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Thyroxine treatment of aged or young rats demonstrates that vascular responses mediated by β-adrenoceptor subtypes can be differentially regulated

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1 Responses to vascular relaxant drugs were obtained on KCl (15 mM)-contracted isolated ring preparations of pulmonary artery and aorta from young (1–2 months old) and aged (> 16 months old) rats. These vessels contain both β_1- and β_2-adrenoceptors.

2 Relaxant responses (i.e. relaxation expressed as a % of the KCl-induced contraction) to isoprenaline, procaterol (β_2-selective partial agonist), fenoterol (β_2-selective) and noradrenaline (β_1-selective) but not those to forskolin, 3-isobutyl-1-methylxanthine, enprofylline or sodium nitrite, were smaller on preparations from aged rats than on those from young rats.

3 Thyroxine (T_4)-treatment (1 mg kg^{-1} s.c. thrice weekly for 3–5 weeks) of aged or young rats enhanced responses to isoprenaline and noradrenaline but reduced those to procaterol, when compared with preparations from age-matched saline-treated control rats.

4 The agonist order of potency, determined in young rats, was isoprenaline>noradrenaline>adrenaline in preparations from T_4-treated rats compared with isoprenaline > adrenaline > noradrenaline in saline-treated control rats.

5 It is concluded (a) that the age-related decline in vascular responses to β-adrenoceptor agonists involves β-adrenoceptor mechanisms specifically and possibly β_2-adrenoceptors more than β_1-adrenoceptors; and (b) that T_4-treatment of rats enhances β_1-adrenoceptor-mediated and reduces, or does not change, β_2-adrenoceptor-mediated responses of preparations of rat pulmonary artery and aorta. In preparations from control rats β_2-adrenoceptors were functionally predominant but in preparations from T_4-treated rats β_1-adrenoceptors appeared to become functionally predominant.

Introduction

There are now several studies, with isolated blood vessels from both rats and rabbits, which show that the ability of the β-adrenoceptor agonist, isoprenaline, to relax contracted arterial preparations declines as the age of the animal from which the preparation was taken increases (Fleisch *et al.*, 1970; Ericsson & Lundholm, 1975; Fleisch & Hooker, 1976; O'Donnell & Wanstall, 1984a). The present study explores three areas related to the effects of aging on vascular responses to β-adrenoceptor agonist drugs.

Firstly, from data in a previous study on isoprenaline, fenoterol and noradrenaline on rat isolated pulmonary artery (a preparation in which both β_1- and β_2-adrenoceptors mediate relaxation, O'Donnell & Wanstall, 1981a), it was speculated that the age-related decline in response to these drugs might involve β_2- more than β_1-adrenoceptors (O'Donnell & Wanstall, 1984a). The effect of aging on β_2-adrenoceptor-mediated vascular relaxation has been further investigated in the present study by comparing responses of isolated blood vessels from aged and young rats to the β_2-selective partial agonist, procaterol.

Secondly, this study has examined whether the age-related decline in vascular responses to β-adrenoceptor agonists might involve β-adrenoceptor mechanisms specifically (as suggested by Ericsson & Lundholm, 1975) or event(s) subsequent to the activation of adenylate cyclase (as suggested by Cohen & Berkowitz, 1974, and Cohen *et al.*, 1977). To study this, the effects of aging on the responses of isolated vascular preparations to the direct adenylate cyclase activator, forskolin, two inhibitors of phosphodiesterase, 3-isobutyl-methyl xanthine (IBMX) and enprofylline, and the vasodilator, sodium nitrite, have been investigated.

Thirdly, although the reason why aging is accom-

panied by a reduction in β-adrenoceptor-mediated vascular relaxation is not known (Fleisch, 1981), Parker *et al.* (1978) suggested that it might be due to an age-related loss in the responsiveness of the blood vessels to thyroid hormones. They observed that the relaxant responses of rat isolated aorta to isoprenaline were reduced not only in preparations from aged rats but also in preparations from thyroidectomized rats. Furthermore, treatment of aged rats with thyroid hormones resulted in restoration of the responsiveness of aortic preparations to isoprenaline. Hence, data have been obtained in the present study on isolated blood vessel preparations taken from both aged and young rats treated with thyroxine (T_4). The relaxant responses of these preparations to isoprenaline or to selective β-adrenoceptor agonists, *viz.* procaterol, fenoterol, adrenaline and noradrenaline, have been examined.

The isolated blood vessel preparations used throughout the present study were from rat aorta (because this preparation was used in the study carried out by Parker *et al.*, 1978), or from rat pulmonary artery (because this vessel was used in our previous studies on aging, O'Donnell & Wanstall, 1984a).

A preliminary account of these data was presented at the 40th meeting of the Australian Physiological and Pharmacological Society, Melbourne, 1984 (Wanstall & O'Donnell, 1984) and at the 9th International Congress of Pharmacology, London, 1984 (O'Donnell & Wanstall, 1984b).

Methods

Male Wistar rats, aged 5 to 10 weeks (young; 100 to 280 g) or > 16 months (aged; 380 to 500 g) at the time of the experiments, were used.

Blood vessel preparations

Isolated single ring preparations of pulmonary artery or aorta were set up for recording changes in tension in the circular muscle as described by O'Donnell & Wanstall (1981a). A resting tension of 1 g was maintained throughout the experiments, and tissues were allowed to equilibrate for at least 1 h before concentration-response curves were started.

All preparations were pre-exposed to phenoxybenzamine 50 μM for 30 min followed by washout, to block α-adrenoceptors and neuronal and extraneuronal uptakes, and were contracted with 15 mM KCl (50 μl of 3M KCl added to 10 ml of Krebs solution in the organ bath). Cumulative concentration-response (relaxation) curves were obtained to vascular relaxant drugs. Responses of pulmonary artery to two of the drugs examined, isoprenaline and forskolin, are illustrated in Figure 1, which also shows the lack of relaxation to ethanol (the solvent used for forskolin). Relaxant responses to all of the drugs are defined as % reversal of the KCl-induced contraction (relaxation back to the basal tone of the tissue is thus 100%). Mean concentration-response curves were obtained by calculating mean relaxant responses, as defined above, to each concentration of relaxant drug used in the experiments.

Thyroxine (T_4)-treatment of rats

Rats were given s.c. injections of the sodium salt of thyroxine (T_4), 1 mg kg^{-1} body weight, three times a week for 3 to 5 weeks. Age- and weight-matched controls were given s.c. injections of saline (0.5 ml kg^{-1} or 1 ml kg^{-1} for aged and young rats, respectively). Animals were killed for the isolated blood vessel experiments 2 or 3 days after the last T_4 injection.

Assessment of thyroid state of T_4-treated rats and saline-treated controls

Body weight was recorded at the start of treatment and on the day of the experiment and the average change in body weight, in g per week, was calculated. Aged rats lost body weight when on T_4-treatment whereas young rats on T_4-treatment gained weight, but less rapidly than the corresponding saline-treated controls. Thyroid glands were dissected out and weighed and thyroid weight was expressed relative to body weight (i.e. mg 100 g^{-1} body weight). T_4-treatment caused a significant decrease in thyroid weight in both young and aged rats (Table 1). A blood sample, taken from each rat by cardiac puncture on the day of the experiment, was centrifuged at 15,000 r.p.m. for 30 s and the serum sample was stored at − 20°C until assayed. Total serum T_4 and triiodothyronine (T_3) levels for each rat were determined by radioimmunoassay using commercially available kits provided by Nuclear Diagnostics, Sydney. T_4-treatment caused significant increases in total serum concentrations of T_4 and T_3 (Table 1).

Drugs and solutions

The following drugs were used:- (−)-adrenaline acid tartrate (Sigma); enprofylline (Astra); (±)-fenoterol hydrobromide (Boehringer Ingelheim); forskolin (Calbiochem-Behring); 3-isobutyl-1-methyl xanthine (IBMX, Sigma); (−)-isoprenaline acid tartrate (Sigma); (−)-noradrenaline acid tartrate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); procaterol (Warner-Lambert); sodium nitrite (B.D.H. Chemicals Australia); L-thyroxine, sodium salt (Sigma).

Stock solutions (100 mM) of adrenaline, fenoterol,

Table 1 Effects of T_4-treatment of aged and young rats on total serum T_4 and T_3 concentrations and thyroid weight

	Aged ($n = 6$)		*Young* ($n = 10$)	
	Saline-treated (controls)	*T_4-treated*[a]	*Saline-treated* (controls)	*T_4-treated*[a]
Total Serum T_4 ($ng\,ml^{-1}$)	46 ± 5.0	208 ± 17.0***	60 ± 2.8	285 ± 31.1***
Total Serum T_3 ($ng\,ml^{-1}$)	1.13 ± 0.11	3.73 ± 0.15***	1.16 ± 0.07	4.80 ± 0.23***
Thyroid weight ($mg\,100\,g^{-1}$ body wt)	5.4 ± 0.2	4.3 ± 0.2**	7.5 ± 0.5	4.8 ± 0.3***

Results shown are mean values ± s.e. Numbers of rats (n) are shown. [a]Rats received $1\,mg\,kg^{-1}$ T_4 sodium salt s.c. 3 times a week for 3–5 weeks.
Significantly different from controls. **$0.01 > P > 0.001$; ***$P < 0.001$ (Student's t test).

isoprenaline and noradrenaline were made up in 0.01 M HCl. Sodium nitrite (M) was made up in deionised water immediately before use. Phenoxybenzamine (100 mM) was dissolved in absolute ethanol containing 0.01 M HCl. Procaterol (10 mM) was made up in Krebs solution immediately before use. IBMX (10 mM) was made up in 0.01 M NaOH and enprofylline (50 mM) in 0.1 M NaOH. Forskolin was dissolved and diluted in absolute ethanol. Dilutions of other drugs were made in Krebs solution and kept on ice during the course of the experiment. Suspensions of thyroxine sodium (1 or $2\,mg\,ml^{-1}$) were made in 0.9% saline.

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.

Statistical analyses

All mean values of serum T_4 and T_3 levels, thyroid weight and increases in tension are quoted together with the standard error (s.e.) of the mean, and the significance of the differences between these mean values was assessed by Student's t test. The significance of the differences between mean values of relaxant response (% reversal of KCl-induced contraction) was assessed by the Mann-Whitney test (Snedecor & Cochran, 1967). For these mean values, standard errors are included in the figures merely as an indication of the extent of the variation in the data.

Results

Relaxant responses to isoprenaline, procaterol, noradrenaline, fenoterol, IBMX, enprofylline, forskolin and sodium nitrite of blood vessels from young and aged rats

Relaxant responses were obtained on KCl (15 mM)-contracted preparations of pulmonary artery and aorta from both young and aged rats (Figures 1, 2 and 3). On pulmonary artery, the relaxant responses to each of the β-adrenoceptor agonists (isoprenaline, procaterol, fenoterol and noradrenaline) were less on preparations from aged rats than on preparations from young rats. In contrast, there was no difference

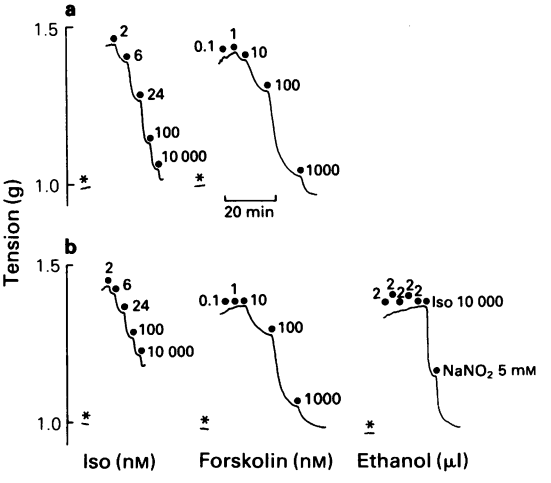

Figure 1 Responses to isoprenaline (Iso) and forskolin on preparations of pulmonary artery from (a) a young and (b) an aged rat. The preparations were pre-exposed to phenoxybenzamine (50 μM for 30 min followed by washout) and contracted with KCl (15 mM). The resting tension before the addition of KCl is shown (*). Concentrations of isoprenaline and forskolin are in nM. In (b, aged rat) the lack of relaxation to successive 2 μl additions of ethanol (the solvent used for forskolin) is shown, together with relaxant responses to maximal concentration of Iso and sodium nitrite ($NaNO_2$). In the preparation from the aged rat, complete relaxation was achieved with forskolin and $NaNO_2$ but not with isoprenaline.

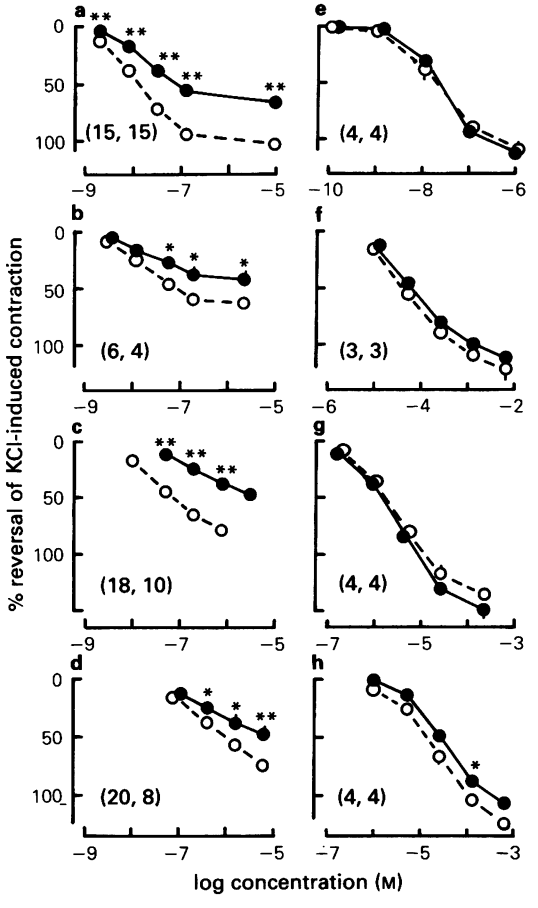

Figure 2 Mean concentration-response curves to isoprenaline (a), procaterol (b), fenoterol (c), noradrenaline (d), forskolin (e), sodium nitrite (f), 3-isobutyl-1-methylxanthine (g) and enprofylline (h) on preparations of pulmonary artery from young (○) and aged (●) rats. Relaxant responses are expressed as a % of the KCl (15 mM)-induced contractions. Standard errors of the mean responses, when larger than the symbols, are shown by the vertical lines to indicate the scatter of the responses. Statistical comparisons between young and aged rats were made using the Mann-Whitney test (* $P<0.05$; ** $P<0.01$). Numbers of rats (young, aged) are shown on each graph in parentheses.

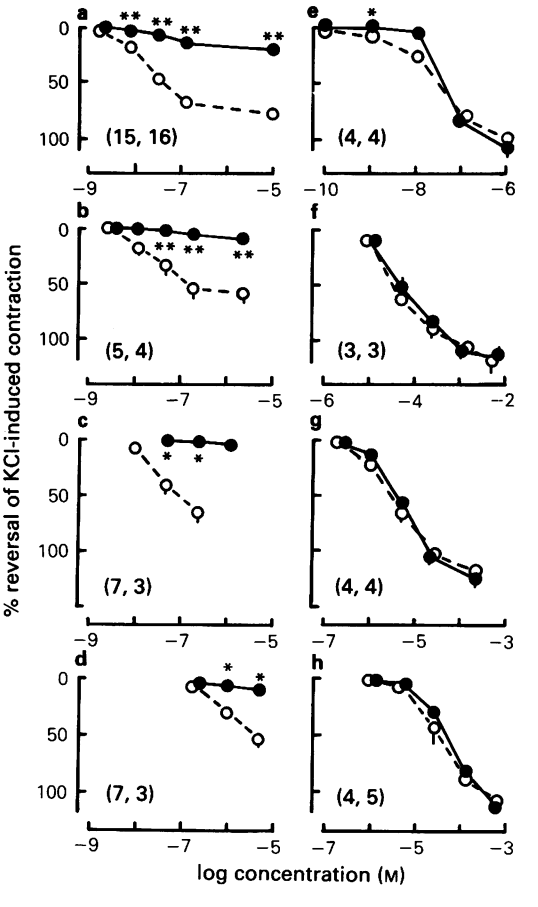

Figure 3 Mean concentration-response curves to isoprenaline (a), procaterol (b), fenoterol (c), noradrenaline (d), forskolin (e), sodium nitrite (f), 3-isobutyl-1-methylxanthine (g) and enprofylline (h) on preparations of aorta from young (○) and aged (●) rats. Relaxant responses are expressed as a % of the KCl (15 mM)-induced contractions. Standard errors of the mean responses, when larger than the symbols, are shown by the vertical lines to indicate the scatter of the responses. Statistical comparisons between young and aged rats were made using the Mann-Whitney test (* $P<0.05$; ** $P<0.01$). Numbers of rats (young, aged) are shown on each graph in parentheses.

between the two age groups in the relaxant responses to forskolin, sodium nitrite, IBMX or enprofylline (Figures 1 and 2), even though the characteristic age-related reduction in the relaxant response to isoprenaline (see above) was always observed on the same preparations (Figure 1). Similar results were obtained on aorta except that the age-related reduction in relaxant responses to the β-adrenoceptor agonists was more pronounced than on pulmonary artery (Figure 3).

The age-related reduction in the relaxant responses to the β-adrenoceptor agonists was not reflecting a change in the magnitude of the KCl-induced increases in tension, since these were not significantly different in the preparations from the two age groups. This was true for the groups of preparations used to obtain data

Table 2 Mean increases in tension (g) induced by 15 mM KCl in preparations of pulmonary artery and aorta from untreated (A) and saline- or T_4-treated young (B) and aged (C) rats

		Increases in tension (g) *Pulmonary artery*	*Aorta*
A *Untreated*	Young	0.28 ± 0.01 (77)	0.51 ± 0.03 (52)
	Aged	0.24 ± 0.02 (57)	0.53 ± 0.03 (46)
B *Young*	Saline-treated (controls)	0.25 ± 0.02 (32)	0.48 ± 0.03 (30)
	T_4-treated[a]	0.27 ± 0.03 (32)	0.42 ± 0.03 (31)
C *Aged*	Saline-treated (controls)	0.19 ± 0.02 (20)	0.49 ± 0.05 (19)
	T_4-treated[a]	0.39 ± 0.05*** (19)	0.48 ± 0.04 (19)

Results shown are mean increases in tension (g) ± s.e. with number of observations in parentheses.
[a] Rats received 1 mg kg^{-1} T_4 sodium salt s.c. 3 times a week for 3–5 weeks.
*** Significantly different from value in pulmonary artery of saline-treated aged rats: $P < 0.001$. Student's t test.

for any individual drug, and also for the preparations overall (Table 2A; untreated rats).

The effects of T_4-treatment of aged rats on relaxant responses of pulmonary artery and aorta to β-adrenoceptor agonists

The only drugs for which the relaxant responses had been apparently affected by aging were the β-adrenoceptor agonists (see above). Thus, for reasons outlined in the Introduction, relaxant responses to the β-adrenoceptor agonists were examined on preparations of pulmonary artery and aorta from aged rats that had been treated with T_4 (see Methods) or with saline (controls). These responses are illustrated in Figure 4. Relaxant responses to isoprenaline (non-selective agonist) and noradrenaline (β_1-selective) were greater in preparations from the T_4-treated rats than in those from saline-treated control rats. In contrast, relaxant responses to the β_2-selective agonist, procaterol, used in concentrations (0.003 to 2 μM) that activate only β_2-adrenoceptors (O'Donnell & Wanstall, 1985), were not enhanced by T_4-treatment on either pulmonary artery (responses to procaterol significantly reduced by T_4-treatment) or aorta (responses to procaterol neglible in preparations from both T_4-treated rats and controls). Relaxant responses to fenoterol, which is also β_2-selective, were little affected by T_4-treatment, except at the highest concentration of fenoterol examined (1.9 μM) where there was a small, but not significant, increase in responses. Note that the β_2:β_1 selectivity of fenoterol is only about 20 fold (O'Donnell & Wanstall, 1981b) and thus, at the two higher concentrations used (0.48 and 1.9 μM), it could have been activating β_1-adrenoceptors as well as β_2-adrenoceptors.

The effects of T_4-treatment of young rats on relaxant responses of pulmonary artery and aorta to β-adrenoceptor agonists

Experiments were carried out to see whether the effects of T_4-treatment seen in preparations from aged rats also occurred in young rats. In this series of experiments the β_2-selective agonist, adrenaline, was also included. T_4-treatment significantly enhanced relaxant responses to noradrenaline and isoprenaline ($P < 0.05$; Mann-Whitney test) but not to adrenaline, fenoterol (responses unchanged) or procaterol (responses significantly reduced, $P < 0.05$, or unchanged). Data from four experiments in which isoprenaline, adrenaline and noradrenaline were compared on the same preparations (Figure 5) showed that T_4-treatment also affected the order of potency of these three catecholamines. In preparations from control rats the order of potency was isoprenaline>adrenaline>noradrenaline whereas in preparations from T_4-treated rats the order of potency was isoprenaline>noradrenaline≥adrenaline.

It is unlikely that the changes in relaxant responses to the β-adrenoceptor agonists described in this and the previous section are due to an effect of T_4-treat-

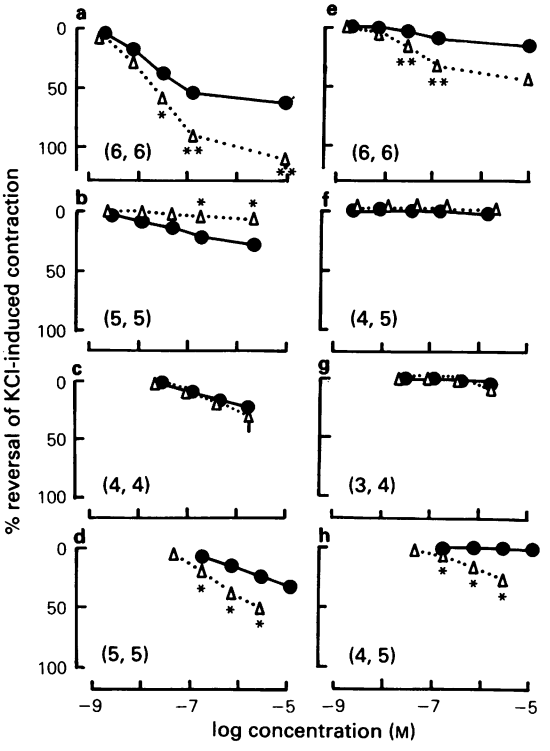

Figure 4 Mean concentration-response curves to isoprenaline (a,e), procaterol (b,f), fenoterol (c,g) and noradrenaline (d,h) on preparations of pulmonary artery (left hand graphs) and aorta (right hand graphs) from aged rats treated with saline (●) or T_4 (Δ). Relaxant responses are expressed as a % of the KCl (15 mM)-induced contractions. Standard errors of the mean responses, when larger than the symbols, are shown by the vertical lines to indicate the scatter of the responses. Statistical comparisons between saline- and T_4-treated rats were made using the Mann-Whitney test (* $P < 0.05$; ** $P < 0.01$). Numbers of rats (saline-treated, T_4-treated) are shown on each graph in parentheses.

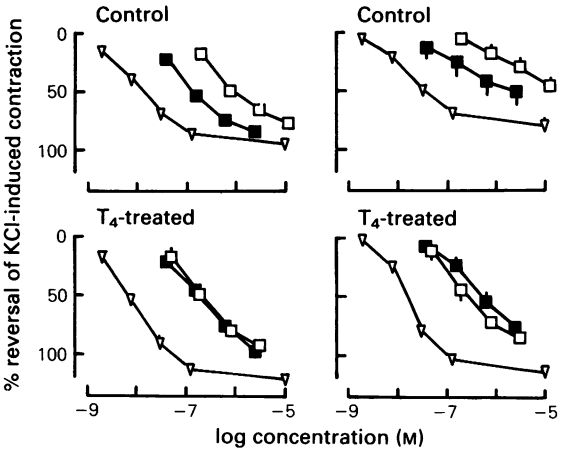

Figure 5 Mean concentration-response curves ($n = 4$) for isoprenaline (▽), adrenaline (■) and noradrenaline (□) on preparations of pulmonary artery (left hand graphs) and aorta (right hand graphs) from saline-treated control (top graphs) and T_4-treated (lower graphs) young rats. Relaxant responses are expressed as a % of the KCl (15 mM)-induced contractions. Standard errors of the mean responses, when larger than the symbols, are shown by the vertical lines to indicate the scatter of the responses.

ment on the magnitudes of the KCl-induced contractions. The KCl-induced contractions were not different in preparations from the T_4- and saline-treated rats, with the exception of the pulmonary arteries from T_4-treated aged rats which appeared to develop significantly more tension than the corresponding controls (Table 2). There was however no consistent or significant difference in individual groups of tissues used for particular agonists.

Discussion

The results of the present study confirmed our previous findings that relaxant responses (expressed as a % of KCl-induced contractions) to isoprenaline, fenoterol and noradrenaline were reduced in preparations of pulmonary artery and aorta taken from aged rats, compared with responses of preparations from young rats (O'Donnell & Wanstall, 1984a). In the present study a reduction in the relaxant responses to the β_2-selective partial agonist procaterol, used in concentrations that activated only β_2-adrenoceptors (O'Donnell & Wanstall, 1985), has also been demonstrated. The reduction was not due to changes in the KCl contractile responses. An affect of aging on relaxant responses to procaterol was also seen in a separate study on rabbit pulmonary artery (which, like rat pulmonary artery and aorta, can relax via both β_1- and β_2-adrenoceptors; O'Donnell & Wanstall, 1985). That study (O'Donnell & Wanstall, unpublished data) showed that preparations from 6–8 week old rabbits relaxed to β_2-adrenoceptor-activating concentration of procaterol (3–200 nM), whereas those from a mature (5.5 months) and an aged (4 years) rabbit relaxed to β_2-adrenoceptor-activating concentrations of procaterol (3–200 nM), whereas those from a and rabbit preparations, support the view that aging can depress vascular responses mediated by β_2-adrenoceptors. Moreover, the data in rabbit are consistent with our previous suggestion that aging may affect β_2-adrenoceptor-mediated responses more than β_1-ad-

renoceptor-mediated responses (O'Donnell & Wanstall, 1984a).

In contrast to the above effects of aging on responses to the β-adrenoceptor agonists, aging did not alter responses to forskolin, IBMX, enprofylline or sodium nitrite. This suggests that the effects of aging specifically involve β-adrenoceptor mechanisms rather than events subsequent to the activation of adenylate cyclase. The possible mechanisms which might be responsible for the age-related change in β-adrenoceptor mechanisms include reduced numbers of β-adrenoceptors (Roth & Hess, 1982), reduced coupling of the β-adrenoceptors to adenylate cyclase (intrinsic efficacy; Shima *et al.*, 1985) and/or reduced adenylate cyclase activity (O'Connor *et al.*, 1981; Scarpace & Abrass, 1983). According to receptor theory, a reduction in either receptor numbers or intrinsic efficacy (coupling) would be expected to reduce the maximum responses to agonists in the absence, but not in the presence, of a receptor reserve (Black *et al.*, 1985). In the present study aging did reduce the maximum response to the partial agonist procaterol, which has no receptor reserve. However, it also reduced the maximum response to the full agonist isoprenaline, despite the presence of a receptor reserve for isoprenaline in preparations from both young and aged rats (receptor occupancy was calculated to be between 65% and 95% for a 100% response, based on a K_A value for isoprenaline of 0.5 μM quoted by Minneman *et al.*, 1979a). Hence, although age-related changes in either receptor numbers or coupling cannot be excluded because they could explain the reduced maximum response to procaterol (see above), an age-related change in adenylate cyclase activity may need to be invoked to explain the reduced maximum response to isoprenaline. The lack of effect of aging on responses to forskolin, a drug that is considered to interact directly with the catalytic subunit of adenylate cyclase (Seamon & Daly, 1981), might seem to contradict this suggestion. However it has been proposed that there are different cellular pools of adenylate cyclase, not all of which may be associated with specific receptors such as β-adrenoceptors (Seamon & Daly, 1981). Hence it is possible that aging could specifically affect the adenylate cyclase linked with β-adrenoceptors without affecting other pools of adenylate cyclase accessible to forskolin.

Because of the similarities between the symptoms of senescence and those of hypothyroidism, a link between the aging process and changes in thyroid function has frequently been suggested (refer Cole *et al.*, 1982). From the study, referred to in the Introduction, which explored this concept (Parker *et al.*, 1978) it cannot be deduced whether thyroid hormone treatment specifically reversed the effects of aging on vascular responses to isoprenaline, or whether it produced an effect that was opposite to, but separate from, the effect of aging on isoprenaline responses. The data obtained in the present study provide evidence that thyroid hormone treatment of aged rats did not simply reverse the effects of aging on vascular β-adrenoceptor-mediated responses. In rat pulmonary artery and aorta, in which aging reduced responses to all the β-adrenoceptor agonists examined, T_4 treatment restored responses to isoprenaline (which acts on β_1- or β_2-adrenoceptors) and noradrenaline (β_1-selective) but not those to procaterol or fenoterol (both β_2-selective). In fact it was observed, on pulmonary artery, that responses to procaterol, which were already reduced by aging, were further reduced if the aged rats had been treated with T_4. These data suggested that β_1-adrenoceptor-mediated responses were enhanced by T_4-treatment whereas β_2-adrenoceptor-mediated responses were, if anything, reduced.

When the experiments with T_4-treatment were repeated in young rats, the same conclusion was reached, since T_4-treatment again enhanced responses to the non-selective agonist (isoprenaline) and the β_1-selective agonist (noradrenaline) but not to the β_2-selective agonists (adrenaline, fenoterol and procaterol). Responses to the latter drugs were either unchanged or reduced. In these experiments on young rats the order of potency of the three catecholamines, isoprenaline, noradrenaline and adrenaline, was obtained and could be used to provide information on the functional β-adrenoceptor populations in the preparations. The order of potency was isoprenaline > adrenaline > noradrenaline in controls but isoprenaline > noradrenaline > adrenaline in preparations from T_4-treated rats suggesting that, whereas β_2-adrenoceptors were functionally predominant in preparations from control rats (confirming O'Donnell & Wanstall, 1981a; 1984a), β_1-adrenoceptors became functionally predominant in preparations from T_4-treated rats. This is compatible with the suggestion that T_4-treatment had enhanced β_1-adrenoceptor-mediated responses and reduced or not changed β_2-adrenoceptor-mediated responses i.e. that responses mediated by β_1- and β_2-adrenoceptors had been differentially regulated.

The concept that responses mediated by the two β-adrenoceptor subtypes may be differentially regulated by thyroid hormones is not incompatible with data on β-adrenoceptor numbers from radioligand binding studies. For example, thyroid hormone treatment of rats increased the number of β-adrenoceptor binding sites in myocardial membranes (Williams *et al.*, 1977; Scarpace & Abrass, 1981), which contain mainly β_1-adrenoceptors (Minneman *et al.*, 1979c), and decreased the number of β-adrenoceptor binding sites in liver (Malbon & Greenberg, 1982), which has a homogeneous population of β_2-adrenoceptors (Minneman *et al.*, 1979a). However in the above examples, unlike the present study, the possibility that the

differential effect represents a difference between tissues rather than between β-adrenoceptor subtypes cannot be excluded. Differential regulation of β-adrenoceptor subtypes within the same tissue has been demonstrated for regulatory factors other than thyroid hormones e.g. in rat cerebral cortex by desmethylimipramine or 6-hydroxydopamine (Minneman *et al.*, 1979b) and in 3T3-L1 adipocytes by dexamethasone (Lai *et al.*, 1982) but these studies were also radioligand binding studies and not functional studies. The data in the present pharmacological study provide functional evidence that, within the same tissue, responses mediated by the different subtypes of β-adrenoceptor may be differentially regulated. The only other pharmacological study which attempted to demonstrate an independent regulation of β_1- and β_2-adrenoceptors in the same tissue, i.e. that of Taylor (1983) on guinea-pig trachea, yielded negative results.

In summary, this study has explored the influence of aging or of T_4-treatment of rats on β-adrenoceptor-mediated relaxation of isolated pulmonary artery and aorta. There was an age-related reduction in the relaxant responses of these vessels to β-adrenoceptor agonists, especially responses mediated by β_2-adrenoceptors. Since there was no age-related reduction in responses to drugs with mechanisms that do not involve β-adrenoceptors, i.e. sodium nitrite, forskolin and phosphodiesterase inhibitors, it appears that this effect of aging involves β-adrenoceptor mechanisms specifically. When rats, young or aged, were treated with T_4, β_1-adrenoceptor-mediated responses of pulmonary artery and aorta were increased whereas β_2-adrenoceptor-mediated responses were reduced or not changed. Hence it is concluded that T_4-treatment of rats can differentially regulate vascular responses mediated by the different β-adrenoceptor sub-types. Consistent with this conclusion was the observation that in preparations from T_4-treated rats β_1-adrenoceptors appeared to be functionally predominant whereas in preparations from controls β_2-adrenoceptors were functionally predominant.

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