J. Pharm. Pharmacol. 1990, 42: 30–34
Received July 20, 1989

Relaxant Effects of α-Adrenoceptor Agonists in the Rat Isolated Gastric Fundus

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Abstract—In rat gastric fundus preparations with tone raised by the addition of barium chloride or carbachol, and in the presence of propranolol (2 μM) to prevent β-adrenoceptor mediated effects, the adrenoceptor agonists noradrenaline, adrenaline, α-methylnoradrenaline, isoprenaline, cirazoline and phenylephrine all caused concentration-related relaxant responses. Relaxations to the catecholamines were poorly antagonized by prazosin (0·01–1 μM) resulting in the slopes of Schild plots being less than unity, low pA_2 values for prazosin against the catecholamines and a clear relaxant effect of the catecholamines even in the presence of 1 μM prazosin. The prazosin-resistant relaxations were unaffected by higher concentrations of prazosin (2 μM) and propranolol (30 μM) or by further additions of idazoxan (1 μM) or haloperidol (30 μM). The relaxations were not due to a non-specific effect of the catechol nucleus since neither dihydroxyphenylethylene glycol (DOPEG) nor dihydroxyphenylacetic acid (DOPAC) produced relaxant effects at concentrations upto 300 μM. In contrast to the results with the catecholamines, prazosin was a potent antagonist of the relaxant effect of cirazoline and phenylephrine, although the antagonism was difficult to quantify due to a lowering of the slope of the concentration response curves to cirazoline and phenylephrine with the higher concentrations of prazosin (0·1 and 1·0 μM). In conclusion postjunctional relaxatory effects of catecholamines in the rat gastric fundus are mediated partly via α_1-adrenoceptors and partly via an atypical adrenoceptor.

In the rat isolated gastric fundus, α-adrenoceptor-mediated responses consist of postjunctional α_1-adrenoceptor-mediated relaxation of the longitudinal smooth muscle and prejunctional α_2-adrenoceptor-mediated inhibition of both excitatory cholinergic and inhibitory non-adrenergic, non-cholinergic (NANC) nerve-induced responses (Verplanken et al 1984; Dettmar et al 1984, 1985, 1986b). In addition, there appears to be a relaxant effect of noradrenaline which persists in the presence of both α- and β-adrenoceptor antagonists (Dettmar et al 1986a, b).

The present study examined the inhibitory effects of a number of α-adrenoceptor agonists postjunctionally in the presence of propranolol in an attempt to determine whether this relaxant effect of noradrenaline in the rat gastric fundus was related to activity at α_1-adrenoceptors. The effects of dihydroxyphenylethyleneglycol (DOPEG) and dihydroxyphenylacetic acid (DOPAC) were also studied since relaxant effects of catecholamines due to a non-specific effect of the catechol nucleus have been reported (Wikberg 1977).

Materials and Methods

Male Sprague-Dawley rats (150–300 g) were killed by cervical dislocation and the stomachs removed. Strips of longitudinal fundal smooth muscle were prepared by the method of Vane (1957) and tissues suspended in 30 mL organ baths containing Krebs medium of the following composition (mM) NaCl, 119; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·0; $NaHCO_3$, 25; KH_2PO_4, 1·2; (+)-glucose, 11·1. The medium was maintained at 37°C and gassed with 5% CO_2 in oxygen. Each tissue was placed under an initial resting tension of 1g and allowed to equilibrate for 1 h before further experimental procedures were carried out. Muscle tension was recorded with Bioscience UF1 isometric transducers and displayed on Washington 400 MD2R oscillographs.

Propranolol (2 μM) was present routinely in the Krebs medium to prevent effects mediated via β-adrenoceptors. In experiments involving the catecholamines, the Krebs medium contained the following: cocaine (3 μM) to block neuronal uptake, hydrocortisone (30 μM) to block extra-neuronal uptake, ethylenediamine tetra-acetic acid (EDTA; 30 μM) and (−)-ascorbic acid (30 μM) to prevent oxidation of the catecholamines.

To examine postjunctional relaxant responses in the rat gastric fundus, tone was raised by the addition of barium chloride to the baths at a concentration (0·5–2 mM) which produced a stable plateau contraction. In these experiments, atropine (2 μM) was present in the Krebs medium to prevent any contractile effects of barium produced via stimulation of cholinergic neurones (Gershon 1967). In one set of experiments, tone was raised by adding carbachol (0·3 μM) to the baths.

The effects of α-adrenoceptor agonists and antagonists on barium chloride-induced, or carbachol-induced contractions of the rat gastric fundus were measured by adding cumulative doses of the agonists every three min to a pre-contracted muscle strip. Responses were expressed as a percentage reduction in barium chloride or carbachol-induced tone. After complete inhibition of induced tone, each tissue was washed three times before any further additions were made. IC50 values were expressed as the concentration of agonist required to cause 50% of the maximum relaxation.

The ability of the α_1-adrenoceptor antagonist prazosin to block the relaxant effects of the agonists was studied by adding increasing concentrations of the antagonist 30 min before repeating the agonist-dose response curve. Concen-

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tration-ratios were calculated from IC50 values and Schild slopes obtained by the method of Arunlakshana & Schild (1959). PA_2 values were calculated for 1 μM prazosin from the following equation (Furchgott 1972):

$$pA_2 = \log (\text{Concn ratio} - 1) - \log (\text{antag. concn}).$$

Drugs used

(−)-Adrenaline bitartrate (Sigma), (−)-α-methylnoradrenaline free base (Sigma), atropine monosulphate (Sigma), barium chloride (Fisons Ltd), carbamylcholine chloride (carbachol; BDH), cirazoline hydrochloride (Synthelabo), cocaine hydrochloride (Ross and McFarlane), 3,4-dihydroxyphenylethyleneglycol (DOPEG; Sigma), 3-4-dihydroxyphenylacetic acid (DOPAC; Sigma), haloperidol (Janssen), hydrocortisone (Sigma), idazoxan hydrochloride (Reckitt and Colman plc), (−)-isoprenaline bitartrate (Sigma), (−)-noradrenaline bitartrate (Sigma), (−)-phenylephrine hydrochloride (Sigma), prazosin hydrochloride (synthesized by Reckitt and Colman plc.), (±)-propranolol hydrochloride (Sigma) and tetrodotoxin (Sigma).

All drugs were prepared fresh daily by dissolving them in distilled water and then kept on ice for the duration of the experiments. Hydrocortisone was first dissolved in absolute ethanol before being added to the Krebs solution. The final ethanol concentration in the Krebs medium did not exceed 60 μM. Ethanol at this concentration had no effect on the rat gastric fundus. All drugs were added to the baths in volumes not exceeding 0·3 mL.

Results

Addition of barium chloride (0·5–1 mM) to the gastric fundus in the presence of atropine (2 μM), produced a tonic contraction. Tetrodotoxin (3 μM) had no effect on the barium chloride-induced tone and therefore agonist-induced relaxations were likely to be postjunctionally mediated, although a prejunctional excitatory effect of the agonists on inhibitory neurons cannot be ruled out. In raised tone and in the presence of propranolol (2 μM), the fundus relaxed in a concentration-dependent manner to noradrenaline, adrenaline, isoprenaline, α-methylnoradrenaline, cirazoline and phenylephrine (Fig. 1). Cirazoline (IC50, 0·07 μM) and adrenaline (IC50, 0·07 μM) were approximately equipotent with noradrenaline (IC50, 0·1 μM) at inducing relaxations. Phenylephrine (IC50, 0·3 μM), α-methylnoradrenaline (IC50, 0·3 μM) and isoprenaline (IC50, 0·2 μM) were slightly less potent.

Prazosin (0·01 and 0·1 μM) caused 4- and 15-fold shifts, respectively, to the right in the concentration-response curve to (−)-noradrenaline. No further shift was obtained by 1·0 μM prazosin (Fig. 2A). The prazosin-resistant relaxations to noradrenaline were much slower in onset than in the absence of prazosin (Fig. 3) and were unaffected by further additions of prazosin (2 μM), propranolol (30 μM), idazoxan (1 μM) or haloperidol (30 μM). A similar prazosin-resistant effect was seen when carbachol was used to induce tone (Fig. 2B).

Concentration-response curves to (−)-adrenaline and (−)-α-methylnoradrenaline were also poorly shifted to the right by prazosin leaving a large prazosin-resistant component of the response (Fig. 4). The effect of prazosin on

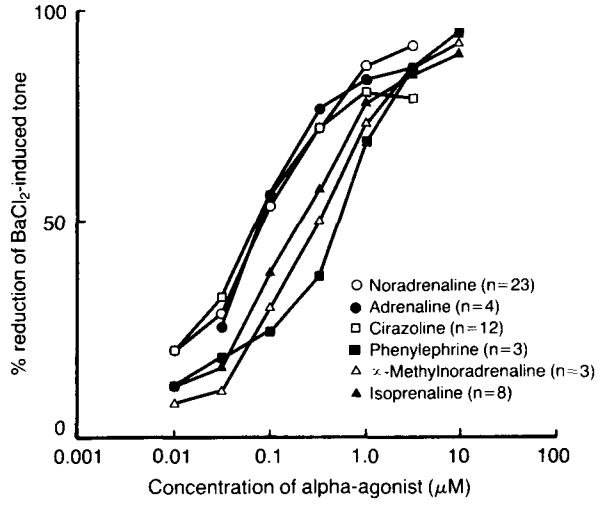


FIG. 1. The effects of α-adrenoceptor agonists on barium chloride-induced tone in the rat gastric fundus. Propranolol (2 μM) was present throughout. O——O Noradrenaline (n = 23), ●——● adrenaline (n = 4), □——□ cirazoline (n = 12), ■——■ phenylephrine (n = 3), △——△ α-methylnoradrenaline (n = 3) and ▲——▲ isoprenaline (n = 8) were added to each preparation in a cumulative manner. Standard errors are omitted for clarity.

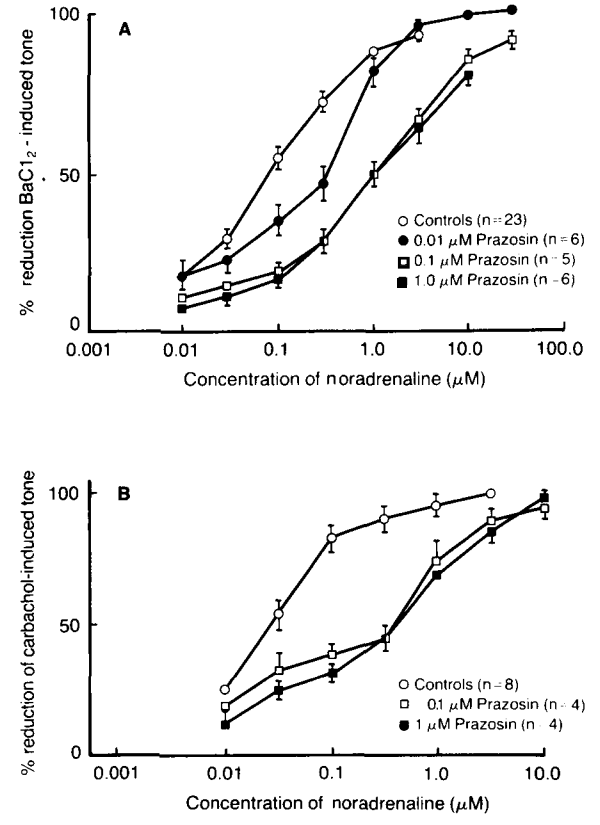


FIG. 2. The effect of prazosin (0·01–1·0 μM) on noradrenaline-induced relaxations in (A) barium-chloride-induced tone in the rat gastric fundus. O——O controls (n = 23), ●——● 0·01 μM prazosin (n = 6), □——□ 0·1 μM prazosin (n = 5) and ■——■ 1·0 μM prazosin (n = 6). (B) illustrates the effect of prazosin on noradrenaline-induced relaxations in carbachol-induced tone. O——O controls (n = 8), □——□ 0·1 μM prazosin (n = 4) and ■——■ 1·0 μM prazosin (n = 4). Results are expressed as percentage reduction of induced tone ± s.e.m.

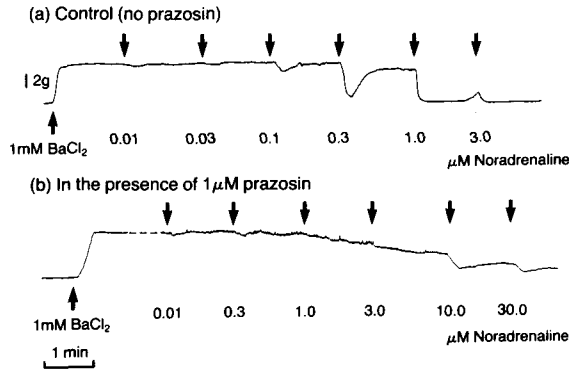
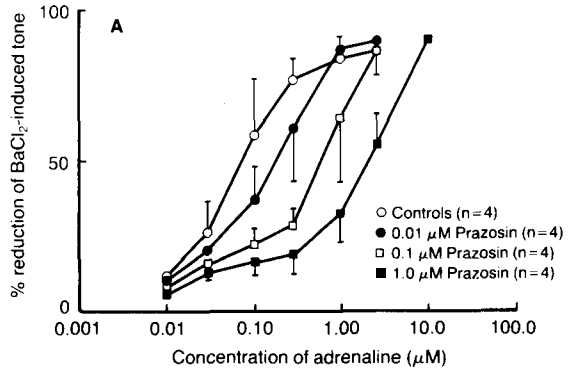


FIG. 3. Traces showing the effect of noradrenaline on barium chloride-induced tone in the rat gastric fundus. The upper trace (a) shows the inhibitory effect of noradrenaline in the absence of 1·0 μM prazosin on induced tone. The lower trace (b) shows the inhibitory effect of noradrenaline in the presence of 1·0 μM prazosin. Propranolol (2 μM) was present throughout.

Table 1. Analysis of the antagonism of catecholamine-induced relaxations of the isolated rat gastric fundus by prazosin. Propranolol (2 μM) was present throughout.

Agonist	n	Slope of Schild plot (±95% C.L.)	pA_2 (±s.e.m.)
Noradrenaline	16	0·32±0·07	7·2±0·17
Adrenaline	11	0·63±0·11	7·5±0·12
α-Methyl-noradrenaline	8	0·37±0·08	6·1±0·19
Isoprenaline	8	—	6·1±0·12[1]

[1] Only one concentration of prazosin tested (1·0 μM).



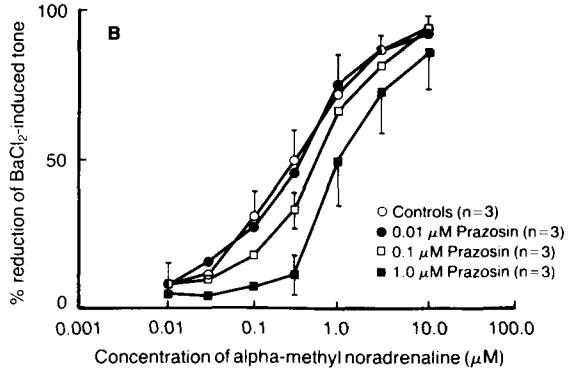


FIG. 4.Cumulative inhibitory concentration-response curves for adrenaline (A) and alpha-methylnoradrenaline (B) on barium chloride-induced tone following exposure to prazosin. In (A) O——O adrenaline alone (n=4), ●——● 0·01 μM prazosin (n=4), □——□ 0·1 μM prazosin (n=4) and ■——■ 1·0 μM prazosin. (B) O——O α-methylnoradrenaline alone (n=3), ●——●0·01 μM prazosin (n=3), □——□ 0·1 μM prazosin (n=3) and ■=■ 1·0 μM prazosin (n=3). Each point is expressed as the mean±s.e.m. from 3–4 experiments.

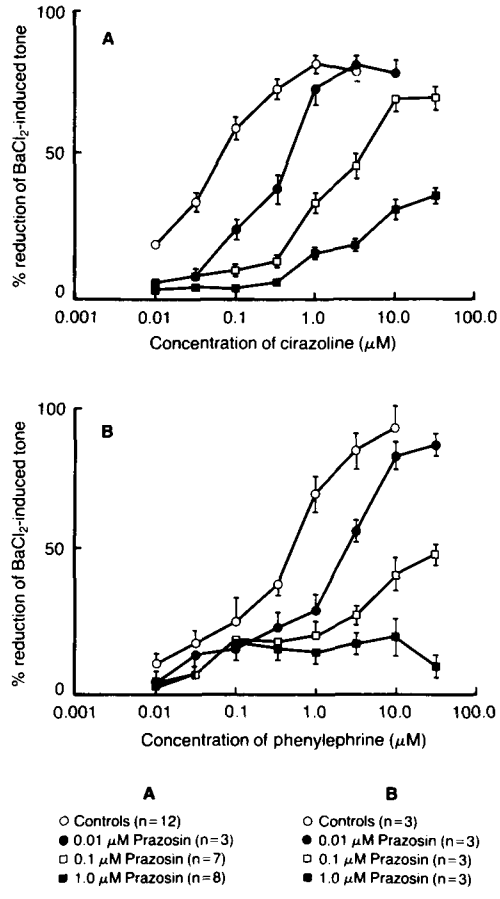


FIG. 5. Cumulative inhibitory concentration-response curves for the α-adrenoceptor agonists cirazoline (A) and phenylephrine (B) following exposure to prazosin is outlined in Fig. 5. (A) O——O cirazoline alone (n=12), ●——● 0·01 μM prazosin (n=3), □——□ 0·1 μM prazosin (n=7) and ■——■1·0 μM prazosin (n=8). In (B), O——O phenylephrine alone (n=3), ●——● 0·01 μM prazosin (n=3), □——□ 0·1 μM prazosin (n=3) and ■——■ 1·0 μM prazosin (n=3). Results are the means±s.e.m. from 3–12 experiments.

isoprenaline-induced relaxations in the fundus was only carried out at the highest concentration of prazosin (1 μM) and this produced less than a two-fold shift in the concentration-response curve to isoprenaline.

Schild analysis of the effects of prazosin on the catecholamine-induced relaxations gave slopes significantly different from unity (Table 1). PA_2 values, calculated from the shifts produced by 1 μM prazosin, were much lower than that

reported from prazosin acting at α_1-adrenoceptors (e.g. Kenakin 1984; pA_2 for prazosin against noradrenaline in the rat anococcygeus, 8·5).

Dihydroxyphenylethyleneglycol (DOPEG) and dihydroxyphenylacetic acid (DOPAC) at concentrations up to 300 μM had no relaxant effects on the rat gastric fundus.

In contrast, prazosin was a more potent antagonist of cirazoline- and phenylephrine-induced relaxations (Fig. 5). The higher concentrations of prazosin (0·1 and 1·0 μM) produced a reduction in the slope of the concentration-response curves which makes it difficult to quantitate antagonism. However, calculation of the pA_2 values from the parallel shifts produced by 0·01 μM prazosin gave values of 8·74 (n = 8) and 8·62 (n = 3) for cirazoline and phenylephrine, respectively.

Discussion

In the presence of propranolol to block β-adrenoceptors,the catecholamines noradrenaline, adrenaline, isoprenaline and α-methylnoradrenaline, and the selective α_1-adrenoceptor agonists cirazoline and phenylephrine, produced concentration-related relaxations of the rat gastric fundus. These relaxations were antagonized to varying extents by prazosin, indicating an action via α_1-adrenoceptors. This supports the suggestion of Verplanken et al (1984) of the presence of postjunctional α_1-adrenoceptors in the preparation.

However, the shifts of the catecholamine concentration-response curves produced by prazosin were poor and less than would be expected for straightforward competitive antagonism, as shown by the values for the slopes of the Schild plots and by the pA_2 values obtained from the plots (Arunlakshana & Schild 1959). Thus, even in the presence of 1μM prazosin, there remained a substantial catecholamine-mediated relaxant effect. This prazosin-resistent component was not due to an action on α_2-adrenoceptors since α_2-adrenoceptor agonists have been shown previously to have no relaxant effect (Dettmar et al 1984) and since idazoxan had no effect on relaxations mediated by noradrenaline. Likewise the relaxations were not mediated by dopamine receptors since haloperidol had no effect. Verplanken et al (1984) also reported non-competitive antagonism of noradrenaline by prazosin in the presence of propranolol in rat gastric fundus and suggested that high concentrations of noradrenaline might be overcoming propranolol blockade. This can be ruled out in the present study since increasing the propranolol concentration more than ten fold failed to produce any further antagonism of the response to noradrenaline.

The prazosin-resistant relaxations were not related to the agent used to produce tone since prazosin antagonized noradrenaline to a similar degree when an alternative agent (carbachol) was used to raise tone.

Catecholamine-induced relaxation of gut smooth muscle in the presence of α- and β-adrenoceptor blockade has previously been reported in guinea-pig ileum and was attributed to a non-specific effect of the catechol nucleus since it was reproduced by dihydroxyphenylethylene glycol (DOPEG) (Wikberg 1977). However, in the present study neither DOPEG nor dihydroxyphenylacetic acid (DOPAC) produced relaxant effects and these non-specific actions can be ruled out.

The prazosin-resistant relaxations were not observed with the non-catecholamine α_1-adrenoceptor agonists cirazoline and phenylephrine and therefore do not appear to be related to activity at α_1-adrenoceptors. The reason for the apparent non-competitive nature of the antagonism by prazosin of these two agonists, with a reduction of the slope of the concentration response curves, is not clear.

Recently evidence has been presented for the presence of an 'atypical' β-adrenoceptor, resistant to conventional β-adrenoceptor antagonists, in guinea-pig ileum (Bond et al 1986a, b; Bond & Clarke 1987, 1988) and in guinea-pig gastric fundus (Coleman et al 1987). The present findings may be explained by an action on such a receptor. In support of this, the order of potency for the catecholamines in the presence of α- and β-adrenoceptor blockade; isoprenaline (3) > noradrenaline (1) > adrenaline (0·5) is similar to that reported by Bond & Clarke (1988); isoprenaline (8) > noradrenaline (1) > adrenaline (0·5).

In conclusion, the present results show that postjunctional relaxatory effects of catecholamines in rat gastric fundus are mediated partly via α_1-adrenoceptors and partly via an atypical adrenoceptor.

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