Vascular Adrenergic Neuroeffector Function Does Not Decline in Aged Rats

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SUMMARY. To investigate adrenergic control of blood vessels during aging, rats aged 6, 12, 20, and 27 months were studied using in vitro techniques. Accumulation of 
$^{3}H$norepinephrine, one index of adrenergic nerve density, did not alter with age in the femoral or renal arteries or renal vein. In the femoral vein $^{3}H$norepinephrine accumulation was greater at 6 and 27 months of age. Norepinephrine sensitivity was determined in both an innervated vessel, the femoral artery, and a non-innervated vessel, the carotid artery. In both cases, sensitivity to norepinephrine did not alter with age. In the renal and femoral arteries and veins, no significant changes in maximum responses to norepinephrine (10$^{-7}$ M), potassium chloride, or transmural nerve stimulation were seen with advancing age. Furthermore, frequency response curves (2–16 Hz, 200 pulses) did not differ with age for any of the four vessels studied, with one exception. The response to stimulation at 4 Hz of the femoral vein from 6-month-old rats was significantly larger than responses at other ages. During nerve stimulation, the renal vein exhibited rapid contractions superimposed upon the maintained contractile response. This type of rapid contraction occurred only rarely (1 out of 5) in the renal vein from 27-month-old rats. In summary, neither adrenergic nerve density as reflected by $^{3}H$norepinephrine accumulation nor norepinephrine sensitivity decline with age. As the net effect of various components, the ability of vascular smooth muscle to respond to adrenergic nerve stimulation is also maintained during advancing age. (Circ Res 56: 109–116, 1985)

WHEREAS some authors maintain that the effectiveness of adrenergic stimulation on the cardiovascular system declines with advancing age (Lakatta, 1980; Wollner and Spalding, 1978), others have referred to the "hyperadrenergic state" of old age (Rowe and Troen, 1980). Most would agree, however, that adrenergic regulation of vascular smooth muscle may potentially play an important role in the pathophysiology and treatment of such age-related problems as postural hypotension, hypertension, and alterations in homeostatic thermoregulation (Balmagiya and Rozovski, 1983; Rowe, 1983; Wollner and Spalding, 1978; Conrad and Bressler, 1982).

Examination of the literature on this controversy over function of the adrenergic system in old age is less than conclusive, as most animal studies of the vasculature during aging have been hampered by methodological problems. For example, as reviewed by Fleisch (1980), few investigators have verified the specificity of alterations in $\alpha$-adrenergic receptor responsiveness by testing another contractile agonist. Thus, alterations in the magnitude of maximum contractile responses could reflect changes in vessel elasticity or smooth muscle structure, rather than specific changes in the adrenergic system. Another problem with many studies of "aging" is that it has been difficult to obtain animals over a wide age range. Immature and adult animals have been compared, but truly senescent animals have not always been available (Duckles, 1983).

For these reasons, we have studied adrenergic responses of the vasculature of the Fischer 344 rat, from a colony maintained by the National Institutes of Aging. In a strain where the mean life span varies from 24 to 29 months depending on diet (Masoro, 1980), we chose to study animals aged 6, 12, 20, and 27 months. Density of adrenergic innervation has been assessed by measuring $^{3}H$norepinephrine ($^{3}H$NE) accumulation in a series of vessels. Sensitivity to exogenous NE as well as to transmural adrenergic nerve stimulation in vitro has been determined under carefully defined conditions in order to answer the question: does the function of the vascular adrenergic neuroeffector alter with advancing age?

Methods

Blood vessels from four groups of Fischer 344 rats were studied: 6, 12, 20, and 27 months of age. Mean weights ($n = 6$) were 324 ± 9, 403 ± 9, 394 ± 10, and 396 ± 6 g, respectively. The animals were obtained from the National Institutes of Health Aging Colony, housed in the Division of Animal Resources in stainless steel hanging cages, and fed with Wayne Lab-Blox rat chow. After being received at the University of Arizona, the animals were retained from 1 to 4 weeks before being studied.

After decapitation, the femoral artery and vein, the left renal artery and vein, and the carotid artery were carefully removed. The vessels were placed in Krebs' solution at room temperature which was composed as follows (mM): Na+, 147.6; K+, 6.4; Ca++, 1.6; Mg++, 1.2; Cl-, 130; HCO3-, 26; SO4-, 1.2; H2PO4-, 1.2; glucose, 11; disodium ethy-
lendamidine tetracacetate, 0.027. Care was taken during dissection so that vessels were not stretched.

**Norepinephrine Accumulation**

Vascular segments were incubated in Krebs' solution containing 0.11 mm ascorbic acid and equilibrated with 95% O₂, 5% CO₂ at 37°C for 60 minutes. To measure non-neuronal accumulation, cocaine (10⁻⁴ M) was present during the last 30 minutes of the equilibration period and during incubation with norepinephrine. The initial equilibration was followed by incubation in Krebs' solution containing [7-³H (N)]-1-NE (10⁻⁴ M, New England Nuclear, lot 1848-041, specific activity 17.2 Ci/mmol) for 30 minutes. At the end of this period, tissues were briefly rinsed in fresh Krebs' solution, blotted, and weighed. Tissues were solubilized in 0.5 ml soluene-100 (Packard), and radioactivity of solubilized tissues and a bath aliquot was determined by liquid scintillation counting. Accumulation was calculated as milliliters of bath fluid cleared per gram wet weight of tissue. Neuronal accumulation was calculated as total accumulation – accumulation in the presence of cocaine.

**Contractile Studies**

With the aid of a dissecting microscope, vessel segments were trimmed to a length of 4 mm, and two pieces of platinum wire (0.13 mm diameter) were passed through the lumen. One wire was connected to a Gould-Statham UC2 Universal transducing cell with microscale accessory for isometric recording of changes in force with a potentiometric recorder. The other wire was attached to a moveable plastic support for adjustment of the resting force. The entire apparatus was then placed in a tissue bath containing 50 ml of prewarmed (37°C), oxygenated (95% O₂, 5% CO₂) Krebs' solution.

Two parallel platinum electrodes (40 mm long) were placed on either side of the vessel segment approximately 5 mm apart for delivery of transmural nerve stimulation (TNS). Electrical stimulation (15 V, 0.3 msec) was delivered from a Grass stimulator and coupling device to provide a low source impedance (Duckies and Silverman, 1980). After equilibration for 60 minutes at 37°C, the bath solution was replaced. For determination of the optimal resting force, tissues were tested at a series of resting forces using a single standard TNS stimulus for 20 seconds at a frequency of 2, 4, or 8 Hz, depending on the responsiveness of the vessel segment. In the case of the carotid artery, 10⁻⁷ M NE was used as the standard stimulus. The resting force at which the largest response to the standard stimulus was produced was then maintained for the remainder of the experiment.

In studies of responses to transmural nerve stimulation, TNS at 2, 4, 8, and 16 Hz for a train duration of 200 pulses was delivered. Ten-minute rest periods were allowed between each stimulus train. This was followed by determination of the maximum contractile response at 16 Hz when vessels were stimulated for at least 2 minutes until a plateau response was obtained. For the renal artery, renal vein, and femoral vein, plateau responses at 32 Hz were smaller than at 16 Hz, 96 ± 4, 75 ± 6, and 71 ± 9%, respectively (n = 3). For the femoral artery, plateau responses at 32 Hz were somewhat larger than responses at 16 Hz, 118 ± 6% (n = 3). Thus maximum responses to TNS are somewhat underestimated by responses at 16 Hz in the femoral artery. In all cases, responses to TNS at 16 Hz were blocked by tetro-}

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dotoxin (10⁻⁴ M), confirming their neurogenic origin. After washout of the tetrodotoxin, deoxyxooctosterone (10⁻⁷ M), desmethylimipramine (10⁻⁷ M), and propranolol (10⁻⁷ M) were added to block extraneuronal and neuronal metabolism and β-adrenergic receptors, respectively. Under these conditions the maximum contractile response to 10⁻⁵ M NE was evaluated as an index of maximum α-adrenergic responsiveness. After washout of the NE, 150 mm KCl was added to the bath.

For determination of sensitivity to NE, in a separate series of experiments, cumulative concentration response curves to NE were determined. Again deoxyxooctosterone, 10⁻⁷ M, desmethylimipramine, 10⁻⁷ M and propranolol, 10⁻⁷ M, were present. Maximum responses to KCl (150 mm) were determined at the end of each experiment. EC₅₀ values (concentration that produces 50% of the maximum response) were determined from concentration-response curves. Geometric means and confidence intervals were calculated (Fleming et al., 1972).

**Drugs Used**

The following drugs were used: deoxyxooctosterone, Aldrich; tetrodotoxin, Calbiochem; l-norepinephrine bitartrate and d,l-propranolol, HCl, Sigma; desmethylimipramine, courtesy of USV Laboratories, Tuckahoe, NY.

**Statistics**

Means and standard errors are reported throughout. Analysis of variance was used for determination of differences between groups.

**Results**

[³H]Norepinephrine Accumulation

Total, extraneuronal (+ cocaine), and neuronal [³H]NE accumulation by the femoral artery and vein and renal artery and vein are illustrated in Figure 1. In the femoral artery and the renal artery and vein, there were no significant differences with age in either total or neuronal NE accumulation. In the femoral vein, however, both total and neuronal [³H]NE accumulation in tissue from 6-month-old rats was significantly greater than in tissue from both 12- and 20-month-old rats (P < 0.01). Accumulation in the femoral vein of 27-month-old rats was significantly greater than in 12-month-old rats.

**Resting Force**

Resting force-response relationships are shown in Figures 2 and 3 for the vessels studied: the femoral artery and vein, the renal artery and vein, and the carotid artery. There were no significant differences with age in the optimum resting forces used, except for the femoral artery, where the optimum force for animals 6 months of age was smaller than the optimum resting forces for vessels from older animals.

**Maximal Contractile Responses**

There were no significant differences with age in the maximal contractile responses to TNS, NE, or KCl in the femoral and renal arteries and veins (Fig. 4). In the carotid artery, however, maximal re-
Responses to NE were significantly smaller in the 6-month-old animals than 20-month-old (*P < 0.001). Maximum responses to NE were 0.52 ± 0.04 g in vessels from 6-month-old rats and 0.63 ± 0.03, 0.77 ± 0.04, and 0.71 ± 0.06 from 12-, 20-, and 27-month-old animals, respectively. The maximal contractile response to TNS, expressed as a percent of the maximal response to NE is shown in Table 1. There were no significant differences with age, as tested by analysis of variance, for any of the four vessels studied. Overall, these findings demonstrate that ability of these vessels to respond maximally to either NE or adrenergic nerve stimulation does not decline with age.

Sensitivity to Exogenous Norepinephrine

Cumulative concentration response curves to NE were determined in an innervated vessel, the femoral artery, as well as in a non-innervated vessel, the carotid artery (Fig. 5). Lack of responsiveness of the carotid artery to transmural nerve stimulation was used as evidence of the lack of adrenergic innervation. As can be seen in Figure 5 and Table 2, sensitivity to NE did not change significantly with age as assessed by the EC50 value.

Sensitivity to TNS

Sensitivity to transmural nerve stimulation can be assessed from the frequency response curve as

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**Figure 1.** [3H]NE accumulation (total, + cocaine and neuronal) in four vessels from rats 6, 12, 20, and 27 months of age. Values are means ± se (n = 6). ** different from 12 and 20 months; * different from 12 months (*P < 0.01).

**Figure 2.** Resting force-response relationship for four vessels studied for responses to TNS. As described in Methods, responses to a single standard frequency of TNS (2, 4, or 8 Hz for 20 seconds) were determined at various resting forces in each vessel segment. Values are means ± se (n as in Table 1).
shown in Figure 6. Comparison of the responsiveness of vessels from animals of various ages demonstrates that, on the whole, adrenergic contractile responsiveness does not alter with age. However, there was one exception: the femoral vein from rats 6 months of age showed a greater response to stimulation at 4 Hz than femoral veins from older animals.
Renal Vein Spiking

During adrenergic nerve stimulation or stimulation with exogenous NE, segments of the renal vein often exhibited rapid contractions superimposed upon the maintained contractile response (Fig. 7). The frequency and magnitude of these rapid contractions, which we have termed spikes, are summarized in Table 3. As can be seen in Table 3 and Figure 7, such spiking activity was common in renal veins from animals younger than 20 months of age, occurring in 13 out of 15 vessel segments studied. However, this type of rapid contraction was rarely seen in renal veins from the oldest group of animals, occurring in only one out of five vessels studied.

Discussion

A major conclusion of the present study is that the function of the adrenergic neuroeffector mechanism of vascular smooth muscle does not decline with age. We took care to circumvent methodological problems that have plagued previous approaches to this problem.

Animals that spanned the full age range from young adult to senescent were studied, using the resources of the National Institutes of Health aging colony. In addition, care was taken to ensure that each vessel segment was studied at the optimum point on the resting force-response curve. In addition, for each vessel studied, the contractile potential of the tissue was assessed using two approaches, maximum responses to both NE and KCl were determined at the end of each experiment. This made it possible to express our results as a percentage of the maximum contraction of which the tissue was capable (Table 1; Fig. 5), allowing us to assess adrenergic mechanisms, independent of possible changes in blood vessel structure or smooth muscle composition (Cox, 1977; Nagasawa et al., 1979). Thus, the major aim of this study was directly addressed, enabling us to conclude that the function of the vascular adrenergic neuroeffector shows only subtle changes with age.

Perhaps the major alteration in adrenergic function seen with age was loss of the rapid pulsatile contractile response of the renal vein during adrenergic nerve stimulation. This loss did not appear to be progressive with age, but was apparent only in the 27-month-old rats. The physiological function and mechanism of this 'spiking' response cannot be determined from our in vitro experiments. The simplest explanation for such a coordinated, relatively fast contractile response would be the presence of pacemaker cell(s) and coordinated electrical activity; however, an electrophysiological approach would be necessary to test this hypothesis.

The best studied vessel showing phasic contractile activity is the rat portal vein, in which multiple pacemaker sites apparently account for coordinated contractions (Hermsmeyer, 1973). In the portal vein phasic contractions occur spontaneously, not in the presence of adrenergic stimulation, as was true in the renal vein that we have studied. It is interesting that this loss of spiking contractions in the 27-month-old rat renal vein is not associated with a decline in maximum responses to nerve stimulation or a shift in the frequency response curve (Figs. 4 and 6). This is true even though, at a stimulation frequency of 4 Hz, the spikes account for as much as 30 or 40% of the total contractile response (Table 3).

For the most part, we saw no change with age in sensitivity to either exogenous NE or to transmural nerve stimulation and no apparent alteration in adrenergic nerve density as assessed by [3H]NE accumulation. The single exception was the femoral vein, where both [3H]NE accumulation and sensitivity to nerve stimulation were increased in vessels from the 6-month-old rats. [3H]NE accumulation was also higher in the 27-month-old femoral vein, but sensitivity to nerve stimulation was not increased. We can only speculate about the reasons for this, which could include alterations in NE release, changes in smooth muscle sensitivity, etc. At any rate, this increased sensitivity to nerve stimulation in the 6-month-old femoral vein does not change our overall conclusion that adrenergic responsiveness does not

### Table 1

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Femoral artery (×10^{-7} m)</th>
<th>Carotid artery (×10^{-4} m)</th>
<th>*% of maximal NE response in rats at age</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>66 ± 4 (11)</td>
<td>101 ± 8 (8)</td>
<td>105 ± 5 (5)</td>
</tr>
<tr>
<td>12</td>
<td>74 ± 4 (6)</td>
<td>89 ± 4 (5)</td>
<td>103 ± 10 (5)</td>
</tr>
<tr>
<td>20</td>
<td>67 ± 6 (7)</td>
<td>90 ± 2 (5)</td>
<td>107 ± 4 (5)</td>
</tr>
<tr>
<td>27</td>
<td>67 ± 7 (6)</td>
<td>88 ± 4 (5)</td>
<td>105 ± 7 (5)</td>
</tr>
</tbody>
</table>

Values are means ± so. Numbers in parentheses = number of animals.

### Table 2

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Norepinephrine EC_{50} in Vessels from Rats of Different Ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Femoral artery (×10^{-7} m)</td>
</tr>
<tr>
<td>12</td>
<td>2.7 (1.1–6.6)</td>
</tr>
<tr>
<td>20</td>
<td>2.0 (0.7–6.0)</td>
</tr>
<tr>
<td>27</td>
<td>0.98 (0.5–2.0)</td>
</tr>
</tbody>
</table>

Geometric mean EC_{50} and confidence intervals are shown.
decline with advancing age, as there was no progressive decline from 12 to 27 months and, as shown by animal weight, 6-month-old rats are not yet fully grown.

In comparing our results with those in the literature, one must keep in mind that function of the adrenergic neuroeffector junction per se has rarely been examined. As reviewed by Fleisch (1980), many authors have neglected to demonstrate that alterations in contractile function are specific to the adrenergic nervous system. In the large blood vessels that have usually been studied, such as the aorta, it is clear that profound structural changes occur with age, and an overall decline in contractile function is not unexpected (Tuttle, 1966; Cohen and Berkowitz, 1976; Simpkins et al., 1983; Yin et al., 1983).

Some authors have seen age-related changes in adrenergic responsiveness per se. For example, in the rabbit, indices of innervation density, NE content, and accumulation, did not change with age, but contractile responses to adrenergic nerve stimulation did decline with age (Duckles, 1983). However, this study compared young rabbits with adults, and did not really study a wide age span. Truly senescent animals have rarely been studied.

Several authors have reported that NE concentration in vascular smooth muscle declines with age in human vessels (Neubauer and Christensen, 1978;...
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**TABLE 3**

Renal Vein Spikes

<table>
<thead>
<tr>
<th>Rat age (mo)</th>
<th>Fraction with spiking</th>
<th>Spike size</th>
<th>% of total response</th>
<th>Spike no.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 Hz</td>
<td>16 Hz</td>
<td>4 Hz</td>
</tr>
<tr>
<td>20</td>
<td>5/5</td>
<td>55 ± 26</td>
<td>77 ± 23</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>12</td>
<td>4/5</td>
<td>111 ± 52</td>
<td>110 ± 35</td>
<td>39 ± 12</td>
</tr>
<tr>
<td>6</td>
<td>5/5</td>
<td>77 ± 16</td>
<td>58 ± 3</td>
<td>31 ± 7</td>
</tr>
</tbody>
</table>

* Total number of spikes in response to stimulation at 4 Hz for 50 seconds and 16 Hz for 12.5 seconds. Values were determined only from those vessels that did show spiking activity.

References

Hermsmeyer K (1973) Multiple pacemaker sites in spontaneously active vascular muscle. Circ Res 38: 244-251

Water et al., 1974), as well as rat vessels (Embree et al., 1981). In contrast to these findings, our results suggest that one index of adrenergic nerve density, [3H]NE accumulation, does not decline with age in vessels of the rat. Further studies will be necessary to determine that [3H]NE accumulation does correlate with NE content, as well as stimulation-evoked NE release.

In support of our findings that the adrenergic responsiveness of vascular smooth muscle does not change with advancing age, it has recently been demonstrated that sensitivity to NE of isolated arteries from humans ranging from 30 to 83 years in age is similar (Scott and Reid, 1982). Furthermore, maximal contractile responses to NE expressed as a percentage of maximal responses to KCl did not change with age in these human vessels. This stability of vascular adrenergic responses with age also supports the findings of two recent clinical studies (MacLennan et al., 1980; Smith and Fasler, 1983). These suggested that postural hypotension in old age is not due to an alteration in adrenergic function per se, but can be accounted for primarily by arteriosclerosis.

This suggests an important point: the current study focuses on a very discrete aspect of the entire functional system for blood pressure control. It is possible that function of the vascular adrenergic neuroeffecter mechanism is maintained in old age, but alterations at other sites, such as baroreceptor sensing, central control or ganglionic transmission, could cause a change in the overall function of adrenergic cardiovascular control.

INDEX TERMS: Transmural nerve stimulation • Norepinephrine • Fischer 344 rat • [3H]Norepinephrine accumulation
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