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Influence of β-adrenoceptor-mediated relaxation on α-adrenoceptor-mediated contraction of rat pulmonary artery to adrenaline or noradrenaline

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Concentration-response (contraction) curves to either adrenaline or noradrenaline were obtained on isolated ring preparations of pulmonary artery from rats. In preparations from young rats the curve for adrenaline was bell-shaped, unless β-adrenoceptors were blocked with propranolol (1×10^{-6} M). The maximum contraction to adrenaline was less in the absence than in the presence of propranolol. In preparations from aged rats the adrenaline curve was no longer bell-shaped, even in the absence of propranolol. This reflected a decrease in the β-adrenoceptor-mediated relaxant responses of preparations from aged rats, seen as a separation between the concentration-response (relaxation) curves to adrenaline on preparations (phenoxybenzamine-treated, KCl-contracted) from young and aged rats. In preparations from young rats the noradrenaline curve was not bell-shaped, but if the preparations were from young rats treated with thyroxine (T_4), then a bell-shaped curve for noradrenaline was obtained, unless β-adrenoceptors were blocked by propranolol. These data could be explained by an increase in β-adrenoceptor-mediated relaxant responses of preparations from T_4-treated rats, seen as a separation in the concentration-response (relaxation) curves to noradrenaline on preparations from control and T_4-treated rats, respectively. Thus (i) α-adrenoceptor-mediated contractile responses of rat pulmonary artery preparations, to adrenaline or noradrenaline, can be attenuated by activation of β-adrenoceptors, mediating relaxation, and (ii) the extent of this attentuation changes under the influence of factors, such as ageing or T_4-treatment, which modify β-adrenoceptor-mediated relaxation in this blood vessel type.

Pulmonary artery preparations from young rats (1–2 months old) contain both α-adrenoceptors, mediating contraction, and β-adrenoceptors (β_1 and β_2), mediating relaxation (O'Donnell & Wanstall 1981, 1984). This vessel contracts to adrenaline or noradrenaline, which are agonists at both α- and β-adrenoceptors, indicating that the α-adrenoceptor-mediated response is dominant. However, for adrenaline, but not for noradrenaline, the contractile concentration-response curve is bell-shaped, unless β-adrenoceptors are blocked by propranolol (O'Donnell & Wanstall 1984). This indicates that contractile responses to the higher concentrations of adrenaline can be attenuated by concurrent β-adrenoceptor-mediated relaxation.

We have previously described two conditions in which β-adrenoceptor-mediated relaxant responses of rat pulmonary artery are modified. Firstly, responses mediated by β-adrenoceptors are reduced if the preparations are taken from aged (>16 months old) rats (O'Donnell & Wanstall 1984, 1986). Secondly, they are enhanced if the preparations are taken from rats treated with thyroxine (T_4, O'Donnell & Wanstall 1986). Thus it was predicted (i) that, in preparations taken from aged rats, β-adrenoceptor-mediated attenuation of contractile responses to adrenaline might be either not observed, or else be less pronounced than that seen in preparations from young rats, and (ii) that β-adrenoceptor-mediated attenuation of contractile responses to noradrenaline might occur in preparations from young rats treated with T_4, even though this was not seen in preparations from untreated young rats (see above). The present study describes experiments in which these predictions have been tested and substantiated. A preliminary account of these data was presented to the 19th meeting of the Australasian Society for Clinical and Experimental Pharmacologists, Brisbane, December 1985 (Wanstall et al 1987).

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MATERIALS AND METHODS

Isolated ring preparations of main pulmonary artery from male, Wistar rats (young, 1–2 months, 105–205 aged 17–19 months, 400–500 g) were set up for recording changes in isometric force in the circular muscle, as described by O'Donnell & Wanstall (1981). A resting force of 10 mN was

applied and maintained during an equilibration period of 1 h, after which the preparations were used to obtain either relaxant or contractile responses to adrenaline or noradrenaline.

Concentration-response curves for relaxant responses were determined on tissues which had been treated with the α-adrenoceptor antagonist, phenoxybenzamine (5×10^{-5} M for 30 min followed by washout), and contracted with $1{\cdot}5 \times 10^{-2}$ M KCl. The relaxant responses were expressed as % reversal of the KCl-induced contractions. The magnitudes of the relaxant responses expressed in this way were independent of the magnitudes of the KCl-induced contractions, which were (mN): young rats, 1·9 ± 0·56 (n = 9); aged rats 3·6 ± 0·98 (n = 5); T_4-treated young rats 3·8 ± 0·86 (n = 4).

On tissues from another group of rats, concentration-response curves for contractile responses were obtained, firstly in the absence of propranolol, and secondly in the presence of 1×10^{-6} M propranolol, after a contact time of 60 min. Absolute values of the maximum contractile responses achieved in the absence or presence of propranolol were determined, and are expressed in mN. Responses were also expressed as a % of the maximum response in the presence of propranolol. Neuronal and extraneuronal uptake inhibitors were not included in these experiments because this has previously been shown to be unnecessary when studying the effects of catecholamines on rat pulmonary artery (O'Donnell & Wanstall 1984). Repeated concentration-response (contraction) curves in the absence of propranolol were reproducible.

The experiments with adrenaline were carried out on preparations taken from untreated young or aged rats. The experiments with noradrenaline were carried out on preparations taken from young rats which had been treated with s.c. injections of either saline (1 mL kg^{-1}) or T_4 (sodium salt, 1 mg kg^{-1}), 7, 5 and 3 days before the experiments. The T_4 treatment regime caused significant increases in total serum levels (ng mL^{-1}, determined in serum samples obtained on the day of the experiment) of T_4 (saline-treated 60·7 ± 4·2, n = 8; T_4-treated 203·9 ± 25·6, n = 9; $P < 0{\cdot}001$, Student's *t*-test) and triiodothyronine (saline-treated 1·11 ± 0·15, n = 8; T_4-treated 2·89 ± 0·34, n = 9, $P < 0{\cdot}001$), and also a significant increase in heart weight (saline-treated 399 ± 10·2 mg/100 g, n = 8: T_4-treated 525 ± 15·1, n = 9, $P < 0{\cdot}001$).

The drugs used were: (−)-adrenaline acid tartrate (Sigma); (−)-noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith Kline and French); (±)-propranolol hydrochloride (ICI); L-thyroxine, sodium salt (Sigma). Radioimmunoassay kits for determining serum concentrations of thyroxine and triiodothyronine were provided by Nuclear Diagnostics, Sydney. Stock solutions of adrenaline and noradrenaline (100 mM) were prepared in 10 mM HCl, of propranolol (10 mM) in deionized water and of phenoxybenzamine (100 mM) in absolute ethanol containing 10 mM HCl. Dilutions of these drugs were made in Krebs solution and kept ice-cold throughout the experiment. Suspensions of thyroxine sodium (1 mg mL^{-1}) were made in 0·9% NaCl (saline) immediately before use.

The composition of the Krebs solution was (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11·7, ascorbic acid 1·1.

Mean values are quoted together with their standard errors. The significance of differences was assessed by Student's unpaired *t*-test, the Mann-Whitney test (Snedecor & Cochran 1967) or paired *t*-test, as indicated in the text.

RESULTS

Responses to adrenaline on preparations of pulmonary artery from young or aged rats

In KCl-contracted preparations of pulmonary artery, in which α-adrenoceptors were blocked with phenoxybenzamine, adrenaline caused concentration-dependent relaxation. The relaxant responses were significantly less in preparations from aged than from young rats, at each of the concentrations of adrenaline examined (Fig. 1A).

In uncontracted preparations from young rats, when β-adrenoceptors were blocked with propranolol, adrenaline (1×10^{-9} to 2×10^{-6} M), caused concentration-related contractions (Fig. 2A). The mean maximum response (to 2×10^{-6} M) was 6·6 ± 1·03 mN (n = 5). On the same preparations, in the absence of propranolol, the concentration-response curve to adrenaline, over the same concentration range, was bell-shaped, i.e. contractions increased with increasing adrenaline concentrations up to 8×10^{-8} M, and thereafter declined as the adrenaline concentration was further increased (Fig. 2A). The mean maximum response, which was achieved with 8×10^{-8} M, was 5·2 ± 0·87 mN (n = 5), and this was significantly less than the maximum response obtained in the presence of propranolol cited above ($0{\cdot}01 > P > 0{\cdot}001$, paired *t*-test). In contrast, in preparations from aged rats, adrenaline caused concentration-related contractions, whether propranolol was present or absent, i.e. the concentration-response curve was not bell-shaped even in the

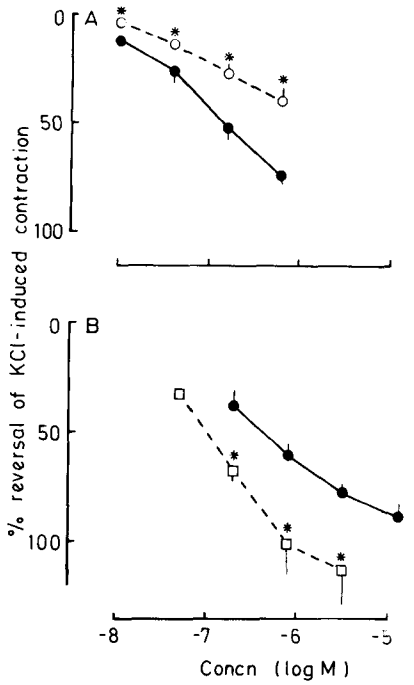

FIG. 1. Mean concentration-response (relaxation) curves on isolated preparations of pulmonary artery pre-exposed to phenoxybenzamine (5×10^{-5} M) and contracted with KCl ($1{\cdot}5 \times 10^{-2}$ M). Responses are expressed as % reversal of KCl-induced contractions. A. Responses to adrenaline on preparations from young (●——● n = 5) or aged (○- - -○ n = 5) rats. B. Responses to noradrenaline on preparations from young rats treated with saline (●——● n = 4) or T_4 (□- - -□ n = 4). Standard errors of mean responses are shown by the vertical bars, except when smaller than the symbols. Asterisks indicate significant differences in responses between young and aged (A) or saline-treated and T_4-treated (B) rats. $^*P < 0{\cdot}05$ (Mann-Whitney test).

absence of propranolol (Fig. 2B). The mean maximum responses (to 2×10^{-6} M) were $11{\cdot}0 \pm 1{\cdot}99$ mN with propranolol present and $13{\cdot}0 \pm 1{\cdot}67$ mN with propranolol absent and were not significantly different ($P > 0{\cdot}05$, paired t-test, n = 4). It was noticed that, in the preparations from aged rats, responses were, if anything, slightly reduced by the presence of propranolol, suggesting that, at this high concentration (1×10^{-6} M), propranolol might have some non-specific depressant activity.

Responses to noradrenaline on preparations of pulmonary artery from saline-treated or T_4-treated young rats

Noradrenaline caused concentration-dependent relaxation of KCl-contracted preparations of pulmonary artery (α-adrenoceptors blocked), and the relaxant responses were significantly greater in preparations taken from T_4-treated young rats than in those taken from age-matched saline-treated rats (Fig. 1B).

In uncontracted preparations from saline-treated rats, when β-adrenoceptors were blocked with propranolol, noradrenaline, studied in the same concentration range as adrenaline (1×10^{-9} to 2×10^{-6} M), caused concentration-related contractions (Fig. 2C) and the mean maximum response (to 2×10^{-6} M) was $5{\cdot}0 \pm 1{\cdot}59$ mN (n = 4). Noradrenaline also caused concentration-related contractions in the absence of propranolol (Fig. 2C), with a maximum response, at 7×10^{-7} M, of $5{\cdot}0 \pm 1{\cdot}53$ mN (n = 4). This was not different from that in the presence of propranolol ($P > 0{\cdot}05$, paired t-test). In contrast, in preparations from rats treated with T_4, the concentration-response curve in the absence of propranolol was clearly bell-shaped whereas that in the presence of propranolol was not (Fig. 2D). The mean maximum responses in the absence and presence of propranolol were $2{\cdot}7 \pm 0{\cdot}34$ mN (noradrenaline concentration 8×10^{-8} M) and $3{\cdot}8 \pm 0{\cdot}61$ mN (noradrenaline concentrations 2×10^{-6} M) and these were significantly different ($0{\cdot}05 > P > 0{\cdot}01$, paired t-test, n = 5).

DISCUSSION

The experiments described in this study support the prediction that, at least for rat pulmonary artery, the contractile concentration-response relationship, and the magnitude of the maximum contractile response, for adrenaline and noradrenaline depend on the balance between α- and β-adrenoceptor activation, and can therefore be influenced by factors which alter β-adrenoceptor-mediated responses.

Rat pulmonary artery was a suitable vessel in which to test this hypothesis because it has previously been shown (a) that it contains predominantly α-adrenoceptors, with a minor population of β-adrenoceptors, (b) that, in preparations from young rats, these β-adrenoceptors can attenuate α-adrenoceptor-mediated contractions to one of the above catecholamines, adrenaline, but not the other, noradrenaline, and (c) that β-adrenoceptor-mediated responses can be either decreased (by ageing) or increased (by T_4 treatment) (O'Donnell & Wanstall 1984, 1986). In the present study, the reduction in β-adrenoceptor-mediated relaxation with ageing, previously observed with various other β-adrenoceptor agonists (O'Donnell & Wanstall 1984, 1986), was demonstrated for the first time for adrenaline, and each of the other observations cited above was confirmed.

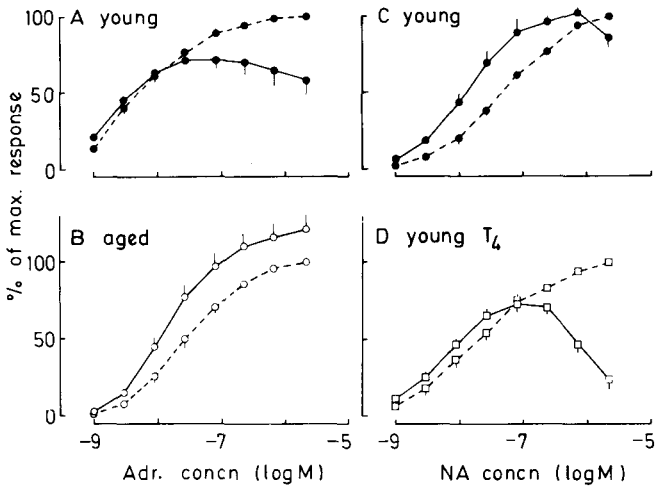

FIG. 2. Mean concentration-response (contraction) curves to adrenaline (A, B) or noradrenaline (C, D) on isolated preparations of pulmonary artery obtained in the absence (——) and presence (– – –) of propranolol (1×10^{-6} M). Responses are expressed as % of the maximum response in the presence of propranolol. A. Data obtained in preparations from young rats (from experiments in O'Donnell & Wanstall, 1984, n = 5). B. Data obtained in preparations from aged rats (n = 4). C. Data obtained in preparations from saline-treated young rats (n = 4). D. Data obtained in preparations from T_4-treated young rats (n = 5). Standard errors of mean responses are shown by the vertical bars, except when smaller than the symbols.

For a given agonist, attenuation of α-adrenoceptor-mediated contractions by β-adrenoceptor-mediated relaxation can be assessed from the effects of propranolol on the shape of the agonist contractile concentration-response curve and on the magnitude of the maximum contractile response to the agonist. If the concentration-response curve is bell-shaped in the absence, but not in the presence, of propranolol, and if the magnitude of the maximum contractile response is less in the absence, than in the presence, of propranolol, this indicates that β-adrenoceptor-mediated relaxation is functionally opposing the α-adrenoceptor-mediated contractions at the higher agonist concentrations.

Using these criteria, there was β-adrenoceptor-mediated attenuation of contractions to adrenaline in preparations from young rats. This did not occur in preparations from aged rats, reflecting a reduction in β-adrenoceptor responses in ageing. In contrast, there was no β-adrenoceptor-mediated attenuation of contractions to noradrenaline in preparations from young rats. This is presumably because the β-adrenoceptor potency of noradrenaline is lower than that of adrenaline in preparations from young rats (O'Donnell & Wanstall 1986; Fig. 1, this paper), and therefore, at any given concentration, noradrenaline-induced relaxations are smaller than adrenaline-induced relaxations. However there *was* β-adrenoceptor-mediated attenuation of noradrenaline contractions in preparations taken from young rats treated with T_4. This reflected an enhancement by T_4 of noradrenaline-induced relaxant responses.

These observations on pulmonary artery from young, aged and T_4-treated rats illustrate how β-adrenoceptor mechanisms can attenuate vascular contractile responses to catecholamines, and how this attenuation can be increased or decreased by various factors. The data can be compared with data obtained on vessels from hypertensive and normotensive rats (Asano et al 1982; Borkowski & Porter 1984). Contractile responses to noradrenaline of femoral artery (Asano et al 1982) and of perfused mesentery (Borkowski & Porter 1984) were greater in preparations from hypertensive than from normotensive rats, and this was attributed, at least in part, to a loss of β-adrenoceptor-mediated attenuation of the responses in hypertension. The present data may also provide an explanation for reports in the literature that vascular preparations from animals treated with thyroid hormones exhibit reduced contractions to noradrenaline (MacMillan & Rand 1962; Coville & Telford 1970; Ress & Field 1980). In the light of the present findings, this could reflect a thyroid hormone-induced increase in β-adrenoceptor responses, resulting in attenuation of the contractile responses.

It is not yet clear whether the changes in β-adrenoceptor-mediated attenuation, reported

here for pulmonary artery, occur in all vessels with both α- and β-adrenoceptors, or whether they are restricted to certain blood vessel types. The latter is a possibility in view of the suggestion that the effects of ageing on β-adrenoceptor-mediated vascular relaxation may be confined to arterial vessels, i.e. not seen in veins (Fleisch & Hooker 1976; Duckles & Hurlbert 1986). Also there is some evidence that the enhancement of vascular β-adrenoceptor responses by T_4 treatment may only occur in vessels in which the β_1-adrenoceptor subtype is present (O'Donnell & Wanstall 1986).

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