamine-treated preparations. Neither the maximum effect (106%) nor sensitivity (dose-ratio = 1.56) of noradrenaline on the anococcygeus was depressed significantly by the high concentration of Sgd 101/75 (Table 1). It is worth noting that the excess of Sgd 101/75 relative to its control EC_{30} value in the mouse (2837 times) is 15 fold greater than in the rabbit (188 times). If Sgd 101/75 was acting on the receptor activated by noradrenaline, the interference with noradrenaline by Sgd 101/75 should have been greater in the mouse than in the rabbit. In fact, there was no interference. Clearly, in these experiments on the mouse anococcygeus, Sgd 101/75 and noradrenaline do not interact with the same receptor. This result is similar to that previously obtained in the rat anococcygeus (Coates *et al.*, 1982).

It is concluded that there are α_{1s}-adrenoceptors in the mouse but not the rabbit anococcygeus. Noradrenaline *can* activate α_{1s}-adrenoceptors: in the rat 31% of the maximum response is sensitive to the low concentration of phenoxybenzamine, and in the mouse 28% of the contractile capacity of the catecholamine is lost in phenoxybenzamine-treated preparations. However, much more phenoxybenzamine has to be employed to depress the maximum response to noradrenaline to any great extent. When a range of phenoxybenzamine concentrations was tested on the mouse anococcygeus preparation, the regression of the maximum response to noradrenaline against concentration of phenoxybenzamine showed three phases. First there was a decline in maximum response, which occurred at the low concentration of phenoxybenzamine that antagonized Sgd 101/75. The second component of the 'regression' was a plateau, where increasing the concentration of phenoxybenzamine failed to produce more block of noradrenaline. Finally, a second concentration-related inhibition of noradrenaline by phenoxybenzamine was obtained. This curve is indicative of noradrenaline activating two receptors of differing sensitivities to phenoxybenzamine. In contrast, in the rabbit anococcygeus, the regression of maximum effect to noradrenaline against concentration of phenoxybenzamine is monophasic, indicating that in this species only the α_1-adrenoceptor is activated by noradrenaline. Thus these results support the conclusions drawn from the interaction of Sgd 101/75 with noradrenaline in preparations treated with low concentrations of phenoxybenzamine. There are two α_1-adrenoceptors that subserve contractions of the mouse anococcygeus to sympathomimetic agents, but only one in the rabbit.

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