Adrenergic Neurotransmission in Vascular Smooth Muscle from Spontaneously Hypertensive Rats

R. Clinton Webb, M.D., Paul M. Vanhoutte, M.D., and David F. Bohr, M.D.

SUMMARY The goal of this study was to compare adrenergic neurotransmission in isolated vascular smooth muscle from spontaneously hypertensive (SHR) and normotensive rats. Tail arteries, excised from adult SHR and normotensive rats, were cut helically into strips that were mounted in organ chambers between two platinum wire electrodes; isometric contractions were recorded. Vascular responsiveness was determined before and after acute denervation with 6-hydroxydopamine or before and after treatment with phentolamine. Release or displacement of endogenous norepinephrine was obtained with electrical stimulation, tyramine, and potassium. The sensitivity to exogenous norepinephrine of innervated vessels was similar for SHR and normotensive rats. Denervation produced a significant shift to the left in the concentration-response curve to norepinephrine only in SHR vessels. Contractile responses to electrical stimulation, tyramine, and potassium were similar in both groups before denervation. Contractile responses to potassium-free solution were greater in SHR than in normotensive vessels. Following denervation, the SHR and normotensive vessels responded similarly to these latter interventions. Blockade of alpha-adrenoceptors with phentolamine reduced contractile responses to all agents in innervated and denerrated vessels. Cocaine caused a slowing of the relaxation following contraction induced by electrical stimulation in both SHR and normotensive vessels. The relaxation of SHR vessels was less affected by cocaine than in normotensive vessels. The tissue content of norepinephrine was similar in SHR and normotensive arterial strips. In arterial strips from SHR the uptake of $^1$H-norepinephrine was significantly larger than in those from normotensive rats. The results suggest that the reactivity of innervated blood vessels to norepinephrine is similar in SHR and normotensive vessels. Important differences in sensitivity to norepinephrine in hypertensive vessels are unmasked when the relationship between the vascular smooth muscle cell and the adrenergic nerve terminal is altered. Apparently, the adrenergic nerve terminals in hypertensive blood vessels can modulate the junctional concentration of norepinephrine so that the contractile response to this agent is similar to that in normotensive blood vessels. (Hypertension 3: 93–103, 1981)

KEY WORDS • norepinephrine • adrenergic denervation • 6-hydroxydopamine • vascular reactivity • vascular sensitivity

VASCULAR hyperresponsiveness to neurogenic or circulating humoral substances has been suggested to play an important role in the maintenance of increased vascular resistance characteristic of hypertension. Vasoconstrictor responses to injected norepinephrine are exaggerated in isolated vascular beds of spontaneously hypertensive rats (SHR) compared to those of normotensive rats. However, vasoconstrictor responses to sympathetic nerve stimulation are unchanged in SHR. This lack of difference to sympathetic nerve stimulation is surprising in view of the increased responsiveness to norepinephrine and suggests that the functional role of the neuroeffector junction in the walls of the blood vessels of SHR may be altered. The purpose of this investigation was to determine the contribution of the sympathetic neuroeffector junction to the responsiveness of vascular smooth muscle of SHR.

Methods

Adult male and female spontaneously hypertensive (SHR), Kyoto Wistar normotensive (WKY), and Wistar normotensive rats (3.5 to 6 months old) were used. The SHR were either provided by Dr. R. Smith of Warner-Lambert/Parke-Davis Company, Ann Arbor, Michigan, or obtained from the SHR colony at the Universitaire Instelling Antwerpen. The WKY and Wistar normotensive rats were obtained either from commercial sources (Charles River Breeding Laboratories, Wilmington, Massachusetts) or from the normotensive rat colony at the Universitaire Instelling Antwerpen.
Instilling Antwerpen. The SHR, WKY, and Wistar rats were maintained on a diet of Purina Laboratory Chow and water, ad libitum. The period of the estrus cycle in female rats was not determined nor controlled in these experiments. The systolic blood pressure as measured by the tail cuff technique was 183 ± 3 mm Hg for the SHR compared to 124 ± 2 mm Hg for the normotensive rats (SHR vs WKY and Wistar normotensive rats, p < 0.05). In each experiment, the rats were paired according to sex and age.

All animals were killed by a blow to the head, and tail arteries (0.7 to 0.8 mm, o.d.) were excised. The arteries were stored in physiological salt solution (PSS) and cut helically into strips (0.7 × 10 mm) under a dissecting microscope. The helical strips were mounted vertically on either a glass or plastic holder in a tissue bath containing PSS. The upper ends of the strips were connected to force transducers (Grass FT-03) and the resting tension was adjusted to 500 mg. The bathing medium was maintained at 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. The pH of the solution was 7.4 and the composition (mM/molar) was as follows: NaCl, 118.3; KCl, 4.7; KH₂PO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25.0; dextrose, 11.1; MgSO₄, 1.2; and CaNa₂ EDTA, 0.03. Potassium-free solutions were of the same composition except that KCl was omitted and 1.2 mM KH₂PO₄ was substituted with 1.2 mM NaH₂PO₄. Higher concentrations of potassium (10 to 75 mM) in the bathing medium were achieved by cumulative addition. Before the start of experiments, the strips were allowed to equilibrate for 60 to 90 minutes in PSS. During the equilibration period, the passive force placed on the strips was readjusted to 500 mg.

Strips of tail artery were electrically stimulated by the use of two platinum wire electrodes placed parallel to the preparations, as described previously. Electrical impulses consisted of square waves (9 V, 2 msec) provided by a direct current power supply and switching transistor (MBLE-BD-139) triggered by a stimulator (Janssen Scientific Instruments SUI). In some experiments, helical strips of tail artery from SHR, WKY, and Wistar normotensive rats were acutely denervated with 6-hydroxydopamine according to the method of Aprigliano and Hermansmyer. The strips were placed in a bicarbonate-free PSS containing 300 μM 6-hydroxydopamine for 10 minutes. The pH of this unbuffered PSS was adjusted to 4.0 by the addition of 20 μM glutathione. The O₂–CO₂ mixture to the bath was turned off during the denervation procedure. Following denervation, the strips were allowed to recover in normal PSS for 2 to 3 hours.

The tissue content of norepinephrine in tail arteries of SHR, WKY, and Wistar normotensive rats was determined radioenzymatically using a commercially available kit (Cat-a-Kit, Upjohn Diagnostics) as described by Vanhouette et al. To determine the tissue uptake of norepinephrine, strips of tail arteries were incubated for 60 minutes in solution containing 10⁻⁷ M l-(7-³H)norepinephrine (specific activity: 9.2 Ci/mmole). After the incubation the strips were repeatedly rinsed with fresh physiological salt solution, blotted dry, and weighed. The radioactivity was extracted by placing each strip in 2.5 ml of ice-cold 0.1 N acetic acid containing 0.03 mM Na₂EDTA and 5 mM ascorbic acid. After 30 minutes, the preparations were transferred to another set of tubes containing the same extraction fluid; after another 30 minutes, the strips were removed, and the two extraction portions were pooled for subsequent determination of ³H content. After 10 ml of Instagel (Packard Instrument Co., Inc.) was added to 1 ml of the extraction fluid, radioactivity was measured in a liquid scintillation counter (Packard, model 2650). Corrections for quenching were made with the external standard method.

The results of these experiments were analyzed by a variety of statistical procedures. Concentration-response curves were calculated as geometrical means. Student's t test and curve fitting analysis (probit analysis) were performed. A p value less than 0.05 was considered to be statistically significant. In all cases, the data for each arterial strip were normalized to its maximal response to exogenous norepinephrine, to allow interpretation of the results in terms of vascular reactivity and sensitivity.

Drugs used were; norepinephrine bitartrate (Winthrop Laboratories or Fluka AG), 6-hydroxydopamine HBr (Sigma Chemical Co.), glutathione (Calbiochem), cocaine HCl (Ciba Pharmaceutical Co.), and tyramine (Sigma Chemical Co.).

Results

Passive Force and Contractile Responses to Norepinephrine

In preliminary experiments, tail artery strips from SHR and normotensive rats were allowed to equilibrate for 60 to 90 minutes with zero passive force (zero resting tension). After this period, the strips were stretched to successively greater levels of tension. At each 100 μg increment of passive force applied to the strips, contractile responses to 3 × 10⁻⁸ M norepinephrine were determined (fig. 1). Contractile responses to norepinephrine increased to a maximum as the passive force applied to the strips increased to approximately 500 mg. The optimum passive force for maximum response to norepinephrine was similar for strips from SHR and normotensive rats. At all levels of passive force, however, the maximum contractile response produced by arterial strips from SHR was less than that of strips from normotensive rats. Based on these experiments, the initial passive force used for all subsequent studies was 500 mg for arterial strips from both SHR and normotensive rats. All contractile responses were normalized to the maximum response to exogenous norepinephrine to account for differences in contractibility between tail artery strips from SHR and those from normotensive rats.

Concentration-Response to Norepinephrine

Cumulative addition of norepinephrine (3 × 10⁻¹² to 3 × 10⁻⁸ M) to the muscle-bath-produced contractile responses in tail artery strips from SHR and normo-
PASSIVE FORCE AND CONTRACTILE RESPONSES TO NOREPINEPHRINE

**FIGURE 1.** Passive force and contractile responses to norepinephrine. Helical strips of tail artery from SHR and normotensive rats were stretched to successively greater levels of tension by the addition of passive force placed on the strip. At each 100 mg increment, contractile responses to $3 \times 10^{-6}$ M norepinephrine were determined. At all levels of passive force, the maximum contractile response of the strips from SHR was less than that of strips from normotensive rats. Asterisks indicate statistical differences between SHR and normotensive rats ($p < 0.05$). Values are the mean ± SEM for six SHR and six WKY or Wistar rats. The magnitude of the contractile response to each concentration of norepinephrine was measured during the plateau phase of the contraction (within 1 to 2 minutes after addition of norepinephrine). There were no detectable differences in the responsiveness of tail artery strips from the two normotensive strains of rats. In the innervated state, the concentration of norepinephrine that produced half-maximum contractile responses ($E_{D0}$) were similar in tail artery strips from SHR and those from normotensive rats (table 1). The maximum contractile force developed was significantly less for innervated tail artery strips from SHR (756 ± 41 mg) as compared to those for normotensive rats (1021 ± 95 mg).

Following denervation with 6-hydroxydopamine, there was a significant shift to the left in the concentration-response relationship to norepinephrine only in arterial strips from SHR. The $E_{D0}$ concentrations of norepinephrine were significantly lower in denervated SHR than in denervated arterial strips from normotensive rats (table 1). Denervation produced no significant change in the $E_{D0}$ concentration of norepinephrine in arterial strips from normotensive animals. The denervation procedure did not alter the magnitude of the maximum contractile response in tail artery strips from SHR (733 ± 50 mg) nor those from normotensive rats (977 ± 94 mg). Denervation caused a significant reduction in the slope of the concentration-response relationship for tail artery strips from SHR; there was no significant change in the slopes of the curves for arterial strips from normotensive rats (table 1).

**FIGURE 2.** Concentration response to norepinephrine. Helical strips of tail artery from SHR and WKY or Wistar rats (fig. 1) were made to contract in response to the cumulative addition of norepinephrine to the muscle bath. Concentration-response curves were performed before and after acute denervation with 6-hydroxydopamine. Asterisks indicate statistical differences between the innervated and denervated state ($p < 0.05$). Values are the mean ± SEM for eight SHR and eight WKY or Wistar rats.
Innervated WKY*Wistar

Dentrvated WKY-Wistar

SHR

240

100

60

20

0

able to contract in response to electrical field stimulation of adrenergic nerve terminals. The contractions were reduced after denervation with 6-hydroxydopamine. Arterial strips from SHR and normotensive rats responded similarly at all frequencies of stimulation. Values are the means ± SEM for four to six SHR and four to six WKY or Wistar rats.

FIGURE 3. Frequency-response relationship. Helical strips of tail artery from SHR and Wistar rats or WKY were made to contract in response to electrical field stimulation of adrenergic nerve terminals. The contractions were reduced after denervation with 6-hydroxydopamine. Arterial strips from SHR and normotensive rats responded similarly at all frequencies of stimulation. Values are the means ± SEM for four to six SHR and four to six WKY or Wistar rats.

Electrical Stimulation

Cumulative frequency-response curves in tail artery strips from SHR and normotensive rats were performed by starting stimulation at 0.1 Hz; the frequency was increased stepwise to 0.25, 0.5, 1, 2, 4, 8 and 16 Hz when the contractile response to the previous stimulation frequency had reached a maximum (fig. 3). Contractile responses to electrical stimulation were abolished by 10⁻⁸ M phentolamine. Following denervation with 6-hydroxydopamine, high frequency electrical stimulation (8 and 16 Hz) produced small contractile responses, which were inhibited by 10⁻⁵ M phentolamine. Contractile responses to electrical stimulation in the tail artery strips of SHR were not significantly different from those in the normotensive rats in either the innervated or denervated state.

Contractile Response to 6-Hydroxydopamine

Treatment of helical strips of tail arteries with 6-hydroxydopamine caused contraction (fig. 4). There were no significant differences in the magnitude nor in the time course of the contractile responses to 6-hydroxydopamine of tail artery strips from SHR and those from normotensive rats. The contractile responses were characterized by a maximum response, which was approximately 60% of the maximum response to exogenous norepinephrine. The development of tension occurred rapidly (maximum at 2 minutes) and then diminished to approximately 15% of the maximal norepinephrine-induced contraction at the end of the denervation procedure. The contractile responses to 6-hydroxydopamine treatment were inhibited by 10⁻⁵ M phentolamine.

TABLE 1. Half Maximum Contractile Responses (ED₅₀) for Norepinephrine in Isolated Rat Tail Arteries

<table>
<thead>
<tr>
<th>Rat</th>
<th>ED₅₀ (M)</th>
<th>Slope (probits/log concentration)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Innervated</td>
<td>Denervated</td>
</tr>
<tr>
<td>SHR</td>
<td>1.0 × 10⁻⁷</td>
<td>1.8 × 10⁻⁸*†</td>
</tr>
<tr>
<td>WKY and Wistar</td>
<td>8.1 × 10⁻⁶</td>
<td>1.2 × 10⁻⁷</td>
</tr>
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</table>

Concentration-response curves were calculated as geometrical means. Individual ED₅₀ values were projected by probit analysis. The asterisks indicate statistical differences between denervated and innervated responses and the dagger indicates a statistical difference between SHR and normotensive rats (Student's t test, p < 0.05).
ADRENERGIC INTERACTION IN SHR/Webb et al.

Concentration-Response to Elevated Potassium

Tail artery strips from SHR and normotensive rats were made to contract in response to the cumulative addition of potassium to the muscle bath in the presence and absence of $10^{-8}$ M phentolamine (fig. 5). In the absence of phentolamine, there was no difference in the contractile responses of tail artery strips from SHR to potassium and those from normotensive rats. Treatment with phentolamine decreased the contractile responses to elevated potassium in both SHR and normotensive arterial strips to the same degree.

Contraction in Potassium-Free Solution

Exposure of tail artery strips from SHR and normotensive rats to potassium-free solution resulted in contraction (fig. 6). These contractions were relatively slow in development, reaching a maximum at 15 to 20 minutes after exposure to the potassium-free environment. The contractions were absent in arterial strips denervated with 6-hydroxydopamine and abolished by $10^{-8}$ M phentolamine. Contractile responses of tail artery strips from SHR were significantly greater than those of normotensive rats at 15, 20, and 25 minutes into the potassium-free cycle.

Concentration-Response to Tyramine

Cumulative addition of tyramine ($10^{-10}$ to $10^{-3}$ M) to the muscle bath produced contractile responses in tail artery strips from SHR and normotensive rats (fig. 7).
Contractile responses to tyramine were reduced in strips denervated with 6-hydroxydopamine and absent in innervated and denervated preparations treated with 10⁻⁶ M phentolamine. There were no statistical differences between responses of tail artery strips from SHR and those from normotensive rats.

Effect of Cocaine on Relaxation Following Electrical Stimulation

Treatment of tail artery strips from SHR and normotensive rats with cocaine (10⁻⁴ to 10⁻⁵ M; 10 minutes) produced a marked slowing of relaxation following contraction induced by electrical stimulation (16 Hz; fig. 8). The interval of time required to reach half maximal relaxation (t½) in untreated arterial strips was 0.13 ± 0.02 minutes for SHR and 0.16 ± 0.02 minutes for normotensive animals. Cocaine increased the interval of time to reach half maximal relaxation to a significantly greater degree in arterial strips from normotensive rats as compared to those from SHR.

In both SHR and normotensive arterial strips, the addition of cocaine to the muscle bath prior to electrical stimulation produced contractile responses. The contractile effect of cocaine was blocked by 10⁻⁶ M phentolamine.

Tissue Content of Norepinephrine

The tissue content of norepinephrine was similar in tail arteries isolated from SHR and those isolated from normotensive rats (fig. 9).

Tissue Uptake of ³H-Norepinephrine

Strips of tail arteries of SHR took up significantly more ³H-norepinephrine than those of normotensive animals. Cocaine (10⁻⁴ M) significantly reduced the tissue uptake in both groups of animals. In the presence of the drug, no significant differences in tissue uptake of ³H-norepinephrine was observed between SHR and normotensive rats (fig. 10).

Discussion

The goal of this investigation was to compare the contribution of the adrenergic neuroeffector interaction to vascular reactivity in spontaneously hypertensive and normotensive rats. Helical strips were used instead of a perfused vascular bed to avoid the differences in vascular reactivity caused by the geometry of the vessel wall. The tail artery contains a dense adrenergic innervation. Contractile responses of the arterial strips were measured before and after acute denervation with 6-hydroxydopamine or before and after treatment with the alpha-adrenoceptor blocking agent, phentolamine. These procedures allowed assessment of the functional role of the adrenergic nerve endings.

Alpha-Adrenoceptor Sensitivity of Vascular Smooth Muscle

It has been suggested that the elevated vascular resistance of hypertension results from increased reactivity to vasoconstrictor influences. Vasoconstrictor responses to injected norepinephrine in the isolated hindlimb, mesentery, and kidney of SHR are exaggerated as compared to normotensive controls. Similarly, isolated vascular preparations from SHR have been shown to be more sensitive to exogenous norepinephrine than those from normotensive rats. We observed that the apparent sensitivity of innervated arterial strips from SHR to exogenous norepinephrine is the same as in innervated strips from normotensive rats. Acute denervation with 6-hydroxydopamine produced a significant shift to the left in the concentration-response curve only in tail artery strips from SHR. Thus, removal of the functional activity of the adrenergic nerve endings (presumably of the neuronal uptake mechanism) revealed that the vascular smooth muscle cells of tail artery strips from SHR are more sensitive to the amine than those from normotensive rats.

Removal of neuronal uptake sites from adrenergically innervated smooth muscle will permit a higher concentration of norepinephrine to be achieved in
the tissue and thereby shift the concentration-response curve to norepinephrine to the left and cause it to be less steep. When the system is saturated with norepinephrine, inhibition of the neuronal pump causes a small shift of the curve to the left and little change in the slope of the curve. This situation probably applies for the normotensive vessels, since the concentrations of norepinephrine used in the present study are within the range of Km's reported for adrenergically innervated rat tissues. The decrease in slope of the concentration-response curve in combination with the change in sensitivity to norepinephrine suggests that higher concentrations of the catecholamine are required to saturate the neuronal pump in SHR than that in normotensive rats.

Collis and Vanhoutte observed that isolated perfused kidneys from SHR were more sensitive to injected norepinephrine than those from normotensive rats. Constrictor responses to renal nerve stimulation were normal in SHR kidneys; however, blockade of the neuronal pump with cocaine potentiated constric tor responses to renal nerve stimulation to a significantly greater extent in SHR than in control kidneys. This earlier work already suggested that a more efficient uptake of norepinephrine by nerve endings in the blood vessel wall may mask the true sensitivity of the vascular smooth muscle cells in SHR.

An interesting observation of this study is that acute denervation of tail artery strips from normotensive rats did not produce a leftward shift of the concentration-response curve to exogenous norepinephrine. This is surprising in view of the dense adrenergic innervation of this artery (fig. 9). Aprigliano and Hermansmeyer have also observed a similar lack of supersensitivity to norepinephrine in acutely denervated rat tail arteries. They suggest that these results may be explained by the geometry of a helically cut strip of artery. Presumably, neuronal uptake of norepinephrine is less effective in a strip preparation because both sides of the strip are exposed when norepinephrine is added to the muscle bath. In support of this hypothesis, they observed that supersensitivity was present when norepinephrine was applied extraluminally to intact, perfused, tail artery segments after denervation but not when norepinephrine was added to the perfusate.

The change in sensitivity that we observed in SHR arterial strips must therefore be related to the fact that the neuronal pump is much more effective in modulating the concentration of norepinephrine at receptor
sites on the vascular smooth muscle cells. It is also possible that a portion of the difference between SHR and normotensive rats may be due to removal of extraneuronal uptake sites by the denervation procedure. The proposed lipid peroxidation mechanism for destruction by 6-hydroxydopamine may not be selective for the neuronal membrane; the results obtained at high concentrations of norepinephrine suggest that removal of uptake at extraneuronal sites might account for some of the curve shift in SHR arterial strips. If this is the case, these results would suggest a greater extraneuronal uptake of the transmitter in SHR tail artery strips as compared to normotensive rats.

The sensitivity of denervated tail artery strips from SHR and from normotensive rats to norepinephrine has been determined previously. Hermmsmeyer \(^7\) reported that the concentration of norepinephrine required to produce a 1/2-maximal response (ED50) was 1.3 \(\times 10^{-7}\) M and 3.0 \(\times 10^{-7}\) M (calculated from his results) for SHR and normotensive arterial strips respectively. The ED50 values are slightly higher than those reported for tail arteries of SHR and normotensive rats in this study (table 1). Reasons for these differences are not apparent, but may be partially due to the following: 1) differences in technique (Hermmsmeyer used a superfusion system and we used a constant volume muscle bath); 2) differences in the age of the rats (Hermmsmeyer used rats that were 6-10 weeks old and we used rats that were 14-24 weeks old); 3) differences in the pairing of animals (Hermmsmeyer paired his animals in each experiment according to weight and sex and we paired our experimental animals according to age and sex); 4) the arteries used in these experiments came from the proximal portion of the rat tail (artery o.d., 0.7 to 0.8 mm) whereas Hermmsmeyer used arterial sections from the distal part of the tail (artery o.d., 0.3 to 0.4 mm); 5) the calcium concentration of the PSS used in our experiments was 2.5 mM whereas Hermmsmeyer used 1.8 mM; and 6) the recovery time after 6-hydroxydopamine reduced these contractile responses to approximately 5% of the maximum contractile response to electrical stimulation of arterial strips from SHR and normotensive rats instead of 1 hour.

The maximum contractile response to norepinephrine was less in tail artery strips from SHR than those from normotensive rats. Other investigators \(^{15-17}\) have observed a similar decreased maximum force generating ability of arterial strips from hypertensive animals. The reasons for the difference in contractility are not clear, but they are not associated with differences in the amount of preload or passive tension placed on the arterial strips from the two groups of rats (fig. 1).

### Release of Norepinephrine from Adrenergic Nerve Endings

The release of transmitter from the adrenergic nerve endings in blood vessels of the intact animal is mediated by nerve impulses in the ganglionic cell. The released norepinephrine that reaches the vascular smooth muscle produces activation of the contractile machinery after binding to receptor sites on the cellular membranes. In this investigation, we used a variety of experimental conditions that cause the release or displacement of transmitter from nerve endings in isolated arterial strips from SHR and normotensive rats (table 2).

#### 6-Hydroxydopamine

We treated tail artery strips from SHR and normotensive rats with 6-hydroxydopamine to produce acute adrenergic denervation. Experimental evidence \(^7\) indicates that this drug produces a functional denervation with a lack of nonspecific effects.

Treatment of innervated vascular smooth muscle with 6-hydroxydopamine causes contraction. These contractions are probably due to catecholamines derived from damaged adrenergic nerve endings. \(^7\) The contractions are blocked with phentolamine and are not reproducible. We observed that the magnitude and time course of 6-hydroxydopamine-induced contractions were similar in tail artery strips from SHR and those from normotensive rats. Since 6-hydroxydopamine occupies the carrier sites for catecholamines on the neuronal membrane, \(^20\) a greater contractile response of arterial strips from SHR would have been predicted due to differences in catecholamine sensitivity. This suggests that either less norepinephrine is displaced or more of the amine is deactivated extraneuronally in SHR than in normotensive rats during the denervation procedure. However differences in the contractile response between SHR and normotensive vessels may have been blunted by the conditions of the bathing medium (bicarbonate-free, pH 4.0) during the denervation procedure. Acidosis is known to inhibit the release of norepinephrine in isolated vascular strips \(^8\) and to alter the contractile properties of vascular smooth muscle. \(^21, 22\)

### Electrical Stimulation

Stimulation of vascular strips with electrical current as used in our experiments causes contraction through activation of adrenergic nerve endings. \(^8\) The exocytotic release of norepinephrine by electrical stimulation most closely resembles that mediated by nerve impulses. \(^8\) In our experiments, tail artery strips from SHR and normotensive rats contracted in response to electrical stimulation. Acute denervation with 6-hydroxydopamine reduced these contractile responses to approximately 5% of the maximum response to exogenous norepinephrine. Phentolamine blocked the contractile responses to electrical stimulation in both innervated and denervated vascular strips. These results suggest that: 1) the contractile response produced by electrical stimulation is due to norepinephrine released from adrenergic nerve endings in the wall of tail artery strips from both SHR and normotensive rats; and 2) the denervation produced with 6-hydroxydopamine is nearly complete.

There were no significant differences in the contractile responses to electrical stimulation of arterial strips from SHR as compared to those from normotensive rats. Similarly, the vasoconstrictor responses
in isolated hindlimbs\(^2\) and kidneys\(^1\) of SHR to stimulation of the sympathetic nerves are the same as those of normotensive controls. During electrical stimulation, the response of the vascular smooth muscle cells is determined by the amounts of transmitter released by the nerve endings.\(^{19}\) The present results are surprising in view of the increased sensitivity of the vascular smooth muscle cells of SHR to norepinephrine. The observations suggest that either less norepinephrine is released at any given frequency of stimulation or that there is a greater disposition of the amine after release in blood vessels of SHR.

### Elevated Potassium Concentration

Elevation of the extracellular concentration of potassium ions is known to cause depolarization of nerve endings resulting in the release of norepinephrine.\(^{23}\) Tail artery strips from SHR and normotensive rats responded similarly to an increase in potassium concentration before and after treatment with phentolamine. This similarity suggests that either less norepinephrine is released during steady-state depolarization of the nerve endings or more norepinephrine is deactivated extraneuronally in SHR since the smooth muscle cells of the rats are more sensitive to the amine. It is unlikely that a greater disposition of norepinephrine by the neuronal pump could account for this similarity because depolarization of the prejunctional membrane inhibits neuronal uptake.\(^{23,18}\) Although less likely, the results may be partially due to a differential effect of an increase in osmolarity on arterial strips from SHR and those from normotensive rats.

### Potassium-Free Solution

Incubation of adrenergically innervated blood vessels in potassium-free solution causes a release of norepinephrine from adrenergic nerve endings.\(^{29}\) The release of norepinephrine under these conditions is probably due to depolarization of the prejunctional membrane.\(^{24}\) However, the release is different from that produced by depolarization with electrical stimulation and elevated potassium. The main difference is that there is no increase in the deaminated metabolites of norepinephrine as accompanies electrical stimulation and elevated potassium concentration.\(^{25}\) This suggests that in low potassium solution, none of the released transmitter is taken up by the

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**Table 2. Release of Norepinephrine in Rat Tail Arteries**

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Mode of action</th>
<th>Neuronal uptake</th>
<th>Extraneuronal deactivation</th>
<th>Response</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exogenous norepinephrine</td>
<td>Stimulates alpha-adrenoceptors</td>
<td>Active in innervated vessels; absent in denervated vessels</td>
<td>Active in both innervated and denervated vessels</td>
<td>No difference between SHR and normotensive vessels during electrical stimulation and elevated potassium.</td>
<td>SHR vascular smooth muscle is more sensitive to norepinephrine; SHR has a greater neuronal uptake system.</td>
</tr>
<tr>
<td>Electrical stimulation</td>
<td>Exocytotic release</td>
<td>Inhibited during the nerve impulse</td>
<td>Active</td>
<td>No difference</td>
<td>Either less norepinephrine is displaced or extraneuronal deactivation is greater in SHR.</td>
</tr>
<tr>
<td>6-hydroxydopamine</td>
<td>Pharmacological displacement</td>
<td>Inhibited due to participation of carrier sites</td>
<td>Active</td>
<td>No difference</td>
<td>Either less norepinephrine is released or more is deactivated extraneuronally in SHR.</td>
</tr>
<tr>
<td>Elevated potassium</td>
<td>Exocytotic release</td>
<td>Inhibited due to depolarization</td>
<td>Active</td>
<td>No difference</td>
<td>SHR contracts to a greater extent.</td>
</tr>
<tr>
<td>Potassium-free solution</td>
<td>Exocytotic release</td>
<td>Inhibited due to depolarization</td>
<td>Inhibited</td>
<td>SHR contracts to a greater extent</td>
<td>Removal of disposition mechanisms unmasks the true sensitivity of SHR vascular smooth muscle to norepinephrine.</td>
</tr>
<tr>
<td>Tyramine</td>
<td>Pharmacological displacement</td>
<td>Inhibited due to participation of carrier sites</td>
<td>Active</td>
<td>No difference</td>
<td>Either less norepinephrine is displaced or more is deactivated extraneuronally in SHR.</td>
</tr>
<tr>
<td>Relaxation following electrical stimulation</td>
<td>Relaxation rate is determined by the disposition of norepinephrine</td>
<td>Active</td>
<td>Active</td>
<td>No difference; after treatment with cocaine, SHR relaxed at a faster rate</td>
<td>Either SHR is less sensitive to the actions of cocaine or there is a greater extraneuronal deactivation.</td>
</tr>
</tbody>
</table>

The interpretation of the results of these experiments is based on the following premises: 1) specific experimental conditions cause the release or displacement of norepinephrine in adrenergically innervated smooth muscle (columns 1 and 2); 2) the concentration of norepinephrine which produces the contractile response (column 5) can be effectively modulated by disposition mechanisms (columns 3 and 4); and 3) the true sensitivity of vascular smooth muscle from SHR is greater than that from normotensive rats. See Discussion for complete explanation.
neuronal pump. In addition, the extraneuronal metabolism of norepinephrine has been shown to be reduced in canine blood vessels incubated in potassium-free solution.

Contractile responses during incubation in potassium-free solution were greater in tail artery strips from SHR than those from normotensive rats. The contractile responses were absent in denervated strips and were abolished by phentolamine, suggesting that the procedure produced release of norepinephrine from adrenergic nerve endings. The increased responsiveness in potassium-free solution of arterial strips from SHR may be attributed to: 1) a difference in the release mechanism during the procedure; 2) impairment of neuronal uptake, which may require an active sodium and potassium extrusion pump; and 3) increased sensitivity to norepinephrine of the vascular smooth muscle cells; and 4) removal of the extraneuronal deactivation of the amine. The magnitude of the difference between SHR and normotensive controls suggests that more than one mechanism is responsible.

Tyramine

Tyramine acts as a false transmitter and displaces norepinephrine from the storage complex of adrenergic nerve endings. Displacement of norepinephrine by tyramine is unlike release produced by prejunctional depolarization in that it is not accompanied by a release of dopamine-beta-hydroxylase and does not depend on an increase in intraneuronal calcium concentration. The indirect sympathomimetic effect of tyramine is greatly attenuated by procedures that inhibit the neuronal pump.

Tail artery strips from SHR and normotensive rats were found to respond similarly to the cumulative addition of tyramine to the muscle bath before and after acute denervation. Phentolamine blocked these contractile responses, suggesting that tyramine-induced contractions are partially due to stimulation of vascular alpha-adrenoceptors. The lack of difference between SHR and normotensive animals is not explained by postjunctional receptor activation by tyramine since denervated strips of SHR responded similarly to those from normotensive animals. Tyramine occupies the carrier sites for neuronal uptake, and it therefore must displace less norepinephrine in adrenergic nerve endings of SHR arterial strips as compared to those from normotensive rats. It is also possible that the results may reflect important differences in the affinity of carrier sites for tyramine on the neuronal uptake pump in blood vessels of SHR.

Blockade of Neuronal Uptake

Sensitization of effector responses in adrenegically innervated tissues by blockade of neuronal uptake is a well-documented observation. Cocaine, the classically used uptake blocker, potentiates vasoconstrictor responses to nerve stimulation in kidneys of SHR to a greater degree than in those from normotensive rats. These results suggest that the disposition of norepinephrine by neuronal uptake is more effective in the blood vessels of SHR. The similarity of concentration-response curves to norepinephrine in innervated tail artery strips of SHR and normotensive rats may also be due to a more efficient disposition masking the increased sensitivity in SHR vessels. This hypothesis is strengthened by the observation that acute denervation produced a significant shift to the left in the concentration-response curve to norepinephrine in strips from SHR.

The rate of relaxation following contraction induced by electrical stimulation is determined by the disposition of the transmitter. Procedures that inhibit neuronal uptake cause a slowing of relaxation following contraction induced by either exogenous norepinephrine or electrical stimulation. We observed that cocaine produced a marked slowing of relaxation in arterial strips from SHR and normotensive rats contracted by electrical stimulation. This effect was dependent upon the concentration of cocaine; the interval of time required to reach half-maximal relaxation was less in SHR than in normotensive controls at all concentrations of cocaine. These experiments demonstrate that arterial strips from SHR are less sensitive to the action of cocaine than those from normotensive rats. If the uptake system in SHR requires more norepinephrine to be saturated, as indicated by the decrease in slope of the concentration-response curve (see above), the lesser effect of cocaine in inhibiting relaxation of SHR arterial strips also suggests that there is a greater neuronal uptake of released norepinephrine as compared to that in normotensive vessels. This conclusion is confirmed by the experiments demonstrating that the cocaine-dependent tissue uptake of \( ^{3} \)H-norepinephrine is greater in arteries from SHR than in vessels from normotensive animals.

Tail artery strips from both SHR and normotensive rats contracted when cocaine was added to the muscle bath prior to electrical stimulation. This effect of cocaine is probably due to the indirect sympathomimetic effect of the drug, the displacement of norepinephrine from extracellular binding sites, or the direct stimulation of postjunctional alpha-adrenergic receptors.

Tissue Content of Norepinephrine

For most blood vessels, the major site of norepinephrine storage is located in the adrenergic nerve endings surrounding the muscle layer in the adventitia. The tissue content of norepinephrine in tail arteries of SHR was similar to that in those from normotensive rats. Thus, it can be concluded that even if the neuronal uptake is more avid in SHR, it does not result in a larger tissue content of norepinephrine.

Conclusions

These functional studies of the interaction between the adrenergic neuroeffector junction and vascular smooth muscle cells suggest that the true sensitivity of
vascular smooth muscle cells to norepinephrine is greater in tail artery strips from SHR as compared to those from normotensive controls. Under most experimental conditions, the junctional concentration of norepinephrine acting on the vascular smooth muscle cells is less in blood vessels of SHR than in those from normotensive rats. The junctional concentration of norepinephrine is maintained at lower levels in SHR: 1) by a greater neuronal uptake of the amine; 2) by a greater extraneuronal deactivation of the transmitter; and 3) possibly by a reduction in the amount of norepinephrine released or displaced from the nerve endings.

Thus, the present experiments suggest that, in contrast with young SHR, the adrenergic neuroeffector junction in blood vessels from adult hypertensive SHR is greater in tail artery strips from SHR than in those from normotensive controls. Under most experimental conditions, the junctional concentration of norepinephrine acting on the vascular smooth muscle cells is less in blood vessels of SHR than in those from normotensive rats. The junctional concentration of norepinephrine is maintained at lower levels in SHR: 1) by a greater neuronal uptake of the amine; 2) by a greater extraneuronal deactivation of the transmitter; and 3) possibly by a reduction in the amount of norepinephrine released or displaced from the nerve endings.

Thus, the present experiments suggest that, in contrast with young SHR, the adrenergic neuroeffector junction in blood vessels from adult hypertensive animals can modulate the junctional concentration of norepinephrine. The reason for this functional adaptation is unknown. In terms of the relationship of our experiments to the maintenance of increased peripheral resistance in hypertension, it appears that during nerve stimulation this long-term autoregulation of the neuroeffector interaction in SHR results in a normal response of the smooth muscle cells to the existing sympathetic tone.

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