to relate to body weight of the animal, it did not yield any significant correlation with CL_p. Therefore, the present results suggest that the age-dependence in the first-pass pulmonary clearance of propranolol may not be largely related to the age-dependent lung blood flow, but predominantly to the immaturity and senescence in pulmonary uptake capacity which has been proposed to be relatively high by Iwamoto et al (1987a).

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Agonist profile of ergometrine (ergonovine) on a population of postsynaptic α-adrenoceptors

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Abstract—Ergometrine (0·02–5 μM) produced concentration-related contractions of the mouse anococcygeus muscle, which were unaffected by cocaine (2 μM) or by pretreatment of mice with 6-hydroxydopamine. Contractions were reduced by α-adrenoceptor antagonists; the rank order of potency was prazosin > phentolamine > yohimbine. With phenoxybenzamine as antagonist, the estimated dissociation constant (K_D) for ergometrine was 0·41 μM. It is concluded that ergometrine causes direct activation of postsynaptic α_1-adrenoceptors, and it is suggested that it acts on the same subtype of the receptor as imidazoline agonists.

Ergometrine (ergonovine) is used therapeutically in the management of postpartum haemorrhage and, more controversially, as a diagnostic agent for the detection of variant forms of angina (Editorial 1982). In both cases, the relevant pharmacological property is smooth muscle contraction, either of the uterus or the coronary arteries. However, the nature of the receptors activated by ergometrine, and in particular the role of α-adrenoceptors, remains a matter of contention (Muller-Schweinitzer & Weidmann 1978; Sakanashi & Yonemura 1980; Brazenor & Angus 1981). Experiments on neural tissue have suggested that ergometrine may act as a partial agonist on α_2-adrenoceptors (Marshall et al 1977; Brown & Caulfield 1979), but few studies have focussed on α-adrenoceptor interactions on smooth muscle. Apart from the uterus and coronary arteries, ergometrine is generally considered to have little contractile effect on smooth muscle (Bowman & Rand 1980). However, during an investigation of α-adrenoceptor function in the mouse anococcygeus muscle, it was found that ergometrine produced strong contractions which were reduced by phentolamine, suggesting α-adrenoceptor activation (Gibson unpublished observation). In the present communication, we examine these contractions in more detail. In particular, the experiments were designed to clarify two points. First, the anococcygeus muscle is very sensitive to contraction by indirect sympathomimetics (Gillespie 1981; Gibson & Wedmore 1981) and therefore experiments were carried out to determine whether ergometrine-induced contractions of the mouse anococcygeus were direct or indirect. Secondly, the nature of the α-adrenoceptor activated by ergometrine was determined.

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Methods

Male mice (LACA strain; 25–35 g) were killed by stunning and exsanguination. The paired anococcygeus muscles were dissected out and set up in series, joined at the ventral bar, in 1 mL glass organ baths containing Krebs-bicarbonate solution (composition mM: NaCl 118·1; KCl 4·7; $MgSO_4$ 1·0; KH_2PO_4 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 25·0; glucose 11·1) maintained at 37°C and gassed continuously with 95% O_2:5% CO_2. A resting tension of 200–400 mg was placed on the tissue and changes in tension recorded with a Grass FTO3 force-displacement transducer attached to a Lectromed pen-recorder. Muscles were allowed to equilibrate for 45 min before the experiment was begun.

Ergometrine was added to the organ bath in volumes not exceeding 50 μL and was left in contact with the tissue for 5 min or until any consequent rise in tone had reached a peak. Following washout, further concentrations of agonist were not added until muscle tone had returned to baseline. pD_2 values (−log of the molar concentration of agonist producing 50% of the maximum response, Ariëns & van Rossum 1957) were calculated by regression analysis of the straight line portion of the concentration-response curve (between 20–80% of the maximum response).

Antagonist drugs of the competitive type (prazosin, phentolamine, yohimbine) were added to the Krebs reservoir at the appropriate concentration and were in contact with the tissue for 30 min before testing their effect on ergometrine sensitivity.

Antagonist pA_2 values were calculated by regression analysis of Schild plots (Arunlakshana & Schild 1959) obtained by repeating ergometrine concentration-response curves in the presence of increasing concentrations of antagonist. Three concentrations of antagonist were studied on each tissue (only one antagonist per preparation), the concentration range being at least ten-fold. In control experiments, it was found that up to four ergometrine concentration-response curves could be repeated without significant alteration in sensitivity. No blockers of uptake processes or of β-adrenoceptors were present; early experiments revealed that ergometrine was not subject to neuronal uptake (see Results), and neither extraneuronal uptake nor β-adrenoceptors are important influences in the mouse anococcygeus (Gibson & Wedmore 1981).

The dissociation constant (K_D) for ergometrine was calculated by the method of Furchgott (1966). After determination of a control concentration-response curve, tissues were incubated with phenoxybenzamine (10–100 nM) for 10 min, and then washed repeatedly for 1 h. A second ergometrine concentration-response curve was then constructed which was to the right of the first, with depressed slope and maximum response. Regression analysis of the plot of 1/[A] upon 1/[A'], where A and A' represent equieffective concentrations of ergometrine before and after phenoxybenzamine incubation, respectively, yielded an estimate of K_D from the equation, $K_D = (\text{slope} - 1)/\text{intercept}$.

Some animals were pretreated with 6-hydroxydopamine (6-OHDA; 2×50 mg kg^{-1} on day 1, 2×100 mg kg^{-1} on day 4, muscle sensitivity measured on day 5). This treatment schedule has been shown to produce an effective sympathectomy of the mouse anococcygeus (Gibson & Wedmore 1981).

Drugs used were: cocaine hydrochloride (May & Baker); ergometrine maleate (Sigma); 6-hydroxydopamine hydrobromide (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); phentolamine mesylate (Ciba); prazosin hydrochloride (Pfizer); yohimbine hydrochloride (Aldrich).

Statistical analysis was by Student's *t*-test.

Results

Ergometrine (0·02–5 μM) produced concentration-related, sustained contractions of the mouse anococcygeus; peak tension increases for each concentration occurred within 3 min and were readily reversed by washout (Fig. 1). Analysis of several such concentration-response curves gave an EC50 of 0·33 μM and a maximum tension increase of 660 mg (Table 1); the maximum response to ergometrine is therefore similar to responses previously recorded for other sympathomimetic drugs in this tissue (Gibson & Yu 1983). The parameters of the concentration-response curve to ergometrine were unchanged in the presence of cocaine (2 μM) or in muscles from mice pretreated with 6-OHDA (Table 1).

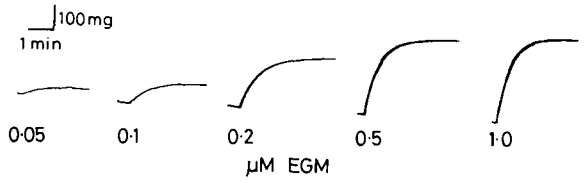

FIG. 1. Contractions of a mouse anococcygeus muscle to increasing concentrations of ergometrine (EGM). The time interval between each response was 30 min.

Table 1. Parameters of the concentration-response curve to ergometrine.

	pD_2	Slope	Maximum response (mg)
Control (9)	6·48 ± 0·03	457 ± 56	660 ± 49
Cocaine (6)	6·51 ± 0·04	477 ± 33	653 ± 28
6-OHDA-pretreated (6)	6·54 ± 0·08	383 ± 51	580 ± 81

Values represent mean ± s.e. Numbers in parentheses represent number of muscles studied in each group.

Ergometrine-induced contractions were reduced by α-adrenoceptor antagonists. The order of potency, in terms of the pA_2 values, was prazosin > phentolamine > yohimbine (Table 2). In all three cases the slope of the Schild plot did not differ from unity.

Table 2. pA_2 values and slopes of Schild plots for some α-adrenoceptor antagonists against ergometrine (n = 6 in each case).

	pA_2 (mean ± s.e.)	Slope (95% confidence limits)
Prazosin	8·82 ± 0·04	1·0 (0·89–1·11)
Phentolamine	8·53 ± 0·16	1·08 (0·92–1·24)
Yohimbine	6·67 ± 0·24	1·06 (0·96–1·16)

Using phenoxybenzamine (Furchgott 1966), the estimated K_D value for ergometrine was 0·41 μM; indeed, there was no significant difference ($P > 0·05$) between the pK_D value (6·39 ± 0·14 n = 5) and the pD_2 value (6·48 ± 0·03 n = 9, Table 1).

Discussion

Pretreatment of mice with 6-OHDA has been shown to reduce greatly the contractions of the anococcygeus to indirectly acting sympathomimetics (Gibson & Wedmore 1981); in addition, such pretreatment, and incubation of the muscle with cocaine, potentiates responses to sympathomimetics which are inactivated by uptake into the presynaptic nerve terminals (Gibson & Wedmore 1981; Gibson & Yu 1983). In the present study, it was found that neither 6-OHDA pretreatment nor cocaine had any effect on contractions of the anococcygeus to ergometrine; thus, ergometrine-induced responses are due to direct activation of postsynaptic α-adrenoceptors and are not subject to modulation by neuronal uptake. Although it has been suggested that 10 μM cocaine might produce a non-selective potentiation of agonist responses in the rat anococcygeus, via a post-junctional mechanism (Doggrell & Waldron 1982), the present results seem to confirm that, in the mouse anococcygeus, 2 μM cocaine does not cause a widespread enhancement of responses, but rather potentiates only those agonists which are removed from the biophase by the presynaptic amine pump (Gibson & Wedmore 1981; Gibson & Yu 1983). In terms of the EC50, ergometrine is 5–10 times less potent than noradrenaline (after uptake block) in causing contractions of the mouse anococcygeus, although the maximum responses produced by the two agonists are similar (Gibson & Yu 1983). Since the pK_D value for ergometrine did not differ from the pD_2 value, it seems that there are few, if any, spare receptors for the drug in the tissue.

As expected, responses to ergometrine were reduced by α-adrenoceptor antagonists. The order of potency of the antagonists (prazosin > phentolamine > yohimbine) and their respective pA_2 values suggests that ergometrine acts on α_1-adrenoceptors (Timmermans & van Zweiten 1981). However, three recent reports have suggested that the α_1-adrenoceptor population of the mouse anococcygeus may consist of two sub-types, one interacting with phenethylamines (noradrenaline, phenylephrine) and the other with imidazolines (oxymetazoline, naphazoline). First, Coates & Weetman (1982) showed that phenoxybenzamine was a more potent antagonist of the imidazole derivative, Sgd 101/75, than of noradrenaline. Secondly, Large (1983) found that contractions of the mouse anococcygeus induced by noradrenaline were generally accompanied by

membrane depolarization, whereas those induced by naphazoline were not. Finally, Gibson & Yu (1983) demonstrated that, while yohimbine was equieffective as an antagonist against phenethylamines and imidazolines, both prazosin and phentolamine could differentiate between the two agonist groups in terms of their respective pA_2 values. We have therefore compared the pA_2 values against ergometrine obtained in the present study with those against noradrenaline and oxymetazoline obtained by Gibson & Yu (1983; Table 3). Clearly, the antagonist profile against ergometrine is the same as that against oxymetazoline, suggesting that ergometrine interacts with the imidazoline subtype.

Table 3. pA_2 values for some α-adrenoceptor antagonists against noradrenaline, ergometrine, and oxymetazoline.

	Noradrenaline	Ergometrine	Oxymetazoline
Prazosin	9·27±0·18 (6)*	8·82±0·04 (6)	8·76±0·09 (6)
Phentolamine	7·83±0·13 (6)*	8·53±0·16 (6)	8·32±0·08 (6)
Yohimbine	6·31±0·14 (5)	6·67±0·24 (6)	6·40±0·05 (6)

Values represent mean±s.e.; those for noradrenaline and oxymetazoline are taken from Gibson & Yu (1983). Numbers in parentheses represent the number of muscles studied in each group. *—values significantly different from ergometrine ($P<0·05$).

To conclude, the results have shown that ergometrine causes contraction of the mouse anococcygeus muscle by a direct activation of post-synaptic α_1-adrenoceptors, and the pA_2 values obtained with prazosin, phentolamine, and yohimbine strongly indicate that ergometrine interacts with a specific sub-type of the α_1-adrenoceptor previously identified as the site of interaction for imidazolines. A similar interaction between ergometrine and imidazoline sites on blood vessels might provide an explanation for the actions of the drug on coronary arteries. Both ergometrine (Kawachi et al 1984; Suyama & Kuriyama 1984) and imidazolines (Egleme et al 1984) produce stronger contractions of blood vessels after removal of endothelial cells; these cells release relaxant factors which offset the direct contractile effects of a variety of drugs on vascular smooth muscle (Bullock et al 1986). It may be that the increased sensitivity of coronary arteries to ergometrine observed in patients with variant angina (Editorial 1982; Freedman et al 1984; Mata Bourcart et al 1985) may reflect a change in the balance between these relaxant factors and the smooth muscle imidazoline receptors in diseased arteries.

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