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Catecholamines act at α_2-adrenoceptors to cause contraction of circular smooth muscle of guinea-pig stomach

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Circular smooth muscle strips taken from the body region of the guinea-pig stomach responded to dopamine and noradrenaline with contraction at lower concentrations followed by relaxation at higher concentrations. A β-adrenoceptor-mediated relaxation response was excluded by propranolol treatment and this allowed the remaining α-adrenoceptor involvement with relaxation and contraction to be incisively differentiated in terms of two distinct α-adrenoceptor mechanisms. Thus, the relaxation responses to the catecholamines were mimicked by phenylephrine and antagonized by prazosin, phentolamine but not by yohimbine or rauwolscine. In contrast, the catecholamine-induced contractions were mimicked by clonidine and antagonized by yohimbine and phentolamine but not by prazosin. It is therefore concluded that the α mechanisms via which dopamine and noradrenaline are able to relax and contract the circular smooth muscle from the body region of guinea-pig stomach are of the α_1- and α_2-type respectively.

Noradrenaline and dopamine can cause both contraction and relaxation of the circular smooth muscle obtained from the guinea-pig stomach. The relaxations can be antagonized by propranolol and phentolamine, which would suggest an α- and β-adrenoceptor involvement, whilst the contractions are antagonized by phentolamine but not propranolol, suggesting only an α-adrenoceptor involvement (Costall et al 1981). In a number of tissues phentolamine exhibits both α_1- and α_2-adrenoceptor blocking action (see Borowski et al 1977) and thus the precise α-mechanisms cannot as yet be defined. Thus, selecting the body region of the stomach for its marked responsiveness to the contractile effects of noradrenaline and dopamine, the present studies were designed to use more selective α_1- and α_2-adrenoceptor agonists and antagonists in an analysis of the contractile-relaxation mechanisms of circular smooth muscle from guinea-pig stomach.

METHODS

Male Dunkin-Hartley guinea-pigs, 350–400 g, were killed by cervical trans-section and smooth muscle strips isolated from the body region of the stomach (15 mm × 5 mm). The strips were dissected in a plane to allow investigation of tension changes in the circular muscle layer. Tissues were bathed in 15 ml oxygenated (95% O_2; 5% CO_2) Krebs and Henseleit solution at 37 °C containing 100 mg litre^{-1} ascorbic acid under a resting tension of 1 g. Tension changes were detected by Grass isometric tension transducers and the response areas integrated (Illingworth & Naylor 1980) in addition to display on a multichannel Grass recorder. Tissues were allowed to equilibrate for 45 min before the addition of drugs.

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Determination of agonist potency

At the commencement of each experiment contraction/relaxation responses to dopamine and noradrenaline and relaxations to phenylephrine and isoprenaline were obtained to ensure tissue viability. For these four agonists there was no indication of changed sensitivity when two concentration-response curves were constructed at an interval of 45–60 min. (±)-Propranolol (5×10^{-7} M known to antagonize isoprenaline relaxation) was then routinely added in the Krebs and Henseleit solution to eliminate β-adrenoceptor-mediated relaxation responses to dopamine and noradrenaline. A contact time of 5 min was allowed for the full spectrum of α-adrenoceptor-mediated contraction and relaxation responses to dopamine, noradrenaline and phenylephrine to develop, and full concentration response analyses were carried out for each tissue used. Fresh tissues were used for each agonist. For clonidine, however, preliminary experiments showed its contraction responses to diminish on repeated treatment, and experiments using this agent were therefore limited both in the numbers of concentrations used and in the repetition of treatments with the selected concentrations (see below).

Determination of antagonist potency
Subsequent to testing tissue viability and carrying out full concentration-response analyses for dopamine, noradrenaline or phenylephrine in the presence of propranolol, the effects of phentolamine, prazosin, yohimbine or rauwolscine on the concentration-response curves to these agonists were determined. A 30 min pretreatment time was employed for each antagonist and a single response curve constructed in each tissue after the antagonist application. The second curve was compared to the first to assess antagonist potential. Fresh tissues were used to assess the effects of the different concentrations of antagonists. In the clonidine studies, the effects of a single dose of clonidine administered before and after antagonist administration to one preparation were compared with responses obtained to two doses of clonidine administered in an identical fashion in another preparation not treated with antagonist.

Statistical methods. Significant differences between data was assessed using the Mann Whitney U test.

Drugs. Dopamine hydrochloride (Koch-Light), noradrenaline hydrogen tartrate (Hoechst), phenylephrine hydrochloride (BDH) and isoprenaline sulphate (Riker) were prepared daily as stock solutions in distilled water containing 0·1% ascorbic acid. Clonidine hydrochloride (Boehringer Ingelheim), prazosin hydrochloride (Pfizer), yohimbine hydrochloride (Sigma) and rauwolscine hydrochloride (Carl Roth GmbH. & Co) were prepared in distilled water and phentolamine mesylate (Rogitine, Ciba-Geigy) was used in the form prepared by the manufacturers.

RESULTS

Dopamine, noradrenaline and phenylephrine action on the circular smooth muscle of stomach body: modification by α-adrenoceptor antagonists
Dopamine (10^{-6} – $2{\cdot}2 \times 10^{-5}$ M) caused concentration-dependent contractions of the circular smooth muscle of the stomach body with decline in contractions and development of small relaxations at higher concentrations (10^{-5} – 10^{-3} M) (Fig. 1); the contractions to noradrenaline were less marked (Fig. 2) and only relaxations were observed to phenylephrine (Fig. 3). Phentolamine at 10^{-8}, 10^{-7} and 10^{-6} M caused parallel shifts to the right in the concentration-response curves for contractions to dopamine (dose ratios 1·8, 5·9 and 22·7 respectively) (Fig. 1A) and markedly reduced the contractions to noradrenaline (Fig. 2A). The relaxations caused by noradrenaline and phenylephrine were only consistently antagonized at the highest concentration of phentolamine, 10^{-6} M (Figs 2A, 3A). In contrast to this finding with phentolamine, prazosin was virtu-

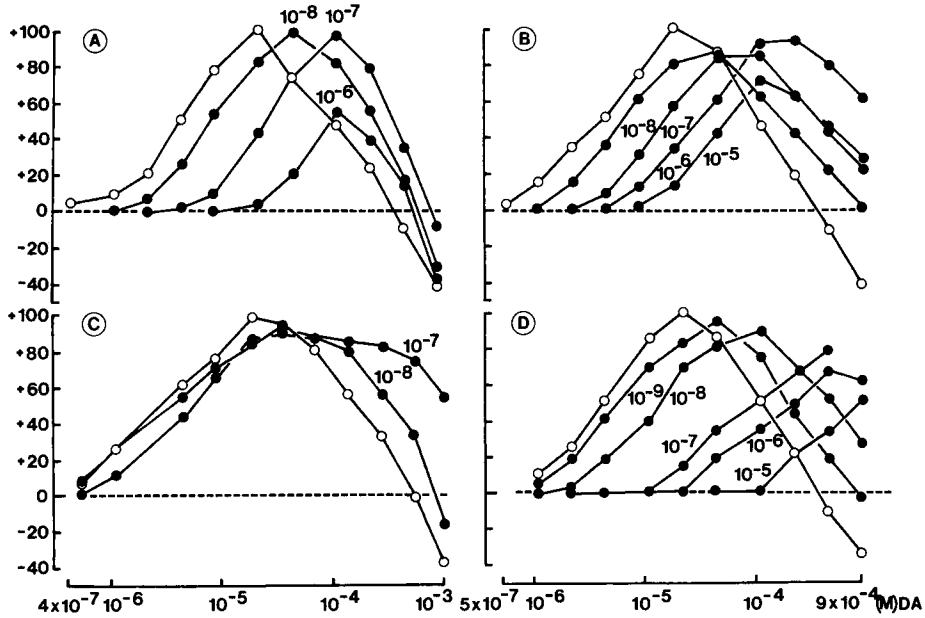

FIG. 1. Effects of A. phentolamine, B. yohimbine, C. prazosin and D. rauwolscine (●——●) on the contraction-relaxation response of the body strip to dopamine (DA) (○——○). The + values on the ordinate indicate contraction, the − values relaxation. The size of response was determined by integration of the area of response: values are expressed as a % of the maximum contraction to dopamine. n = 6, s.e.m.s <11%.

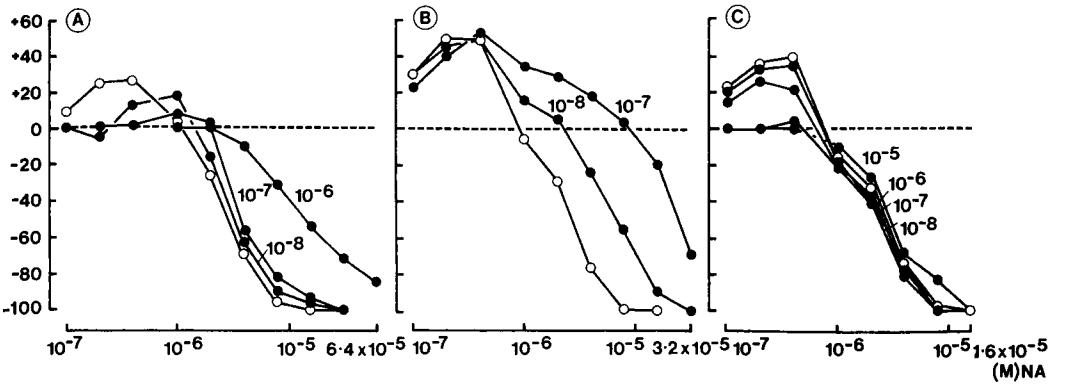

FIG. 2. Effects of A. phentolamine, B. prazosin and C. yohimbine (●——●) on the contraction-relaxation response of the body strip to noradrenaline (NA) (○——○). The + values on the ordinate indicate contraction, the − values relaxation. The size of response was determined by integration of the area of response: values are expressed as a % of the maximum relaxation to noradrenaline. n = 6. s.e.m.s <15%.

ally without effect to antagonize the dopamine and noradrenaline contractions but, at 10^{-8} and 10^{-7} M, did antagonize the decline in contraction and relaxations caused by both dopamine and noradrenaline (although the biphasic nature of the dopamine and noradrenaline responses confounds comment as to a possibility that the curves are subject to a parallel shift; for dopamine the dose ratios are 2·0 and 7·1 and for noradrenaline 2·5 and 9·3) (Figs 1C, 2B). The selective action of prazosin against the relaxation responses was particularly marked for phenylephrine whose response curves were shifted to the right (dose ratios 2·4 and 12·8) and for which the relaxations to lower concentrations (5×10^{-7} – 8×10^{-6} M phenylephrine) were actually reversed to contractions (Fig. 3B).

Yohimbine (10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) caused parallel shifts to the right of the concentration-response curves for contraction to dopamine (dose ratios 1·5, 3·5, 7·2 and 13 respectively); the displacement of the contraction curves to the right obscured the relaxant effect (Fig. 1B). A similar antagonist action was recorded for rauwolscine (dose ratios of 1·3, 3·3, 26, 56 and 209 for 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M respectively) (Fig. 1D). Yohimbine (10^{-8} – 10^{-5} M) also antagonized the modest noradrenaline contractions without affecting its relaxation potential (Fig. 2C). The phenylephrine-induced relaxations were also shown to be resistant to yohimbine antagonism excepting at the highest yohimbine concentration (10^{-5} M), although even this effect was modest.

Clonidine action on the body of the stomach: modification by adrenoceptor antagonists

Clonidine caused contraction of the circular smooth muscle from the body of the stomach, the concentrations producing a threshold response being in the order of 5×10^{-9} M. The decline in response to repeated administration precluded the construction of reproducible concentration-response curves. For example, in 6 tissues, the contractions to a submaximal concentration of $1·3 \times 10^{-7}$ M clonidine decreased by 10–15, 35–40 and 50–60% to the second, third and fourth exposures respectively. Relaxation responses to single or repeated administrations were never observed. The reduced responsiveness of the tissues to repeated clonidine treatment restricted an assessment of antagonistic action to the use of a single submaximal concentration of clonidine ($1·3 \times 10^{-7}$ M). Using 6 tissues per antagonist concentration, yohimbine and rauwolscine (10^{-9} – 10^{-7} M) were shown to antagonize the clonidine contraction response (Table 1); prazosin (10^{-7} M), haloperidol (10^{-6} M) and (±)-propranolol (10^{-7} M) were ineffective. In a study of the dopamine-clonidine interaction, a concentration-dependent contraction curve was initially established to dopamine (10^{-6} – 10^{-5} M) and the tissues then subjected to a clonidine (10^{-7} M) treatment for 45 min. Subsequent treatment with dopamine (10^{-6} – 10^{-4} M) failed to elicit any contraction response (data not shown).

DISCUSSION

Isolated preparations of the circular smooth muscle taken from the body of the guinea-pig stomach respond with a contraction to low concentrations of dopamine and noradrenaline, but with the development of relaxation at higher concentrations. The potential to induce a biphasic response complicates a characterization of the receptor types involved in

that measures of antagonist affinity defined as pA_2 values cannot be satisfactorily determined (Furchgott 1972). A second difficulty relating to pA_2 determinations is that both the biphasic and monophasic (relaxation to phenylephrine) responses to the agonists, whilst invariably modified in a competitive manner (i.e. parallel shifts in response curves and attainment of maximum responses), gave Arunlakshana & Schild (1959) plot regressions which were generally linear but, in all cases, having slopes considerably less than unity. This divergence may be related to the experimental protocol where agonist action on uptake sites, for example, varying at different concentrations, may influence agonist availability for receptor activation; the importance of this possibility is presently being assessed. However, such factors relate to difficulties in obtaining a *precise* measure of antagonist affinity and do not detract from the use of data obtained in a clarification of the nature of the α-adrenoceptors mediating both the relaxation and contraction of the stomach musculature under investigation.

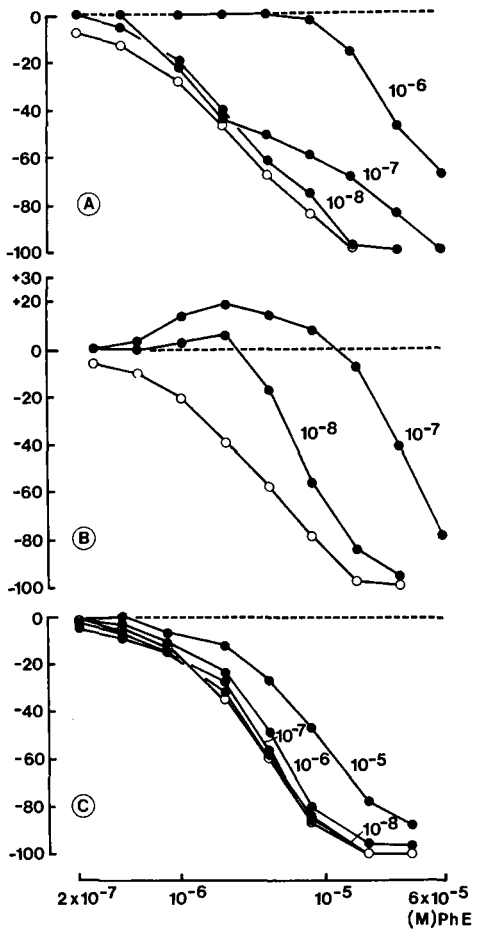

FIG. 3. Effects of A. phentolamine, B. prazosin and C. yohimbine (●——●) on the relaxation response of the body strip to phenylephrine (PhE) (○——○). The + values on the ordinate indicate contraction, the − values relaxation. The size of response was determined by integration of the area of response: values are expressed as a % of the maximum contraction to dopamine. n = 6. s.e.m.s <13%.

Table 1. Antagonist action against clonidine-induced contraction of the circular smooth muscle taken from the body region of guinea pig stomach.

Antagonist	Concentration (M)	% reduction in contraction response
—	—	9[a]
Yohimbine	10^{-9}	8
	10^{-8}	39**
	10^{-7}	88**
	10^{-6}	100**
Rauwolscine	10^{-9}	23*
	10^{-8}	64**
	10^{-7}	100**
Prazosin	10^{-6}	14
Haloperidol	10^{-6}	11
Propranolol	10^{-6}	13

[a] This reduction reflects a decline in tissue sensitivity (see text). Vehicle treatments did not cause significant change ($P > 0.05$) from the control value. Reductions by antagonists significant to * $P < 0.01$, ** $P < 0.001$ (Student's *t*-test).

In the present characterization of adrenoceptor mechanisms it is emphasized that any potential β-mediated relaxation response was excluded by propranolol treatment. The remaining α-adrenoceptor involvement with relaxation and contraction could then be incisively differentiated in terms of two distinct α-adrenoceptor mechanisms. Firstly, considering the relaxation response, this was obtained not only with dopamine and noradrenaline but also with phenylephrine which is accepted as having a highly selective α_1-agonist action in a number of tissues (Drew 1977). That an α_1-adrenoceptor type may be involved with noradrenaline and dopamine-induced relaxation was thus investigated using agents with relative selectivity for α_1- and α_2-adrenoceptors, prazosin and yohimbine/rauwolscine respectively (Starke et al 1977; Weitzell et al 1979). On the circular smooth muscle of the guinea-pig stomach body, prazosin, at a concentration of 10 nM, was shown to cause significant antagonism of the phenylephrine relaxation curve, indeed, the relaxation was reversed to a modest contraction response. At the same low concentration prazosin was shown to antagonize the noradrenaline and dopamine-induced relaxations. Phentolamine also antagonized the relaxations caused by all three

agonists but was required in higher (10 to 100 fold) concentrations. These antagonistic activities of prazosin and phentolamine contrast markedly with the ineffectiveness of yohimbine, notwithstanding the use of micromolar concentrations. Only at the very highest concentration of 10 μM yohimbine, where its specificity for α_2-adrenoceptors is very doubtful, was the phenylephrine relaxation modestly reduced. Even at this concentration, however, noradrenaline-induced relaxations were not antagonized and, notwithstanding the complex spectrum of the dopamine contraction-relaxation response, there was no indication that yohimbine or rauwolscine could preferentially antagonize its relaxation potential. It is therefore concluded that dopamine and noradrenaline relax the circular smooth muscle from the body region of guinea-pig stomach by stimulating α_1-adrenoceptors.

In contradistinction to the relaxation component, the marked contractile response to dopamine and the more modest contractile response to noradrenaline were resistant to prazosin but sensitive to yohimbine; the dopamine contraction response was also shown to be sensitive to rauwolscine. The nanomolar potency of yohimbine and rauwolscine to antagonize the contractions is entirely consistent with the order of potency of these two agents to inhibit α_2-type receptor sites in other tissues (see Drew 1977; Weitzell et al 1979). Further support for the presence of an α_2-type adrenoceptor within the body of the stomach was obtained using clonidine. Clonidine has potent agonist/partial agonist action (and a reasonable degree of selectivity) on the α_2-adrenoceptor and was shown to cause contraction of the circular smooth muscle of the guinea-pig stomach; relaxations were never recorded. The apparent loss of response to clonidine upon repeated treatment may reflect the partial agonist potential. Yohimbine antagonized the clonidine-induced contractions when used in concentrations similar to those required to antagonize the contractions to noradrenaline and dopamine. The clonidine contractions were resistant to prazosin, notwithstanding the use of concentrations clearly shown to antagonize the relaxation component. It is interesting that phentolamine which possesses α_1- and α_2-adrenoceptor blocking action was also effective in antagonizing noradrenaline, dopamine and clonidine contractions, indeed, phentolamine was at least ten times more potent in antagonizing the contractions than the relaxations. It is therefore concluded that the contractile responses to noradrenaline, dopamine and clonidine are mediated via α_2-adrenoceptors.

In summary, noradrenaline and dopamine can cause both contraction and relaxation of circular smooth muscle strips taken from the body of the guinea-pig stomach. In the presence of a β-adrenoceptor blockade, the relaxation and contraction responses are mediated via α_1- and α_2-type adrenoceptors respectively. Whilst many tissues contain α_1-adrenoceptors to mediate relaxation-contraction responses, the present studies indicate that the circular smooth muscle from the body of the guinea-pig stomach additionally contains an α_2-type adrenoceptor. This preparation may thus provide a convenient extravascular 'smooth muscle' model for the assessment of α_2-agonist-antagonist potential.

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