

Evidence for an Increase in Adrenergic Nerve Function in Blood Vessels from Experimental Hypertensive Rabbits

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ABSTRACT

The possibility of changes in the adrenergic innervation of blood vessels in experimental hypertension was investigated by measuring arterial norepinephrine content, neuronal uptake of norepinephrine, and the neurogenic contractile response in rabbits made hypertensive by partial constriction of the abdominal aorta proximal to the kidneys. Two to 3 weeks after surgery, norepinephrine content was increased in the arteries above the ligature, where arterial blood pressure was increased, but not in the arteries below the ligature, where arterial blood pressure was normal, in the heart, or in the veins. Neuronal norepinephrine uptake per unit length of vessel and the neurogenic contractile response increased with the rise in arterial blood pressure. The neurogenic contractile response can be taken as an indication of an increase in transmitter release. These results taken together suggest an increase in the function and possibly the amount of the adrenergic neuronal terminal in hypertension. Since the distributions of the changes in the adrenergic innervation and the increases in smooth muscle cell proliferation in hypertension are similar, these two processes may be interrelated.

■ Despite extensive investigation of changes in the cardiovascular system associated with hypertension in both man and experimental animals, comparatively little information is available concerning changes in the adrenergic innervation of the peripheral vascular bed in this condition. Changes in the function of the adrenergic innervation of the heart have received considerable attention (1), but such detailed study has not been extended to blood vessels even though some evidence of alteration has been documented (2, 3). There is no reason to assume that the changes which take place in the innervation to the heart also happen in the vascular tree; the changes do not necessarily occur in parallel. For example, in early, established, uncomplicated, essential hypertension, there is frequently an elevation in peripheral resistance with little or no change in cardiac output (4, 5).

The extent and the nature of the reported changes in the neurogenic response of blood vessels in experimental hypertension are not consistent (6-9). None of the studies to date have explored the possibility that there is a change in the vascular

innervation. Many changes in parameters associated with the adrenergic innervation of blood vessels in experimental hypertension have been described, including increases in the vesicular content of the adrenergic varicosity (10, 11), the amount of transmitter released from the nerve terminal (8), the density of adrenergic fluorescence (12, 13), and the activity of various enzymes that influence adrenergic anabolic and catabolic processes (14). Although there has been some speculation relating these changes to altered activity of the sympathetic nervous system (12, 15), no cohesive picture of the alteration of the adrenergic terminal in hypertension has emerged.

The present study presents evidence that supports the concept of altered neuronal function in blood vessels in experimental hypertension. Because of the complexities of the adrenergic nerve terminal mechanism, several lines of evidence were explored. We suggest that the reported changes can be explained as an increase in either the function of existing neuronal tissue or the amount of nerve tissue present in the vessel wall. The distribution of the neuronal changes indicates a causal interrelationship with the medial smooth muscle proliferation that occurs in hypertension (16).

Methods

Experimental hypertension was induced in rabbits by partial constriction of the abdominal aorta above the kidneys (17). Observations were made 2-3 weeks after surgery when the carotid arterial blood pressure had stabilized at an average of 40 mm Hg above the preoperative level and the femoral arterial blood pressure was within preoperative levels. Thus, the proximal arterial

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vasculature was hypertensive, and the vascular tree distal to the constriction was normotensive. It has been reported that plasma renin is normal in this model at this time (18). Tissues were removed from exsanguinated rabbits, cleaned of extraneous loose fatty material, and examined *in vitro*. The following measurements were made.

NOREPINEPHRINE CONTENT

After blotting with filter paper, the tissues were weighed and homogenized in 5% trichloroacetic acid with a Willens Polytron homogenizer. After centrifugation, the supernatant fluid was neutralized to pH 8.4 in the presence of alumina, and the latter was eluted with 0.1N acetic acid. Norepinephrine in the eluate was assayed spectrofluorometrically by the trihydroxyindole method (19).

TRITIATED NOREPINEPHRINE UPTAKE

The method employed to determine tritiated norepinephrine uptake was essentially the same as one described previously (20). Paired adjacent segments of each vascular specimen were equilibrated in Krebs-bicarbonate solution at 38°C and bubbled with 95% O₂-5% CO₂ for 90 minutes. During the last 30 minutes of the equilibration period, one segment of each paired specimen was treated with cocaine (10⁻⁴M). Both the cocaine-treated and the control segment were then soaked in *l*-7-³H-norepinephrine hydrochloride (10⁻⁸M) for 60 minutes. After a rapid rinse, the tissues were blotted and weighed, and their lengths were measured on a calibrated microscope slide; the tissues were then digested. Tissue radioactivity was measured by scintillation spectrometry, and uptake was expressed as milliliters of bath fluid cleared per millimeter length of wet tissue.

CONTRACTILE RESPONSE TO TRANSMURAL NERVE STIMULATION

Ring preparations of the ear artery were mounted as previously described (21). Two platinum electrodes placed on either side of 5-mm lengths of a vessel segment subjected to a resting tension of 500 mg and exposed to propranolol (10⁻⁶M) and desmethylinipramine (10⁻⁷M) were used to selectively stimulate the intramural nerves (pulse duration 0.3 msec, frequency 10 Hz, and supramaximum voltage). After recovery from nerve stimulation, the contractile response to *l*-norepinephrine (20 µg/ml) (a maximum dose) was recorded.

DATA ANALYSIS

The relationship between tissue parameters and carotid arterial blood pressure was examined by plot-scatter diagrams. Pearson's product-moment correlation coefficient was computed whenever a linear trend was indicated. The significance of relationships between grouped data from hypertensive and normotensive (sham-operated) rabbits was determined using an unpaired *t*-test. The *P* value for testing the statistical significance of the correlation coefficient was determined from Table A-30a of Dixon and Massey (22); a probability of less than 0.05 was considered significant.

Results

Three measurements were made in the vascular tissue which reflect the major functions of the

adrenergic terminal neuron: norepinephrine content, neuronal uptake of norepinephrine, and the contractile response to neuronal activity.

NOREPINEPHRINE CONTENT OF BLOOD VESSEL

Norepinephrine in the blood vessel wall except during and immediately after neuronal activity is essentially confined to the adrenergic nerve plexus (23). Since a rise in arterial blood pressure over a period of several weeks is known to be associated with increases in wall thickness and length (17), if norepinephrine content is to be used to indicate the extent of adrenergic innervation it should be expressed in terms of the whole blood vessel. Thus, tissue to be utilized for the estimation of norepinephrine content should be biologically defined; namely, the tissue between two anatomical landmarks should be considered. For this reason, the whole left common carotid artery from the level of the first rib to its bifurcation, the saphenous arteries from their origin to the level of the knee joint, and the portal vein from the confluence of the main mesenteric veins to its prehepatic branching were utilized. These vessels represent the hypertensive, normotensive, and lower pressure segments of the circulation, respectively. The norepinephrine content of the heart was also measured.

In Table 1, the norepinephrine contents of vessels from hypertensive and sham-operated rabbits 2-3 weeks after surgery are shown. Although there was no change in the norepinephrine content of the whole heart, the saphenous artery, or the portal vein, there was a significant increase of 70% in the norepinephrine content of the whole carotid artery. There was no significant change in the mean wet weight of the saphenous artery or the portal vein; in contrast, the carotid artery significantly increased in size. When the norepinephrine content was expressed on a wet weight basis, there was no significant alteration in this parameter in the carotid artery with hypertension. In a previous study of the same model, it has been argued that the increase in arterial wall thickness in vessels from the proximal hypertensive part of the circulation is in part due to an increase in cellular, probably smooth muscle, volume (17). When expressed per unit wet weight of tissue, the norepinephrine content of hearts from hypertensive rabbits is significantly lower, due presumably to cardiac hypertrophy in the hypertensive animal (24).

NEURONAL UPTAKE OF ³H-NOREPINEPHRINE

The terminal adrenergic plexus takes up norepinephrine from the surrounding extracellular space

TABLE 1

Norepinephrine Content of Blood Vessels and Heart of Hypertensive and Normotensive Rabbits

	Hypertensive (131.5 mm Hg)*		Normotensive (89.5 mm Hg)*	
	$\mu\text{g}/\text{vessel or organ}$	$\mu\text{g}/\text{g}$	$\mu\text{g}/\text{vessel or organ}$	$\mu\text{g}/\text{g}$
Carotid artery	0.162 ± 0.026 (10)	3.50 ± 0.8 (10)	$0.094 \pm 0.01^\dagger$ (10)	2.92 ± 0.3 (10)
Saphenous artery	0.078 ± 0.013 (10)	2.32 ± 0.44 (10)	0.066 ± 0.032 (10)	1.71 ± 0.26 (10)
Portal vein	0.066 ± 0.008 (8)	1.28 ± 0.152 (8)	0.063 ± 0.007 (8)	1.35 ± 0.13 (8)
Heart	8.25 ± 1.12 (10)	0.78 ± 0.11 (10)	8.67 ± 0.77 (10)	$1.38 \pm 0.12^\ddagger$ (10)

All values are means \pm SE; the number of vessels or organs tested is given in parentheses.

* Mean value of mean carotid artery pressure.

† Significantly less than the corresponding value for the hypertensive group, $P < 0.01$.

‡ Significantly greater than the corresponding value for the hypertensive group, $P < 0.01$.

by a specific transporting mechanism. This mechanism represents the major route of adrenergic transmitter disposition in blood vessels and is specifically blocked by cocaine. In Figure 1, neuronal (cocaine-sensitive) uptake of norepinephrine per unit length of blood vessel is plotted against mean carotid artery pressure. There is a significant positive correlation between the two parameters ($r = 0.615$, $P < 0.01$; 95% confidence limits, 0.18 and 0.87). As mentioned previously, since blood vessel wall thickness alters in hypertension and the adrenergic plexus is an essentially two-dimensional perimedial structure, it is not appropriate to express uptake per unit weight of blood vessel. The mean increase in norepinephrine uptake capacity derived from the data shown in Figure 1 is greater than 100% over the range from 100 to 160 mm Hg.

In contrast, neuronal norepinephrine uptake in the saphenous artery and in the cephalic and saphenous veins could not be correlated with the carotid artery pressure. The latter parameter was utilized in this study as a measurement of the level of hypertension. The correlation coefficients for these three vessels were 0.033, 0.073, and 0.308, respectively (P in all cases > 0.1).

Nonneuronal uptake of norepinephrine expressed on a tissue weight basis did not correlate significantly with carotid or femoral artery pressures.

RESPONSE TO NEUROGENIC NERVE STIMULATION

Another functionally important feature of adrenergic nerve activity is transmitter release. Because of the small size of the segments of ear artery available for study in these experiments, adrenergic transmitter release could not be measured directly. In Figure 2, the size of the equilibrium contractile response of the ear artery to electrical

excitation of its intramural nerves at 10 Hz is plotted against vessel wall thickness and against the maximum response to norepinephrine of that particular vessel. The neurogenic response can be correlated positively with both parameters ($r = 0.72$ [0.33–0.90] and 0.62 [0.15–0.85], respectively; $P < 0.01$ in both cases). These data show that the response to neurogenic activation increases proportionately with artery wall thickness and the amount of contractile material in the vessel wall as indicated by the response to a maximum dose of norepinephrine. It will be argued in the Discussion that, for this relationship to hold, there must be a concomitant increase in the amount of transmitter released with an increase in medial tissue in the

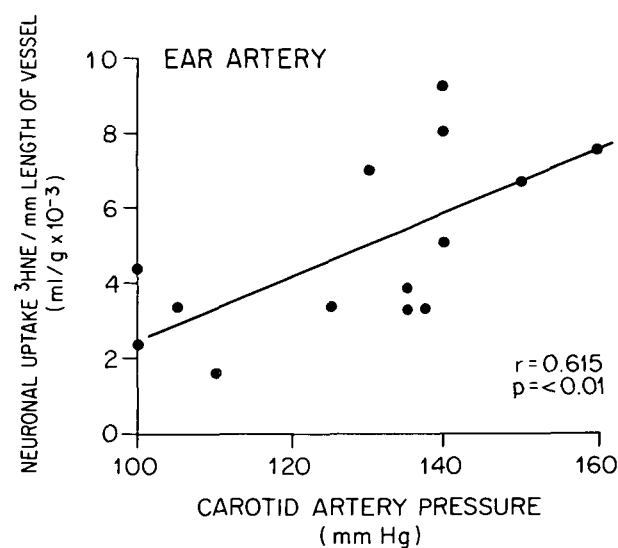


FIGURE 1

Relationship between neuronal uptake of ^3H -norepinephrine (^3HNE) per unit length of ear artery expressed as milliliters of bath solution cleared of tritiated norepinephrine per gram wet weight of tissue and mean carotid artery pressure.

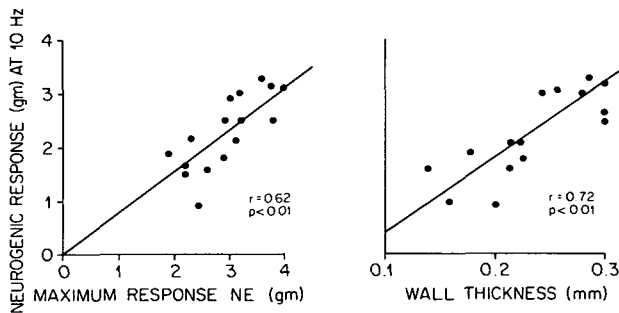


FIGURE 2

Relationship between the neurogenic response of isolated rabbit ear artery segments and the maximum response to l-norepinephrine (NE) and also the total vessel wall thickness.

blood vessel wall. It has been shown that smooth muscle sensitivity to norepinephrine is independent of arterial blood pressure in this experimental preparation (17).

Discussion

In this model of hypertension, the arteries from the proximal hypertensive part of the vascular bed and also the veins are hyperresponsive to norepinephrine and to sympathetic nerve stimulation (17, 25). Hyperresponsiveness of the arteries to norepinephrine may be due to increased muscle mass in the vessel wall. Hyperresponsiveness of the veins may be due to increased sensitivity of the smooth muscle cells, a mechanism which can also explain the increased responsiveness of the veins to nerve stimulation (25). Changes that occur in the arterial neurogenic response are more complex; and they are the result of many factors. Evidence presented in this paper indicates that there may be an increase in the adrenergic innervation of hypertensive arteries, which must contribute to the altered responsiveness to nerve activity.

Norepinephrine in the blood vessel is predominantly stored in the vesicles of the adrenergic varicosity from whence it is released. The increased amount of norepinephrine in the wall of the hypertensive arteries indicates an increase in either the number of vesicles, the norepinephrine per varicosity, the number of varicosities, or a combination of these changes. In this respect, it is of interest that Burnstock et al. (11) and Graham et al. (10) have demonstrated an increase in the number of vesicles per varicosity in the adrenergic innervation of hypertensive sheep and rats, respectively. Ichijima (12) has described an increase in the size of the adrenergic varicosities in prehypertensive and the initial hypertensive stages of spontaneously hypertensive rats. Unfortunately, the quality of the

photomicrographs in all of these studies is poor, and an independent objective interpretation of the data is difficult.

Norepinephrine uptake mechanisms in regenerating and adult adrenergic neurons have similar kinetic properties (26). Establishment of this uptake process in regenerating neurons precedes maturation of storage and release mechanisms. Thus, if a change in the innervation takes place, perhaps in the form of neuronal sprouting or growth, neuronal uptake of norepinephrine might be considered a better measure of the extent of the change. When it is expressed per unit length of vessel, neuronal uptake of norepinephrine is increased in hypertension. It is not known whether this change indicates an increase in the number of uptake channels per varicosity or an increase in the number of varicosities. Alterations in adrenergic transmitter disposition mechanisms in this model of hypertension have been discussed elsewhere (25).

Finally, an increase in the neurogenic response proportionate to the increase in wall thickness and the maximum response to norepinephrine is cited as evidence of neuronal change. This increase occurred in a vessel in which vascular muscle sensitivity is not altered by a rise in arterial blood pressure (17). In another paper (25), it has been argued that an increase in wall thickness tends to diminish the effective proportion of released transmitter that enters the vessel wall and to increase the likelihood of its extraneuronal disposition. Thus, if the response to neurogenic stimulation increases along with the increase in muscle mass, transmitter entry would have to increase concomitantly. To ensure this parallel change, a disproportionately greater increase in transmitter release might be expected. Confirmation of this speculation must await further experimentation.

That norepinephrine content did not increase in the heart, veins, and the saphenous artery denotes that the increase in norepinephrine content is not generalized. Similar arguments apply to the increase in neuronal uptake of norepinephrine and, taken together, suggest a local basis for these alterations. Since the distribution of neuronal changes among those vessels studied was similar to that of vascular smooth muscle hyperplasia, both being confined to hypertensive arteries, it might be speculated that some interrelationship exists between smooth muscle proliferation and neuronal alteration. However, since the neurons are situated on the outside of the muscle vessel coat and the smooth muscle proliferation is generalized throughout the thickness of the vessel wall (14), the

mechanism of such an interrelationship is obscure. The muscular artery thickens mainly by an increase in the outer vessel circumference. In these experiments, the increase in outer circumference of the ear artery can be calculated to be approximately 60% (15). Thus, stretch of the neuronal plexus resulting from growth of the blood vessel might be a causal link in the neuronal change.

The findings of Tarver et al. (2) and Trajkov et al. (14), who observed a decrease in tyrosine hydroxylase and dopamine- β -hydroxylase in mesenteric arteries from spontaneously hypertensive rats, contrast with those of this study which indicate an alteration in neuronal function in the direction of an increase in neuronal function. Trajkov et al. (14) have suggested that in established hypertension there is an alteration of regulating mechanisms secondary to the rise in arterial blood pressure which tends to diminish the consequences of sympathetic activity. Marked differences in the time course of the arterial blood pressure rise occur in these two models. Moreover, it is difficult to relate the findings of DeQuattro and Alexander (3) on SAD rabbits with our own, particularly since the changes they found differed at different levels of the same vascular bed, the mesenteric arterial tree. Furthermore, in the SAD model, increased sympathetic activity seemed to play a primary role in the development of hypertension; in the model used in the present study, changes in the vessel wall may be the result of a rise in intravascular pressure.

Studies of the alteration in the neurogenic response in hypertension are conflicting and inconsistent. Baum and Shropshire (7) have studied rat blood vessels by perfusing their hind limbs; although they have demonstrated an increased responsiveness to norepinephrine and other vasoconstrictors, they have seen significant increases in the neurogenic response only with lower frequencies of stimulation and then only in early hypertension. The changes disappear in older animals. Finch (9) has found significant increases in the vasoconstrictor response particularly at higher frequencies in both deoxycorticosterone-NaCl and renal hypertensive rats. In renal hypertensive dogs, Zimmerman et al. (8) have found increased norepinephrine release in cutaneous but not in muscular beds, particularly at high frequencies of stimulation; they have tentatively concluded that transmitter inactivation mechanisms are depressed. Furthermore, they have observed a fall-off in transmitter output during prolonged nerve stimulation in hypertensive but not in normotensive animals.

The role of the sympathetic nervous system and

its possible alteration in function in various forms of hypertension has been extensively studied (1, 27). Utilizing comparatively recently developed sensitive techniques for norepinephrine estimation (27, 28), plasma norepinephrine in various subgroups of essential hypertension (29-31) has been found to be elevated in various proportions of the patients. In patients with raised systolic arterial blood pressure, the norepinephrine content of an excised specimen of the vas deferens and associated blood vessels is increased in proportion to the rise in arterial blood pressure (32). This increase is significantly correlated with increases in tyrosine hydroxylase, dopa decarboxylase, and dopamine- β -hydroxylase activity. These multiple findings suggest increased biosynthesis of norepinephrine. As a result of many studies, it has been concluded that hypertensive patients have an increased release of newly synthesized norepinephrine. Yet, existing evidence, sparse as it is, does not support any conclusion of increased sympathetic nerve traffic (33). If these observations are correct, then any alteration or dysfunction of the adrenergic innervation must be at the level of the vascular neuroeffector complex. Although the interpretation of evidence obtained from man is very difficult, some investigators have suggested a primary role for neurogenic alteration in human hypertension.

In a variety of animal models, alteration of the adrenergic mechanism in the heart has been shown. Because of the dissociation of changes in the heart and the blood vessels in hypertension and the results of this study, showing that changes can occur in the blood vessels in the absence of similar changes in the heart, the relevance of these studies to the vasculature is questionable.

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