Sympathectomy Fails to Reveal Prominent Vasodilation by Atrial Natriuretic Factor

R. Wayne Barbee, Lisa M. Harrison-Bernard, Robert S. Zimmerman, Nick C. Trippodo, and Edward D. Frohlich

Reflex activation of the sympathetic nervous system may conceal direct vasodilatory actions of atrial natriuretic factor and mediate atrial natriuretic factor-induced increases in total peripheral resistance. We determined whether peripheral sympathectomy would enhance the hypotensive actions of atrial natriuretic factor and convert the increase in total peripheral resistance to peripheral vasodilation. Sympathectomized rats studied included 1) conscious rats treated with 6-hydroxydopamine alone (partially sympathectomized) and 2) conscious anephric rats sympathectomized with adrenal demedullation and 6-hydroxydopamine (totally sympathectomized), with vascular tone returned to levels of sham-operated (control) rats with norepinephrine infusion. Sympathectomized rats and appropriate control rats received rat atrial natriuretic factor infusion (0.5 μg/kg/min) or vehicle for 1 hour. Atrial natriuretic factor infusion lowered mean arterial pressure and increased hematocrit in control rats but not in partially sympathectomized rats. Changes in cardiac output and total peripheral resistance were not significantly different between control and partially sympathectomized rats. In totally sympathectomized rats, atrial natriuretic factor lowered mean arterial pressure more than in control rats; changes in cardiac output were nearly identical in both groups, but there were no changes in total peripheral resistance from control levels in the totally sympathectomized group. Changes in plasma volume and central venous pressure were similar in totally sympathectomized rats and control rats. These findings suggest that reflex sympathetic activity largely mediated atrial natriuretic factor-induced increases in total peripheral resistance but failed to reveal an atrial natriuretic factor-mediated sustained vasodilation in the absence of sympathetic reflexes. Furthermore, atrial natriuretic factor decreased cardiac output, central venous pressure, and plasma volume independent of the sympathetic nervous system. (Hypertension 1990;15:888–893)

Atrial natriuretic factor (ANF) reduces arterial blood pressure in a variety of animal species. However, the mechanism of this hypotensive effect remains unclear. Early in vitro studies demonstrated relaxation of vessels precontracted with a variety of substances; these findings suggested arteriolar vasodilation in intact animals. Although bolus administration of this peptide generally causes a transient decrease in vascular resistance, acute infusions normally lower blood pressure primarily by reducing cardiac output. Total peripheral resistance may decrease, remain constant, or actually increase. The increased vascular resistance seen with ANF may be due to reflex effects. However, studies addressing this possibility have yielded equivocal results. Various manipulations of the sympathetic nervous system have been shown to partly blunt, prevent, or reverse ANF-induced increases in vascular resistance.

We wished to test the hypothesis that sympathectomy would unmask a prominent vasodilatory action of ANF and questioned whether reductions in cardiac output and plasma volume were partly mediated by reflex activation of the sympathetic nervous system. The effect of ANF on systemic hemodynamics was assessed in chemically sympathectomized rats with or without adrenal demedullation and control of vascular tone.

Methods

Animals

Male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Indiana), weighing 300–400 g, were kept in a temperature-controlled room with a 12-hour light/dark cycle, with food and water available ad
libitum. Rats were housed in groups until instrumentation, after which they were housed individually in a laminar flow unit. All experiments were approved by the Institutional Animal Care and Use Committee.

Doppler Flow Measurement

Pulsed ultrasonic Doppler velocity transducers (10 MHz) were constructed by using modifications of the technique reported by Haywood et al. Transducers were implanted around the ascending aorta with modifications of the surgery described previously for continuous wave Doppler transducers.

Experimental Protocols

Protocol 1. Approximately 2 weeks after Doppler transducer implantation, rats were anesthetized with ether. Polyvinyl catheters (SV-31, Critchley Electrical Products, Auburn, New South Wales, Australia) were placed in the left femoral artery for blood sampling and measurement of mean arterial pressure and in the left femoral vein for infusions. Rats were pretreated with phentolamine (2.5 mg/kg i.v.) and then injected with 100 mg/kg i.v. 6-hydroxydopamine (6-OHDA) to destroy sympathetic nerve endings. Control rats received an equal volume of vehicle (1% ascorbic acid in saline). Catheters were routed subcutaneously, exteriorized at the back of the neck, and placed in a protective plastic container sutured to the skin.

The following day, rats were placed in clear ventilated Plexiglas boxes (25×15×11 cm³) with bedding and were allowed 1 hour for acclimatization to the environment. Changes in cardiac output in the conscious rat were assessed after connecting the transducer wires to a custom-built directional pulsed Doppler velocity meter (Instrumentation Development Laboratories, Baylor College of Medicine, Houston, Texas). The phasic output of the Doppler meter was connected to a polygraph (model 79D, Grass Instrument Co., Quincy, Massachusetts), and the range gate was set to obtain a maximal Doppler shift, free of artifact with the end-diastolic signal equal or close to the electronic zero. Because the Doppler system does not measure absolute flow, changes in cardiac output were assessed by recording changes in mean Doppler shift with the control period set at 100%. Total peripheral resistance was calculated by dividing mean arterial pressure by the corresponding shift (in kilohertz) and was also expressed as a percent of the control value. The arterial catheter was connected to a disposable transducer (COBE Laboratories, Arvada, Colorado), and mean arterial pressure was recorded continuously along with mean Doppler shift on the Grass recorder. The phasic arterial pressure or Doppler signal was processed by an amplifier (Grass Tachograph) to record heart rate. To assess the completeness of sympathectomy, the rats were tested for a pressor response to tyramine (250 μg/kg i.v.). Forty minutes after the tyramine test, 15 minutes of control data were obtained. After control measurements, rat ANF-(99–126) (Peninsula Laboratories, Inc., Belmont, California) was infused continuously at a dose of 0.5 μg/kg/min. Variables were recorded continuously, tabulated each minute, and averaged for each 15-minute period. Approximately 50 μl blood was removed for hematocrit determinations at 15, 30, and 60 minutes of the infusion period. A separate group of rats received vehicle (0.9% saline, 15–20 μl/min i.v.) infusion alone.

Protocol 2. Rats weighing approximately 200 g were anesthetized with ether, and a midline abdominal incision was made. Demedullation was accomplished by isolating the adrenal glands, making a horizontal incision into the cortex with a No. 11 scalpel blade, and then carefully extruding the medulla by squeezing the cortex with silastic-tipped forceps. Control rats received sham surgery. The spleen was also removed at this time in all rats to avoid an effect of splenic contraction or dilation on blood volume. Two to 3 weeks later, Doppler transducers were implanted as described earlier. Two weeks after thoracotomy, rats were anesthetized with ether and injected with phentolamine and 6-OHDA through a tail vein as in protocol 1. The next day, the rats were again anesthetized with ether, and a bilateral nephrectomy protocol 1. The next day, the rats were again anesthetized with ether, and a bilateral nephrectomy performed to obviate any effect of the kidneys on ANF-induced changes in plasma volume. Catheters were placed as described above, with the addition of a PE-50 catheter for determination of central venous pressure placed just cephalad to the level of the diaphragm. All rats were allowed at least 3 hours to recover from ether anesthesia before beginning experiments. Erythrocytes labeled with 51Cr were injected for the measurement of blood volume as described previously. Plasma volume was calculated by the formula: [(1–(hematocrit/100))]×blood volume. Sympathectomy was confirmed with tyramine as described previously. Because resting blood pressure was lower in medullectomized and sympathectomized (MSX) rats, arterial vascular tone was returned to levels of control rats in these experiments by means of an infusion of norepinephrine (0.1–1.0 μg/kg/min). After a 15-minute control period (40 minutes after tyramine injection), rat ANF or vehicle was infused in MSX and control rats as described in protocol 1, except that the infusion rate was 5–10 μl/min. The norepinephrine infusion rate was not adjusted after rat ANF or vehicle infusion began.

Statistical Analysis

Data are expressed as mean±SEM. Hemodynamic and body fluid variables in response to ANF or vehicle were analyzed by two-way analysis of variance with repeated measures, followed by Tukey’s test when a significant F value was encountered. Comparisons were made between both control and sympathectomized rats, for both ANF and vehicle treatments. Blood pressure before norepinephrine infusion and responses to tyramine injection were compared by one-way analysis of variance. The level of significance was set at p<0.05.
Results

Protocol 1

Chemical sympathectomy was confirmed by a lack of pressure response to tyramine in rats treated with 6-OHDA (2±1 mm Hg) compared with control rats (43±4 mm Hg). The hemodynamic and hematocrit responses to rat ANF infusion are summarized in Figure 1. Resting levels of mean arterial pressure were lower in 6-OHDA–treated rats than in control rats (94±5 vs. 108±4 mm Hg, p<0.05). Mean arterial pressure decreased by 10±4 mm Hg with rat ANF infusion in control rats but did not change significantly in 6-OHDA–treated rats. Cardiac output tended to decrease less with rat ANF in rats treated with 6-OHDA (18±2%) than in control rats (28±4%), but the overall difference was not significant (p=0.07 for interaction). The increase in total peripheral resistance with rat ANF infusion was not significantly different between groups; total peripheral resistance increased 29±7% in control rats and 28±4% in 6-OHDA–treated rats. Resting heart rates were not different between control rats (351±11 beats/min) and 6-OHDA–treated rats (381±26 beats/min) and did not change with rat ANF infusion. Control levels of hematocrit were lower in 6-OHDA–treated rats (41.8±2%) than in control rats (44.1±0.3%, p<0.05). Hematocrit increased from 44.1±0.3% to 48.3±1% in control rats (p<0.05) but did not change significantly in 6-OHDA–treated rats (from 41.8±1.6% to 42.7±1.3%). Vehicle infusion had no effect on any hemodynamic parameters in either group but did cause a slight but significant fall in hematocrit in rats treated with 6-OHDA (from 35.6% to 34.3% at 60 minutes, p<0.05).

Protocol 2

Because an intact adrenal medulla could contribute to rat ANF–induced changes in total peripheral resistance, we infused rat ANF in a second group of rats subjected to both adrenal demedullation and 6-OHDA treatment. Nephrectomy and splenectomy were performed to avoid renal and splenic effects on hematocrit during rat ANF infusion. The MSX rats also failed to respond to tyramine; the change in mean arterial pressure was 2±2 mm Hg in MSX rats and 42±2 mm Hg in control rats. Resting mean arterial pressure of MSX rats was 79±3 mm Hg; therefore, norepinephrine infusion was used to ensure that MSX rats had control levels of mean arterial pressure similar to control rats. The hemodynamic responses to rat ANF infusion are illustrated in Figure 2. Under these conditions, MSX rats had a statistically greater fall in mean arterial pressure with rat ANF infusion than control rats (−30±5 vs. −12±2 mm Hg at 60 minutes, p=0.0004). However, the changes in cardiac output were nearly identical in control and MSX rats (−24±3% vs. −27±4%, respectively). Total peripheral resistance increased 21±7% in control rats after 45 minutes of rat ANF infusion but did not change significantly from control levels in MSX rats. Nonetheless, the change in total peripheral resistance from 15 to 60 minutes in the MSX rats was significant (a 20% increase in total peripheral resistance from 88% to 108% of control). In addition, the initial 12% drop in total peripheral resistance in MSX rats was not significant. MSX rats experienced a slight but significant increase in heart rate during ANF infusion (289±15 to 328±8 beats/min, p<0.05). Mean levels of central venous pressure were lower in MSX rats than in control rats during control (−0.6±0.5 vs. 0.7±0.3 mm Hg) and ANF infusion periods, but the decreases in central venous pressure were similar in both groups during rat ANF infusion (maximum decreases of 0.8±0.2 mm Hg in control rats vs. 1.1±0.3 mm Hg in MSX rats).

The body fluid effects of rat ANF in MSX and control rats are shown in Table 1. There were no group differences in blood volume, hematocrit, or plasma volume, and both groups responded to rat ANF with similar reductions in blood volume, plasma volume, and increases in hematocrit. Vehicle infusion produced no
significant changes in any of the hemodynamic parameters, but it did lead to a small but significant decrease in hematocrit (approximately 1% in both groups).

**Discussion**

ANF possesses modest hypotensive properties when infused at pharmacological doses in conscious normotensive rats. The drop in blood pressure is normally due to a decrease in cardiac output. However, the mechanism of this effect is still unclear. Direct cardiac depression is unlikely; a number of investigators have reported that atriopeptin III does not alter cardiac work in anesthetized rats, anesthetized dogs, or isolated perfused rat hearts. Dilation of venous capacitance vessels is also unlikely. Most veins are relatively unresponsive to ANF.

TABLE 1. Body Fluid Effects of Rat Atrial Natriuretic Factor Infusion in Medullectomized and Sympathectomized Rats or Sham-Operated Control Rats

<table>
<thead>
<tr>
<th>Period</th>
<th>Blood volume (ml)</th>
<th>Hematocrit (%)</th>
<th>Plasma volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MSX rats (n=11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17.2±0.7</td>
<td>47.1±1.1</td>
<td>9.1±0.4</td>
</tr>
<tr>
<td>15 min</td>
<td>16.7±0.6*</td>
<td>48.2±1.1*</td>
<td>8.7±0.4*</td>
</tr>
<tr>
<td>30 min</td>
<td>16.5±0.6*</td>
<td>48.9±1.0*</td>
<td>8.4±0.4*</td>
</tr>
<tr>
<td>60 min</td>
<td>16.5±0.6*</td>
<td>49.2±0.9*</td>
<td>8.4±0.4*</td>
</tr>
<tr>
<td><strong>SH rats (n=9)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17.3±0.4</td>
<td>45.3±1.3</td>
<td>9.6±0.2</td>
</tr>
<tr>
<td>15 min</td>
<td>16.5±0.3*</td>
<td>46.9±1.3*</td>
<td>8.8±0.2*</td>
</tr>
<tr>
<td>30 min</td>
<td>16.3±0.3*</td>
<td>47.3±1.3*</td>
<td>8.7±0.2*</td>
</tr>
<tr>
<td>60 min</td>
<td>16.2±0.3*</td>
<td>47.8±1.1*</td>
<td>8.6±0.2*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Rat atrial natriuretic factor was infused at a rate of 0.5 μg/kg/min. MSX, medullectomized and sympathectomized; SH, sham-operated.

More, infusion of ANF in dogs with autonomic blockade does not change venous compliance.

ANF could decrease cardiac output by increasing resistance to venous return. Speculated that the sympathetic nervous system mediated this increase in resistance to venous return, and studies by Sasaki et al support this concept. Anesthetized rats sympathectomized with 6-OHDA treatment alone experienced attenuated decreases in cardiac output and mean arterial pressure after ANF infusion. We have demonstrated similar findings with regard to mean arterial pressure in conscious rats (protocol 1) and also found a blunted decrease in hematocrit in 6-OHDA-treated rats after rat ANF infusion. In those experiments, the adrenal medulla was left intact. Increased epinephrine release from the adrenal medulla could have attenuated ANF-induced increases in resistance to venous return by means of a preferential interaction at α-receptors or β-adrenergic receptors. It is also possible that the increase in total peripheral resistance observed in sympathectomized rats in protocol 1 after ANF infusion was due to epinephrine release. In protocol 2, the adrenal medulla was removed to avoid an effect of epinephrine on ANF-induced changes in hemodynamics, and vascular tone was returned to normal with norepinephrine. The data from those experiments do not provide evidence for a role of the sympathetic nervous system in mediating the cardiac output and plasma volume changes induced with rat ANF infusion. The changes in cardiac output during rat ANF infusion were not significantly different between control or 6-OHDA-treated rats in protocols 1 or 2. Furthermore, although central venous pressure was lower in MSX rats than control rats during control and ANF infusion, the decrease in central venous pressure with rat ANF infusion was
similar in both groups. If decreases in central venous pressure during ANF infusion are mediated by increases in resistance to venous return, the sympathetic nervous system does not seem to influence this response. In addition, rat ANF infusion caused similar decreases in plasma volume in control and MSX rats in protocol 2. The effects of ANF on plasma volume and central venous pressure could be due to changes in capillary permeability; however, increases in capillary permeability were observed at concentrations of ANF ranging from 0.1 to 10 μM,27,28 concentrations far greater than those achieved in the present study. Furthermore, increased capillary permeability as a mechanism for ANF-induced reductions in plasma volume is not consistent with the observation that ANF attenuated capillary absorption.29 ANF could also reduce plasma volume by causing constriction of large venules or small veins, which could lead to increases in resistance to venous return25 and capillary hydrostatic pressure. Faber et al26 have suggested that ANF-induced increases in resistance to venous return occur by means of a relaxation of α1-mediated, but not α2-mediated, adrenergic constriction.

Although our data support the hypothesis that sympathetic reflexes caused the increases in total peripheral resistance during rat ANF infusion, they are not compatible with the concept that rat ANF is a general systemic vasodilator. Although sympathomimeticized rats experienced initial decreases in total peripheral resistance of 5% and 12% in protocols 1 and 2, respectively, this change was not significant and was only transient. Breuhaus et al14 and Woods et al40 also failed to reveal a vasodilator effect of ANF in conscious animals after ganglionic blockade or neurohormonal blockade. It is possible that ANF-induced increases in resistance to venous return occur by means of a relaxation of α1-mediated, but not α2-mediated, adrenergic constriction.

In conclusion, rat ANF infusion at a pharmacological dose decreased central venous pressure, cardiac output, and plasma volume in the conscious rat with controlled sympathetic tone; these changes were independent of reflex sympathetic nerve activity. The increase in total peripheral resistance during ANF infusion was largely due to autonomic reflexes. There was no evidence of an ANF-mediated sustained vasodilation in these acute experiments.

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