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)
libitum. Rats were housed in groups until instrumenta-
tion, 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.6

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.17
Control rats received an equal volume of vehicle (1%
ascorbic acid in saline). Catheters were routed sub-
cutaneously, 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 venti-
lated Plexiglas boxes (25×15×11 cm³) with bedding
and were allowed 1 hour for acclimatization to the
environment. Changes in cardiac output in the con-
scious rat were assessed after connecting the trans-
ducer wires to a custom-built directional pulsed
Doppler velocity meter (Instrumentation Develop-
ment 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 trans-
ducer (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., Bel-
mont, California) was infused continuously at a dose
of 0.5 µg/kg/min. Variables were recorded continu-
ously, 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 phen-
tolamine and 6-OHDA through a tail vein as in
protocol 1. The next day, the rats were again anes-
ethetized 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.¹⁸ 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 sympathecto-
mized (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. Compar-
isons were made between both control and sympa-
thetomized rats, for both ANF and vehicle treat-
ments. 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 than in control rats (41.8±2% vs. 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 vs. 43±4 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,19 anesthetized dogs, or isolated perfused rat hearts.20 Dilation of venous capacitance vessels is also unlikely. Most veins are relatively unresponsive to ANF.21-22 Furthermore, infusion of ANF in dogs with autonomic blockade does not change venous compliance.23 ANF could decrease cardiac output by increasing resistance to venous return.24-25 Chien et al25 speculated that the sympathetic nervous system mediated this increase in resistance to venous return, and studies by Sasaki et al15 support this concept. Anesthetized rats sympathectomized with 6-OHDA treatment alone experienced attenuated decreases in cardiac output and mean arterial pressure after ANF infusion.15 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-receptors26 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 more, infusion of ANF in dogs with autonomic blockade does not change venous compliance.23
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, 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. ANF could also reduce plasma volume by causing constriction of large venules or small veins, which could lead to increases in resistance to venous return and capillary hydrostatic pressure. Faber et al. 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 sympathomimetic 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 al. also failed to reveal a vasodilator effect of ANF in conscious animals after ganglionic blockade or neurohormonal blockade. It is possible that ANF could directly lower vascular resistance in some beds, and have no effect or even increase resistance in others; thus, the overall outcome could be transient decreases in total peripheral resistance. Lappe et al. have shown that ANF lowered vascular resistance in renal and hindquarter, but not mesenteric, beds after 6-OHDA treatment. In protocol 2, total peripheral resistance actually increased 20% in MSX rats from 15 to 60 minutes of rat ANF infusion (from 88% to 108% of control value). This increase in total peripheral resistance could represent changes in hormones not blocked by sympathectomy or could be due to direct vasoconstrictor effects of ANF. Atlas et al. have previously demonstrated vasoconstriction in the isolated perfused kidney in response to auriculin B infusion; Young et al. and Woods and Anderson have suggested a direct peripheral vasoconstrictor role for ANF.

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.

Acknowledgments

The excellent technical assistance of Wanda W. Myers and Nell R. Taylor is gratefully acknowledged. We thank Dr. Merrill B. Kardon, Dr. Craig J. Hartley, and Dr. Julianna E. Szilagyi for advice on Doppler transducer construction and Jean Wood for excellent secretarial assistance.

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KEY WORDS • hemodynamics • vascular resistance • central venous pressure • blood volume • sympathectomy, chemical • hematocrit • Doppler effect • rat studies
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Hypertension. 1990;15:888-893
doi: 10.1161/01.HYP.15.6.888

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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World Wide Web at:
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