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Supersensitivity of atherosclerotic rabbit aorta to ergometrine is mediated by 5-HT_2 receptors

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Abstract—The concentration response curve of ergometrine in aortae from rabbits fed a high cholesterol diet for 12 weeks is biphasic. The first phase of the biphasic curve is antagonized by ketanserin, spiperone and cyproheptadine, but not by prazosin. pK_B values are compatible with a 5-HT_2 receptor mediated effect. The second phase is shifted to the right by prazosin, ketanserin and spiperone but not by cyproheptadine. In this case the pK_B values are compatible with an α_1-adrenoceptor mediated effect. The concentration response curves for ergometrine and phenylephrine in aortae from control rabbits are monophasic and pK_B values again indicate an α_1-adrenoceptor mediated response. Thus, ergometrine contracts the aortae of normal and cholesterol-fed rabbits via activation of α_1-adrenoceptors. The supersensitivity observed in atherosclerotic strips seems to reflect the appearance of a high affinity component mediated by 5-HT_2 receptors.

Persistent hypercholesterolaemia in rabbits is known to induce atherosclerosis and to alter vascular responsiveness to ergometrine (ergonovine) (Henry & Yokoyama 1980; Yokoyama et al 1983; Heric & Tackett 1985). The hypersensitivity of atherosclerotic aortae to ergometrine was selectively antagonized by cyproheptadine (Yokoyama et al 1983). Since cyproheptadine binds with high affinity to the recognition site designated as 5-HT_2 (Leysen et al 1982), and since the receptor subtype mediating contraction of the rabbit aorta has been characterized as 5-HT_2 (Maayani et al 1984), these results would be consistent with hypersensitivity to ergometrine being mediated by 5-HT_2 receptors. This suggestion is based, however, on the results with a single 5-HT receptor antagonist, which apart from 5-HT_2 receptors also antagonizes 5-HT_{1C} receptors (Hoyer 1988). We now report the antagonistic effects of cyproheptadine and other 5-HT receptor antagonists on the contractile response to ergometrine of atherosclerotic rabbit aortae. The choice of the antagonists was such, that a distinction between a 5-HT_2 and a 5-HT_{1C} receptor mediated event would be possible. Ergometrine is used in clinical practice as an oxytocic agent and in a provocation test for Prinzmetal-type of angina. Since in both cases the receptor subtype involved in the clinical effect has not been defined, the present investigation could contribute to the understanding of the mode of action of ergometrine.

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Materials and methods

Male New Zealand white rabbits, with an initial weight between 2·0 and 2·5 kg, were randomized to two dietary groups of 15 animals each. One group was fed normal chow while the other group received pellets containing 2% cholesterol for 12 weeks.

The rabbits were killed with an overdose of pentobarbitone. The thoracic aorta was promptly excised and cleaned of surrounding tissue. Spiral strips of approximately 2·5 cm length and 3 mm width were cut and the endothelial layer removed. Strips were mounted under 2 g tension in Krebs solution bubbled with 95% O_2 and 5% CO_2 at 37°C. The composition of the Krebs solution was as follows [mM]: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $NaHPO_4$ 1·2, $NaHCO_3$ 25, glucose 11. Changes in tension were recorded with Gould force-displacement transducers attached to a pen-recorder (Texas Instruments).

After equilibration (2 h), the organs were repeatedly stimulated with a submaximal concentration of noradrenaline (0·1 μM) until constant contractions were obtained. Acetylcholine (3 μM) was administered during the last contraction to verify the absence of a functional endothelium (Furchgott & Zawadzki 1980). The tension was reduced to 1·5 g and a cumulative concentration response curve to ergometrine was determined. From one aorta 8 strips were obtained. One was used as control; the remaining 7 were randomly allocated to be incubated in cyproheptadine, ketanserin, spiperone (all at 0·1 μM) or prazosin (0·01 μM) 15 min before ergometrine. This parallel set-up had to be chosen, since between killing the animal and the completion of the ergometrine concentration response curve it took one complete working day. A variation in sensitivity across the 8 different strips was noted and therefore a full randomization of the experimental protocol was ensured. Responses to ergometrine were plotted as % of the contraction to the last administration of noradrenaline.

In a separate experiment, ergometrine (0·1 μM) was tested as an antagonist of 5-HT in aortae from control rabbits. In this case, prazosin (0·1 μM) was added to the Krebs solution, since it has been reported that in rabbit aortae high concentrations of 5-HT stimulate α_1-adrenoceptors (Purdy et al 1987).

Curves were fitted by computer using Feldman's equations for complex ligand-binding systems at equilibrium (Feldman 1972). These equations allow the determination of pD_2 values and

maximum contractile responses of single- or two (or more) superimposed concentration response curves. The improvement of the goodness of the fit, obtained by calculating a double instead of a single concentration response curve, was evaluated with the "extra sum of squares" principle (Rodbard 1974). From parallel shifts of the concentration response curve, pK_B values were calculated according to Furchgott (1972).

Drugs used were: ergometrine maleate (Sandoz); noradrenaline bitartrate (Hoechst); phenylephrine HCl and cyproheptadine HCl (Sigma); prazosin HCl (Pfizer); ketanserin and spiperone (spiroperidol) (Janssen Pharmaceutica).

Results

At the end of the feeding period the mean body weight of cholesterol-fed animals was $3{\cdot}30 \pm 0{\cdot}11$ kg and was not significantly different ($P > 0{\cdot}05$) from that of the control group ($3{\cdot}48 \pm 0{\cdot}09$ kg). During the preparation of aortae from cholesterol-fed animals atherosclerotic plaques were macroscopically visible. In control strips, the concentration-response curve to ergometrine was monophasic ($pD_2 = 6{\cdot}22 \pm 0{\cdot}07$; n = 9) with a mean threshold concentration [−logM] of $7{\cdot}12 \pm 0{\cdot}14$ (n = 9). In 10 out of 15 aortae from cholesterol-fed rabbits, the concentration-response curve to ergometrine clearly consisted of two phases (pD_2-high $= 8{\cdot}22 \pm 0{\cdot}11$; pD_2-low $= 5{\cdot}87 \pm 0{\cdot}09$; n = 10) and the threshold concentration was reduced significantly ($P < 0{\cdot}01$) to $8{\cdot}91 \pm 0{\cdot}10$ (n = 10). The maximum of the high affinity component amounted to approximately 20% of the maximum that ergometrine could achieve in the preparation (see Fig. 1). Aortae from cholesterol-fed animals in which the biphasic fit was not significantly improved over the monophasic fit (5 from 15), were not investigated further.

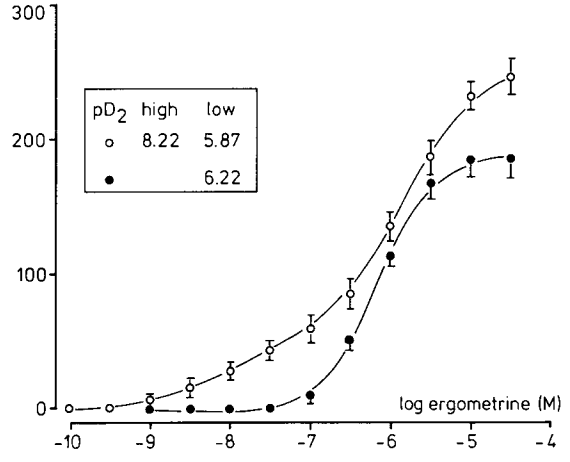

FIG. 1. Cumulative concentration-response curves for ergometrine on aortae from rabbits fed normal chow (●) or a 12 weeks diet containing 2% cholesterol (○). Data represent means ± s.e.m. (n = 9–10). The contractions are expressed as percentage of the response to noradrenaline (0·1 μM).

Monophasic concentration response curves to ergometrine and phenylephrine in control strips were shifted to the right by prazosin, ketanserin and spiperone and the effect of the antagonist was surmountable, thus allowing the calculation of pK_B values. The individual maximal effects of the two phases of the concentration response curve to ergometrine in atherosclerotic strips in the presence and the absence of antagonists were also investigated in order to assess whether the antagonist activity was surmountable. The first phase of the biphasic concentration response curve to ergometrine was shifted to the right by preincubation with all 5-HT receptor antagonists, but not by prazosin. A comparison of the calculated maxima of the first phase in the presence and the absence of antagonists is given in Table 1. With one antagonist (ketanserin) the calculated maximal response appeared to be significantly increased. The second phase was shifted to the right by prazosin, ketanserin and spiperone and was clearly surmountable (not shown). $-\text{Log } K_B$ values calculated from these shifts are given in Table 2. In the presence of prazosin (0·1 μM), ergometrine (0·1 μM) shifted the concentration response curve of 5-HT to the right in a parallel manner. From this displacement a pK_B value of $7{\cdot}67 \pm 0{\cdot}13$ (n = 6) was calculated.

Table 1. Maximal effects, expressed as percentage of the contraction to noradrenaline (0·1 μM), calculated for the first contractile component of the concentration response curves of ergometrine in aortae from cholesterol-fed rabbits in the presence or absence of antagonist.

	Control strip	Preincubated strip	*P*
Prazosin	23·9 ± 4·6 (7)	16·9 ± 6·0 (8)	0·167
Ketanserin	18·2 ± 7·2 (5)	44·5 ± 7·2 (6)	0·021
Spiperone	19·5 ± 8·7 (4)	45·2 ± 11·8 (5)	0·202
Cyproheptadine	14·7 ± 3·9 (3)	25·8 ± 8·2 (5)	0·076

Discussion

Acetylcholine produces relaxation of the rabbit aorta via the release of an endothelium derived relaxing factor (EDRF; Furchgott & Zawadzki 1980). Data by Griffith et al (1984) indicate that ergometrine can also relax rabbit aorta via EDRF release. It is conceivably possible that endothelium derived relaxation and high affinity contraction potentially could balance each other so that under circumstances in which endothelial function is unimpaired, the first phase of the contractile response to ergometrine would appear to be absent. However, in aortic strips from control rabbits in which the absence of a functional endothelium was established, the concentration response curve to ergometrine was monophasic and not biphasic.

Table 2. $-\log K_B$ values (± s.e.m.) for antagonist effects against ergometrine (EM) or phenylephrine (PE) in control and atherosclerotic aortae.

	Atherosclerotic (EM)			Control (EM)	Control (PE)
	high	low***	(n)	monophasic (n)	monophasic (n)
Prazosin		*/8·03 ± 0·16	(9)	8·48 ± 0·11 (8)	8·35 ± 0·11 (8)
Ketanserin	8·85 ± 0·32	/7·48 ± 0·22	(6)	7·52 ± 0·14 (8)	7·32 ± 0·16 (7)
Spiperone	9·02 ± 0·12	/7·71 ± 0·34	(5)	7·35 ± 0·16 (8)	7·01 ± 0·13 (8)
Cyproheptadine	8·10 ± 0·28	/*	(8)	7·01 ± 0·10 (8)**	6·52 ± 0·07 (8)**

* no significant shift in the concentration response curve.
** determined after preincubation with cyproheptadine (1 μM).
*** pK_B values for high affinity, respectively, low affinity component of the contractile response to ergometrine.

In the aortae of control animals the contractile response to ergometrine was antagonized by prazosin, ketanserin and spiperone, but only weakly by cyproheptadine. pK_B values calculated from the parallel shifts in the concentration response curves are in reasonable agreement with the pK_B values obtained from the antagonism of the selective α_1-adrenoceptor agonist, phenylephrine (Table 2). pA_2 values for these antagonists against α_1-adrenoceptor stimulants in rabbit aorta reported in the literature are also similar: prazosin $pA_2 = 8{\cdot}4 - 8{\cdot}8$ (summarized in Agrawal et al 1984); ketanserin $pA_2 = 7{\cdot}8$ (Feniuk et al 1983); spiperone $pA_2 = 7{\cdot}8$ (Feniuk et al 1983) and cyproheptadine $pA_2 = 6{\cdot}7$ (Apperley et al 1976). The predominant response of atherosclerotic aortae (approximately 80% of the total contraction) also seems to be mediated by α_1-adrenoceptors since pK_B values calculated from the parallel shift of this phase of the concentration response curve again yielded pK_B values similar to those obtained with phenylephrine as the agonist.

Our main interest however, was, to define the mechanism of the high affinity contractile response to ergometrine which is evident only in atherosclerotic aortae. Using atherosclerotic aortae, Henry & Yokoyama (1980) and Yokoyama et al (1983) observed that the contraction to a low concentration (1 nM) of ergometrine was resistant to blockade with α-adrenoceptor antagonists, but was inhibited by cyproheptadine (0·1 μM). As indicated in the introduction, this finding suggests that the hypersensitivity is mediated by 5-HT_2 receptors. However, on the basis of these data, a 5-HT_{1C} receptor mediated effect cannot be excluded. Furthermore, a non-specific inhibition (as can be seen with this concentration of cyproheptadine in the rabbit coronary artery; Yokoyama et al 1983), could not be ruled out either. For these reasons, we investigated the effects of other 5-HT receptor antagonists and tested whether the antagonism was surmountable. From receptor affinities (Hoyer 1988) the following 5-HT_2/5-HT_{1C} selectivity ratios can be calculated: cyproheptadine 4×, ketanserin 60× and spiperone 675×. With these antagonists it should thus theoretically be possible to determine the 5-HT receptor subtype involved.

Ketanserin, spiperone and cyproheptadine antagonized the high affinity phase with pK_B values close to 9. pA_2 values similar to the pK_B values from the present study, have been reported by Feniuk (1984) for 5-HT antagonism on the rabbit aorta and the receptor involved in this effect has been characterized as 5-HT_2 (Maayani et al 1984). The results thus indicate that the supersensitivity of atherosclerotic rabbit aortae to ergometrine is mediated by 5-HT_2 receptors. In one instance the antagonism of the first phase of the concentration response curve of ergometrine was found to deviate from parallel. After incubation with ketanserin the maximal effect of the first phase appeared to be increased (Table 1). This result is most likely an artefact due to the curve fitting procedure since this becomes less accurate when the ED50 values for the individual phases approach each other.

Why the 5-HT_2 receptor agonistic activity of ergometrine is only evident in atherosclerotic aortae is a matter of speculation. Data reported by Nanda & Henry (1982) may give an answer to this question. Those authors performed receptor binding experiments on membranes from rabbit aorta and observed that cholesterol-feeding led to a four-fold increase in the total number of 5-HT receptors; their affinity was not significantly altered.

Ergometrine, in concentrations which in atherosclerotic aortae induced a contraction, in control strips theoretically will occupy the 5-HT_2 receptor, but, without producing sufficient stimulus to induce contraction. In other words, in control strips ergometrine should be a 5-HT_2 antagonist. This was indeed observed. Ergometrine (0·1 μM) shifted the concentration response curve of 5-HT parallel to the right with a pK_B value of $7{\cdot}67 \pm 0{\cdot}13$ ($n = 6$). This pK_B value is lower than the pD_2 value of the agonist effect of ergometrine. However, both parameters cannot be simply compared since only the pK_B value represents a true receptor dissociation constant.

In summary, the present results suggest that ergometrine contracts the aortae of normal- and cholesterol-fed rabbits mainly via activation of α_1-adrenoceptors. The supersensitivity observed in atherosclerotic strips occurs independently of changes in endothelial function and appears to be mediated by 5-HT_2 receptors.

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