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THE EXISTENCE OF A NEW SUBTYPE OF α-ADRENOCEPTOR ON THE RAT ANOCOCCYGEUS IS REVEALED BY SGD 101/75 AND PHENOXYBENZAMINE

JILL COATES, U. JAHN* & D.F. WEETMAN

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Sunderland Polytechnic, Sunderland SR1 3SD, Tyne and Wear and Department of Pharmacology*, Siegfried AG, CH-4800 Zofingen, Switzerland

1 Noradrenaline and Sgd 101/75 (4(2-imidazoline-amino)-2-methylindazol-chlorhydrate) acted as full agonists in contracting the rat anococcygeus.

2 Very low concentrations of phenoxybenzamine (0.3 nM for 2–30 min) reduced preferentially the effects of Sgd 101/75. Preparations made insensitive to Sgd 101/75 by phenoxybenzamine still contracted to noradrenaline, this contraction occurring in the presence of high concentrations (400 μM) of Sgd 101/75.

3 It is concluded therefore that the α_1-adrenoceptor in this preparation is not a homogeneous entity and must be subdivided into at least two subtypes.

Introduction

Sgd 101/75 (4(2-imidazoline-amino)-2-methylindazol-chlorhydrate), a derivative of clonidine, is a full agonist (rat anococcygeus), partial agonist (guinea-pig taenia caecum) or antagonist (coaxially stimulated guinea-pig ileum) on the α-adrenoceptors of various smooth muscle preparations (Ismail, Jahn & Weetman, 1981). We now describe the interaction of Sgd 101/75 and phenoxybenzamine on the rat anococcygeus preparation.

Methods

Anococcygeal muscles were dissected from Sprague-Dawley rats (230–270 g) by the method of Gillespie (1972) and suspended in isolated organ baths (10 ml) containing 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 phenoxybenzamine was administered (2–30 min incubation); preparations were then washed 20 times over 30 min before a contractile drug was administered.

Individual concentration-response curves for agonists were plotted and the EC_{50} value (concentration producing a tension equivalent to 50% of the control maximum) and maximum response were measured. The slope of control curves was measured as the ratio of the concentrations causing 80 and 20% maximal effects. 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, after checking the homogeneity of the variances (Snedecor & Cochran, 1967).

Drugs

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

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 1 M HCl to remove turbidity. Subsequent dilutions were made in distilled water.

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.

Results

A comparison of the concentration-response curves

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for the two agonists on 25 paired preparations indicated that the anococcygeus was more sensitive to Sgd 101/75 than to noradrenaline. The EC_{50} values for noradrenaline and Sgd 101/75 were respectively: 438 ± 5 nM, and 130 ± 5 nM, ($P < 0.05$), whereas the slopes (2.31 ± 0.2 and 2.66 ± 0.21, $P > 0.05$) and maxima (6.75 ± 0.4, 6.53 ± 0.44, $P > 0.05$) were not significantly different (Figure 1a). Preliminary experiments revealed that the response of the anococcygeus to Sgd 101/75 was particularly sensitive to phenoxybenzamine (< 1 nM), and that the antagonism could not be reversed by washing (120 washes over 3 h, $n = 4$).

When untreated preparations were exposed to a low concentration of phenoxybenzamine (0.3 nM for 2–30 min), the response of the anococcygeus to Sgd 101/75 was blocked to a greater extent than that to noradrenaline (Figure 1b and c). After 2 min contact with phenoxybenzamine, the maximum response to noradrenaline was unchanged (6.80 ± 0.69 g compared with a control value of 6.75 ± 0.4 g, $n = 6, 25$), whereas that to Sgd 101/75 (4.26 ± 0.7 g, control 6.53 ± 0.44 g, $n = 6$, 25, $P < 0.02$) was reduced. There was a reduction in the maximum response to both drugs when the incubation was increased to 30 min (noradrenaline 4.66 ± 0.57 g, $n = 5$, $P < 0.02$: Sgd 101/75 1.1 ± 0.3 g, $n = 5$).

Contraction of the anococcygeus to Sgd 101/75

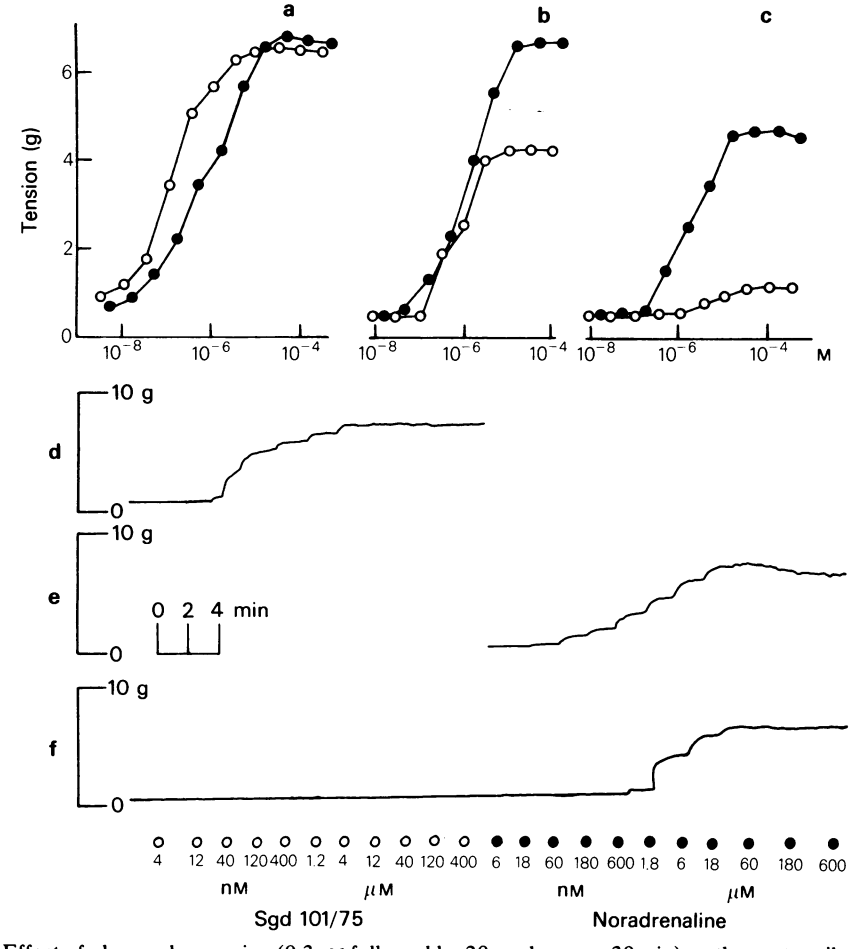

Figure 1 Effect of phenoxybenzamine (0.3 nM followed by 20 washes over 30 min) on the contractile response of the rat anococcygeus to noradrenaline (●) and Sgd 101/75 (○): (a) mean responses in 25 untreated paired preparations; (b) responses in 6 paired preparations treated with phenoxybenzamine for 2 min; (c) as (b) but after 30 min treatment with phenoxybenzamine, $n = 5$. The traces show control concentration-response curves for Sgd 101/75 (d) and noradrenaline (e). (f) The anococcygeus was first treated with phenoxybenzamine (0.3 nM for 30 min), washed (20 times over 30 min), then Sgd 101/75 (○) was added, and finally noradrenaline (●), without washing out the Sgd 101/75. This experiment was taken from a series not quoted in the text but illustrates the ability of noradrenaline to contract preparations that are totally unresponsive to Sgd 101/75.

was abolished by 3 nM phenoxybenzamine for 30 min ($n=4$), but with noradrenaline a much higher concentration was required (3 μM for 30 min, $n=3$). A 52% reduction in the maximum contraction of the anococcygeus to noradrenaline was obtained after 300 nM phenoxybenzamine for 30 min, ($n=4$). Thus to obtain approximately equal degrees of antagonism, it was necessary to employ over 1000 times more phenoxybenzamine for noradrenaline than for Sgd 101/75.

Finally, the effect of noradrenaline was examined in preparations made relatively insensitive to Sgd 101/75 by treatment with phenoxybenzamine (0.3 nM for 30 min). Although this treatment markedly reduced the maximum response to Sgd 101/75 (to 1.02 ± 0.28 g, $n=5$), subsequent addition of noradrenaline without washing out the Sgd 101/75 (400 μM) resulted in a further contraction of the anococcygeus (noradrenaline maximum effect 4.63 ± 0.6 g, $n=5$: Figure 1f), and the sensitivity was only slightly reduced (EC_{50} 8.35 ± 0.32 μM, compared with 4.3 ± 0.23 μM in preparations treated with phenoxybenzamine).

Discussion

Sgd 101/75 exerted a full agonist effect on the α-adrenoceptors of the rat anococcygeus preparation, producing a maximum effect not significantly different from that of noradrenaline, but with 3 times the sensitivity. Ismail *et al.* (1981) concluded that Sgd 101/75 contracted the rat anococcygeus by an action on α-adrenoceptors, because phentolamine yielded the same pA_2 value with both stimulant drugs. We have since shown that the contractions of the anococcygeus to Sgd 101/75 are blocked by prazosin (pA_2 8.7, slope not significantly different from 1, $P > 0.05$, $n=12$: Coates & Weetman, unpublished). However, it is now clear that Sgd 101/75 and noradrenaline contract the anococcygeus by different mechanisms, by virtue of the specific block of the contractions to the former by low concentrations of phenoxybenzamine. The intensity of this treatment (0.3 nM phenoxybenzamine for 2–30 min) is much less than that previously employed by Foster & Jones (1981) to block α_1-adrenoceptors: they found that 6 nM phenoxybenzamine for 30 min reduced contractions of the anococcygeus to noradrenaline by 50%. It is unlikely that phenoxybenzamine is interacting with other adrenergic mechanisms in blocking the contractions of the anococcygeus to Sgd 101/75 because of the low concentration of the antagonist employed. Blockade of α_2-adrenoceptors only occurs after incubation of the anococcygeus with 20 nM phenoxybenzamine (65 times higher than the concentration that blocks contractions to Sgd 101/75: Leighton, Butz & Parmeter, 1979), whereas inhibition of neuronal uptake of noradrenaline requires 0.5 μM (1500 times higher), and extraneuronal 4 μM (13000 times higher: Foster & Jones, 1981).

It could be argued that our results are due to differing efficacies of Sgd 101/75 (low) and noradrenaline (high) in activating a single receptor. For this to be so, there would have to be a receptor reserve for noradrenaline, to allow for its lower occupancy relative to Sgd 101/75. This is not the case since when the receptor reserve was measured as the increase in noradrenaline EC_{50} before the maximum response of the anococcygeus to noradrenaline was depressed, the increase was less than two. The critical result was the demonstration that noradrenaline was able to exert a considerable contraction (maximum effect 69% of the untreated preparations, and 99% of the paired control tissues treated with the low concentration, 0.3 nM for 30 min, of phenoxybenzamine) in preparations with greatly reduced responsiveness to Sgd 101/75. Furthermore, the very high concentration of Sgd 101/75 (3000 times the control EC_{50} value) used in these experiments reduced the sensitivity of the anococcygeus to noradrenaline less than two fold. Such a low affinity of Sgd 101/75 for the receptors activated by noradrenaline in contracting the anococcygeus precludes the conclusion that the two drugs interact with a common receptor. It is also unlikely that Sgd 101/75 contracts the anococcygeus by an indirect sympathomimetic effect because desmethylimipramine (350 nM) failed to increase the sensitivity of the tissue to Sgd 101/75 (reciprocal dose-ratio (RDR) = 1.1, $n=6$), whereas the sensitivity to noradrenaline was increased (RDR = 84.5, $n=6$).

The adrenoceptor subtype activated by Sgd 101/75 must next be considered. A stimulant action by Sgd 101/75 on α_2-adrenoceptors is not possible for three reasons. First, the concentrations of phenoxybenzamine found to block Sgd 101/75-induced contractions of the anococcygeus are too low to block α_2-adrenoceptors (Leighton *et al.*, 1979). Secondly, Sgd 101/75 is a competitive antagonist of the α_2-adrenoceptors on the coaxially stimulated guinea-pig ileum (Ismail *et al.*, 1981), and the rat vas deferens (Turner & Weetman, unpublished). Finally, there are few α_2-adrenoceptors on the smooth muscle of the rat anococcygeus (Docherty & Starke, 1981). Thus Sgd 101/75 contracted the rat anococcygeus by an action on a new subtype of α_1-adrenoceptor. This structure, which we would like to designate α_{1s}, is characterized by high sensitivity to Sgd 101/75 and phenoxybenzamine. Phentolamine does not discriminate between the α_1-adrenoceptor subtypes. The small part of the contractile response of the anococcygeus to noradrenaline that is blocked by the very low concentration of phenoxybenzamine (i.e.

about 30% of the maximal response) is achieved via an action on the α_{1s}-adrenoceptor, but the remainder of the contraction is due to activation of distinct α-adrenoceptors.

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