Norepinephrine Overflow in Perfused Mesenteric Arteries of Spontaneously Hypertensive Rats

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We examined the overflow of endogenous norepinephrine with electrical stimulation, the associated pressor response, and rate of initial neuronal uptake of \[^3H\]norepinephrine in perfused mesenteric arteries of 7- and 13-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. The tissues of two rats, a spontaneously hypertensive and a WKY control rat, were simultaneously processed and subjected to the same electrical stimulation. Both absolute and fractional overflow of endogenous norepinephrine during periarterial nerve stimulation (5 and 10 Hz for 1 minute) in the tissue of 7-week-old SHR was significantly greater whereas overflow of 13-week-old SHR was equivalent as compared with that of the age-matched WKY rats. The tissue content of norepinephrine was 20-25% higher in SHR of both ages. There was significantly enhanced \[^3H\]norepinephrine uptake in the tissues of young SHR, but no difference was observed in the older SHR. The pressor response to periarterial nerve stimulation was significantly enhanced in 7-week-old SHR and much more so at the older age as compared with the WKY control rats. Exogenous norepinephrine dose–response curves in the tissues of 7-week-old SHR exhibited a parallel leftward shift, characteristic of a change in sensitivity, whereas that of 13-week-old SHR showed a much steeper slope as compared with the respective WKY control rats. This finding suggests that in addition to smooth muscle supersensitivity, structural alterations had occurred in vasculature of 13-week-old SHR. These data indicate that in SHR both the exocytotic release of norepinephrine and the responsiveness of the vascular smooth muscle cells are enhanced in the developmental stage of hypertension whereas smooth muscle supersensitivity to norepinephrine and nonspecific structural alterations primarily contribute to the maintenance of hypertension at 13 weeks of age. (Hypertension 1989;14:44–53)

There is increasing evidence to suggest that the sympathetic nervous system plays an important role in the development and maintenance of hypertension in spontaneously hypertensive rats (SHR).1-4 Several investigators have reported a vasoconstrictor hyperresponsiveness to sympathetic nerve stimulation in isolated organs from adult SHR.5-7 Both Eikenberg et al8 and Ekas and Lokhandwala9 reported a significantly greater overflow of total tritium \(^{({}^{3}H)}\) from the sympathetic adrenergic nerve terminals in the mesenteric vasculature of adult (18-week-old) SHR as an indication of \[^3H\]norepinephrine release. Blockage of neuronal reuptake with cocaine increased responses in both SHR and Wistar-Kyoto (WKY) control rats, although the potentiation appeared to be slightly greater in SHR.

There also was a significantly greater overflow of total tritium in the isolated perfused kidney from the same aged group of SHR.10 Westfall et al11 reported that there was no difference between SHR and WKY control rats at 5-6 weeks of age either in the release of \[^3H\]norepinephrine or in the developed tension of the portal vein to any frequency of field stimulation, but there was a significantly greater release of \[^3H\]norepinephrine and developed tension in veins of SHR in response to low (1 or 2 Hz), but not high (5 or 10 Hz), frequencies in the older groups. In these experiments, desipramine and metanephrine were added to the superfusion buffer to block neuronal and extraneuronal uptake of norepinephrine.

In contrast, Cassis et al12 reported that responses to high (10-15 Hz), but not low (0.5–5 Hz), frequen-
cies of sympathetic nerve stimulation were increased in the perfused caudal artery of adult (16–20-week-old) SHR. The authors also observed that the increases of perfusion pressure were potentiated in the presence of cocaine, but the response of caudal arteries from SHR and WKY rats to injected noradrenaline in the presence and absence of cocaine was similar. Release by K+ of endogenous noradrenaline from the coccgeal artery of SHR at all ages was also significantly greater than in WKY rats. However, both Collis and colleagues14,15 and Vanhoutte and coworkers16–18 found an age-related effect with nerve stimulation. More endogenous noradrenaline was released from the kidney of young SHR (46-day-old)15 but not in the kidney of adult animals (4–6 months) when compared with the normotensive controls.18 These authors also observed that the effects of cocaine on responses to renal nerve stimulation and noradrenaline exhibited similar leftward shift, but neither the magnitude of this shift nor the prolongation of the response, caused by cocaine, were significantly different between kidneys from SHR and sex-matched control rats.

Zsoter et al19 also found significantly more [3H]norepinephrine overflow upon electrical stimulation in tail artery from 7–9-week-old SHR than the WKY control rats. Recently, one of us20 examined the Ca2+ dependency of noradrenaline release and vascular responsiveness in the perfused mesenteric preparations of SHR and deoxycorticosterone acetate (DOCA)-salt hypertensive rats and found that norepinephrine overflow was enhanced only in young SHR (7–8-week-old) and in the chronic phase of DOCA-salt hypertension.

Although all of these observations have implicated the sympathetic system in the development of spontaneous as well as other forms of experimental hypertension, there still remains a question as to whether the above increased noradrenaline release alone contributes to vascular hyperresponsiveness in SHR. Theoretically, synaptic cleft concentrations of noradrenaline depend on both the magnitude of exocytotic release and the rate of disposition of the released transmitter.11,14,16 The greater effect in SHR may be a consequence of an imbalance or an excessive quantity of noradrenaline that results from enhanced release per nerve impulse and an inadequate neuronal or extraneuronal uptake of noradrenaline.

In an attempt to assess the contribution of these two noradrenergic synaptic processes to initiation and maintenance of hypertension, we have measured overflow of endogenous noradrenaline during electrical stimulation, the associated mesenteric arterial pressor response, and the rate of neuronal uptake of [3H]norepinephrine in isolated perfused mesenteric vessels. Using the same experimental solutions and electrical stimulation, we simultaneously processed tissues of two rats, a SHR and an age-matched WKY control rat. Combined investiga-

gations, where the same mesenteric bed is examined for both release and reuptake, offer the best way of quantitating the two processes and establishing the degree to which vascular tone is generated by net effect. Both young (7-week-old) and sustained hypertensive rats (13-week-old) were compared with the age-matched WKY control rats.

**Materials and Methods**

**Blood Pressure and Body Weight Measurements**

Two age groups of male SHR of the Okamoto-Aoki strain at 6 and 12 weeks of age21 and age-matched WKY rats were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) and were housed in groups of four or five in 12-hour light/dark cycles for 1 week. Rats were habituated before blood pressure measurements. At 1 day before the preparation of the tissue, systolic blood pressures were measured after the rats were heated and restrained with Narco Rat Holder (Harvard Apparatus, South Natick, Massachusetts) according to the procedure described by Bunag.22

**Preparation of Tissue and Electrical Stimulation**

After the rats were anesthetized with sodium pentobarbital anesthesia (60 mg/kg i.p.), the intestinal loop containing the mesenteric artery was prepared by modification of the method of Castellucci et al.23 The abdominal cavity was opened, the superior mesenteric artery was located, and the proximal segment was cleaned of surrounding tissue in the area of the aorta. A cannula (PE50; Becton Dickinson and Co., Parsippany, New Jersey) was inserted distally into the main trunk of the mesenteric artery at its origin from the aorta and tied in place, and the pancreaticoduodenal, ileocolic branches of superior mesenteric artery were tied. The mesenteric vein was also cannulated selectively from the portal vein with an 18-gauge cannula. The mesenteric vasculature was flushed with approximately 3 ml heparinized Krebs-Henseleit solution, and the intestine was separated from stomach, duodenum, and large intestine. The mesenteric artery-intestinal loop was cut and quickly connected to the perfusion apparatus. The preparations were perfused with Krebs-Henseleit solution by use of a peristaltic pump (model 1201, Harvard Apparatus).

Optimum perfusion conditions were determined for the mesenteric arteries from both strains by stepwise increases in perfusion flow rate.15 Electrodes placed around the periarterial plexus of the mesenteric artery were used to stimulate the sympathetic nerves. A standard electrical stimulus (15 Hz, 5-msec pulse width, 10-second durations every 15 minutes) was given at each flow rate. The minimum perfusion flow rate that permitted a maximal constrictor response amplitude, which was measured by perfusion pressure increase, was taken as optimum (Table 1), and we subsequently
TABLE 1. Optimal Flow Rate in Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>Optimal flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>6</td>
<td>2.35±0.04</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>7</td>
<td>2.27±0.06</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>5</td>
<td>2.62±0.11</td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>5</td>
<td>2.60±0.11</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

used 3 ml/min in tissues of both SHR and WKY rats.

Constituents of the solution were as follows (mM): NaCl 144.5, KCl 4.6, KH2PO4 1.4, MgSO4 2.4, CaCl2 2.5, NaHCO3 25, and glucose 5.6. The solution was continuously oxygenated with the gas mixture of 95% O2 and 5% CO2 at 37° C. The perfusion pressure was recorded by means of a side arm with a pressure transducer (MP-15D, Micron Instrument, Wilmington, Delaware) connected to a polygraph (TR-200 A, Gulton Industries, Inc., Greenwich, Rhode Island). A platinum electrode was placed around the periartrial plexus of the mesenteric artery. A 30-minute equilibrium period was allowed before starting an experiment. The tissues of two rats, a SHR and a WKY rat, were simultaneously processed with the same experimental solutions.

Release of Endogenous Norepinephrine and Vascular Reactivity by Electrical Stimulation

- The platinum plate electrode was placed around the periartrial plexus of mesenteric artery. Endogenous norepinephrine was released by electrical stimulation (Grass S II stimulator, Grass Instr. Co., Quincy, Massachusetts) of the intramural sympathetic nerves at supramaximal voltage for 1 minute with rectangular pulses of 5-msec duration at frequencies of 5 and 10 Hz at 15-minute intervals. The perfusate through the mesenteric-loop preparation in the presence or absence of neuronal uptake blocker, cocaine (10^-6 M), was collected into tubes containing 10 mg EDTA for each 5-minute period before and after nerve stimulation. The associated pressor response was recorded on a polygraph recorder (TR-200 A, Gulton Industries Inc.). The perfusate was immediately frozen in acetone–dry ice bath and stored at −70° C until the time of assay.

When assayed, internal standard 3,4-dihydroxybenzylamine (10 ng) was added to each tube. Norepinephrine in the perfusate was adsorbed on 50 mg alumina by adding 500 µl 1.5 Tris-HCl buffer (pH 8.5) and mixing it for 2 minutes, eluted with 200 µl 0.1N perchloric acid, and then assayed with high-pressure liquid chromatography with an electrochemical detector (Bioanalytical system LC-4, West Lafayette, Indiana). Recovery of norepinephrine was routinely 97–98%. The 3-µm ODS column (4.5 mm×100 mm Altech Associates, Inc., Deerfield, Illinois) was used. A mobile phase chloroacetic acid (0.1 M) containing 0.07 M NaOH, 1.5 mM EDTA, and 0.17 mM sodium octyl sulfate was pumped through the column at a rate of 1.5 ml/min by high-speed pump (3500B, Spectra-Physics, Bedford, Massachusetts).

Norepinephrine overflow by nerve stimulation in the presence of deoxycorticosterone (10^-3 M), a specific uptake II blocker,24 is defined as the difference between norepinephrine content of perfusates for 5-minute periods before and after the nerve stimulation. Fractional norepinephrine overflow is given as the ratio of the amount of norepinephrine present in the perfusate divided by the norepinephrine content of the tissue.

Pressor Response to Exogenous Norepinephrine

Vasoconstrictor responses to l-norepinephrine were studied at three different dose levels, 0.5, 1.0, and 2.0 µg. After the endogenous norepinephrine overflow experiment, a 10-minute equilibrium period was allowed. All doses were injected into the perfusion tube immediately before the arterial cannula. The injection volume was 0.1 ml, and all dilutions of the norepinephrine were made with Krebs-Henseleit solution. Ten-minute intervals were allowed between the injection of different doses of norepinephrine.

Norepinephrine Uptake Measurement

After the pressor response experiment, a 15-minute equilibrium period was allowed before the [3H]norepinephrine was added to the perfusion fluid. Thus, after an equilibrium period, the two mesenteric arteries, one from the spontaneously hypertensive rat and the other from the age-matched WKY rat, were perfused in parallel for 5 minutes with Krebs-Henseleit solution containing [3H]norepinephrine (1.0×10^-7 M), deoxycorticosterone (10^-5 M), pargyline (1.5×10^-4 M), and ascorbic acid (0.1 mM). After a 30-minute washout period with amine-free perfusion solution, mesenteric vessels were removed from the perfusion system. After the fluid in the intestine was blown out by air stream, the tissues were blotted dry and weighed. They were placed in 10 ml ice-cold 0.4N perchloric acid, minced into small pieces, and homogenized for 2 minutes by Polytron (PT 10/35, Brinkmann Instrs., Inc, Westbury, New York) at 0° C. The test tubes were centrifuged at 15,000g for 10 minutes at 4° C. The residue was homogenized with 10 ml 0.4N perchloric acid again, and the supernatants were pooled. One milliliter supernatant was then removed to determine the total radioactivity. Under the combined conditions of monoamine oxidase inhibition and blockage of uptake II process, a short exposure time (5 minutes) to [3H]norepinephrine permitted assessment of the neuronal uptake of [3H]norepinephrine. Both the mesenteric artery of the hypertensive rat and that of an appropriate control rat were simultaneously exposed to the perfusion fluid containing exactly the same concentration of [3H]norepinephrine.
Application of Cocaine

To determine specific neuronal norepinephrine uptake, the effect of cocaine on norepinephrine uptake was evaluated by the same protocol described above for norepinephrine uptake. Thus, after an initial 30-minute equilibrium period, cocaine at a concentration of $5 \times 10^{-3}$ M was added to the perfusion fluid, and the tissue was perfused for a 15-minute period. The two mesenteric arteries, one control artery and one with cocaine, were then perfused for 5 minutes with the perfusion fluids containing $[^3]$H]norepinephrine ($1.0 \times 10^{-7}$ M). After a washout period of 30-minute perfusion with amine-free solution, the mesenteries were processed for the measurement of $[^3]$H]norepinephrine uptake with the same procedure described above.

Measurement of Norepinephrine Content

For the determination of content of endogenous norepinephrine in the mesenteric arteries, norepinephrine in 100 µl supernatant solution, which was used for the determination of total radioactivity in the norepinephrine uptake measurement, is first neutralized with 1 ml 0.1 M phosphate buffer, pH 7.0, and an internal standard 3,4-dihydroxybenzylamine (10 ng) was added to each tube. Norepinephrine in the solution was extracted by the procedure described for analysis of released norepinephrine in the perfusate of norepinephrine overflow experiment and assayed by high-pressure liquid chromatography with electrochemical detector.

Statistical Analysis

Values were presented as mean±SEM. Statistical significance was determined by one-way and two-way analyses of variance, which were followed by Bonferroni’s method for multiple group comparison. Difference of $p<0.05$ was considered to be significant.

Results

The average weights of the animals at 7 and 13 weeks of age were (mean±SD) 146±17 g and 251±13 g for SHR and 152±19 g and 246±14 g for WKY rats, respectively. The average systolic blood pressures of the animals at 7 and 13 weeks of age were 148.0±4.6 mm Hg ($n=18$) and 173.2±4.1 mm Hg ($n=19$) for SHR and 110.1±4.1 mm Hg ($n=19$) and 118.7±3.1 mm Hg ($n=19$) for WKY rats. The average tissue weights of the animals at 7 and 13 weeks of age were 9.59±0.73 g and 12.84±0.63 g for SHR and 10.57±0.28 g and 11.57±0.88 g for WKY rats.

Effect of Field Stimulation on Perfusion Pressure

Periarterial nerve stimulation (5 and 10 Hz) was applied to the perfused mesenteric vasculatures of two age groups of animals each comprised of equal numbers of SHR and WKY control rats. The optimal flow rate of mesenteric arteries from SHR did not differ significantly from their age-matched controls (Table 1). Also, there was no significant difference in basal perfusion pressures at a flow rate of 3 ml/min in mesenteric arteries in SHR and WKY control rats (Table 2). The vasoconstrictor responses to periarterial nerve stimulation (5 and 10 Hz) of the two age groups over their basal perfusion pressure are shown in Figure 1. The tissues of two rats, a spontaneously hypertensive rat and a WKY control rat, were simultaneously processed and subjected to the same electrical stimulation with the same experimental solution. The constrictor responses evoked by 5- and 10-Hz stimulation were of significantly greater amplitude in mesenteric vasculatures from both age groups of SHR than from WKY control rats ($p<0.01$).

Overflow and Contents of Endogenous Norepinephrine

The perfusate from mesenteric vasculature of both SHR and WKY rats was collected for 5 minutes before and 5 minutes after periarterial nerve stimulation. The basal overflow of norepinephrine in the absence of electrical stimulation from the mesentery of SHR and WKY rats at 7 and 13 weeks of age is shown in Table 2. The basal perfusion pressures and norepinephrine overflow are shown in Table 2. The basal perfusion pressures and norepinephrine overflow at 5 and 10 Hz were significantly higher than that in WKY in both age groups (two-way analysis of variance).

TABLE 2. Basal Perfusion Pressures and Norepinephrine Overflow

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>Basal perfusion pressure (mm Hg)</th>
<th>Basal NE Overflow (pmol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>9</td>
<td>51.71±1.56</td>
<td>0.43±0.08</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>10</td>
<td>54.85±2.40</td>
<td>0.49±0.15</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>10</td>
<td>49.00±3.72</td>
<td>0.30±0.09</td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>10</td>
<td>52.00±1.47</td>
<td>0.31±0.08</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

FIGURE 1. Frequency-response curves to periarterial nerve stimulation in isolated perfused mesentery from spontaneously hypertensive rats (SHR) (7-week-old, n=10) and 13-week-old, n=10; and Wistar-Kyoto (WKY) (7-week-old, n=9; 13-week-old, n=10) normotensive (control) rats. The circles and bars indicate mean±SEM. The perfusion pressor response in SHR (electrical stimulation [E.S.] at 5 and 10 Hz) was significantly higher than that in WKY in both age groups (two-way analysis of variance).
13 weeks of age is shown in Table 2. There was no significant difference in basal norepinephrine overflow between the same age groups of SHR and WKY control rats. In both groups, electrical stimulation (5 and 10 Hz) evoked an increase in the overflow of endogenous norepinephrine (Table 3); at both frequencies, the overflow above the basal value that was induced by the stimulation was more significantly enhanced in the mesenteries from 7-week-old SHR (63.8% with 10 Hz stimulation) than from normotensive WKY control rats. In contrast, the extent of norepinephrine overflow from mesenteric arteries of 13-week-old SHR was slightly higher than that of age matched WKY rats, but it was not significantly different. This overflow from the mesenteric arteries of 13-week-old SHR was also significantly reduced from the level of norepinephrine overflow from 7-week-old SHR. In a separate experiment, we also assessed the norepinephrine overflow under a partial inhibition of the uptake mechanism. Blockade of neuronal uptake with cocaine (10^-6 M) increased the response in both SHR and WKY control rats (Table 4), but the overflow was significantly enhanced only in the mesenteric arteries from 7-week-old SHR (41.9% with 10 Hz stimulation) compared with that of normotensive WKY control rats. The overflow of endogenous norepinephrine and pressor response induced by electrical stimulation were completely abolished by addition of 2x10^-6 M guanethidine to the perfusate. There was no significant change in tissue weight before and after the perfusion experiment, which indicated minimal edema formation.

The norepinephrine content of mesenteric arteries from SHR was significantly higher than the WKY control rats at both age groups as shown in Table 5. There was significant difference not only between SHR and WKY rats at each age group but also between 7- and 13-week-old SHR.

Because of differences in norepinephrine content between SHR and WKY rats, fractional overflow of norepinephrine was also determined (Table 6). When norepinephrine overflow was expressed this way, that is, norepinephrine in overflow per content of norepinephrine in the tissue, electrical stimulation with both frequencies also resulted in a significantly higher overflow of norepinephrine (45.6% with 10 Hz stimulation) in the mesenteries from 7-week-old SHR than in the age-matched WKY control rats. The fractional overflow of norepinephrine from the mesenteric arteries of 13-week-old SHR, however, was insignificantly reduced as compared with that of WKY control rats but rather significantly reduced from the level of 7-week-old SHR.

### Pressor Response to Exogenous Norepinephrine

Vasoconstrictor responses to exogenous norepinephrine in SHR and WKY rats of both age groups are illustrated in Figure 2. The concentrations that were used for analysis of the norepinephrine concentration-response relations in SHR and WKY rats were 0.5, 1.0, and 2.0 µg per 0.1 ml injection

### Table 3. Norepinephrine Overflow by Electrical Stimulation

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>NE overflow by electrical stimulation (pmol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 Hz</td>
</tr>
<tr>
<td>WKY</td>
<td>7</td>
<td>9</td>
<td>8.62±0.84</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>10</td>
<td>12.34±0.80*</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>10</td>
<td>7.87±0.53</td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>10</td>
<td>8.26±0.47</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*Significant difference between 7-week-old WKY and SHR by two-way analysis of variance.

†Significant difference between 7- and 13-week-old SHR by Student's t test.

### Table 4. Effect of Cocaine on Norepinephrine Overflow

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>NE overflow (pmol/g wet wt, 10 Hz)</th>
<th>Δ NE overflow (pmol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cocaine (-)</td>
<td>Cocaine (+)</td>
</tr>
<tr>
<td>WKY</td>
<td>7</td>
<td>8</td>
<td>18.48±0.74</td>
<td>24.38±1.69*</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>8</td>
<td>25.89±1.46</td>
<td>34.59±2.67</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>5</td>
<td>17.49±1.93</td>
<td>23.03±1.08*</td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>6</td>
<td>18.81±0.74</td>
<td>22.15±1.66*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Cocaine concentration was 10^-6 M. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*Significant difference between cocaine(-) and cocaine (+).

†Significant difference between SHR and WKY.

### Table 5. Endogenous Norepinephrine Content in Isolated Mesenteric Artery

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>NE content (pmol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>9</td>
<td>949.23±35.76</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>10</td>
<td>1,149.23±66.39*</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>10</td>
<td>1,035.78±21.66</td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>10</td>
<td>1,263.50±53.17†</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

*Significant difference between WKY and SHR in each age group by Student's t test.

†Significant difference between 7- and 13-week-old SHR by Student's t test.
volume for mesenteric arteries of both SHR and WKY rats. Responses to these submaximal doses of exogenous norepinephrine showed dose-related increases and were significantly greater than control in mesenteric arteries from both age groups of SHR. The norepinephrine-evoked responses were antagonized by both phentolamine (10^-6 M) and prazosin (10^-6 M). These results indicate that the response is mediated by postsynaptic α-adrenergic receptor activation.

### Discussion

Noradrenergic hyperresponsiveness, observed in various isolated organs of SHR, could result from greater transmitter release during sympathetic stimulation or alterations in the transmitter reuptake and structural changes. 5,7,25-28 Although some data is available on certain discrete parameters of uptake and release of norepinephrine, it is still not known how extensively these individual neuronal processes contribute to the exaggerated sympathetic activity, often observed during the initiation of primary hypertension. The present study is the first to examine both endogenous norepinephrine release and its reuptake as well as the content of norepinephrine in the same mesenteric artery along with the associated pressor response. This protocol was carried out simultaneously in an intact mesenteric-intestinal loop from the hypertensive and from the normotensive control rat.

The results of the present study indicate that the pressor response observed during 1-minute periaortic nerve stimulation of perfused mesentery was high in both 7- and 13-week-old SHR. This could be due to 1) a greater release of endogenous norepinephrine per nerve pulse, 2) supersensitivity of the postsynaptic α-adrenergic receptor, or 3) a reduced rate of norepinephrine disposition.
A greatly enhanced overflow of endogenous norepinephrine was indeed observed in the tissues from 7-week-old SHR with or without neuronal uptake blockade as compared with the age-matched WKY rats, but the difference in overflow of norepinephrine in the tissues of the 13-week-old rats was not statistically significant (Tables 3 and 4), despite highly elevated contractile force and about 22% higher norepinephrine content in SHR than that obtained in control rats (Table 5). Because of the difference in norepinephrine content between SHR and WKY rats, fractional overflow of norepinephrine was also expressed. Although the extent of enhancement in norepinephrine overflow was slightly reduced from 63.7 to 45.6% with 10 Hz stimulation, the fractional overflow was still significantly higher in the tissues of 7-week-old SHR, and the difference in the tissues of 13-week-old rats was not statistically significant (Table 6).

The results of our present study are in agreement with those of Masuyama et al.\textsuperscript{20} and in line with those reported by Collis et al.,\textsuperscript{15} who found more endogenous norepinephrine release on electrical stimulation from the kidney of young SHR (46 days old) than that of normotensive control rats. By use of tissue in vitro labeled with [3H]norepinephrine, Vanhoutte et al.\textsuperscript{16-18} also found significantly more endogenous norepinephrine release on electrical stimulation from the kidney of young SHR but not in the kidney of adult rats (4–6 months of age) when compared with the normotensive controls whereas Zsoter et al.\textsuperscript{19} observed more tritium overflow from the tail artery of 7–9-week-old SHR as compared with the age-matched WKY control rats.

Our results, however, differ considerably from those of Elkenberg et al.\textsuperscript{8} and Ekas and Lokhandwala,\textsuperscript{9} who reported significantly increased norepinephrine release, measured as total tritium overflow, from the perfused mesenteric vasculature of 14–18-week-old SHR during periaortial nerve stimulation as compared with the WKY control rats. These authors used in vitro prelabeled tissues with [3H]norepinephrine. This discrepancy could easily result from three major differences in the experimental systems: 1) The mesenteric vasculature used by the above authors was cut free from the small intestines (McGregor’s method\textsuperscript{55}), and thus most of mesenteric capillary vessels were eliminated from their preparation. We, on the other hand, used the entire mesenteric-intestinal loop (modified Castellucci’s method\textsuperscript{20}) and kept the mesenteric capillary vessels intact. 2) The perfusion flow rate used by the above authors was 7.5 ml/min, but we used 3.0 ml/min flow rate, which we found to be the optimal rate in tissues of both SHR and WKY rats. 3) We determined norepinephrine overflow by directly measuring endogenous norepinephrine released into the perfusate by means of extraction and assaying by high-pressure liquid chromatography whereas the above authors used the release of total tritium from the tissues prelabeled with [3H]norepinephrine as an indication of transmitter release. Because prelabeling nerve terminals with [3H]norepinephrine in the mesenteric vasculatures may not be the same degree in SHR and WKY rats, it may be erroneous to assume a uniformity of prelabeling and use total release of

### Table 7. Tritiated l-Norepinephrine Uptake in Isolated Perfused Mesenteric Artery After Electrical Stimulation Experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>[3H]-NE uptake/5 min (pmol/g wet wt)</th>
<th>Cocaine (5×10^-3 M) Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>5</td>
<td>26.79±0.34</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>5</td>
<td>30.92±0.41*</td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>5</td>
<td>29.86±0.58†</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>5</td>
<td>29.57±1.13</td>
<td></td>
</tr>
</tbody>
</table>

*Values are given as mean±SEM. Electrical stimulation was at 5 and 10 Hz. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.
†Significant difference between 7- and 13-week-old WKY by one-way analysis of variance.

### Table 8. Tritiated l-Norepinephrine Uptake Without Prior Electrical Stimulation

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>[3H]-NE uptake/5 min (pmol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>5</td>
<td>26.79±0.34</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>5</td>
<td>30.92±0.41*</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>5</td>
<td>29.86±0.58†</td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>5</td>
<td>29.57±1.13</td>
</tr>
</tbody>
</table>

*Values are given as mean±SEM. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.
†Significant difference between 7-week-old WKY and SHR by one-way analysis of variance.

### Table 9. Fractional Tritiated l-Norepinephrine Uptake in Isolated Mesenteric Artery

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (Weeks)</th>
<th>Number</th>
<th>Fractional NE uptake (×10^-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>7</td>
<td>9</td>
<td>27.85±0.75</td>
</tr>
<tr>
<td>SHR</td>
<td>7</td>
<td>10</td>
<td>26.57±2.97</td>
</tr>
<tr>
<td>WKY</td>
<td>13</td>
<td>10</td>
<td>29.01±0.83</td>
</tr>
<tr>
<td>SHR</td>
<td>13</td>
<td>10</td>
<td>24.03±1.04</td>
</tr>
</tbody>
</table>

*Values are given as mean±SEM. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.
†Significant difference between 13-week-old WKY and SHR by one-way analysis of variance.
tritium as an indicator solely of endogenous norepinephrine release. Our results are also different from those reported by Westfall et al., who reported no difference in the evoked fractional release of total tritium measured as a reliable marker for the release of \[^3H\]norepinephrine upon any frequency of field stimulation from portal vein of SHR or WKY rats at 5–6 weeks of age. They also reported a significantly greater release of tritium and developed tension of veins of SHR in response to low (1 or 2 Hz), but not high (5 or 10 Hz), frequencies at 8–10, 16–18, and 28 weeks of age. Westfall and coworkers used the fractional release of total tritium as a marker for the release of \[^3H\]norepinephrine from the prelabeled portal vein with \[^3H\]norepinephrine, assuming a uniformity of prelabeling the tissues of SHR and WKY control rats, but we determined chemically endogenous norepinephrine released into perfusate of mesenteric arteries. Thus, the discrepancies might have been created by the two different experimental designs. Also, Cassis et al. reported that responses to sympathetic nerve stimulation at high (10–15 Hz), but not at low (0.5–3 Hz), frequencies were increased in the perfused caudal artery of adult (16–20-week-old) SHR.

Although several explanations for the discrepancies among the published reports are possible, our present results are consistent with the idea that there is an enhanced release of endogenous norepinephrine in young SHR. A considerable difference has also been reported in sympathetic nerve activity between young and adult SHR. It is enhanced mainly in young SHR, at the time when the rise in blood pressure is particularly pronounced.

Enhanced release can be consequent to a greater number of sympathetic nerve endings or an enhanced presynaptic responsiveness to stimuli in SHR. The results of the present study indicate that norepinephrine content is significantly higher in both age groups of SHR than in the normotensive WKY control rats. The higher absolute concentration in the mesenteric artery of SHR could be due to several factors like more dense innervation, an accelerated biosynthesis of norepinephrine, more norepinephrine per storage vesicle, or efficient reuptake of norepinephrine. It is of interest to note that differences in norepinephrine biosynthetic enzyme, dopamine-\(\beta\)-hydroxylase activity has been observed to be elevated in peripheral blood vessels from 3-week-old SHR. Also, Cassis et al. reported a greater number of nerve axon bundles in the caudal artery of 16–20-week-old SHR as compared with the age-matched WKY rats. In our previous study, we found a greater incorporation of \[^3H\]norepinephrine into isolated storage vesicular fractions of mesenteric arteries of adult SHR as well as those of 4-week-old SHR when compared with the age-matched WKY rats. Consistent with our observations, other investigators also suggested an apparently greater uptake of \[^3H\]norepinephrine into sympathetic nerves within blood vessels from SHR when compared with those from WKY control rats.

The results of norepinephrine overflow in the present study, however, cannot be explained totally by norepinephrine content because the fractional overflow was still significantly higher in tissues from 7-week-old, but not 13-week-old, SHR than in tissues from the age-matched WKY control rats when norepinephrine overflow was expressed on the basis of the content of norepinephrine in the tissue. It is well known that norepinephrine release is inhibited by presynaptic \(\alpha_2\)-receptor activation and facilitated by presynaptic \(\beta\)-receptor. The experimental results with the presynaptic \(\alpha_2\)-receptor of SHR are, however, somewhat conflicting. Westfall et al. showed that the selective \(\alpha_2\)-antagonist yohimbine caused an equal enhancement of the field stimulation-induced release of \[^3H\]norepinephrine from the portal vein of SHR and age-matched WKY rats at 6, 10, and 16 weeks of age, but the ability to enhance the release of \[^3H\]norepinephrine from the veins of 28-week-old SHR was significantly attenuated compared with the veins of normotensive control rats. However, one of us observed that a facilitatory effect of yohimbine was less in the mesenteric artery of young SHR. The \(\beta\)-adrenergic agonist isoproterenol also produced a similar degree of enhancement of the field stimulation-induced release of \[^3H\]norepinephrine from vessels obtained from SHR and WKY rats at all ages.

The cause for this enhanced efflux of norepinephrine cannot be completely determined from the present experiments. However, several papers report that there is an increased sodium permeability in erythrocytes of SHR and that fractional sodium excretion (percent of amount ingested) by SHR was significantly less during weeks 4–7 but did not differ appreciably between SHR and WKY control rats after 8 weeks of age. Additionally, the increased ion turnover that has been reported in vascular tissue of SHR could result in a more labile calcium pool. Moreover, the \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchange system that is present in plasma membranes of many types of cells, including neurons, vascular smooth muscle cells, and renal tubule epithelial cells, mediates an increase in intracellular \(\text{Ca}^{2+}\) concentration in response to a rise in intracellular \(\text{Na}^+\) concentration. The implication is that a high intracellular \(\text{Na}^+\) concentration resulting from an increased sodium permeability in young SHR membrane may cause intracellular \(\text{Ca}^{2+}\) concentration to increase and thereby enhance \(\text{Ca}^{2+}\)-dependent exocytotic release of norepinephrine from nerve terminals in response to electrical stimulation of the mesenteric sympathetic nerves. At later age, as sodium retention in SHR becomes normalized, intracellular \(\text{Ca}^{2+}\) concentration will also be normalized, and thereby norepinephrine release in SHR would become equivalent to that in normotensive control rats.

Because the overflow of endogenous norepinephrine by periarterial stimulation was highly enhanced in young SHR, we tested the possibility that the
excess amounts of norepinephrine could have resulted from decreased reuptake of norepinephrine in young SHR. We have assessed the norepinephrine overflow under a partial inhibition of the neuronal uptake system with cocaine. Blockade of the reuptake increased the overflow in both SHR and WKY control rats, but the net increase of norepinephrine overflow was significantly higher only in the tissues of 7-week-old SHR than that of the age-matched control WKY rats. Moreover, the initial neuronal uptake of \(^{3}H\)norepinephrine was rather significantly increased (15.7\%) in the mesenteric artery of young SHR. It is, therefore, unlikely that the greater transmitter overflow (63.8\% with 10 Hz stimulation) observed in young SHR was due to reduction in neuronal uptake mechanisms. We feel that the alteration in norepinephrine uptake would be a compensatory mechanism for transient increase of norepinephrine caused by an enhanced release of norepinephrine and that this alteration in norepinephrine uptake plays a role in modulating the concentration of norepinephrine in the synaptic cleft of mesenteric artery of young SHR. These results suggest that the percentage of an enhanced release greatly exceeds the extent of an increased uptake of norepinephrine and that an imbalance between release and uptake processes would result in more endogenous norepinephrine overflow in the arteries of young SHR and would, in turn, cause a greater constriction response as compared with the normotensive control rats. This gross enhancement of the exocytotic process may indeed be one of the major factors causing neurogenic hypertension.

Although there were no significant differences either in norepinephrine overflow or in its uptake between the hypertensive and normotensive animals at 13 weeks of age, the periartrial nerve stimulation with 10 Hz frequency caused significantly higher pressor response in the SHR. Because pressor response as well as overflow of endogenous norepinephrine were completely abolished by addition of guanethidine (\(2 \times 10^{-6} \) M) to the perfusion fluid, the periartrial electrical stimulation in these experiments must be activating sympathetic nerves. The present findings indicate that submaximal doses of exogenous norepinephrine evoked significantly greater response amplitudes in mesenteric arteries from both age groups of SHR (Figure 2); the norepinephrine dose–response curves exhibited a simple leftward shift in the tissues of 7-week-old SHR and much steeper slope in those of 13-week-old hypertensive rats as compared with those of WKY control rats. An increase in vascular reactivity can be caused by smooth muscle supersensitivity, though by a structural vascular change, or by both factors. A parallel leftward shift of the norepinephrine dose–response curve in the tissues of 7-week-old SHR reflect pure supersensitivity whereas an increased norepinephrine dose–response curve slope and maximal response in the older animal tissues appear to represent a combined effect of smooth muscle super-sensitivity and structural alteration that is due to medial hypertrophy, as others have reported. Because the pressor response was markedly antagonized by both phentolamine and prazosin, the vascular supersensitivity to norepinephrine is clearly mediated by postsynaptic \(\alpha\)-adrenergic receptor activation. Although the exact mechanism for this hypervascular reactivity is at present not known, it is possible that in the vascular smooth muscle of SHR there is a membrane alteration either in density of norepinephrine receptor or in the excitation-contraction coupling system.

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References


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T Hano and J Rho

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