Sensitivity of Caudal Arteries and the Mesenteric Vascular Bed to Norepinephrine in DOCA-Salt Hypertension

PENEOLE A. LONGHURST, PETER J. RICE, DAVID A. TAYLOR, AND WILLIAM W. FLEMING

SUMMARY This study was undertaken to determine what factors might contribute to arterial supersensitivity to norepinephrine associated with deoxycorticosterone acetate (DOCA)-salt hypertension in the rat. Experimental groups of male rats were uninephrectomized and 1 week later began receiving twice weekly injections of DOCA (20 mg/kg s.c. in sesame oil) plus 1% NaCl and 0.2% KCl in their drinking water. For each experimental group, a group of age-matched male rats underwent a sham operation and received injections of sesame oil and the NaCl-KCl drinking water. Perfused caudal arteries from 3-week-hypertensive rats were supersensitive to intraluminal and extraluminal norepinephrine administration. However, this difference in sensitivity between hypertensive and control caudal arteries was demonstrable at low rates of perfusion, 0.5 to 1.0 ml/min, but not at rates of 2.0 to 2.6 ml/min. The supersensitivity was not due to differences in neuronal uptake or to inhibition of extraneuronal uptake by DOCA. The perfused mesenteric vascular bed from 3- or 6-week-hypertensive rats was also supersensitive to intraluminal norepinephrine. However, the demonstration of supersensitivity in the mesenteric vasculature was independent of perfusion rate (2.3–6.8 ml/min) and perfusion pressure in the range of 30 to 60 mm Hg. There was little or no supersensitivity to transmural nerve stimulation in either the caudal artery or the mesenteric vasculature, a finding consistent with the observed decrease in endogenous norepinephrine content. Microelectrodes were used to determine resting membrane potential in the smooth muscle cells. No differences in resting membrane potential were detected between caudal or mesenteric arteries from hypertensive compared with control rats 2, 3, or 6 weeks after initiation of the DOCA-salt regimen. It is concluded that 1) the perfusion rate is a critical factor in designing experiments to test the sensitivity of caudal arteries to drugs, 2) the perfused mesenteric vascular bed is a useful preparation for studying sensitivity of blood vessels in hypertension, 3) the supersensitivity of blood vessels in the DOCA-salt model may be of greater importance relative to circulating catecholamines than to sympathetic innervation, and 4) the supersensitivity of blood vessels to norepinephrine in the DOCA-salt model is not due to changes in neuronal uptake, extraneuronal uptake, or membrane potential of the vascular smooth muscle cells. (Hypertension 12: 133–142, 1988)

KEY WORDS • DOCA-salt hypertension • vascular sensitivity • norepinephrine • arteries

The literature on sensitivity of blood vessels to norepinephrine and other agonists is extensive and often conflicting. For example, Hansen and Bohr1 reported a shift to the left and a depression of maximum of the dose-response curve for epinephrine in femoral artery strips from 4- to 5-week-hypertensive deoxycorticosterone acetate (DOCA)-salt rats. In contrast, Hermsmeyer et al.2 found no difference in sensitivity or maximum response to norepinephrine in caudal artery strips from control and 6-week-hypertensive DOCA-salt rats. A number of factors may contribute to such discrepancies, including the model of hypertension chosen, the artery chosen, the duration of hypertension, differences in neuronal or extraneuronal uptake of catecholamines, and the experimental conditions under which sensitivity is measured. In the course of a preliminary investigation of perfused
intravascular preparations from DOCA-salt hypertensive rats to norepinephrine.

An endogenous inhibitor of the Na⁺-K⁺ pump has been proposed to contribute to hypertension of the volume expansion type.²⁻⁵ It has also been demonstrated that rubidium uptake of isolated blood vessels and Na⁺,K⁺-adenosine triphosphatase (ATPase) activity of ventricles are depressed in several animal models of hypertension, including DOCA-salt.⁶ Evidence indicates depression of Na⁺-K⁺ pump activity in leukocytes of patients with "uncomplicated" essential hypertension.⁶ Such evidence of reduced Na⁺-K⁺ pumping in vitro suggests that any endogenous inhibitor of the pump binds tenaciously to the pump or that, in addition to such an endogenous inhibitor, there is an innate deficiency of the pump in certain types of hypertension.

One consequence of depressed Na⁺-K⁺ pump activity can be a decrease in electrogenic pumping, its contribution to resting membrane potential, and a partial depolarization.⁷ Indeed, in the smooth muscle of the guinea pig vas deferens and the saphenous artery of the rabbit, a clear association has been established among the loss of electrogenic Na⁺-K⁺ pumping, membrane potential, and supersensitivity to agonists.⁸⁻¹³ Therefore, a second objective of this work was to determine if resting membrane potential is altered in arteries from DOCA-salt hypertensive rats. Preliminary results of these studies have been published.¹²,¹³

Materials and Methods

Procedures for Inducing Hypertension

Male Sprague-Dawley rats (Hilltop Laboratory Animals, Scottsdale, PA, USA), weighing approximately 150 g, were anesthetized with Innovar, 0.1 ml/100 g s.c. (0.25 mg droperidol and 5 g fentanyl/100 g). An uninephrectomy was performed on the left side of rats in the experimental group. Simultaneously, a group of rats, matched in age, weight, and time of receipt from the supplier, received a sham operation in which the left kidney was exposed but not removed. One week after operation, the experimental rats began to receive DOC A, 20 mg/kg s.c. in sesame oil twice weekly. Sham-operated rats received vehicle injections on the same schedule. Also beginning 1 week after operation, all rats received drinking water containing 1% NaCl and 0.2% KCl.

Beginning 1 week before operation, all rats were weighed and their blood pressure measured at weekly intervals until they were killed to remove tissues. Blood pressure was measured by means of a Narco Bio-Systems PE-300 programmed electrosphygmomanometer (Houston, TX, USA).

Rats were killed by a blow on the head followed by decapitation, and arteries or other organs (or both) were removed for further study 2, 3, or 6 weeks after the initiation of treatment with DOCA (or vehicle) and drinking water containing NaCl/KCl.

Perfusion of Caudal Arteries

Segments of the proximal portion of the caudal artery (1.5 cm long) were removed, cleaned, and cannulated at each end as described by Nicholas¹⁴ and by Venning and de la Lande.¹⁵ The arterial segments were perfused intraluminally from a reservoir by means of a peristaltic pump. The perfusion fluid was Krebs solution bubbled with 95% O₂, 5% CO₂ and maintained at 37 °C. Composition of the solution, in mM, was NaCl, 113; KCl, 4.7; MgSO₄, 1.2; CaCl₂, 2.5; KH₂PO₄, 1.2; NaHCO₃, 25; and glucose, 5.6. Perfusion pressure was monitored at a point between the pump and the artery by means of a transducer and Grass polygraph (Quincy, MA, USA). The artery was immersed in a water-jacketed bath filled with Krebs solution, bubbled with 95% O₂, 5% CO₂ at 37 °C, and passed through a pair of ring electrodes to allow transmural nerve stimulation. Drugs were administered intraluminally through a T-junction between the transducer and the artery or extraluminally by addition to the bathing medium.

Perfusion of the Mesenteric Vascular Bed

A midline incision was made after the rats were killed, and the superior mesenteric artery was cannulated with PE-90 tubing near its origin at the aorta. The entire intestine with attached mesentery was removed and placed in Krebs solution. Krebs solution was used to flush the intestine, and the entire intestine-mesentery preparation was placed in a water-jacketed 50-ml organ bath. Through the cannulated mesenteric artery, the preparation was perfused with Krebs solution, which was aerated with 95% O₂, 5% CO₂ and maintained at 37 °C. The perfusion fluid was allowed to flow out of the severed mesenteric veins, bathe the tissues, and leave the bath by overflowing. A ring electrode around the mesenteric artery and a second electrode in the bath allowed for transmural stimulation of the nerves to the vasculature. A Grass Model S9 stimulator delivered 1-msec pulses at a voltage determined to be supramaximal in each experiment. The system for delivering the perfusion fluid, measuring perfusion pressure, and administering intraluminal norepinephrine was similar to that described for the caudal artery. This perfused mesenteric vascular preparation is similar to that described by Castellucci et al.¹⁶ and by Longhurst and Head.¹⁷

Dose-Response and Concentration-Response Curves

Intraluminal administration of norepinephrine was by bolus injections, and doses are expressed in
nanograms or micrograms. Dose-response curves were developed by twofold increments in dose, each dose delivered only after complete recovery from the preceding dose. Extraluminal administration of drugs in the caudal artery was accomplished by adding the drug to the bath. Concentration-response curves were obtained by twofold steps in a cumulative fashion, each increase in concentration being added when the preceding response had reached a steady state.

The effects of cocaine or DOCA (in vitro) on the sensitivity to norepinephrine were tested in some experiments with caudal arteries. This was done by obtaining dose-response (intraluminal) or concentration-response (extraluminal) curves with norepinephrine in the absence of uptake inhibitors. Cocaine (to inhibit neuronal uptake) or DOCA (to inhibit extraneuronal uptake) was then added to the bathing medium, and the dose-response or concentration-response curve with norepinephrine was repeated.

Determination of Endogenous Norepinephrine

Norepinephrine concentrations in vasa deferentia, ventricles, kidneys, spleens, and caudal arteries were measured using an adaptation of the high performance liquid chromatography method of Kisinger et al., as modified by Stitzel et al. Tissues were frozen in perchloric acid (400 mM) before analysis. Tissues were homogenized in an all-glass homogenizer. Known concentrations of the internal standard, dihydroxybenzylamine, were added to the homogenate. The homogenates were centrifuged, and to a 1-ml aliquot of the supernatant, the following were added: 140 mg of alumina and 0.2 ml of sodium metabisulfite (2.5%) containing EDTA (5%) and Tris buffer (3 M, pH 9.6), to give a pH of 8.4. The tubes were shaken for 10 minutes, the supernatant was discarded and the alumina washed with 0.03 M phosphate buffer and deionized water. The catecholamines were eluted in two fractions from the alumina by agitation for 10 minutes with 300 μl of 1 M acetic acid containing 0.01 M boric acid. The two eluates were pooled and evaporated to dryness using a Buchler vortex evaporator (Fort Lee, NJ, USA), and the residue was redisissolved in 250 μl of 0.05 M acetic acid. The catecholamine content of samples was determined by high performance liquid chromatography in conjunction with electrochemical detection. Chromatography was performed using a Model M45 pump, a WISP Model 710B automatic sample injector, and a Bondapak C8 column (10-μm particle size range, inside diameter, 30 cm × 3.9 mm; all from Millipore Waters Associates, Milford, MA, USA). The signal was recorded on a Waters Model 720 data module, and peak areas were determined. The mobile phase was 2.5% methanol in water containing 0.1 M Na2HPO4 heptahydrate, 0.1 M citric acid, 0.27 mM Na2 EDTA, and 0.34 mM sodium octyl sulfate at a flow rate of 1 ml/min. The detector was a Model LC 4A electrochemical detector, with a glassy carbon electrode (Bioanalytical Systems, West Lafayette, IN, USA). The potential was maintained at +0.74 V against a silver-silver chloride reference electrode. Each sample was corrected for loss of catecholamines during isolation by the use of the internal standard.

Electrophysiology

Caudal or mesenteric arteries were removed, pinned out as tubes in a chamber, and superfused with Krebs solution bubbled with 95% O2, 5% CO2 maintained at 37 °C. In each experiment, an artery from a control animal was pinned in the bath with an artery from a hypertensive animal, allowing alternating measurements from control and experimental preparations. Impalements were made from the adventitial surface using microelectrodes filled with 3 M KCl. Membrane potentials were viewed on a Tektronix Model 5110 oscilloscope (Beaverton, OR, USA) fed by a WPI model M-707 microprobe system (New Haven, CT, USA) and recorded on a Brush recorder (Gould, Cleveland, OH, USA). The methods employed have been used in this laboratory for some time.6-11

Calculations and Statistics

Body weight, blood pressure, norepinephrine content of tissues, membrane potential, perfusion pressure, and changes in perfusion pressure are given as arithmetic means. Doses producing an increase in perfusion pressures of 50 mm Hg were calculated by linear regression from individual dose-response curves. These values are termed ED50 mm Hg. Such values are log-normally distributed, not arithmetically normally distributed, as shown by Fleming et al.20 Thus, mean logs were determined. Statistical analyses were performed on the mean logs. The mean logs were then converted to antilogs to provide the geometric mean ED50 mm Hg with 95% confidence interval as described by Fleming et al.20 Statistical tests included analysis of variance followed by Student–Newman Keuls test or Student’s t test as appropriate. A p value of 0.05 was accepted as the level of significance.

Results

Figure 1 presents the weight gain and blood pressure changes with time in a typical group of control and DOCA-salt rats. The uniphrectomy or sham operation on each animal was done at −1 week. DOCA or oil injections and addition of NaCl and KCl to the drinking water began at Time 0. As shown in Figure 1, weight gain of the control and treated groups in all experiments remained similar from −2 weeks until about 3 weeks, at which time the experimental animals began to lag behind. A significant difference in blood pressure was always present by the end of 2 weeks and sometimes by the end of 1 week and was maintained through 6 weeks, the longest period studied. All blood vessels or
vascular beds were removed at either 3 or 6 weeks after Time 0, with the exception of a few mesenteric arteries removed at 2 weeks for electrophysiology.

With the perfused caudal artery, the relative sensitivity to norepinephrine in arteries from hypertensive compared with control animals depended on the infusion rate. Figure 2 demonstrates this relationship for intraluminal bolus injections of norepinephrine. With a perfusion rate of 0.5 ml/min, the norepinephrine dose-response curve was shifted to the left in the caudal arteries from 3-week-hypertensive rats. As the flow rate was increased, the difference in sensitivity between the two groups of arteries diminished until there was no difference at a flow rate of 2.6 ml/min.

This inverse relationship between difference in sensitivity and flow rate is also indicated by the ratio of equieffective intraluminal doses obtained with different perfusion rates (Table 1), decreasing from 3.8 at the lowest rate to 1.0 at the highest rate. The sensitivity of both the experimental and control caudal arteries, as indicated by the geometric means of doses producing an increase in perfusion pressure of 50 mm Hg, increased as flow rate increased. However, this trend was much more marked in the control arteries, such that they were as sensitive to norepinephrine as the experimental arteries were at the highest perfusion rate.

A very similar pattern was observed with extraluminal norepinephrine administration (see Table 1). Again, a difference in sensitivity between caudal arteries from control and 3-week-hypertensive animals tended to decline as perfusion rate increased. However, the supersensitivity to norepinephrine was less marked with extraluminal administration, and a significant difference at the level of the \( ED_{50} \) occurred only at the 0.5 ml/min perfusion rate. Table 1 also demonstrates that basal perfusion pressure (i.e., the pressure before administration of drugs) increased as perfusion rate increased to 2.0 ml/min. This trend was more marked and consistent in the hypertensive group. An additional increase in perfusion rate to 2.6 ml/min did not cause any further increase in basal perfusion pressure.

Experiments were performed in arteries with responses to transmural nerve stimulation. Mean frequency-response curves in caudal arteries from control animals were virtually superimposed on mean curves obtained with arteries from 3-week-hypertensive animals. Figure 3 (left panel) illustrates this at a perfusion pressure of 1.0 ml/min. Thus, there was no indication of supersensitivity of arteries from hypertensive rats to nerve stimulation. This finding pertains to all four perfusion rates.

No evidence was found for changes in neuronal uptake contributing to differences in sensitivity between arteries from control and hypertensive rats. Cocaine (10^{-3} M) produced small shifts to the left in the dose-response curves obtained with intraluminal norepinephrine and larger shifts in the concentration-response curves obtained with extraluminal norepinephrine. However, the potentiation...
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of norepinephrine by cocaine was virtually identical in four control caudal arteries (intraluminal, 3.1-fold; extraluminal, 9.3-fold) compared with six arteries from 3-week-hypertensive rats (intraluminal, 3.5-fold; extraluminal, 8.9-fold). Cocaine also produced equal shifts to the left in frequency-response curves generated by nerve stimulation in caudal arteries from hypertensive (5.3-fold) and control animals (5.5-fold).

The hypertensive rats received injections of DOCA, and the controls did not. This difference raises the possibility that, by inhibiting extraneuronal uptake, DOCA might be contributing directly to the supersensitivity of caudal arteries from the hypertensive rats. However, short-term exposure of five control arteries to DOCA (3 × 10⁻⁵ M), a concentration adequate to inhibit extraneuronal uptake,21 had no effect on responses to intraluminal norepinephrine (see Figure 3, right panel).

The sensitivity of the isolated mesenteric vascular bed to norepinephrine was also examined. Figure 4 presents dose-response curves to intraluminal norepinephrine in mesenteric vasculature from control and 3-week-hypertensive rats. There was a significant shift to the left of the mean dose-response curve at each of the three perfusion rates used in the vasculature from hypertensive rats. The nature of the perfused mesenteric bed preparation does not lend itself to experiments with extraluminal norepinephrine.

There was little sign of supersensitivity to transmural electrical stimulation in the mesenteric vasculature from 3-week-hypertensive animals (Figure 5). There was a significantly greater response at one frequency (8 Hz) in vessels perfused at 4.5 ml/min and at two frequencies of stimulation (4 and 8 Hz) in vessels perfused at 6.8 ml/min. However, the frequency-response curves clearly showed little overall difference between preparations from control and hypertensive animals at any perfusion rate.

Very similar results were obtained with mesenteric preparations from 6-week-hypertensive rats and matched controls (Figure 6). The vasculature from the hypertensive rats was supersensitive to intraluminal norepinephrine at all three perfusion rates. The magnitude of supersensitivity was similar in the 3- and 6-week-hypertensive groups, and in both it was not affected by perfusion rates between 2.2 and 6.8 ml/min. Also at 6 weeks, the vasculature of hypertensive rats provided only marginal indications of supersensitivity to transmural nerve stimulation (Figure 7).

### Table 1. Sensitivity and Basal Perfusion Pressure of Perfused Caudal Arteries from Control and Age-Matched 3-Week-Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraluminal NE (ng)</td>
<td>Control (n = 7)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6</td>
</tr>
<tr>
<td>EP max Hg (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose ratio (mg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraluminal NE (μg/ml)</td>
<td>Control (n = 7)</td>
<td>0.44 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Hypertensive (n = 10)</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>EP max Hg (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose ratio (mg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal perfusion pressure (mm Hg ± SE)</td>
<td>Control (n = 7)</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Hypertensive (n = 10)</td>
<td>4.9 ± 1.3*</td>
</tr>
</tbody>
</table>

Data were obtained in same experiments and arteries as in Figure 2. EP max Hg = doses producing a 50 mm Hg increase in perfusion pressure; NE = norepinephrine; CI = confidence interval.

*p < 0.05, compared with control values.

### Figure 3. Left panel: Frequency-response curves obtained with transmural nerve stimulation in perfused caudal arteries from six 3-week-hypertensive DOCA-salt rats and five age-matched control rats. The perfusion rate was 1.0 ml/min. Open circles are mean control values, and closed circles are mean values obtained in arteries from hypertensive rats. Right panel: Dose-response curves to intraluminal norepinephrine in perfused caudal arteries (1.0 ml/min) from five control rats before (open circles) and after (closed circles) the addition of DOCA (3 × 10⁻⁵ M) to the bathing medium. In both panels, vertical bars represent SEM. There were no significant differences at any frequency or any dose.

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**NOREPINEPHRINE SENSITIVITY IN DOCA-SALT HYPERTENSION/Longhurst et al.**
FIGURE 4. Dose-response curves to intraluminal norepinephrine in perfused mesenteric vascular beds from eight 3-week-hypertensive DOCA-salt rats and six age-matched control rats. A dose-response curve was determined in each preparation at each of three perfusion rates, indicated at the top of the figure. Open circles are mean control values, and closed circles are mean values obtained in arteries from hypertensive rats. Vertical bars represent SEM. Asterisks indicate mean responses that are significantly different (p < 0.05) from control responses at a given dose and perfusion rate.

FIGURE 6. Dose-response curves to intraluminal norepinephrine in perfused mesenteric vascular beds from eight 6-week-hypertensive DOCA-salt rats and six age-matched control rats. A dose-response curve was determined in each preparation at each of three perfusion rates, indicated at the top of the figure. Open circles are mean control values, and closed circles are mean values obtained in arteries from hypertensive rats. Vertical bars represent SEM. Asterisks indicate mean responses that were significantly different (p < 0.05) from control responses at a given dose and perfusion rate.

FIGURE 5. Frequency-response curves obtained with transmural nerve stimulation in perfused mesenteric vascular beds from eight 3-week-hypertensive DOCA-salt rats and six age-matched controls. A frequency-response curve was obtained in each preparation at each of three perfusion rates, indicated at the top of the figure. Open circles are mean control values, and closed circles are mean values obtained in arteries from hypertensive rats. Vertical bars represent SEM. Asterisks indicate mean responses that were significantly different (p < 0.05) from control responses at a given frequency and perfusion rate.

FIGURE 7. Frequency-response curves obtained with transmural nerve stimulation in perfused mesenteric vascular beds from eight 6-week-hypertensive DOCA-salt rats and six age-matched controls. A frequency-response curve was obtained in each preparation at each of three perfusion rates, indicated at the top of the figure. Open circles are mean control values, and closed circles are mean values obtained in arteries from hypertensive rats. Vertical bars represent SEM. Asterisk indicates mean response that was significantly different (p < 0.05) from control responses at a given frequency and perfusion rate.
TABLE 2. Basal Perfusion Pressure in Isolated Mesenteric Vasculature at Different Rates of Perfusion

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>2.3</th>
<th>4.5</th>
<th>6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>27.9±1.3</td>
<td>32.4±1.6</td>
<td>37.7±1.0</td>
</tr>
<tr>
<td>Hypertensive (n=8)</td>
<td>28.8±1.7</td>
<td>36.8±2.0</td>
<td>44.0±2.5*</td>
</tr>
<tr>
<td>Ratio</td>
<td>1.03</td>
<td>1.14</td>
<td>1.17</td>
</tr>
<tr>
<td>6-week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>43.6±5.8</td>
<td>41.3±6.5</td>
<td>51.0±7.3</td>
</tr>
<tr>
<td>Hypertensive (n=8)</td>
<td>40.0±7.2</td>
<td>52.0±9.2</td>
<td>59.6±8.3</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.91</td>
<td>1.3</td>
<td>1.17</td>
</tr>
</tbody>
</table>

Values represent mean basal perfusion pressure (mm Hg). *p < 0.05, compared with control value.

In the perfused mesenteric vascular bed, increasing the perfusion pressure between 2.3 and 6.8 ml/min increased basal perfusion pressure in preparations from both control and hypertensive rats (Table 2). In contrast to the results in caudal arteries (see Table 1), there was little difference in basal perfusion pressure between control and experimental preparations. There was a significant difference only at the highest perfusion rate in the 3-week-hypertensive group.

Endogenous levels of norepinephrine were lower in several tissues from the hypertensive animals relative to control (Table 3). This trend was not found in the vas deferens. However in spleen, ventricle, and kidney, the norepinephrine content was significantly lower by 30 to 50% in 6-week-hypertensive animals than in matched controls. In the caudal artery from hypertensive rats, norepinephrine content was 25% lower than in controls, but this difference was not significant. The endogenous levels of norepinephrine were not measured in the mesenteric artery because the mesenteric vasculature from this set of animals was used for dose-response experiments.

The development of hypertension was not associated with changes in resting membrane potential in either the caudal artery or the mesenteric artery (Table 4). In the caudal artery the comparison was made between 3-week-hypertensive rats and matched controls. In the mesenteric artery comparisons were made at 2, 3, and 6 weeks. In no instance was there a significant difference in resting membrane potential between arteries from control and hypertensive rats.

**TABLE 3. Norepinephrine Content of Tissues from Control and 6-Week-Hypertensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Vas deferens</th>
<th>Spleen</th>
<th>Ventricle</th>
<th>Kidney</th>
<th>Caudal artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.1±0.8</td>
<td>0.67±0.07</td>
<td>0.70±0.05</td>
<td>0.11±0.01</td>
<td>6.3±0.9</td>
</tr>
<tr>
<td>DOCA-salt</td>
<td>19.9±0.73</td>
<td>0.45±0.06*</td>
<td>0.35±0.03*</td>
<td>0.05±0.01*</td>
<td>4.6±0.76</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E. from eight to 15 tissues, each from separate animals. *p < 0.05, compared with control values.

**Discussion**

The results of the present study demonstrate that the determination of a difference in sensitivity to norepinephrine between perfused caudal arteries from DOCA-salt hypertensive rats and matched controls is highly dependent on the conditions of perfusion. At a very low rate, 0.5 ml/min, the caudal arteries from 3-week-hypertensive rats were more sensitive to either intraluminal or extraluminal norepinephrine than were those from controls. However, as the flow rate was increased, this difference gradually decreased, and at a perfusion rate of 2.6 ml/min, the dose-response curves for norepinephrine were virtually identical.

Venning and de la Lande,22 in a study of DOCA-salt rats, reported that caudal arteries from the hypertensive rats were more sensitive to norepinephrine compared with control arteries. They used a perfusion rate of 2 ml/min. Our data indicate that flow rate influences the demonstration of a difference in sensitivity between control and experimental vessels. At any given flow rate, the presence or lack of a statistical difference will depend on the number of experiments and, possibly, other experimental factors, such as the severity of hypertension and the types of controls. Thus, the findings of Venning and de la Lande22 do not necessarily conflict with our data generated with several different perfusion rates while all other experimental conditions were held constant.

The sensitivity, as measured by geometric mean values of doses required to increase the perfusion pressure by 50 mm Hg, increased as flow rate increased in caudal arteries from both control and hypertensive rats.
hypertensive rats. However, the relationship between perfusion rate and sensitivity was much steeper in the control arteries. For example, for intraluminal norepinephrine, the sensitivity of control arteries increased sevenfold as the perfusion rate was raised from 0.5 to 2.6 ml/min. In the arteries from hypertensive rats, the increase in sensitivity was only twofold.

These changes are not related in any obvious way to basal perfusion pressure. At any perfusion rate, the basal perfusion pressure was greater in the caudal arteries from hypertensive rats. There was a general trend for basal perfusion pressure to increase as perfusion rate increased. This relationship was more clearly defined for the arteries from hypertensive animals. Note, in particular, that control arteries were four times more sensitive to intraluminal norepinephrine at a perfusion rate of 2.6 ml/min than at a perfusion rate of 1.0 ml/min. However, the basal perfusion pressures in control arteries at these two perfusion rates were nearly identical.

Sensitivity of isolated caudal arteries and mesenteric resistance vessels increases with increases in preload.23'24 Our finding of increasing sensitivity of caudal arteries to norepinephrine with increasing flow rate is consistent with those results. However, acceptance of increasing preload as an explanation of the effect of flow rate is tempered by the lack of relationship between basal perfusion pressure and sensitivity.

Although there is no obvious explanation for this relationship between perfusion rate and sensitivity in the caudal artery, it may explain some discrepancies in the literature. Hermmsmeyer25 reported that caudal artery strips from spontaneously hypertensive rats (SHR) were supersensitive to norepinephrine, while Cassis et al.26 found no difference in sensitivity between perfused caudal arteries from SHR and control rats. The perfusion rate used in the latter study was 2.0 ml/min. In the present study using DOCA-salt rats, there was no difference in sensitivity to norepinephrine between hypertensive and control caudal arteries at that perfusion rate. The experimental procedures of Cassis et al.26 were very similar to ours.

Frequency-response curves to supramaximal voltage transmural nerve stimulation were also determined in caudal arteries. Regardless of the perfusion rate, frequency-response curves obtained with arteries from hypertensive rats were virtually superimposed on curves obtained with control arteries. The absence of supersensitivity to nerve stimulation under conditions in which there is supersensitivity to norepinephrine may be due to depletion of norepinephrine. The norepinephrine content of heart, kidney, and spleen was significantly decreased in 6-week hypertensive DOCA-salt rats. The norepinephrine content of the caudal arteries was 25% less than control. Although this difference was not significant, depletion of norepinephrine in arteries of DOCA-salt hypertensive rats has been reported by others.27

The nerves to larger arteries are generally located in the adventitial layer and do not penetrate into the media. For this reason, neuronal uptake plays a more important role in regulating norepinephrine concentration in the biophase when the norepinephrine is applied from the extraluminal surface rather than the intraluminal surface of arteries.28 As a consequence, cocaine potentiates extraluminal norepinephrine much more than intraluminal norepinephrine administration.22'28 The present results demonstrate such a relationship for the caudal artery, cocaine (10^{-5} M) producing a ninefold potentiation of extraluminal norepinephrine and only a threefold potentiation of intraluminal norepinephrine.

Similar to earlier studies of Venning and de la Lande,22 the effects of cocaine were nearly identical in control and hypertensive caudal arteries, indicating that no change in neuronal uptake is associated with DOCA-salt hypertension. In this regard, the DOCA-salt hypertensive rat is quite in contrast to the SHR. In the SHR, there is a hypernoradrenergic innervation of blood vessels26 that results in enhanced neuronal uptake of norepinephrine in the arteries of the hypertensive animals.26'29 In contrast, norepinephrine content is decreased in the DOCA-salt hypertensive rat.27

DOCA is an effective inhibitor of extraneuronal uptake of catecholamines.21 Under some conditions, in some tissues, extraneuronal uptake is great enough to modify the apparent sensitivity of the tissue to catecholamines.29 It is important, therefore, to determine if long-term administration of DOCA to the hypertensive rats could be inducing an apparent supersensitivity to norepinephrine by inhibiting extraneuronal uptake. This possibility is disproven because DOCA, in a concentration of 3 \times 10^{-3} M in the bath, a concentration adequate to inhibit extraneuronal uptake,21 had no effect on the dose-response curve of norepinephrine in control caudal arteries.

The relationship of perfusion rate to sensitivity to norepinephrine and nerve stimulation was also studied in the perfused mesenteric vasculature. This preparation offered advantages over the caudal artery. It presents an entire vascular bed, including small arteries and arterioles, which are more representative of vessels contributing most to total peripheral resistance. With rates of perfusion that overlapped the range used with the caudal artery, basal perfusion pressures were produced in the mesenteric vasculature (30–60 mm Hg) that were much closer to the physiological range.

Mesenteric vascular beds from both 3- and 6-week hypertensive rats were compared with matched controls. There was no difference between the 3- and 6-week groups. In both groups, the vasculature from hypertensive animals was supersensitive to norepinephrine (2.5-fold to fourfold) relative to that of controls. Furthermore, this difference in sensitivity was independent of a perfusion rate between 2.3 and 6.8 ml/min. Although basal perfusion pressure increased as perfusion rate increased, there was, with
one exception, no significant difference between basal perfusion pressures in preparations from hypertensive and control rats.

A decrease in the ratio of internal radius to wall thickness favors constriction of arteries. Thus, a decrease in internal radius or an increase in wall thickness (or both) would explain the increase in sensitivity to norepinephrine in both the caudal arteries and mesenteric vascular bed in the present results. The finding that, in the mesenteric preparation, at any given flow rate, basal perfusion pressures were the same between mesenteric preparations from hypertensive and control rats suggests that the resistance arteries from the hypertensive rats did not differ markedly in internal radius from the control vessels. On the other hand, it is quite possible that the arterial walls of the hypertensive animals were thicker. Thus, the greater sensitivity of the hypertensive arteries to norepinephrine may be due to a physical change in the arterial walls, a cellular change in the effects of norepinephrine, or both.

Similar to the results with the caudal artery, there was little indication of supersensitivity to transmural nerve stimulation at any of the perfusion rates in the mesenteric vasculature. Although there was an occasional significantly greater response in the vasculature from hypertensive animals, the differences were 1) not related to perfusion rate, 2) confined to the lower portion of the frequency–response curve, and 3) small compared with the differences in responses to norepinephrine. As with the caudal artery, the combination of supersensitivity to norepinephrine with little or no supersensitivity to neurally released transmitter is consistent with evidence of depletion of endogenous norepinephrine in mesenteric arteries of DOCA-salt hypertensive rats. The supersensitivity to intraluminally applied norepinephrine without supersensitivity to nerve stimulation draws attention to the potential role of circulating catecholamines and, perhaps, other vasoconstrictors in DOCA-salt hypertension.

There was no indication of a partial depolarization of either caudal or mesenteric arteries. In the caudal artery, membrane potential was compared between arteries from 3-week-hypertensive rats and matched controls. Membrane potential was compared between mesenteric arteries from hypertensive rats and matched controls at 2, 3, and 6 weeks. In no instance was there a difference in mean resting membrane potential between the experimental and the control arteries. Thus, even very early in the development of hypertension, depolarization of the membrane does not appear to contribute to the supersensitivity to norepinephrine. In this regard, our data are in agreement with those of Hermssmeyer et al.,2 who found similar membrane potentials between the smooth muscles of caudal arteries from 6-week-hypertensive DOCA-salt rats and control rats. Thus, we find no electrophysiological evidence for depression of the Na⁺-K⁺ pump in vitro in this model of hypertension.

There is evidence in the literature that Na⁺-K⁺ pump activity of cells is decreased in vitro in models of volume-expanded hypertension, including the DOCA-salt model, and in human essential hypertension. However, recent experiments indicate this may not be so in the DOCA-salt model. Rather, sodium permeability and the activity of the pump may both be increased in arteries of DOCA-salt hypertensive rats. An increase in pump activity would fit with the report of Knox and Sen that aldosterone, a hormone closely related to DOCA, can stimulate the rate of formation of Na⁺,K⁺-ATPase. An increase in sodium permeability would tend to depolarize, and an increase in Na⁺-K⁺ pump activity, through enhanced electrogenic current, would tend to hyperpolarize. The net result could be no significant change in resting membrane potential, as found by Hermssmeyer et al. and in the present report.

In conclusion, the caudal artery and mesenteric vascular bed of the rat are supersensitive to norepinephrine in DOCA-salt hypertension. There is little supersensitivity to nerve stimulation, probably because of the depletion of transmitter from the noradrenergic nerves. The supersensitivity to norepinephrine is not due to changes in neuronal or extraneuronal uptake of catecholamines. The demonstration of hypertension-related supersensitivity in the perfused caudal artery is highly dependent on the rate of perfusion but is not correlated with basal perfusion pressure. The perfused mesenteric vascular bed appears to be a more dependable as well as a more physiologically relevant preparation for studying vascular sensitivity in hypertension. The hypertension and arterial supersensitivity in DOCA-salt hypertension are not associated with changes in membrane potential in the arterial smooth muscle.

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