Sympathetic neurotransmission in the tail artery of aging rats

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1 Age-related changes in noradrenergic neurotransmission in the tail arteries of three rat strains: outbred Wistar (WI/Ico), inbred Wistar (WAG/Rij) and inbred Fischer (F344) have been compared in the present study.

2 The arterial noradrenaline content varied from 5 to 10 ng mg⁻¹ wet weight amongst young (3 to 6-month old) representatives of each strain, but did not change with age. As protein content increased in senescent rats (24-month old) by 30–40%, arterial tissue growth would not appear to receive a concomitant increase in sympathetic growth leading to relative, age-related, structural sympathectomy in all strains.

3 The vasoconstrictor response to transmural electrical stimulation was diminished in adult and senescent rats of all strains.

4 As far as could be judged from the increase in noradrenaline release following perfusion with the α -adrenoceptor antagonist, phentolamine (1 μ M), the presynaptic α_2 -adrenoceptor-mediated inhibition of noradrenaline release was intact in old representatives of all strains.

5 With blockade of the two main systems which control noradrenaline release in the rat tail artery, *viz.* neuronal reuptake with cocaine (4 μ M) and presynaptic α_2 -adrenoceptors with phentolamine (1 μ M), stimulation-evoked release of noradrenaline was similar at all ages and in all strains. This suggests that in the rat tail artery the basic mechanism of neuronal release of noradrenaline is not functionally modified by aging.

6 We conclude that as sympathetic nerve terminals are apparently intact in all three strains of senescent rats used, the age-associated deficit of α -adrenergic control of vascular function is postsynaptic in nature.

Keywords: Noradrenaline; vasoconstriction; rat tail artery; strain of rat; age; electrical stimulation

Introduction

Although the study of the physiology of the aging vascular system in animals is fraught with many difficulties (Bennett & Gardiner, 1988; Vanhoutte, 1988), at first sight the rat would appear to be a good choice for the evaluation of changes in noradrenergic regulation of the vascular system with advancing age in the absence of pathological complications. Thus, for instance, the rat does not develop hypertension with age (Lartaud *et al.*, 1993) and is resistant to atherosclerosis (Gill *et al.*, 1989). Yet experiments carried out in the rat have often given contradictory data (Docherty, 1986, 1990). There are several reasons for this, and Docherty (1990) suggested that the strain of the rat used – generally Fischer 344 or, more recently, Wistar – may be important.

Apart from certain exceptions, such as α_2 -adrenoceptor-mediated inhibition of release, noradrenergic neuroeffector function is maintained with advancing age in the Fischer 344 strain (Duckles *et al.*, 1985), which is the strain most frequently used in this type of study. This may not be the case in other strains (Fouda & Atkinson, 1986; Wanstall & O'Donnell, 1988).

The main objective of the current study, therefore, was to ascertain the effect of aging on noradrenergic, neuroeffector mechanisms by comparing age-related changes in different strains of rats (Wistar and Fischer 344). Two Wistar strains were used, the first an outbred Wistar strain (WI/Ico), with which the methodology was developed (Thorin & Atkinson, 1994), and the second an inbred Wistar strain (WAG/Rij)

which was used in a recent multicentric study on vascular aging (Heudes *et al.*, 1994; Michel *et al.*, 1994).

Experiments were performed *in vitro* on the richly innervated tail artery (Handa & Duckles, 1987). This artery was used rather than preparations such as the aorta in which structural rather than functional modifications may explain the altered reactivity (Duckles *et al.*, 1985; Docherty, 1990).

Changes in responses to nerve stimulation could arise from a modification of the presynaptic noradrenaline release mechanism(s) and/or of the postsynaptic reactivity to noradrenaline. The technique we used (Thorin & Atkinson, 1994) allows simultaneous measurement of noradrenaline release and vasoconstriction.

Finally, in so-called aging studies, young and adult animals have often been used but truly senescent animals have not always been available. As sexual maturity is attained at 2–3 months and the increase in body weight tails off at around 9 months (Handa & Duckles, 1987; Weihe, 1987), we used 'young' rats aged 3–6 months. Senescent rats were 24-months old, a commonly accepted age for senescence in rats (Docherty, 1990; Wadsworth, 1990). Longevity, as judged from the median length of life, is similar in all 3 strains: 21–29 months in outbred Wistar (Masoro, 1980), 22–29 months in Fischer 344 (Masoro, 1980), and 27 months in Wistar WAG/Rij (Heudes *et al.*, 1994).

In experiments on the Wistar strain which, as stated above, has been less investigated than the Fischer 344 strain, an intermediate age group of 12 months (= adult) was used. The ages of the Fischer 344 rats (3–4 and 24 months) are the same as those taken by Martinez *et al.* (1981) as representative of 'young' and 'aged' rats and include those, 6 and 20 months, suggested by Buchholz *et al.* (1992) as representing the extremes of the greatest age-related difference in noradrenergic function in this strain.

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Four criteria were used for the evaluation of age-related differences in noradrenergic, vascular neuroeffector mechanisms. They were: (1) the density of sympathetic innervation, as indicated by the noradrenaline content of the tail artery relative to wet weight or protein content; (2) the efficiency of the postsynaptic vasoconstrictor mechanism as indicated by the increase in perfusion pressure following transmural nerve stimulation; (3) the efficiency of the α_2 -adrenoceptor-mediated inhibition of noradrenaline release, as indicated by the increase in noradrenaline spillover following perfusion with the α -adrenoceptor antagonist phentolamine, and (4) the efficiency of the basic mechanism of neuronal release of noradrenaline, as indicated by the noradrenaline spillover into the perfusate in the presence of the neuronal uptake blocker, cocaine, and phentolamine.

Methods

Animals

Male rats were used in all experiments. The strains and ages used were: (1) outbred Wistar WI/Ico, 3–4 ($n = 10$), 12 ($n = 12$) and 24 months ($n = 14$); (2) inbred Fischer 344, 3–4 ($n = 8$) and 24 months ($n = 8$); and (3) inbred Wistar WAG/Rij, 6 ($n = 9$), 12 ($n = 13$) and 24 ($n = 10$) months. The Wistar WI/Ico and Fischer 344 rats (Iffa-Credo SA, L'Arbresle, France) received food and water *ad libitum*, and were subjected to standard housing and lighting conditions for at least 10 days before each experiment. Twelve-month old Wistar WI/Ico were used in our previous publication which describes the methodology (Thorin & Atkinson, 1994).

The origin, housing and treatment of the Wistar WAG/Rij has been described in detail elsewhere (Heudes *et al.*, 1994).

Anaesthesia, tissue sampling, noradrenaline and protein contents

Under anaesthesia (sodium pentobarbitone, 50 mg kg⁻¹, i.p.), a 1 ml blood sample was taken by aortic puncture (heparin 10 i.u. ml⁻¹) for determination of plasma noradrenaline levels. Following centrifugation (1000 g, 4°C, 5 min), perchloric acid (50 μ l, 0.3 N) and 3–4, dihydroxybenzylamine (36 nM, internal standard) were added to the plasma (100 μ l) which was stored at -80°C (≤ 1 week; loss of noradrenaline is undetectable when samples are stored at -80°C for ≤ 2 months, unpublished results).

Twice distilled water (500 μ l), aluminium oxide (15 mg), and 100 μ l of Na₂EDTA (0.13 M) plus Na₂S₂O₅ (53 mM) were added to the plasma, and pH adjusted to 8.6 with 0.25 ml of Tris-HCl (2 M, pH 8.8). Samples were shaken at 4°C for 15 min and the supernatant discarded. The alumina was washed 3 times with twice distilled water (2 ml) at 4°C, and noradrenaline eluted with perchloric acid (50 μ l, 0.3 N) prior to high performance liquid chromatography. Plasma noradrenaline concentration is expressed as pg ml⁻¹. It should be noted that values are for samples obtained under pentobarbitone anaesthesia.

Samples of the left ventricular myocardium, cerebral cortex, kidney, and 2 segments (1 cm each) of the caudal artery were removed, weighed and stored at -80°C. Loss of tissue noradrenaline under these conditions is undetectable for at least 2 months (unpublished results). For the determination of tissue noradrenaline levels, samples were homogenized in perchloric acid (510 μ l, 0.3 N) containing 3–4 dihydroxybenzylamine as internal standard. After centrifugation at 4°C and 8000 g for 30 min, the supernatant was filtered (microfilters 0.45 μ m), and 25 μ l placed on the chromatographic column. The perchloric acid-soluble protein content of the supernatant was determined; tissue protein content is expressed as μ g mg⁻¹ wet weight. Tissue noradrenaline content is expressed as ng mg⁻¹ wet weight and ng μ g⁻¹ protein.

For the determination of tissue water content (% w/w) samples were thawed then heated to constant dry weight at 100°C for ≥ 18 h.

Tail artery perfusion technique

A third caudal artery segment was removed and immediately perfused and superfused at 2 ml min⁻¹ in a specially constructed system with modified Krebs-bicarbonate (mM): NaCl 118, KCl 4.7, MgCl₂ 1.2, NaH₂PO₄ 1, CaCl₂ 2.6, NaHCO₃ 25 and glucose 11.1; pH 7.4 $\pm$ 0.2, 37 $\pm$ 0.2°C) oxygenated with 5% CO₂/95% O₂ (Fouda *et al.*, 1987, 1991; Thorin & Atkinson, 1994). The rat tail artery is a small diameter (451 $\pm$ 13 μ m) muscular (wall thickness 96 $\pm$ 5 μ m) artery ($n = 7$, 12 month old WI/Ico rats, unpublished results).

Cocaine (4 μ M) was added to inhibit neuronal uptake. This concentration produces maximal blockade of uptake in our preparation (Thorin & Atkinson, 1994). Furthermore as (i) neuronal uptake accounts for $\geq 90\%$ of the uptake of noradrenaline in the tail artery (Webb *et al.*, 1981), (ii) uptake function is not altered by age (Buchholz & Duckles, 1990), and (iii) a similar concentration (10 μ M) has been used in Fischer 344 rat tail arteries (Buchholz & Duckles, 1990), this concentration of cocaine (4 μ M) was used in all strains and ages.

The arterial segment was placed under a longitudinal tension of 0.4 g which acted both as a tether and restored the *in vivo* length. Platinum electrodes were placed around the artery at either end of the segment. Segments were perfused and superfused for 30 min prior to electrical stimulation (1 Hz for 4 s, pulse duration 0.2 ms, 5 stimulations, at 5 min intervals for 25 min; pulse generator Alvar SA, Montreuil, France). By the 3rd or 4th stimulation the vasoconstrictor response was stable.

Increases in perfusion pressure (mmHg) following stimulation were measured with a low volume, strain gauge, pressure transducer (Statham P23Db, Statham Ins., Puerto Rico). All pressure values were corrected by the subtraction of the pressure created by the tubing of the perfusion system (= 4 to 6 mmHg) and the basal perfusion pressure created by the resistance of the unstimulated arterial segment (= 10 to 12 mmHg).

Following equilibration, tail artery segments were stimulated (1 Hz, 30 s, square wave pulses, 0.2 ms duration, supramaximal voltage), the frequency of electrical stimulation chosen being approximately half-maximal (Fouda *et al.*, 1987). A relatively low stimulation frequency was used as the effectiveness of presynaptic α_2 -adrenoceptor-mediated inhibition of noradrenaline release (see below) is inversely correlated to the intensity of nerve activation (Duckles & Budai, 1990). Furthermore, pressures of ≤ 160 mmHg were used in order to avoid possible tissue damage (Hynes & Duckles, 1987).

Following 10 min perfusion and superfusion with the α -adrenoceptor antagonist, phentolamine (1 μ M), stimulation was repeated. The increase in noradrenaline release following presynaptic α_2 -adrenoceptor blockade was expressed as a S2/S1 ratio where S2 represents noradrenaline release in the presence of phentolamine and S1 that in the absence. This high concentration of phentolamine (Fouda *et al.*, 1991) completely abolished the vasoconstrictor response in all strains and ages. Selective α_2 -adrenoceptor blockade potentiates vasoconstriction and may modify flow rate at the high pressures developed. This could indirectly modify noradrenaline spillover. This was the reason for preferring a non-specific α -adrenoceptor antagonist to a selective α_2 -adrenoceptor antagonist.

As cocaine was present throughout the experiment, noradrenaline spillover into the perfusate, in the presence of the uptake blocker and the antagonist of presynaptic α_2 -adrenoceptor-mediated inhibition of noradrenaline release (phentolamine), was taken as an indication of the total neuronal capacity for release.

Noradrenaline release was estimated from noradrenaline spillover into the perfusate, which was collected before stimulation for the measurement of basal noradrenaline release, and then during the 30 s stimulation. Basal release was subtracted from stimulation-induced release. The perfusate was collected over aluminium oxide (15 mg), perchloric acid (0.3 N), 3-4 dihydroxybenzylamine (36 nM), Na₂EDTA (0.13 M) and 53 mM Na₂S₂O₅ (53 mM) (4°C, pH 8.6). Samples were shaken (4°C, 15 min), the supernatant discarded, and the alumina washed 3 times with 2 ml of bidistilled water at 4°C. Noradrenaline was eluted with 50 µl of 0.3 N perchloric acid then measured by high performance liquid chromatography.

Fractional noradrenaline release was expressed as pg pg⁻¹ per pulse, viz. the amount of noradrenaline (pg) collected during the 30 s stimulation period divided by (i) the noradrenaline content of the tail artery (pg) and (ii) the number of pulses.

Drugs

Sodium pentobarbitone was purchased from Sanofi Pharma, Paris, France. Noradrenaline bitartrate, bovine serum albumin, cocaine hydrochloride, 3-4 dihydroxybenzylamine hydrobromide and sodium octyl sulphate, were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. Aluminium oxide was purchased from Millipore-Waters, Bedford, MA, U.S.A. All other chemicals were purchased from Merck AG, Darmstadt, Germany. Phentolamine was a gift from Ciba Geigy, Basel, Switzerland.

Statistics

Results are expressed as means ± s.e.mean. Significant differences between means were determined by either Scheffe or Student's paired *t*-test. The probability level chosen was *P* < 0.05 (*, NS = not significant).

Results

Body weight, tissue noradrenaline and protein contents

Body weight tripled in outbred Wistar WI/Ico between the ages of 3-4 and 12 months (young 207 ± 1 g, adult 611 ± 17 g, *P* < 0.05) but did not increase thereafter (senescent 600 ± 12 g). In WAG/Rij, the increase was much smaller (young 301 ± 9 g, adult 340 ± 8 g, *P* < 0.05); once again there was no further increase in body weight between 12 and 24 months (senescent 347 ± 18 g). Body weight in senescent F344 was double that of young F344 rats (406 ± 8 g versus 199 ± 2 g, *P* < 0.05).

Plasma noradrenaline levels increased to a similar extent in senescent animals. Values for WI/Ico were 437 ± 86, 340 ± 29 and 807 ± 66 pg ml⁻¹ at 3-4, 12 and 24 months. Values for WAG/Rij were 287 ± 23, 405 ± 30 and 849 ± 76 pg ml⁻¹ at 6, 12 and 24 months. Values for F344 were 488 ± 75 and 1017 ± 86 pg ml⁻¹ at 3-4 and 24 months.

Tail artery noradrenaline content expressed as ng mg⁻¹ wet weight did not vary with age in any of the 3 strains (Figure 1). As soluble protein content increased with age by 30 to 40%, indicating hypertrophy and/or hyperplasia, noradrenaline content expressed as ng µg⁻¹ soluble protein decreased by a proportional amount. There were no differences in the tissue water content amongst strains or ages (results not shown).

Left ventricular myocardial noradrenaline and protein contents expressed per mg wet weight decreased with maturity but did not change with age in Wistar rats (Figure 2). The decrease in noradrenaline content relative to protein content was greater in WI/Ico and F344 such that in these two strains - as in the tail artery - noradrenaline content expressed as ng µg⁻¹ protein significantly decreased. There were

Figure 1 Tail artery noradrenaline content (a: ng ml⁻¹ wet weight, c: ng µg⁻¹ protein) and acid-soluble protein content (b: µg mg⁻¹ wet weight) in Wistar (WI/Ico: open columns, WAG/Rij: horizontally hatched columns) and Fischer 344 rats (vertically hatched columns) of various ages. For the sake of ease of comparison results are given as percentages of values in young animals which were, for WI/Ico, WAG/Rij and Fischer 344 respectively: (a) 5.4 ± 0.5, 10.7 ± 1.2, 10.4 ± 1.5; (b) 5.4 ± 0.6, 5.3 ± 0.5, 5.1 ± 0.6; (c) 1.0 ± 0.1, 2.0 ± 0.2, 2.0 ± 0.2. **P* < 0.05 versus young.

no differences in myocardial water content amongst strains or ages (results not shown).

Renal noradrenaline content was similar in young representatives of all 3 strains (WI/Ico 0.17 ± 0.02, WAG/Rij 0.17 ± 0.01, and F344 0.18 ± 0.02 ng mg⁻¹ wet weight, as was protein content (WI/Ico 6.1 ± 0.5, WAG/Rij 5.4 ± 0.4, and F344 6.8 ± 0.4 µg mg⁻¹ wet weight). There was no significant change with either maturity or senescence. Cerebral cortex noradrenaline content was similar in young representatives of all 3 strains (WI/Ico 0.17 ± 0.02, WAG/Rij 0.30 ± 0.02, F344 0.28 ± 0.03 ng mg⁻¹ wet weight) as was protein content (WI/Ico 1.4 ± 0.02, WAG/Rij 1.7 ± 0.1, F344 2.3 ± 0.1 µg mg⁻¹ wet weight). There was no significant change with either maturity or senescence. There were no differences in either renal or cerebral cortical water content amongst strains or ages (results not shown).

Tail artery vasoconstriction and noradrenaline release

Whereas the increase in perfusion pressure following electrical stimulation was lower in adult and senescent rats, noradrenaline release (in the presence of cocaine) was similar at all ages (Figure 3). Inhibition of the presynaptic α₂-adrenoceptor negative feedback control of release, by phentolamine, doubled noradrenaline release (Figure 3). This latter effect was of similar intensity in all strains and at all ages.

Discussion

Body weight, tissue noradrenaline and protein contents

The results for body weight in the 3 strains of rats are in line with more detailed data (Wistar: Weihe, 1987; WI/Ico: Iffa-

Figure 2 Left ventricular myocardium noradrenaline content (a: ng mg^{-1} wet weight, c: $\text{ng } \mu\text{g}^{-1}$ protein) and acid-soluble protein content (b: $\mu\text{g mg}^{-1}$ wet weight) in Wistar (Wi/Ico: open columns, WAG/Rij: horizontally hatched columns) and Fischer 344 rats (vertically hatched columns) of various ages. For the sake of ease of comparison results are given as percentages of values in young animals which were, for Wi/Ico, WAG/Rij and Fischer 344 respectively: (a) 0.72 ± 0.05 , 1.11 ± 0.13 , 0.83 ± 0.10 ; (b) 2.5 ± 0.2 , 2.6 ± 0.6 , 2.6 ± 0.2 ; (c) 0.29 ± 0.02 , 0.43 ± 0.05 , 0.32 ± 0.04 . * $P < 0.05$ versus young.

Credo, 1988; WAG/Rij: Burek, 1978; F344: Masoro, 1980) which show relatively rapid growth up to 8–9 months, with a tailing off thereafter. Thus the young representatives of each strain were still in the growing phase. The Wistar rats reached a stable body weight by 12 months as there was no further increase. The weights of the Fischer 344 rats were in line with those of (approximately) corresponding ages used by Duckles and coworkers (Hynes & Duckles, 1987; Duckles, 1991), and Schmidlin *et al.* (1992).

The senescent WI/Ico was 1.7 times larger than the age-matched WAG/Rij, and 1.5 times larger than the age-matched F344. This may suggest that tail artery dimensions (diameter, wall to lumen ratio, etc.) are different in senescent WI/Ico compared to the two other strains. However, we observed a similar increase in the acid-soluble protein content of the tail artery of all 3 strains of senescent rats. The age-related change in noradrenaline spillover could be due to a change in the length of the diffusion pathway from the synapse to the lumen following wall remodelling. Structural changes would also modify the vasoconstrictor response but it should be noted that we used low levels of sympathetic stimulation at which structural effects on vasoconstriction are less important (Folkow, 1978). If wall remodelling with smooth muscle hypertrophy had occurred in senescent rats, an increased vasoconstrictor response is to be expected, not a decreased response as observed.

Plasma noradrenaline levels increased approximately 2.2 fold with aging (12 to 24 months) but not with maturation (3–4 and 12 months) in Wistar rats. Likewise in senescent Fischer 344 rats, plasma noradrenaline was 2.1 times that of young rats. Many authors have reported that plasma

Figure 3 Responses to electrical stimulation in the tail artery of Wistar (Wi/Ico: open columns, WAG/Rij: horizontally hatched columns) and Fischer 344 rats (vertically hatched columns) of various ages. Responses are in (a) increase in perfusion pressure (mmHg); (b) release of noradrenaline ($\text{pg pg}^{-1} \text{ p}^{-1} \times 10^{-6}$); (c) increase in noradrenaline release in the presence of phentolamine (S_2/S_1 ratios). For the sake of ease of comparison results are given as percentages of values in young animals which were, for Wi/Ico, WAG/Rij and Fischer 344 respectively: (a) 128 ± 10 , 151 ± 15 , 160 ± 9 ; (b) 4.9 ± 0.3 , 2.3 ± 0.2 , 2.8 ± 0.4 ; (c) 2.4 ± 0.2 , 2.2 ± 0.3 , 2.1 ± 0.3 . * $P < 0.05$ versus young.

levels of noradrenaline are increased in man (Docherty, 1990), but the methodology used in many reports has been criticised (Bennett & Gardiner, 1988). There are fewer reports in animals but others (Hoffman *et al.*, 1985) have found that plasma noradrenaline levels increase with age in the rat. It should be noted that figures are given for venous levels in man and arterial levels in rats.

An age-related increase in the plasma level of noradrenaline could be due to an increased rate of appearance in the plasma and/or a decreased plasma clearance. As the basic mechanism of neuronal release of noradrenaline does not appear to be modified by aging (see below), and others have reported that noradrenaline reuptake is not modified by age (Buchholz & Duckles, 1990), we would propose, that, although others have stressed the importance of the increased rate of appearance of noradrenaline (Docherty, 1990), a decreased plasma clearance cannot be excluded.

Although there is some evidence that in relatively poorly innervated human (Waterson *et al.*, 1974; Neubauer & Christenson, 1978) and rat (Handa & Duckles, 1987) arteries, noradrenaline content decreases with age, the consensus concerning the richly innervated rat tail artery is that noradrenaline levels are similar in young and senescent animals (Handa & Duckles, 1987; Buchholz & Duckles 1990; Duckles, 1991).

We could find only two exceptions to this rule: the Dawson & Meldrum paper (1992) and one of our previous papers (Fouda & Atkinson, 1986). In both of these studies extremely old rats (up to 30–31 months) were used. It is possible that when dealing with animals whose age is beyond

that of the median length of life (see Introduction) problems of biological variability and selection of survivors become important.

As acid-soluble protein content was greater in the tail artery of senescent rats of all 3 strains the noradrenaline content relative to protein significantly decreased. If we hypothesize that acid-soluble protein represents mainly smooth muscle cells, then arterial tissue growth would not appear to receive a concomitant increase in sympathetic growth leading to 'sympathectomy'. A similar phenomenon has been previously described in the brain microvessels of the Sprague-Dawley rat (Embree *et al.*, 1981).

Changes in the noradrenaline content of the myocardium were more marked than those in the tail artery. There was a decrease in noradrenaline content relative to wet weight in adult and senescent rats. An age-related decrease in noradrenaline content relative to protein was observed in the left ventricular myocardium in two of the strains studied (W1/Ico and F344). This pattern has already been reported in Fischer 344 rats (Martinez *et al.*, 1981; Dawson & Meldrum, 1992; Schmidlin *et al.*, 1992). It is generally assumed that cardiac noradrenergic density declines with age and that this may explain the reduction in stimulation-evoked noradrenaline release (Roberts & Tumer, 1987; Daly *et al.*, 1988; Docherty, 1990).

In our experiments the reduction in noradrenergic innervation as judged from noradrenaline content, would appear to be restricted to cardiovascular tissues as there were no changes in cerebral cortex or kidney. Results in the latter tissue confirm these reported by Eikenburg (1991) in F344 rats. Two other groups have reported a slight (25%) decrease in renal noradrenaline content in senescent F344 rats (Martinez *et al.*, 1981; Dawson & Meldrum, 1992). It should be noted that levels in kidney are 50 times lower than in a richly innervated tissue such as the tail artery, and so in the former, relatively poorly innervated tissue, it would be more difficult to demonstrate statistically any slight change, be it age-related or otherwise.

Tail artery vasoconstriction and noradrenaline release

In all three strains of rats the vasoconstrictor response to electrical stimulation was lower in adult rats but there was no further change with aging. This is in agreement with the majority of previously reported values for blood vessels (Duckles *et al.*, 1985; Fouda & Atkinson, 1986; Hynes & Duckles, 1987; Wanstall & O'Donnell, 1988; Eikenburg, 1991; Atkinson *et al.*, 1994) except the aorta (Tuttle, 1966; Simpkins *et al.*, 1983; Hyland *et al.*, 1987; Moritoki *et al.*, 1988; Wanstall & O'Donnell, 1988). It should be noted, however, that in the latter vessel, there may be a global decrease in reactivity to many agents and not specifically to noradrenaline (Tuttle, 1966; Simpkins *et al.*, 1983; Docherty, 1990).

In our experiments, there was no evidence for an age-related decrease in stimulation-evoked noradrenaline release from the tail artery. Published reports on noradrenaline

release are contradictory. In the Fischer 344 rat heart (Daly *et al.*, 1988; Tumer *et al.*, 1990) and the rabbit ear artery (Duckles, 1983) an age-related decrease was observed, whereas in the Fischer 344 rat kidney an increase in noradrenaline release was seen (Eikenburg, 1991). In the Fischer 344 rat tail artery, one group has reported an increase in noradrenaline release which they explained – at least partially – on the basis of a decrease in the sensitivity of the presynaptic α_2 -adrenoceptor inhibitory mechanism (Buchholz & Duckles, 1990; Buchholz *et al.*, 1992). Another group reported the opposite, *viz.* that stimulation-evoked noradrenaline release and the efficiency of the presynaptic α_2 -adrenoceptor inhibition of release were not modified by age in the Fischer 344 rat tail artery (Dawson & Meldrum, 1992). Our results are in agreement with those of the latter group.

When the two main systems which control noradrenaline release (neuronal uptake and presynaptic α_2 -adrenoceptor-mediated inhibition) were blocked (with cocaine and phenolamine, respectively), stimulation-evoked release of noradrenaline was similar at all ages. A similar result was obtained by the Duckles group (Buchholz & Duckles, 1990) in the Fischer 344 rat tail artery, suggesting that the basic mechanism of neuronal release of noradrenaline is not functionally modified by aging.

In summary, our results are consistent with the hypothesis that vascular noradrenergic nerve terminals are structurally and functionally intact, but, in spite of this the vasoconstrictor response is attenuated. We suggest two explanations for this observation. Firstly, structural changes with increasing medial fibrosis and a decreasing number of small muscle cells (Bouissou *et al.*, 1975) could explain why a muscular artery is less capable of reacting to a noradrenergic stimulus. Secondly, the efficiency of the coupling of the signal transduction mechanism(s) may decline with age (Roberts & Tumer, 1987; Vanhoutte, 1988; Docherty, 1990; Johnston & Wray, 1990). Evidence for such a functional modification has recently been provided by an experiment in Wistar WAG/Rij rat tail arteries in which intracellular calcium (using the fluorescent calcium-sensitive dye, fura-2) and vasoconstriction were measured simultaneously. When stimulated with noradrenaline, arteries from senescent rats showed a diminished vasoconstrictor response with no change in the intracellular calcium signal (Oster *et al.*, 1993).

In conclusion, we suggest that sympathetic nerve terminals are intact in the tail artery of senescent rats, and that the age-associated deficit of α -adrenergic control of vascular function is largely postsynaptic. In studies of age-related changes in noradrenergic vascular regulation in rats, the strain used would appear to be of minor importance.

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