British Journal of Pharmacology (1997) 121, 1017–1023

α_1-Adrenoceptor vasoconstriction in the tail artery during ageing

[1]Elisabet Vila, Nuria M. Vivas, Antonia Tabernero, *Jesús Giraldo & †Silvia M. Arribas

Departament de Farmacologia i Terapèutica, *Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain and †Clinical Research Initiative in Heart Failure, Institute of Biomedical & Life Sciences, University of Glasgow, Glasgow

1 We have studied the α_1-adrenoceptor-mediated responses in intact tail artery rings from 3–4 and 20–22 months old Sprague-Dawley rats, focusing on possible endothelial alterations. The influence of nitric oxide released by the endothelium, the number of α_1-adrenoceptors and the functional receptor reserve were evaluated to determine their contribution to the contractile response mediated by this receptor. The state of the endothelial layer was assessed by confocal microscopy.

2 Noradrenaline (1 nM–100 μM) induced concentration-dependent vasoconstriction. The maximum contractions to noradrenaline ($P<0.05$) and to 75 mM KCl ($P<0.01$) were higher in young than in old animals.

3 The density (B_{max}) of α_1-adrenoceptors and the dissociation constant (K_D) obtained in [^{3}H]-prazosin binding experiments were unchanged by age.

4 The apparent affinity (pK_A) and the percentage of functional receptors (qx100) remaining after phenoxybenzamine (0.03 μM) were similar in both age groups.

5 After partial α_1-adrenoceptor inactivation with phenoxybenzamine, N^G-nitro-L-arginine methylester (30 μM) significantly potentiated the E/[A] curve to noradrenaline in young rats. However, only responses to 0.1 to 1 μM noradrenaline were significantly potentiated in old animals. In addition, 94% of the vessels from young, but only 52% from old rats were relaxed by 80–100% of the noradrenaline (0.03 μM) contraction, with 1 μM acetylcholine.

6 No modifications in the area (μm^2) or in the number of endothelial nuclei (per mm^2) were observed between age groups. An elongation of the nuclei of endothelial cells was observed in the old animals.

7 These data suggest that the noradrenaline-induced contraction is decreased in old rats probably due to differences in either the contractile machinery or postreceptor mechanisms. These alterations may be accompanied by an impairment of the release or production of NO from endothelial cells.

Keywords: α_1-Adrenoceptor responsiveness; ageing; endothelium; tail artery

Introduction

Ageing is associated with marked changes in the cardiovascular system, especially in the structure and function of arteries (Docherty, 1990; Wadsworth, 1990; Marín, 1995). Responses to α_1-adrenoceptor agonists in blood vessels have been shown to increase (Cox *et al*., 1976; Olah & Rahwan, 1987), decrease (Fouda & Atkinson, 1986; Johnson & Wray, 1990; Gurdal *et al*., 1995b) or remain the same (Stevens *et al*., 1982; Scott & Reid, 1992; Hüsken *et al*., 1993; McDonald *et al*., 1995; Tabernero & Vila, 1995) with ageing. The variations observed in vascular α_1-adrenoceptor-mediated responses from old animals have been ascribed to different factors. Among them, changes in the number of receptors, in the receptor reserve or in the uptake mechanisms have been described (Docherty, 1990). Furthermore, some (Shimizu & Toda, 1986; Lüsher *et al*., 1992) but not all (Hynes & Duckles, 1987) studies have observed that endothelial-mediated relaxations are impaired in several vascular beds from aged animals, probably due to a defect in the release and/or production of relaxing factors, mainly nitric oxide (NO) (Hadju *et al*., 1993; Atkinson *et al*., 1994; Paterno *et al*., 1994). Modifications in the shape, size and axial orientation of endothelial cells (Yin, 1980; Epstein, 1992) also seem to appear with ageing.

In previous studies, we have shown that removal of the endothelium (Tabernero & Vila, 1995) and N^G-nitro-L-arginine methylester (L-NAME) (Tabernero *et al*., 1996a) do not affect α_1-adrenoceptor-mediated responses in tail artery rings from 3–4 month old rats, probably due to a high functional α_1-adrenoceptor reserve (Tabernero *et al*., 1996a). However, in tail artery from old rats, the absence of endothelium reduced the noradrenaline-induced contraction, suggesting a different role of endothelium in young and old animals (Tabernero & Vila, 1995). The aim of this study was to examine further the effect of ageing on α_1-adrenoceptor-mediated responses in tail artery rings, focusing on possible endothelial alterations. The role of NO released by the endothelium, the number of α_1-adrenoceptors and the functional reserve have been evaluated to determine their contribution to the contractile response mediated by this receptor. The state of the endothelial layer was also assessed by laser scanner confocal microscopy (LSCM).

Methods

The experiments were performed on 3–4 month (408 ± 16 g, $n=51$) and 20–22 month (821 ± 26 g, $n=63$) old male Sprague-Dawley rats. Nine young and nine old rats, that had not been used for *in vitro* studies, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and the systolic and diastolic arterial pressures were measured by means of a Statham transducer connected to a cannula placed in the right carotid artery. Pulse arterial pressure was taken as the difference between systolic and diastolic pressure. Heart rate was derived from the arterial pulse. Blood pressure and heart rate values used for comparison between age groups were obtained after a 15 min stabilization period.

Functional studies

The animals were decapitated and the tail artery was quickly removed, cleaned of adherent tissue and placed in physiological salt solution (PSS) of the following composition (in mM):

[1] Author for correspondence.

NaCl 112.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1. Desipramine (0.1 μM), normetanephrine (1 μM), Na_2 EDTA (23 μM), propranolol (1 μM) and yohimbine (0.1 μM) were present throughout the experiment to block neuronal and extraneuronal uptake, to prevent noradrenaline oxidative degradation and stimulation of β-(Minneman *et al*., 1983; Sallés *et al*., 1994) and α_2-adrenoceptors (Bao *et al*., 1993), respectively.

Rings of proximal tail artery (2–3 mm) were set up in a 5 ml organ bath containing PSS maintained at 37 ± 0.5°C and continuously gassed with 95% O_2, 5% CO_2. A resting tension of 7.35 mN was placed and changes in tension recorded with a PIODEN (UF-1) isometric transducer attached to an Omniscribe pen recorder. The preparations were allowed to equilibrate for 30 min and tension was readjusted if necessary. The tissues were contracted 4 times with KCl 75 mM every 5 min until the amplitudes of the contractile responses were similar in magnitude. After a 30 min equilibration period, each ring was contracted with noradrenaline (0.03 μM) and relaxed with acetylcholine (ACh, 1–30 μM) to verify the functionality of the endothelium. Only preparations that relaxed by 80% or more were used. Rings were washed with PSS and after a further 30 min equilibration, a cumulative agonist concentration-effect (E/[A]) curve to noradrenaline (1 nM–100 μM) was constructed. The bath was then washed repeatedly with PSS until preparations reached their initial tension. Afterwards, tissues were exposed for 10 min to the alkylating agent, phenoxybenzamine (0.03 μM; Tabernero *et al*., 1996a) to allow partial α_1-adrenoceptor inactivation. The rings were then washed successively every 5 min for 30 min, and a second E/[A] concentration-response curve was repeated. Control experiments were run in parallel to check the reproducibility over time between the two curves performed under the above mentioned conditions.

The effect of L-NAME was studied on contractions elicited by noradrenaline alone and after partial alkylation of α_1-adrenoceptors with 0.03 μM phenoxybenzamine. The protocol of partial inactivation was similar to the one described above but the incubation was done before the first E/[A] curve. Once the agonist was washed out, L-NAME (30 μM) was incubated for 30 min before a second E/[A] curve with noradrenaline was run. Control experiments were carried out in parallel under the aforementioned conditions. In some experiments, the effect of indomethacin (100 μM), without pretreatment with phenoxybenzamine, on E/[A] curves to noradrenaline was studied by use of the same protocol as described for L-NAME.

A series of preliminary experiments were performed to determine to which extent the blockers included in the PSS (yohimbine, propranolol, desipramine and normetanephrine) influenced the noradrenaline-induced vasoconstriction in both groups of rats. The procedure followed was similar to the one described above in the absence of phenoxybenzamine. Once the presence of endothelium was proved, two concentrations E/[A] curves to noradrenaline in the absence and in the presence of these blockers were carried out in young and old animals. The presence of the antagonists at the concentrations used shifted to the left the E/[A] curve to noradrenaline to a similar extent in young (1.99 ± 0.11 times, $n = 6$) as in old (1.58 ± 0.29 times, $n = 7$) animals. These results allowed us to use the same concentration in both age groups.

Structural analysis

Morphology was studied in intact tail artery segments from young and old rats. The arteries were fixed with formalin (10% saline solution). To determine the general structure of the wall the vessels were stained for connective tissue and elastin with Van Gieson (1889) and Miller (1971) methods, respectively, and visualized with a bright field microscope. To study endothelial layer in more detail a LSCM was used. The vessels were longitudinally cut to expose the endothelial layer and incubated for 30 min at 4°C with the nuclear dye propidium iodide (10 μM) and washed overnight in distilled water. The segments were placed on a microscope slide with the endothelial layer exposed and visualized by LSCM (Odyssey, Noran Instruments, Middleton, Wisconsin, U.S.A; argon ion 529 nm line with 550 nm LP barrier filter) with a $\times 40$ oil immersion objective (NA 1.3, Nikon, Kingston on Thames, Surrey, U.K.). Metamorph software was used to acquire 10 images of the endothelial layer along each segment. The area (μm^2), the number of nuclei per mm^2 and the shape factor of the nuclei (value from 0 to 1 representing how closely the object resembles a circle) of endothelial cells were determined.

[^{3}H]-prazosin binding assay

Saturation binding studies were performed by incubation of rat tail artery rings (1.5–2 mg wet weight) under conditions similar to those previously described (Tabernero *et al*., 1996b). Six rats were used in each individual experiment to obtain sufficient material. The arteries were cut into rings (4 mm) and pooled. One ring of tail artery per tube was incubated for 60 min with increasing concentrations of [^{3}H]-prazosin (0.05–8 nM) in a final volume of 0.25 ml buffer, pH 7.4 (composition in mM: HEPES 8.3, NaCl 130, KCl 5.6, $CaCl_2$ 2, $MgCl_2$ 0.24 and glucose 11) maintained at 37 ± 0.5°C and gassed with a mixture of 95% O_2, 5% CO_2. At the end of the incubation period each ring was washed in buffer solution (5 s), dried on filter paper, weighed and dissolved in 0.1 ml of a mixture of perchloric acid:H_2O_2 (1 : 1). Radioactivity of the tissue was determined by liquid scintillation spectrometry with a counting efficiency of 60%. Specific receptor binding of [^{3}H]-prazosin was defined as the binding inhibited by the presence of 100 μM phentolamine.

Data analysis

Pragmatic logistic curve fitting Each individual set of E/[A] curve data was fitted to a logistic function of the form:

$$E = \frac{\alpha [A]^m}{[EC_{50}]^m + [A]^m} \quad (1)$$

in which E and [A] are the pharmacological effect and the concentration of agonist, respectively; α, EC_{50} and m are the asymptote, location and slope parameters, respectively. Location parameters were actually estimated as pEC_{50} (i.e. the negative logarithm of the concentration required to cause 50% of the maximal response).

Nested hyperbolic null method Data obtained from receptor inactivation experiments were analysed by the nested hyperbolic null method (James *et al*., 1989). This method involves fitting the control E/[A] curve data to equation 1 whilst simultaneously fitting the post-inactivation E/[A] curve data to the following equation:

$$E = \frac{\alpha}{\left(\frac{EC_{50}}{qK_A[A']}(K_A + [A'](1-q))\right)^m + 1} \quad (2)$$

where q represents the fractional receptor concentration which remains following inactivation.

Statistics

Experimental points and results from pragmatic logistic curve fitting are expressed as mean $\pm$ s.e.mean. The number of animals for contractile and LSCM studies and the number of experiments performed in triplicate in binding assays (n) are indicated in the legends of the figures and tables. Contractile responses are expressed as a percentage of the maximum (α) of the first curve. To obtain the E/[A] curves, experimental data were directly fitted to the mathematical models described above with the 'AR' programme (derivative-free, non-linear regression analysis) within the BMDP statistical software package (Dixon, 1990), implemented on a Vax 6610 computer. It was assumed that estimates of K_A and EC_{50} were log-nor-

mally distributed; therefore, each of these is expressed as a logarithmic value. The statistical significance for the estimated parameters (pEC_{50}, α, pK_A, q, m) and the differences between blood pressure measurements were assessed by the two-tailed Student's t test for paired or unpaired observations, respectively. Two-way analysis of variance for repeated measures followed by an orthogonal contrast test was applied to analyse the E/[A] curves. This statistical analysis was carried out with the SAS statistical package (Littell *et al*., 1991) by use of a general linear model (PROC GLM) which allows for the occurrence of unbalanced designs. A probability level of 0.05 or less was considered significant.

Ligand binding data were analysed with computerized curve-fitting programmes as described by McPherson (McPherson, 1983). The dissociation constant (K_D) and the total number of binding sites (B_{max}) were estimated by non-linear regression of saturation curves.

Drugs and isotopes

Acetylcholine HCl, desipramine HCl, indomethacin, (−)-noradrenaline bitartrate, normetanephrine HCl, N^G-nitro-L-arginine methylester, (±)-propranolol HCl, yohimbine HCl and propidium iodide were purchased from Sigma Chemical Co; [^{3}H]-prazosin from Amersham International; phentolamine mesylate and phenoxybenzamine HCl from Research Biochemical Incorporated (RBI). All drugs were prepared in physiological salt solution except noradrenaline, which was prepared in 23 μM Na_2 EDTA. Stock phenoxybenzamine solution (1 mM) was dissolved in 2 mM tartaric acid and kept at −20°C. Further dilutions of phenoxybenzamine were prepared in physiological salt solution. All other chemicals used were of analytical grade.

Results

Systolic (young: 136.9±2.9 mmHg; old: 150.8±9.7 mmHg; $n=9$) and diastolic (young: 114.5±2.4 mmHg; old: 115.0±9.6 mmHg; $n=9$) arterial blood pressure did not change but pulse pressure (young: 22.5±1.6 mmHg; old: 35.8±3.1 mmHg; $n=9$) increased ($P<0.01$) with age. Heart rate was smaller ($P<0.001$) in old (341.8±8.3 beats min^{-1}, $n=9$) compared to young (421.0±8.9 beats min^{-1}, $n=9$) animals.

Functional studies

The experiments were performed only in vessels with functional endothelium. Among them the ability of ACh to relax the noradrenaline-induced contraction showed that most (94%) of the vessels from young rats relaxed by 80–100% with 1 μM ACh. However, only half of the rings (52%) from old animals relaxed by 80–100% with the same concentration of ACh. In aged animals, concentrations of 3 μM and 10 μM ACh were needed to attain the same relaxation in 37% and 11% of the vessels, respectively.

Noradrenaline contracted the tail artery rings in a concentration-dependent manner. The results are presented in Figure 1 and Table 1. Maximum responses to noradrenaline (α) and KCl were significantly higher ($P<0.001$) in young than in old animals. However, neither the sensitivity (pEC_{50}) nor the slope (m) of the noradrenaline E/[A] curve were affected by ageing.

The effect of irreversible antagonism by phenoxybenzamine on the noradrenaline E/[A] curve was examined in rings from young and old animals. The averaged data are also illustrated in Figure 1 (c, d) and Table 1. The presence of the alkylating agent shifted to the right ($P<0.001$) the E/[A] curve to the agonist in both age groups. Furthermore, the maximum responses to noradrenaline were depressed by 41.73±3.91% ($n=11$) in young and by 32.25±3.13% ($n=14$) in old animals (Figure 1, c, d). Quantitative evaluation of the phenoxybenzamine effect was carried out by use of the nested hyperbolic method. Each pair of E/[A] curves in the absence and presence of phenoxybenzamine depicted in Figure 1 were analysed simultaneously to obtain the respective pK_A and q values. Neither the estimated pK_A values for noradrenaline (young: 5.10±0.05, $n=11$; old: 5.16±0.08, $n=14$) nor the theoretical percentage of functional receptors remaining after phenoxy-

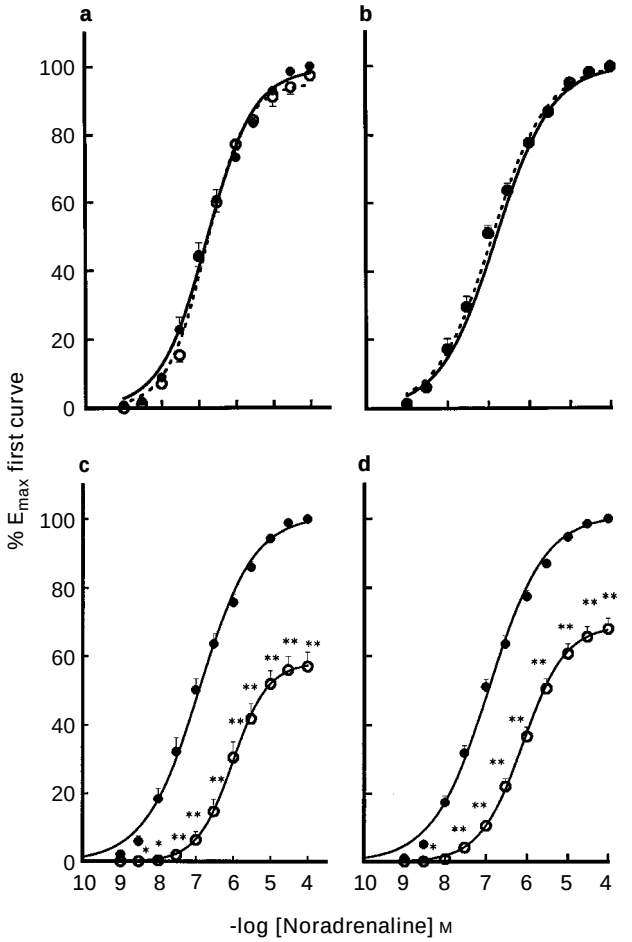

Figure 1 Effect of preincubation with 0.6 μM tartaric acid (○, vehicle, a, b) and 0.03 μM phenoxybenzamine (○, c, d) on the contraction induced by noradrenaline (NA) in tail artery rings from (a and c) young and (b and d) old Sprague-Dawley rats. (●) Control responses to NA. Results are expressed as percentage of the maximum response obtained in the first curve. The lines drawn through the data were obtained by pragmatic logistic curve fitting (see Methods). Results are the mean of 11 (young rats) and 14 (old rats) experiments; vertical lines show s.e.mean. *$P<0.05$, **$P<0.01$.

Table 1 Parameter estimates for noradrenaline obtained by pragmatic logistic fitting of agonist E/[A] curves performed in intact tail artery rings from young and old rats

	pEC_{50}	α (mN)	m
3–4 months			
Noradrenaline	6.91±0.09	16.52±0.52[b]	0.60±0.06
Noradrenaline + Pbz	5.93±0.09[a]	9.55±0.44[a]	0.88±0.13
KCl 75 mM	–	11.95±0.60[b]	–
20–22 months			
Noradrenaline	6.89±0.12	12.33±0.55	0.63±0.09
Noradrenaline + Pbz	6.08±0.10[a]	8.43±0.42[a]	0.80±0.12
KCl 75 mM	–	8.68±0.26	–

[a]$P<0.001$ compared to noradrenaline (paired t test). [b]$P<0.001$ compared to old rats (unpaired t test); $n=11$ for young and $n=14$ for old rats. Pbz, phenoxybenzamine; pEC_{50}, the concentration required to cause 50% of the maximum response; α, the maximum response and m, the slope of the concentration-response curve.

benzamine inactivation ($q \times 100$) were affected by age (young: 4.50 ± 1.43, $n=11$; old: 7.69 ± 1.58, $n=14$). No difference between E/[A] curves to noradrenaline were observed in the presence of the vehicle (0.6 μM tartaric acid) used to dissolve phenoxybenzamine either in young or old animals (Figure 1, a, b).

L-NAME did not induce a contractile response in tail artery rings from either age group. The evaluation of the effect of L-NAME (30 μM) on noradrenaline-induced responses, showed no difference in the potency (pEC_{50}) in either young (control: 6.84 ± 0.05; L-NAME: 6.64 ± 0.10, $n=6$) or old (control: 6.66 ± 0.14; L-NAME: 6.65 ± 0.19, $n=6$) rats. The maximum effect induced by noradrenaline was unchanged by the NO-synthase inhibitor in both age groups (results not shown). Since we have previously observed that in tail artery rings from 3 month old rats (Tabernero *et al*., 1996a), the effect of L-NAME on contractile responses to a full agonist should be studied when the population of receptors has been substantially decreased, these experiments were repeated in the presence of phenoxybenzamine. After partial alkylation of α_1-adrenoceptors, L-NAME (30 μM) significantly potentiated the E/[A] curve for noradrenaline in young (Figure 2a) animals. In contrast, in old animals the effect of the NO-synthase inhibitor was observed on some but not all of the concentrations of noradrenaline used (Figure 2b). When the same experiment was run in parallel with PSS instead of L-NAME no difference was observed between the first and the second curve for noradrenaline, in the presence of phenoxybenzamine.

The addition of indomethacin (100 μM) to the incubation medium of rings from young animals induced a slight but not significant shift to the right of the E/[A] curve for noradrenaline (control: 6.91 ± 0.09, $n=11$; indomethacin: 6.70 ± 0.04, $n=6$). Old animals also showed a similar midrange sensitivity in the absence (pEC_{50}: 6.89 ± 0.10, $n=14$) or presence (pEC_{50}: 6.90 ± 0.06, $n=6$) of indomethacin. The maximum contraction to noradrenaline was unaffected by the cyclo-oxygenase inhibitor in both young (control: 16.35 ± 0.80 mM, $n=11$; indomethacin: 15.10 ± 2.80 mM, $n=6$) and old (control: 12.26 ± 1.03 mM, $n=14$; indomethacin: 12.50 ± 0.87 mM, $n=6$) rats.

Structural analysis

Intact segments of tail artery, other than those used in functional studies but belonging to the same young and old rats, were analysed to determine the wall structure. There were no differences in the general wall structure between old and young rats, e.g. wall thickness, number of smooth muscle cell (SMC) layers and elastic lamina (data not shown). Furthermore, endothelial structure was assessed with LSCM. There was no difference either in the nucleus area (μm^2) or the number of endothelial cells per mm^2 between young and old rats (Table 2). The shape factor for endothelial nuclei was smaller ($P<0.001$) in segments from old rats when compared to young animals. This indicates that the nuclei of endothelial cells are elongated by ageing (Table 2 and Figure 3). LSCM also allowed for visualization of the first layer of SMCs underneath the endothelium. This layer was similar in young and old rats and the SMC nuclei showed similar shape, area and orientation (results not shown).

Binding studies

Saturation studies were carried out to determine the K_D and the B_{max} values in our experimental conditions. We observed that [^{3}H]-prazosin labelled a homogeneous population of binding sites in rings of tail artery from either age group. [^{3}H]-prazosin binding in intact rings of tail artery from young and old animals was saturable and of high affinity. The specific binding represented approximately 50–60% of the total binding at a concentration of [^{3}H]-prazosin close to its K_D. Non-linear regression analysis of the saturation data was

Table 2 Endothelial nuclei area, number and shape factor in tail artery segments from young and old rats

	Young	*Old*
Area (μm^2)	238.6 ± 15.0	260.4 ± 16.8
Number per mm^2	762.0 ± 52.8	746.0 ± 46.9
Shape factor	0.503 ± 0.012**	0.408 ± 0.012

Data are mean ± s.e.mean, $n=7$ rats. **$P<0.001$ with respect to old rats.

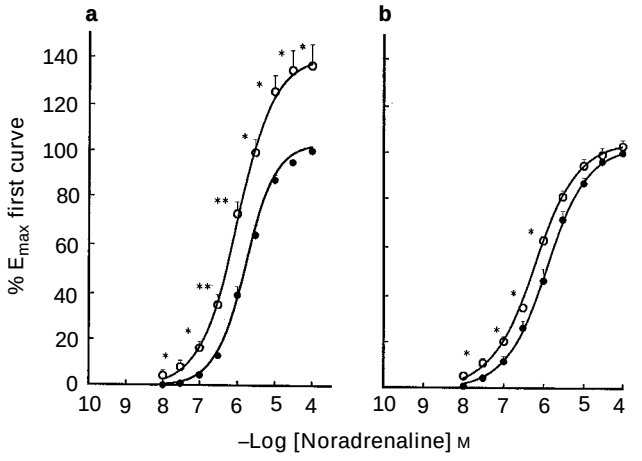

Figure 2 Effect of preincubation with 30 μM L-NAME (○) on the contraction induced by noradrenaline, in presence of 0.03 μM phenoxybenzamine in tail artery rings from (a) young and (b) old Sprague-Dawley rats. (●) Control responses to noradrenaline. Results are expressed as percentage of the maximum response obtained in the first curve. The lines drawn through the data were obtained by pragmatic logistic curve fitting (see Methods). Results are the mean of 6 experiments; vertical lines show s.e.mean. *$P<0.05$, **$P<0.01$.

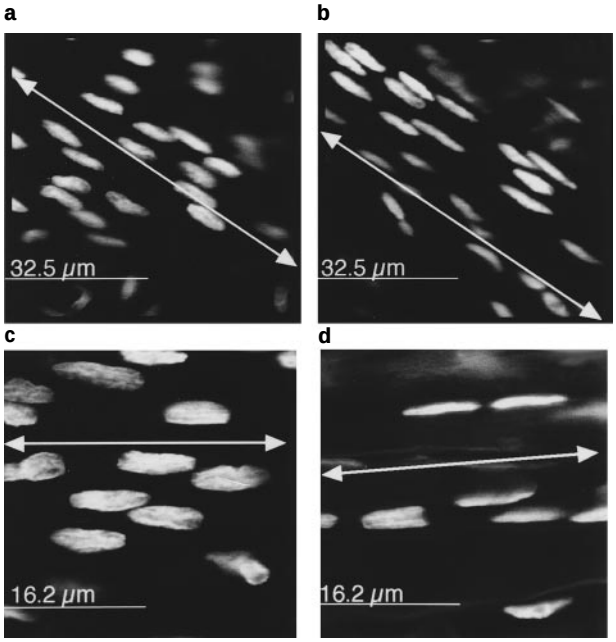

Figure 3 Images of the endothelial layer from segments of (a and c) young and (b and d) old tail arteries. The vessels were longitudinally cut and the endothelial layer was stained with 10 μM of the nuclear dye propidium iodide. The images were taken from slide-mounted vessels with a ×40 oil immersion objective, zoom ×1 (a and b) or zoom ×2 (c and d) with a laser scanning confocal microscope. Lines with arrows show the longitudinal axis of the vessel. Image dimensions are shown in each figure.

consistent with the presence of a single population of sites. The derived K_D (young: 3.26 ± 0.77 nM, $n=7$; old: 3.23 ± 0.78 nM, $n=9$) and B_{max} (young: 16.23 ± 3.08 fmol mg^{-1} tissue, $n=7$; old: 15.61 ± 2.70 fmol mg^{-1} tissue, $n=9$) were not changed by age.

Discussion

This study was designed to assess the influence of receptor density, functional receptor reserve and endothelium on α_1-adrenoceptor-mediated vasoconstriction by noradrenaline in the ageing process.

The Sprague-Dawley rats used in our study did not show any difference of diastolic and systolic blood pressure but an increase in pulse arterial pressure with age. Conflicting results on blood pressure changes on ageing have been presented in the literature. Thus, an increase in diastolic and systolic blood pressure in Sprague-Dawley rats (Hashimoto *et al*., 1995) and in mean arterial pressure from Wistar Kyoto and spontaneously hypertensive rats (Hüsken *et al*., 1993) were found in ageing. In contrast, studies in Ivanos or Wistar and Fisher rats have also shown that ageing is not accompanied by an increase in mean (Fouda & Atkinson, 1986) or pulse pressure (Lartaud *et al*., 1993), respectively. It is worthy of note that chronic elevations of pulse pressure may be more important than mean pressure as a determinant of vascular structure (Ryan *et al*., 1995). In addition, heart rate was reduced in old anaesthetized rats. Previous studies have shown a decreased heart rate during maturation (Lartaud *et al*., 1993) or no change in the anesthetized aged rats (Docherty, 1990). This alteration has been attributed to the attenuation of tachycardic responses to β-adrenoceptor stimulation (Folkow & Svanborg, 1993).

The present study shows that age produces a decrease in the maximum response with no alteration in the potency (pEC_{50}) and affinity (pK_A) of noradrenaline induced vasoconstriction on intact tail artery rings. β-Adrenoceptor-mediated responses in the cardiovascular system of man and animals usually seem to be reduced with age (Marín, 1995) but no differences in postjunctional α_2-adrenoceptor-mediated vasoconstriction have been observed between tail artery rings from old and young Wistar rats (Tsai *et al*., 1993). The rat tail artery is a richly innervated vessel where neuronal uptake accounts for more than 90% of the uptake of noradrenaline (Webb *et al*., 1981). Nevertheless, there is no consensus on the effect of age on neuronal uptake mechanisms in blood vessels. Data showing no change (Bucholz & Duckles, 1990) or a decrease (Docherty & Hyland, 1986; Borton & Docherty, 1989) in the uptake mechanisms with ageing have been obtained. Our experiments were carried out in the presence of yohimbine, propranolol, desipramine and normetanephrine to avoid the effect of α_2- and β-adrenoceptors and neuronal and extraneuronal uptake, respectively. In previous experiments, we observed that the concentration used of all these blockers affected to the same extent the noradrenaline-induced vasoconstriction in young and in old animals (see Methods). Thus, in our experimental conditions, responses to noradrenaline are due to stimulation of α_1-adrenoceptors and should not be affected by possible differences in either uptake mechanisms or α_2- and β-adrenoceptors.

There are several factors that could account for the smaller development of maximum contraction to noradrenaline observed in old animals: (i) alterations in the wall morphology, (ii) a decrease in α_1-adrenoceptor density, (iii) an impairment of the contractile machinery, (iv) differences in endothelial functionality and (v) a modification of the post-receptor events.

Changes in vascular structure appear to occur during ageing (Fornieri *et al*., 1992; Tracy, 1995; Robert, 1996) and seem to be paralleled by an increase in pulse pressure (Ryan *et al*., 1995). However, analysis of vessel wall structure showed similar thickness and SMC characteristics in young and old rats, suggesting that the decrease in contractile responses in the tail artery from aged rats is not due to a morphological alteration of the tissue.

Binding experiments showed a similar receptor density between age groups, indicating that a decrease in receptor number does not account for the alteration of maximum response to noradrenaline observed in old animals. In accord with our results, no difference in α_1-adrenoceptor density has been previously observed, between young and old animals, in the carotid artery from Wistar (Benetos *et al*., 1993) and in the aorta from Fisher 344 (Gurdal *et al*., 1995a) rats.

Arteries from old animals tend to show a reduced maximum contraction to several agonists including catecholamines and KCl, suggesting that basic excitation-contraction links and/or the contractile machinery *per se* have become modestly altered with age (Folkow & Svanborg, 1993). Furthermore, the vasoconstriction obtained with KCl was greater in young than in old rats ($P<0.01$). Potassium-induced vasoconstriction in tail artery is mediated by smooth muscle depolarization and release of endogenous noradrenaline (Fouda *et al*., 1991; Xiao & Rand, 1991). The fact that KCl-induced responses were impaired in aged rats may be related to an alteration in either the basic contractile machinery or a diminished stimulation of α_1-adrenoceptors by the noradrenaline released by KCl.

To test the role of NO released from the endothelium on the decrease of maximum contraction observed in old animals, the effect of L-NAME on this response was studied. L-NAME was without effect on noradrenaline vasoconstriction in either age group. These results agree with previous observations in young rats (Tabernero *et al*., 1996a), where we have demonstrated that in rat tail artery rings, due to the high efficiency of coupling exhibited, the influence of the NO-synthase inhibitor could not be seen unless the population of α_1-adrenoceptors was diminished by an alkylating agent. However, other authors (Thorin & Atkinson, 1994), using perfused tail artery from Wistar rats, observed that removal of endothelium enhanced the vasoconstrictor response to 1 μM but not to 3 μM noradrenaline. We do not have a clear explanation for the difference between this and our studies other than variations in the rat strain and the experimental model. The lack of effect of L-NAME observed in old animals can also be attributed to a high functional reserve since the percentage of receptors remaining after partial alkylation ($q \times 100$) and the affinity (pK_A) were similar in old and young rats, giving an overlap of the occupancy-response curves (results not presented). As expected from our previous observations, in the presence of phenoxybenzamine, L-NAME significantly potentiated the noradrenaline-induced vasoconstriction in rings from young rats (Tabernero *et al*., 1996a). Nevertheless, the influence of the NO-synthase inhibitor was only observed on the lower part of the E/[A] curve to noradrenaline in old animals indicating a decreased effect of NO on the α_1-adrenoceptor-mediated responses. Moreover, when we checked the ability of ACh to relax rings precontracted with noradrenaline (0.03 μM), only 52% of the vessels from old but 94% from young rats relaxed by more than 80% to 1 μM ACh. The present study supports the hypothesis of an impairment of endothelial function in blood vessels during ageing reported by several authors (Marín, 1995; Gerhard *et al*., 1996). Some studies have shown that the increase in pulse pressure is related to an impairment of synthesis or release of endothelium-derived relaxing factor (Hutcheson & Griffith, 1991; Ryan *et al*., 1995) and also to structural changes in the vasculature (Baumbach & Heidstad, 1993; Robert, 1996). Thus, we could speculate that the alteration of the endothelial function in tail artery from aged rats could be due to an elevation of pulse pressure that is accompanied by a physical abnormality. Our results with LSCM showed more elongated nuclei in the endothelium of old rats. This is in agreement with previous findings in aorta from 25 months old Wistar rats where elongation and thinning of endothelial cells have been described (Fornieri *et al*., 1992). Nevertheless, if differences in NO affect the maximum contraction to noradrenaline, an impairment would be manifested as an increased α_1-adrenoceptor-mediated response rather than

the observed decrease. To exclude the possibility that an alteration of cyclo-oxygenase-dependent contracting factors released by endothelial cells, could account for the smaller noradrenaline response in old rats, E/[A] curves for noradrenaline were carried out in the presence of indomethacin, a cyclo-oxygenase inhibitor. This compound was without effect on E/[A] curves to the agonist in either young or old rats, excluding the possibility that a difference in the release of an endothelium contractile factor could counteract the vasodilatation due to NO. Again, these results agree with those observed previously (Ryan *et al*., 1995) where an increase in pulse pressure did not modify the effect of indomethacin on acetylcholine-induced relaxations.

Differences in transductional mechanisms could also explain an impairment of maximum response to agonists. The α_1-adrenoceptor-mediated contraction depends on both intra- and extracellular calcium but the participation of each mechanism varies depending on the agonist used. We have demonstrated, in tail artery rings from 3 month old Sprague-Dawley rats, that noradrenaline-induced vasoconstriction utilizes predominantly, but not exclusively, intracellular calcium via inositol phosphate turnover (Tabernero *et al*., 1996b). Important alterations in the function of G proteins have been suggested to occur in hypertension and ageing (Feldman *et al*., 1995; Johnson *et al*., 1995). Nevertheless, in previous experiments ageing did not change the phosphatidylinositol hydrolysis to noradrenaline in tail artery and aorta (Tabernero & Vila, 1995). These results would suggest that the G protein linked to this mechanism is not altered by age. However, we cannot exclude the possibility that the ageing process could modify either the G protein linked to the entry of extracellular calcium or later steps on the post-transductional pathway implicated in the final vasoconstriction.

In summary, the present study shows that the noradrenaline-induced contraction is decreased in older rats probably due to differences in either the contractile machinery or post-receptor mechanisms. These alterations may be accompanied by an impairment of the release or production of NO from endothelial cells.

This study was supported by DGICYT (UE94-0018; PB95-0624); the European Community BIOMED Program (BMH1-CT94-1357) and Generalitat de Catalunya, CIRIT (GRQ93-2036). We would like to thank Carme Alemany and Cristina Rubio for their technical assistance and Dr Ian Montgomery for the morphological studies.

References

ATKINSON, J., RABELAIS, T.T. & CAPDEVILLE-ATKINSON, C. (1994). Reduction of endothelial function with age in the mesenteric arterial bed of the normotensive rat. *Br. J. Pharmacol*., **111,** 1184–1188.

BAO, J.X., GONON, F. & STJÄRNE, L. (1993). Frequency-dependent and train length-dependent variation in the role of postjunctional α_1-adrenoceptor and α_2-adrenoceptor for the field stimulation-induced neurogenic contraction of rat tail artery. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **347,** 601–616.

BAUMBACH, G.L. & HEIDSTAD, D.D. (1993). Effects of increased pulse pressure on cerebral structures. *FASEB J*., **7,** A750.

BENETOS, A., HUGUET, F., ALBADALEJO, P., BRISAC, A.M., PAPPO, M., SAFER, M.E. & LEVY, B.I. (1993). Role of adrenergic tone in mechanical and functional properties of carotid artery during aging. *Am. J. Physiol*., **265,** H1132–H1138.

BORTON, M. & DOCHERTY, J.R. (1989). The effects of ageing on neuronal uptake of noradrenaline in the rat. *Naunyn-Schmiedeberg's Arch. Pharmacol*., **340,** 139–143.

BUCHOLZ, J. & DUCKLES, S.P. (1990). Effect of age on prejunctional modulation of norepinephrine release. *J. Pharmacol. Exp. Ther*., **252,** 159–164.

COX, R.H., JONES, A.W. & SWAIN, M.L. (1976). Mechanisms and electrolyte composition of arterial smooth muscle in developing dogs. *Am. J. Physiol*., **23,** 77–83.

DIXON, W.J. (1990). *BMDP Statistical software*. Berkeley, Los Angeles, London: University of California Press.

DOCHERTY, J.R. (1990). Cardiovascular responses in ageing: a review. *Pharmacol. Rev*., **42,** 103–125.

DOCHERTY, J.R. & HYLAND, L. (1986). α-Adrenoceptor responsiveness in the aged rat. *Eur. J. Pharmacol*., **126,** 75–80.

EPSTEIN, F.H. (1992). Age and the cardiovascular system. *N. Eng. J. Med*., **327,** 1735–1739.

FELDMAN, R.D., TAN, C.M. & CHORAZYCZEWSKI, J. (1995). G protein alterations in hypertension and aging. *Hypertension*, **26,** 725–732.

FOLKOW, B. & SVANBORG, A. (1993). Physiology of cardiovascular aging. *Physiol. Rev*., **73,** 725–764.

FORNIERI, C., QUAGLINO, D. Jr. & MORI, G. (1992). Role of extracellular matrix in age-related modifications of the rat aorta. *Arteriosclerosis Trombosis*, **12,** 1008–1016.

FOUDA, A.K. & ATKINSON, J. (1986). Reduced sensitivity to noradrenaline and electrical stimulation of the caudal artery of the aged rat. *Arch. Mal Coeur*, **79,** 919–922.

FOUDA, A.K., KAUFMANN, A., THORIN, E., HENRION, D., CAPDEVILLE-ATKINSON, C. & ATKINSON, J. (1991). The role of endogenous norepinephrine release in potassium-evoked vasoconstriction of the rat tail artery. *Eur. J. Pharmacol*., **205,** 63–72.

GERHARD, M., RODDY, M.A., CREAGER, S.J. & CREAGER, M.A. (1996). Aging progressively impairs endothelium-dependent vasodilatation in forearm resistance vessels of humans. *Hypertension*, **27,** 849–853.

GURDAL, H., FRIEDMAN, E. & JOHNSON, M.D. (1995a). Effects of dietary restriction on the change in aortic α_1-adrenoceptor mediated responses during aging in Fisher 344 rats. *J. Gerontol*., **50,** B67–B71.

GURDAL, H., TILAKARATNE, R., DALE BROWN, R., FONSECA, M., FRIEDMAN, E. & JOHNSON, M.D. (1995b). The expression of alpha$_1$ adrenoceptor subtypes changes with age in the aorta. *J. Pharmacol. Exp. Ther*., **275,** 1656–1662.

HADJU, M.A., MCELMURRY, R.T., HEISTAD, D.D. & BAUMBACH, G.L. (1993). Effect of ageing on endothelium-dependent vascular relaxation of isolated human basilar artery to thrombin and bradykinin. *Stroke*, **21,** 1039–1043.

HASHIMOTO, M., SHINOZUKA, K., BJUR, R.A., WESTFALL, D.P., HATTORI, K. & MASUMURA, S. (1995). The effects of age on the release of adenine nucleosides and nucleotides from rat caudal artery. *J. Physiol*., **489,** 841–848.

HÜSKEN, B.C.P., HENDRIKS, M.G.C., PFAFFENDORF, M. & VAN ZWIETEN, P.A. (1993). Effects of aging and hypertension on the reactivity of isolated conduit and resistence vessels. *Microvasc. Res*., **48,** 303–315.

HUTCHESON, I.R. & GRIFFITH, T.M. (1991). Release of endothelium-derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. *Am. J. Physiol*., **261,** H257–H262.

HYNES, M.R. & DUCKLES, S.P. (1987). Effect of increasing age on the endothelium-mediated relaxation of rat blood vessels in vitro. *J. Pharmacol. Exp. Ther*., **241,** 387–392.

JAMES, M.K., MORGAN, P.H. & LEIGHTON, H.J. (1989). A new method for estimation of agonist dissociation constants (K_A): directly fitting the postinactivation concentration-response curve to a nested hyperbolic equation. *J. Pharmacol. Exp. Ther*., **249,** 61–69.

JOHNSON, M.D. & WRAY, A. (1990). Alpha-1 adrenergic receptor function in senescent Fisher 344 rat aorta. *Life Sci*., **46,** 359–366.

JOHNSON, M.D., ZHOU, Y., FRIEDMAN, E. & ROBERTS, J. (1995). Expression of G protein α subunits in the aging cardiovascular system. *J. Gerontol*., **50A,** B14–B19.

LARTAUD, I., BRAY-DES-BOSC, J.M., CHILLON, J.M., ATKINSON, J., CORMAN, B. & CAPDEVILLE-ATKINSON, C. (1993). In vivo cerebrovascular reactivity in Wistar and Fisher 344 rat strains during aging. *Am. J. Physiol*., **33,** H851–H858.

LITTELL, R.C., FREUND, R.J. & SPECTOR, P.C. (1991). *SAS System for Linear Models*. Cary N.C.: SAS Institute Inc.

LÜSHER, T.F., DOHI, Y. & TSCHUDI, M. (1992). Endothelium-dependent regulation of resistance arteries: alterations with ageing and hypertension. *J. Cardiovasc. Pharmacol*., **19,** S34–S42.

MARÍN, J. (1995). Age-related changes in vascular responses: a review. *Mech. Ageing Dev*., **79,** 71–114.

MCDONALD, A., MACDONALD, E., FULTON, J.D., WADSWORTH, R.M., SCOTT, P.J.W. & HOWIE, K.A. (1995). No evidence for a general change in contractile responsiveness of the mesenteric artery with ageing. *J. Gerontol.*, **50A,** B20 – B25.

MCPHERSON, G.A. (1983). A practical computer-based approach to the analysis of radioligand binding experiments. *Computer Progr. Biomed.*, **17,** 107 – 113.

MILLER, P.J. (1971). An elastin stain. *Med. Lab. Technol.*, **28,** 148 – 149.

MINNEMAN, K.P., FOX, A.W. & ABEL, P.W. (1983). Occupancy of α_1-adrenergic receptors and contraction of rat vas deferens. *Mol. Pharmacol.*, **23,** 359 – 368.

OLAH, M.E. & RAHWAN, R.G. (1987). Age-related changes in the responsiveness of the rat aortae to depolarizing and receptor-mediated contractile stimuli and to calcium antagonist. *Pharmacology*, **35,** 163 – 173.

PATERNO, R., FARACI, F.M. & HEISTAD, D.D. (1994). Age-related changes in release of endothelium-derived relaxing factor from the carotid artery. *Stroke*, **25,** 2459 – 2462.

ROBERT, L. (1996). Aging of the vascular wall and atherigenesis: role of the elastin-lamini receptor. *Atherosclerosis*, **123,** 169 – 179.

RYAN, S.M., WAACK, B.J., WENO, B.L. & HEISTAD, D.D. (1995). Increases in pulse pressure impair acetylcholine-induced vascular relaxation. *Am. J. Physiol.*, **268,** H359 – H363.

SALLÉS, J., GIRALDO, J. & BADIA, A. (1994). Analysis of agonism at functional prejunctional α_2-adrenoceptors of rat vas deferens using operational and null approaches. *Eur. J. Pharmacol.*, **258,** 229 – 238.

SCOTT, P.W. & REID, J.L. (1992). The effect of age on the responses of human isolated arteries and veins to noradrenaline. *Br. J. Clin. Pharmacol.*, **13,** 237 – 239.

SHIMIZU, I. & TODA, N. (1986). Alterations with age of the response to vasodilator agents in isolated mesenteric arteries of the beagle. *Br. J. Pharmacol.*, **89,** 769 – 778.

STEVENS, M.J., LIPE, S. & MOULDS, R.F.W. (1982). The effect of age on the responses of human isolated arteries and veins to noradrenaline. *Br. J. Clin. Pharmacol.*, **14,** 750 – 752.

TABERNERO, A. & VILA, E. (1995). Effect of age on noradrenaline response in rat tail artery and aorta: role of endothelium. *J. Auton. Pharmacol.*, **15,** 327 – 333.

TABERNERO, A., GIRALDO, J. & VILA, E. (1996a). Effect of N^G-nitro-L-arginine methyl ester (L-NAME) on functional and biochemical α_1-adrenoceptor-mediated responses in rat blood vessels. *Br. J. Pharmacol.*, **117,** 757 – 763.

TABERNERO, A., VIVAS, N.M. & VILA, E. (1996b). Sources of calcium and α_1-adrenoceptor-mediated contraction in rat tail artery. *Br. J. Pharmacol.*, **118,** 2067 – 2072.

THORIN, E. & ATKINSON, J. (1994). Modulation by the endothelium of sympathetic vasoconstriction in an *in vitro* preparation of rat tail artery. *Br. J. Pharmacol.*, **111,** 351 – 357.

TRACY, R.E. (1995). Declining density of intimal smooth muscle cells and age as preconditions for atheronecrosis in the basilar artery. *Virchows Arch.*, **427,** 131 – 138.

TSAI, H., BUCHOLZ, J. & DUCKLES, S.P. (1993). Postjunctional α_2-adrenoceptors in blood vessels: effect of age. *Eur. J. Pharmacol.*, **237,** 311 – 316.

VAN GIESON, J. (1889). Laboratory notes on technical methods for the nervous system. *New York Med. J.*, **50,** 57 – 69.

WADSWORTH, R.M. (1990). Calcium and vascular reactivity in ageing and hypertension. *J. Hypertens.*, **8,** 975 – 983.

WEBB, R.C., VANHOUTTE, P.M. & BOHR, D.F. (1981). Adrenergic neurotransmission in vascular smooth muscle from spontaneously hypertensive rats. *Hypertension*, **3,** 93 – 103.

XIAO, X.H. & RAND, M.J. (1991). Mechanisms of vasoconstrictor responses to KCl in rat isolated perfused tail arteries: interaction with the α_2-adrenoceptor agonist UK14304. *Eur. J. Pharmacol.*, **196,** 133 – 136.

YIN, F.C.P. (1980). The aging vasculature and its effects on the heart. In *The Aging Heart*, ed. Weisfeldt, M.J., pp 137 – 214, New York: Raven Press.

(Received October 4, 1996
Revised February 7, 1997
Accepted March 18, 1997)