Atrial Natriuretic Factor–Induced Systemic Vasodilation in Conscious Dogs, Rats, and Monkeys

You-Tang Shen, Mark A. Young, Jacqueline Ohanian, Robert M. Graham, and Stephen F. Vatner

This study addresses the hypothesis that atrial natriuretic factor (ANF) is a primary vasodilator, which reduces arterial pressure directly and increases total peripheral resistance secondarily by reflex mechanisms. The effects of 30-minute infusions of ANF (0.3 μg/kg/min i.v.) were examined in conscious dogs, rats, and monkeys before and after ganglionic blockade with hexamethonium. In seven intact, conscious dogs, ANF reduced mean arterial pressure by 7±1% and cardiac output by 19±3% and increased total peripheral resistance by 15±3%. After ganglionic blockade, ANF reduced mean arterial pressure by 7±2% but still increased total peripheral resistance by 15±3%. Similar results were observed in four dogs with total cardiac denervation and in six dogs with arterial baroreceptor denervation. Furthermore, in two dogs, combined ganglionic and \( \alpha \text{-adrenoceptor} \) blockades failed to alter the rise in total peripheral resistance observed with ANF. In six intact, conscious rats, ANF reduced mean arterial pressure by 8±2% and cardiac output by 27±2% and increased total peripheral resistance by 27±5%. After ganglionic blockade, ANF still increased total peripheral resistance by 13±3%. In six intact, conscious monkeys, ANF reduced mean arterial pressure by 14±2% and cardiac output by 26±3% and increased total peripheral resistance by 17±3%. However, after ganglionic blockade, ANF decreased total peripheral resistance by 11±2%. These data provide evidence for a fundamental species difference in the vascular actions of ANF. In conscious dogs, ANF elicits "direct" vasoconstriction, which increases total peripheral resistance, even in the presence of denervation of reflexes or autonomic blockade. In conscious rats, ANF elicits both direct and reflexly mediated vasoconstriction. In conscious monkeys, although a component of direct vasoconstriction may also be present, the most prominent component appears to be reflexly mediated, since it was abolished by ganglionic blockade. (Circulation Research 1990;66:647–661)
plish this, the effects of ANF infusions on systemic hemodynamics were examined before and after autonomic blockade with hexamethonium in conscious dogs, in conscious dogs after total cardiac denervation, and in conscious dogs after arterial baroreceptor denervation. The hemodynamic effects of ANF were also compared with those induced by the known vasodilators, nitroglycerin and hydralazine. Experiments were also conducted in chronically instrumented conscious rats and monkeys to determine if there were major species differences in the systemic vascular effects of ANF. Although effects of ANF on cardiac output and arterial pressure have been examined previously in conscious rats,7,21,25,26–30 sheep,11 and humans,8,16–20,23 they have not been assessed in experimental studies in conscious primates with direct and continuous measurements of cardiac output.

Materials and Methods

Seventeen mongrel dogs, weighing 17–22 kg, were sedated with xylazine (0.5 mg/kg i.m.) and anesthetized with pentobarbital sodium (30 mg/kg i.v.). Operations were performed using sterile surgical techniques. Through a left lateral thoracotomy in the fourth intercostal space, Tygon catheters (Norton Plastics, Akron, Ohio) were implanted in the aorta and right atrium for measurement of their respective pressures, and an electromagnetic flow transducer (Zepeda Instruments, Seattle, Washington) was implanted on the ascending aorta for measurement of cardiac output (minus coronary blood flow). The wires and catheters were externalized between the scapulae, the incision was closed, and the chest was evacuated. During the same surgical session, the spleen was removed through a midline laparotomy. Four of the dogs underwent total cardiac denervation by the intrapericardial technique of Randall et al.31 and six other dogs underwent arterial baroreceptor denervation by stripping the aortic arch and major cephalic branches and, during a second procedure 10 days later, inducing bilateral carotid sinus denervation. Each dog was treated with 1 g cephalothin (Keflin, Eli Lilly and Company, Indianapolis, Indiana) intraoperatively and for 2–3 days after surgery.

Six adult Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Massachusetts), weighing 310–430 g, were anesthetized with pentobarbital sodium (50 mg/kg i.p.) after pretreatment with atropine (1 mg/kg i.p.). A rodent respirator (Harvard Apparatus, South Natick, Massachusetts) maintained adequate ventilation during the thoracotomy. Operations were performed using sterile surgical techniques. Through a right thoracotomy at the third intercostal space, the ascending aorta was dissected free from its surrounding tissue, and then a Transonic flow probe (Transonic Systems, Ithaca, New York) was implanted on the ascending aorta for measurement of cardiac output (minus coronary blood flow). Negative intrapleural pressure was restored with a chest tube. Heparinized saline-filled Tygon catheters (0.96 mm o.d.) were inserted in the abdominal aorta and inferior vena cava via the right femoral artery and vein for measurement of arterial pressure and for injection of drugs, respectively. The transducer leads and catheters were tunneled subcutaneously and externalized at the back of neck.

Six monkeys (Macaca fascicularis), weighing 7–13 kg, were anesthetized with ketamine (6–8 mg/kg i.m.) followed by halothane (0.5–1.5 vol%). Operations were performed using sterile surgical techniques. Through a left thoracotomy at the fourth intercostal space, a Transonic flow probe was implanted on the ascending aorta for measurement of cardiac output (minus coronary blood flow), and heparinized saline-filled Tygon catheters were implanted into the descending thoracic aorta and in the left atrium for measurements of arterial and left atrial pressures, respectively, while a catheter was implanted in the right atrium for infusion of drugs. The transducer leads and catheters were tunneled subcutaneously and externalized in the interscapular region. The monkeys were treated with cephalothin (30 mg/kg i.m.) for 2 days after surgery. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services publication No. [NIH] 85-23, revised 1985).

The experiments in conscious dogs were conducted 2–4 weeks after surgery, during which time the dogs were trained to lie quietly on their right side on the experiment table. The experiments in conscious rats were conducted 3–7 days after surgery, while the rats were upright in a plastic cylinder. The experiments in conscious monkeys were conducted 2–4 weeks after surgery, with the monkeys supine in a chair. The experimental protocol consisted of three 15-minute control periods, one 30-minute experimental period for the infusion of ANF or vehicle (saline), and two 15-minute recovery periods. Hemodynamic data were monitored continuously. After the control periods, ANF (0.3 μg/kg/min) or saline was infused for 30 minutes at a rate of 0.4 ml/min in dogs or monkeys and at a rate of 0.04 ml/min in rats. ANF-(102–126) (Wyeth Pharmaceutical, Philadelphia, Pennsylvania) was used for the dogs, whereas ANF-(99–126) (Peninsula Laboratories, Belmont, California) was used for the rats and monkeys. Although prior work by our laboratory32 and others33 has shown that the effects of these ANF peptides are similar, one dog was examined with ANF-(99–126).

On separate days, the effects of ANF or saline were examined in the same animals after ganglionic blockade. After stabilization of hemodynamics, but before the control collection periods, hexamethonium bromide (30 mg/kg i.v.) and methyl atropine bromide (0.1 mg/kg) were infused over a 20-minute period for conscious dogs. In three dogs, the infusion of ANF was repeated in the presence of atropine methyl bromide (0.1 mg/kg) alone to determine whether differences before and after ganglionic
blockade could be attributed to the atropine. In conscious rats, ganglonic blockade with hexamethonium was administered in three or four bolus injections to give a total dose of 15 mg/kg, followed by an infusion of hexamethonium (0.45 mg/kg/min) and methyl atropine bromide (0.005 mg/kg/min) at a rate of 0.01 ml/min throughout the experiment. In conscious monkeys, hexamethonium bromide (15 mg/kg i.v.) and methyl atropine bromide (0.05 mg/kg) were infused over 45 minutes. When hemodynamic parameters had stabilized, the control collection periods were begun as described above. Ganglonic blockade was verified at the end of the experiment by the absence of reflex heart rate responses to phenylephrine (5 μg/kg i.v.) in all species and to nitroglycerin (5 μg/kg i.v.) in dogs or monkeys and (25 μg/kg i.v.) in rats.

The effects of ANF (0.3 μg/kg/min) were also examined in six dogs with chronic arterial baroreceptor denervation and in four dogs with chronic cardiac denervation. The completeness of arterial baroreceptor denervation was confirmed by the elimination of reflex heart rate changes in response to alterations in arterial pressure by injections of phenylephrine (5 μg/kg i.v.) and nitroglycerin (10 μg/kg i.v.). To verify whether the cardiac receptors were functionally denervated, veratrine alkaloid (5 μg/kg) was injected in the left atrium; no changes were observed in mean arterial pressure and heart rate in dogs with cardiac denervation, whereas decreases in arterial pressure and heart rate were observed in dogs with intact cardiac nerves. In two conscious dogs, α1-adrenoreceptor blockade (1 mg/kg i.v. prazosin) was administrated in the presence of ganglonic blockade. In these experiments, the pressor as well as the reflex bradycardiac responses to phenylephrine were eliminated by the combined blockade.

Hemodynamics were recorded continuously on a 14-channel magnetic tape recorder (Honeywell, Denver, Colorado) and on an eight-channel ink-writing oscillograph (Gould-Brush, Cleveland, Ohio). Aortic and right atrial pressures were measured by strain gauge manometer (Statham Instruments, Oxnard, California) connected to the respective catheters. Cardiac output for dogs was measured with a square-wave electromagnetic flowmeter (Benton Instruments, Cupertino, California). Cardiac output for rats or monkeys was measured with the Transonic flowmeter. Because of the markedly different body weights among the different species, cardiac output was normalized for body weight, and values of the cardiac index are included in the tables. Mean pressures and cardiac output were determined by using resistance-capacitance filters with 2- or 8-second time constants, respectively. Total peripheral resistance was calculated as the quotient of mean arterial pressure and cardiac output, normalized for body weight.

Arterial blood samples were collected before and at the end of a 30-minute infusion of ANF in dogs and monkeys. The blood samples were placed in iced Na+ EDTA tubes containing aprotonin (500 kIU) and 50 μl phenylmethylsulfonyl fluoride (50 mM). Blood samples for plasma renin and vasopressin were collected in tubes containing EDTA. All blood samples were kept on ice until centrifuged at 4°C for plasma separation after each experiment, and plasma was stored at −70°C for analysis. For radioimmunoassay of ANF, the plasma was thawed, and the ANF was extracted on a Sepak C18 column, after initial washing with 100% methanol and H2O and then further washing with 100 mM KPO4 (pH 3–5) and 1% trifluoroacetic acid, before elution with 50% CH3CN. Assay for ANF levels was conducted as previously described by Waldman et al.34 The sensitivity of the ANF assay is 2 pg/tube (95% confidence limits), and inter- and intra-assay variability is 9.2% and 6.1%, respectively. The standard curve is linear up to 1,500 pg/ml, with half-maximal displacement of tracer binding at 140 pg/ml. Samples stored at −70°C are stable for greater than 6 months and were generally assayed within 2 months. Plasma vasopressin was determined by a specific radioimmunoassay procedure developed by Cowley et al.35 Plasma renin activity was determined by a standard radioimmunoassay kit (New England Nuclear, Billerica, Massachusetts) adapted from the method originally described by Haber et al.36 These blood samples were also used for measurements of hematocrit in dogs and monkeys. Because of the small blood volume in the rats, arterial blood was sampled for hematocrit before and at the end of a 30-minute infusion of ANF in the presence and absence of ganglonic blockade during separate experiments.

All data were stored on an IBM-AT computer. The comparison between baseline, response to ANF, and recovery after ANF was performed using one-way analysis of variance for repeated measurements and analysis of linear contrasts.37 The profiles of the response to ANF for the intact animals and the animals with ganglonic blockade were compared at each point by paired Student’s t test. Statistical significance for these data was accepted at the level of 0.05. All values are expressed as mean±SEM.

Results

In all experiments, ANF was infused for 30 minutes and was followed by a 30-minute recovery period. The absolute values during baseline and the change from baseline at the end of the 30-minute infusion of ANF and 15 minutes later during the recovery period are presented in the tables. Averaged data are presented as percent change from control at 10-minute intervals during ANF infusion and at 15-minute intervals during the recovery period in the figures. Examples of the phasic data are shown in Figure 1 (conscious dog), Figure 2 (conscious rat), and Figure 3 (conscious monkey).

Effects of ANF on Hemodynamics in Conscious Dogs

The effects of infusions of ANF on hemodynamics in intact conscious dogs (Figure 1) with and without
Hematocrit rose significantly ($p<0.01$) by 2.6±0.3% from a baseline of 38±1.7%. In one of the seven conscious dogs studied, human ANF-(99–126) was used instead of the ANF-(102–126). In that dog, ANF reduced mean arterial pressure by 8% and cardiac output by 21% and increased total peripheral resistance by 17%. To verify that the vasoconstrictor effects of ANF were not unique to the dose of ANF chosen for study, a lower dose (0.1 μg/kg/min×30 min) was infused in five intact dogs. This dose reduced mean arterial pressure (−4±3%) and cardiac output (−12±1.0%) and increased total peripheral resistance (+10±4%). Infusion of vehicle (saline) did not alter mean arterial pressure.

**Figure 1.** Tracings showing the effects of 0.3 μg/kg/min atrial natriuretic factor (ANF) on phasic and mean measurements of aortic pressure and aortic blood flow and on computed total peripheral resistance in a conscious dog before (left tracings) and 30 minutes after (right tracings) infusion of ANF. Note that ANF reduced mean aortic blood flow (cardiac output) more than mean arterial pressure, indicating a rise in peripheral resistance.
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Aortic Pressure (mmHg)

Mean Aortic Pressure (mmHg)

Aortic Blood Flow (L/min)

Mean Aortic Blood Flow (L/min)

Total Peripheral Resistance (mmHg/L/min)

Control

ANF 0.3 μg/kg/min

FIGURE 2. Tracings showing the effects of 0.3 μg/kg/min atrial natriuretic factor (ANF) on phasic and mean measurements of aortic pressure and aortic blood flow and on computed total peripheral resistance in a conscious rat before (left tracings) and 30 minutes after (right tracings) infusion of ANF. Note that ANF reduced mean aortic blood flow (cardiac output) more than arterial pressure, indicating a rise in peripheral resistance.

(-1±2%), cardiac output (+2±3%), or total peripheral resistance (-3±2%) in intact dogs.

After ganglionic blockade, baseline levels of mean arterial pressure, cardiac output, and total peripheral resistance were similar to those observed in intact dogs without blockade, while heart rate (123±3.2 beats/min) was significantly increased (p<0.01). Infusion of ANF reduced (p<0.025) mean arterial pressure (-7±1.9%) and cardiac output (-19±3.0%) and increased (p<0.01) total peripheral resistance (+15±3.1%) by amounts similar to those observed in intact dogs. Cardiac output remained depressed, and total peripheral resistance remained elevated during the recovery period. In one of the seven conscious dogs, human ANF-(99–126) was used instead of the ANF-(102–126). In that dog, ANF reduced mean arterial pressure by 8% and cardiac output by 23% and increased total peripheral resistance by 19%. Hematocrit rose (p<0.025) by 1.5±0.5% from the baseline of 38±2.2%. Infusion of vehicle (saline) after ganglionic blockade also did not alter mean arterial pressure (-3±2%), cardiac output (-1±3%), or total peripheral resistance (-3±3%).

Infusion of ANF in three dogs pretreated with atropine alone decreased mean arterial pressure (-8±3%) and cardiac output (-19±5%) and increased total peripheral resistance (+13±6%).
These changes were similar to those observed in intact dogs.

Figures 5 and 6 illustrate the responses to infusion of ANF in cardiac denervated dogs (n=4) and arterial baroreceptor denervated dogs (n=6), respectively, before and after ganglionic blockade. The responses to ANF in these groups of dogs were similar to those observed in intact dogs reported in Table 1. Interestingly, the fall in arterial pressure was not enhanced during the infusion of ANF, and the sustained reduction in cardiac output and elevation in total peripheral resistance were still observed during recovery. ANF increased hematocrit in both groups of denervated dogs in the presence and absence of ganglionic blockade.

Effects of α1-Adrenoceptor Blockade With Prazosin on the Response to ANF

In two conscious dogs, the hemodynamic responses to ANF were examined after ganglionic blockade with hexamethonium and atropine with the addition of α1-adrenoceptor blockade with prazosin. The results for these two experiments were averaged and are shown in Figure 7. In these two dogs, ANF still induced sustained increases in total peripheral resistance.
Table 1. Effects of Atrial Natriuretic Factor in Seven Conscious Dogs

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ANF infusion*</th>
<th>Recovery†</th>
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<tr>
<td><strong>Mean Arterial Pressure (mm Hg)</strong></td>
<td></td>
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<tr>
<td>Intact</td>
<td>94±2.8</td>
<td>−6.6±1.0‡</td>
<td>−1.1±1.7</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>91±4.2</td>
<td>−7.1±1.9‡</td>
<td>−4.6±2.0</td>
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<tr>
<td><strong>Cardiac index (ml/min/kg)</strong></td>
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<tr>
<td>Intact</td>
<td>96.5±5.0</td>
<td>−18.0±2.1‡</td>
<td>−17.6±2.6‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>101.2±6.5</td>
<td>−20.2±4.2‡</td>
<td>−18.9±4.3‡</td>
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<tr>
<td><strong>Total peripheral resistance (mm Hg/ml/min/kg)</strong></td>
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<tr>
<td>Intact</td>
<td>0.99±0.07</td>
<td>+0.16±0.04‡</td>
<td>+0.22±0.05‡</td>
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<tr>
<td>Ganglionic block</td>
<td>0.92±0.07</td>
<td>+0.14±0.03‡</td>
<td>+0.15±0.03‡</td>
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<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td></td>
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<tr>
<td>Intact</td>
<td>92±2.3</td>
<td>−5.3±1.8‡</td>
<td>−2.7±2.6</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>123±3.2‡</td>
<td>−2.6±1.2</td>
<td>−3.3±2.9</td>
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<tr>
<td><strong>Hematocrit (%)</strong></td>
<td></td>
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<tr>
<td>Intact</td>
<td>38±1.7</td>
<td>+2.6±0.3‡</td>
<td>...</td>
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<tr>
<td>Ganglionic block</td>
<td>38±2.2</td>
<td>+1.5±0.5‡</td>
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<tr>
<td><strong>ANF (pg/ml)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Intact</td>
<td>79±14</td>
<td>+4,514±884‡</td>
<td>...</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>94±11</td>
<td>+4,579±380‡</td>
<td>...</td>
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</table>

*Values presented are at end of 30-minute infusion of 0.3 μg/kg/min×30 min atrial natriuretic factor (ANF).
†Values presented are at 15 minutes of recovery after infusion of ANF.
‡Significant change from baseline at p<0.05.
§Significant difference from intact, conscious dogs at p<0.05.

Effects of Infusion of ANF on Blood Levels of ANF

Table 1 reports the arterial levels of ANF achieved during infusion of the peptide before and after ganglionic blockade. Baseline levels and those at the end of infusion of ANF did not differ in the absence and presence of ganglionic blockade. During infusion of ANF, plasma levels rose significantly (p<0.01) by 4,514±884 pg/ml from a baseline of 79±14 pg/ml in the intact dogs. After ganglionic blockade, the increases in the plasma levels of ANF were similar to those observed in intact dogs. ANF levels returned to control during the recovery period. Infusion of vehicle (saline) did not change the plasma levels of ANF in the absence or presence of ganglionic blockade.

Effects of Infusion of ANF on Plasma Renin Activity and Vasopressin Levels

In the four intact conscious dogs, ANF infusion did not alter either plasma renin activity (0.7±0.1 ng angiotensin I/hr) or vasopressin levels (1.8±0.3 pg/ml) from baseline values of 0.9±0.3 ng angiotensin I/hr and 1.7±0.2 pg/ml, respectively. After ganglionic blockade, ANF also did not increase plasma renin activity (0.4±0.1 ng angiotensin I/hr) or vasopressin levels (4.0±0.7 pg/ml) from baseline values of 0.4±0.1 ng angiotensin I/hr and 5.9±1.4 pg/ml, respectively.

Effects of Infusion of Nitroglycerin and Hydralazine on Hemodynamics

To compare the hemodynamic actions of ANF with other vasodilators, nitroglycerin (1 μg/kg/min) and hydralazine (2.5 μg/kg/min) were infused for 30 minutes before and after ganglionic blockade with hexamethonium and atropine (Figure 8). In three intact conscious dogs, nitroglycerin and hydralazine reduced mean arterial pressure by 7±3.0% and 3±1.3%, respectively, which was similar to the hypotensive action of ANF. The reductions in right atrial pressure with nitroglycerin (−1.3±0.6 mm Hg) and hydralazine (−1.5±0.5 mm Hg) were also similar to those observed with ANF. In contrast to results during infusion of ANF, total peripheral resistance decreased with nitroglycerin (−3.3±1.7% and hydralazine (−16±0.9%). After ganglionic blockade, the reductions in total peripheral resistance were greater with both nitroglycerin (−21±5.9%) and hydralazine (−26±0.8%) than those observed in intact dogs. Infusion of saline instead of ANF failed to elicit significant hemodynamic effects in either the presence or absence of ganglionic blockade (Figure 8).

Effects of ANF on Hemodynamics in Conscious Rats

The effects of infusion of ANF on hemodynamics in six intact conscious rats (Figure 2) with and without ganglionic blockade are shown in Table 2 and Figure 9. The hemodynamic effects of ANF were also slow in onset. Mean arterial pressure fell (p<0.01) and remained depressed by 8±1.8% at the end of ANF infusion and then gradually returned to control. Cardiac output fell (p<0.01) by 27±2.5% at the end of the infusion and remained depressed for the following 30-minute recovery period. Total peripheral resistance rose (p<0.01) by 27±4.7% at the end of the infusion and remained elevated during the recovery period.
Heart rate was unchanged (-2±2.8%) from a baseline of 440±24 beats/min. Hematocrit rose (p<0.01) by 2.3±0.5% from a baseline of 46±2.3%. Infusion of vehicle (saline) did not affect mean arterial pressure (-3±1%), cardiac output (-1±1%), or total peripheral resistance (-1±1%) in intact rats.

After ganglionic blockade, baseline levels of mean arterial pressure, total peripheral resistance, and heart rate were significantly reduced (p<0.01); cardiac output was lower, but not significantly, than that observed in intact rats. Infusion of ANF in the presence of ganglionic blockade still reduced (p<0.025) mean arterial pressure (-5±1.5%) and cardiac output (-17±1.9%) and increased (p<0.01) total peripheral resistance (+13±2.7%), while heart rate was unchanged (-4±1.6%). However, in contrast to the results in dogs, the decreases in cardiac output and increases in total peripheral resistance with ANF were attenuated (p<0.01) in the presence of ganglionic blockade. These differences observed between the intact rats and the rats with ganglionic blockade were no longer apparent during the recovery period. Hematocrit rose (p<0.025) by 1.4±0.3% from a baseline of 42±1.6%. After ganglionic blockade, infusion of vehicle (saline) did not alter mean arterial pressure (0±3%), cardiac output (+3±3%), or total peripheral resistance (-2±2%).

**Effects of ANF on Hemodynamics in Conscious Monkeys**

The effects of infusions of ANF on hemodynamics in intact conscious monkeys (Figure 3) with and without ganglionic blockade are shown in Table 3 and Figure 10. In intact conscious monkeys, the hemodynamic effects of ANF were also slow in onset. Mean arterial pressure fell (p<0.01) by 14±2.1% and remained depressed at the end of infusion and throughout the recovery period. Cardiac output fell (p<0.01) by 26±2.8% at the end of infusion and remained depressed for the following 30-minute
recovery period. Total peripheral resistance rose ($p<0.01$) by $17\pm3.4\%$ at the end of infusion period and remained elevated during the recovery period. Left atrial pressure fell ($p<0.05$) by $1.4\pm0.4$ mm Hg from $5.5\pm0.5$ mm Hg. Heart rate was increased ($p<0.01$) by $4\pm0.9\%$ from a baseline of $174\pm3.9$ beats/min. Hematocrit rose significantly ($p<0.01$), by $1.8\pm0.3\%$ from baseline of $38\pm0.9\%$. Infusion of vehicle (saline) did not affect mean arterial pressure ($0\pm1.0\%$), cardiac output ($+3\pm1.0\%$), or total peripheral resistance ($-3\pm1.0\%$).

After ganglionic blockade, baseline levels of mean arterial pressure, cardiac output, and heart rate were significantly reduced ($p<0.01$), while total peripheral resistance was unchanged, compared with those values observed in intact monkeys. Infusion of ANF still reduced ($p<0.05$) mean arterial pressure ($-10\pm3.1\%$), and then arterial pressure gradually returned to control. However, in contrast to dogs and rats, cardiac output did not fall (+1$\pm2.6\%$) throughout the ANF infusion or recovery period. Total peripheral resistance fell ($p<0.01$) by $11\pm1.8\%$ at the end of infusion and then gradually returned to control. Heart rate was decreased ($p<0.05$) by $3\pm1.2\%$ from a baseline of $107\pm9.3$ beats/min. ANF increased ($p<0.025$) hematocrit by $1.9\pm0.4\%$ from a baseline of $33\pm4.0\%$. Infusion of vehicle (saline) after ganglionic blockade did not affect mean arterial pressure (+2$\pm2\%$), cardiac output ($-1\pm3\%$), and total peripheral resistance ($+1\pm3\%$). Table 3 reports the arterial level of ANF achieved during infusion of ANF before and after ganglionic blockade in conscious monkeys. At the end of the 30-minute infusion, plasma levels of ANF rose ($p<0.025$) by $4,007\pm1,023$ pg/ml in intact monkeys. After ganglionic blockade, the increases in plasma levels of ANF were also similar to those observed in intact monkeys. The increases in ANF levels were similar to those observed in conscious dogs. Infusion of vehicle (saline) did not change the plasma levels of ANF, in either the presence or absence of ganglionic blockade.
action for ANF in conscious dogs. Rather, they suggest that the increase in total peripheral resistance in the conscious dog is due to a direct vasoconstrictor action of ANF. The term “direct” is used to indicate that the ANF-induced vasoconstriction was not reflexly mediated. The increase in peripheral resistance induced by ANF may actually involve another, as of yet undefined, messenger. The finding that ANF increases total peripheral resistance does not exclude the possibility that vasodilation may still be observed in selective regional circulations or in large arteries, since the calculation of total peripheral resistance reflects resistance to blood flow across the entire peripheral arteriolar circulation. It is also conceivable that ANF-induced systemic vasodilation is only observed with massive pharmacological levels achieved by bolus administration of ANF, well above the dose level of ANF (0.3 μg/kg/min) used in the present study. However, it is noteworthy that the dose used in the present study is well within the range that consistently results in reductions in arterial pressure both in our laboratory and in other laboratories. This dose also elicited a 50-fold increase in blood levels of ANF to over 4,000 pg/ml, values considerably higher than would be observed under any physiological or pathological conditions. Furthermore, a lower dose also increased total peripheral resistance in the present study.

The conclusions from prior studies on the vasoactive effects of ANF remain controversial. Although the lack of direct vasodilator activity of ANF or the presence of direct vasoconstrictor actions has been postulated by several studies in vitro as well as

![FIGURE 8. Bar graphs comparing the effects of saline (0.4 ml/min x 30 min), nitroglycerin (NTG, 1 μg/kg/min x 30 min), and hydralazine (HYDRAL, 2.5 μg/kg/min x 30 min) on mean arterial pressure and total peripheral resistance in intact dogs (open bars) and in dogs after ganglionic blockade (solid bars). NTG and HYDRAL induced greater decreases in arterial pressure and total peripheral resistance after ganglionic blockade. Time controls did not induce any effect in either the presence or absence of ganglionic blockade.](image-url)

**Discussion**

The results of the present investigation do not support a primary peripheral arteriolar vasodilator

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**Table 2. Effects of Atrial Natriuretic Factor in Six Conscious Rats**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ANF infusion*</th>
<th>Recovery†</th>
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<tbody>
<tr>
<td><strong>Mean arterial pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>115±4.9</td>
<td>−9±2.4‡</td>
<td>−8±2.9‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>75±2.7§</td>
<td>−5±1.5‡</td>
<td>−4±2.3</td>
</tr>
<tr>
<td><strong>Cardiac index (ml/min/kg)</strong></td>
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<tr>
<td>Intact</td>
<td>269.5±17.9</td>
<td>−75.7±10.4‡</td>
<td>−61.8±11.2‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>232.1±25.1</td>
<td>−44.6±7.2‡§</td>
<td>−37.9±5.9‡</td>
</tr>
<tr>
<td><strong>Total peripheral resistance (mm Hg/ml/min/kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>0.44±0.03</td>
<td>+0.12±0.02‡</td>
<td>+0.09±0.02‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>0.34±0.04§</td>
<td>+0.05±0.01§</td>
<td>+0.06±0.01‡</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>440±24.1</td>
<td>−10±13.2</td>
<td>−6±16.2</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>334±16.7§</td>
<td>−15±5.7†</td>
<td>−22±8.2‡</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>46±2.3</td>
<td>+2.3±0.5†</td>
<td>...</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>42±1.6</td>
<td>+1.4±0.3§</td>
<td>...</td>
</tr>
</tbody>
</table>

*Values presented are at end of 30-minute infusion of 0.3 μg/kg/min x 30 min atrial natriuretic factor (ANF).
†Values presented are at 15 minutes of recovery after infusion of ANF.
‡Significant change from baseline at p<0.05.
§Significant difference from intact, conscious rats at p<0.05.
The lack of contribution from autonomic reflexes to the systemic constrictor response during infusion of ANF in the present study is supported by the experiments in dogs with cardiac or arterial baroreceptor denervation. These studies demonstrate that in the absence of afferent input from either cardiac or arterial baroreceptors, ANF still did not result in vasodilation. It is conceivable that, in the absence of either the cardiac or arterial baroreceptors, the alternate system could compensate and prevent the expected fall in resistance. Yet, the addition of ganglionic blockade to either group of dogs still did not unmask a vasodilator effect of ANF. In contrast, known vasodilators, nitroglycerin and hydralazine, were infused before and after ganglionic blockade. The fact that ganglionic blockade enhanced the fall in total peripheral resistance with nitroglycerin and hydralazine supports the well-established concept that peripheral reflexes counteract the fall in pressure and resistance with nitroglycerin and hydralazine. Failure of ganglionic blockade to unmask a fall in total peripheral resistance with ANF mitigates against a direct vasodilator effect of ANF in the conscious dogs.

It is of further interest that neither ganglionic blockade, arterial baroreceptor denervation, nor cardiac denervation potentiated the fall in arterial pressure with ANF in conscious dogs. These results also suggest that afferent or efferent nerves to the ventricles or atria do not modify the hemodynamic responses to ANF in conscious dogs. Similarly, the fact that ANF did not alter plasma renin activity or vasopressin levels suggests that these indirect hormonal mechanisms are also not responsible for the rise in total peripheral resistance.

The involvement of autonomic reflexes in the hemodynamic response to infusion of ANF has been examined in several previous studies. Lappe et al.21 infused ANF in conscious normotensive and hypertensive rats and observed a 10–30% increase in total peripheral resistance and greater increases in regional vascular resistances. In a second study, conducted in spontaneously hypertensive rats,49 the increases in regional vascular resistance with ANF were abolished by chemical or surgical sympathectomy, suggesting that sympathetic reflexes counteract the direct effect of the peptide to vasodilate peripheral beds. There are three major differences between the experimental design of that work49 and the present investigation: 1) The prior study in which sympathetic mechanisms were examined49 did not measure cardiac output or calculate total peripheral resistance and reported only regional resistance. 2) The prior study was performed in hypertensive animals. 3) The prior study did not determine potential species difference. Breuhaus et al.11 also reported that the ganglionic blocker, trimethaphan camsylate, abolished the increase in total peripheral resistance but not the fall in cardiac output observed with atriopeptin II in conscious sheep. In that study, ganglionic blockade administered during ANF infu-

**Figure 9.** Graphs showing the effects of a 30-minute infusion of atrial natriuretic factor (ANF) on mean arterial pressure, cardiac output, and total peripheral resistance in six conscious rats. Values shown are the percent change from baseline levels in the absence and presence of ganglionic blockade.

In vivo,15,24 most previous studies either in isolated vessel segments,1–3 regional vascular beds,2–4,9 or intact animals9–14 have reported smooth muscle relaxation, increases in blood flow, and reductions in arterial pressure with administration of ANF, suggesting that the primary action of atrial peptides is vasodilation. Other studies have suggested that the reduction in arterial pressure after ANF is due to a fall in cardiac output21,22,48 and that total peripheral resistance rises reflexly to counteract the drop in arterial pressure.10,11,21,22 This was not observed in the present investigation in conscious dogs. After ganglionic blockade, the depressor response to ANF and the rise in total peripheral resistance were nearly identical to those responses observed in the absence of ganglionic blockade. These findings indicate that the major fraction of the rise in total peripheral resistance in the conscious dog is not reflexly mediated but, instead, is a direct vascular effect of ANF.
sion unmasked a greater fall in arterial pressure than that observed in intact sheep, suggesting a compensatory role of sympathetic nerves. The reasons for the apparent discrepancy between the present study and that of Breuhaus et al are not clear. However, there were differences both in species and in method of administration of the ganglionic blocker. In the study by Breuhaus et al, the ganglionic blocker was administered during infusion of ANF, whereas in the present investigation the ganglionic blockade was administered to the same animals prior to ANF and on a separate day from the experiment without blockade. Furthermore, we also examined the effects of ANF in the presence of α-adrenergic blockade, which confirmed the findings with ganglionic blockade (i.e., that sympathetic mechanisms were not involved in mediating the ANF-induced vasoconstriction in conscious dogs).

Our results demonstrating the lack of autonomic involvement in the vascular response to ANF in conscious dogs are consistent with a recent study in humans but are contrary to those of Ebert and colleagues, who reported that infusion of ANF in humans produced systemic activation of the sympathetic nervous system and release of norepinephrine resulting in elevated total peripheral resistance. Although we did not measure plasma levels of catecholamines, the results in two dogs studied with prazosin rule out the possibility that the increased resistance with ANF is due to the release of catecholamines via a reflex or direct mechanism. The discrepancy between our results and those of Ebert and colleagues most likely represents a difference in the response of dogs versus humans to infusion of ANF. Other studies in humans have demonstrated increased or no change in circulating levels of catecholamines in response to infusion of ANF. The fact that studies in rats and dogs have concluded that ANF induces withdrawal of sympathetic tone points to a fundamental difference in the autonomic response to ANF in humans and other species.

To investigate the potential role of species differences in response to ANF, we also compared the effects of the same dose of ANF in six conscious rats and six conscious monkeys. Reductions in arterial pressure and cardiac output and increases in total peripheral resistance, similar to those observed in dogs, were observed both in intact rats and monkeys. After ganglionic blockade, ANF still reduced cardiac output and increased total peripheral resistance in conscious rats; however, these changes were attenuated significantly during the infusion of ANF but not during the recovery period. In contrast, in conscious monkeys with ganglionic blockade, the fall in cardiac output and rise in total peripheral resistance were abolished both during infusion of ANF and in the recovery period. These results suggest that the ANF-induced vasoconstriction is mediated in part by cardiovascular reflexes in rats and to a greater extent in monkeys. However, other factors must also be considered. If ANF were a potent arteriolar vasodilator, it would be expected to increase cardiac output in the presence of a minor reduction in arterial pressure, unless it also induced venodilation. However, even in

### Table 3. Effects of Atrial Natriuretic Factor in Six Conscious Monkeys

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ANF infusion*</th>
<th>Recovery†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean arterial pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>106±2.6</td>
<td>-14.3±2.1‡</td>
<td>-15.0±3.0‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>60±1.0§</td>
<td>-6.0±2.0§§</td>
<td>-2.3±2.0§</td>
</tr>
<tr>
<td><strong>Cardiac index (ml/min/kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>114.0±6.7</td>
<td>-30.2±4.7‡</td>
<td>-36.3±6.1‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>62.3±5.3§</td>
<td>+0.4±1.6§</td>
<td>-2.5±1.9§</td>
</tr>
<tr>
<td><strong>Total peripheral resistance (mm Hg/ml/min/kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>0.96±0.08</td>
<td>+0.16±0.03‡</td>
<td>+0.24±0.05‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>1.01±0.10</td>
<td>-0.11±0.02§§</td>
<td>+0.01±0.02§</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>174±3.9</td>
<td>+7.5±1.5‡</td>
<td>+10.3±2.0‡</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>107±9.3§</td>
<td>-3.0±0.8§§</td>
<td>-4.0±1.0§§</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>38±0.9</td>
<td>+1.8±0.3‡</td>
<td>...</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>33±4.0</td>
<td>+1.9±0.4‡</td>
<td>...</td>
</tr>
<tr>
<td><strong>ANF (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>64±20</td>
<td>+4.007±1.023‡</td>
<td>...</td>
</tr>
<tr>
<td>Ganglionic block</td>
<td>50±9</td>
<td>+5.083±1.572‡</td>
<td>...</td>
</tr>
</tbody>
</table>

*Values presented are at end of 30-minute infusion of 0.3 μg/kg/min×30 min atrial natriuretic factor (ANF).
†Values presented are at 15 minutes of recovery after infusion of ANF.
‡Significant change from baseline at p<0.05.
§Significant difference from intact, conscious monkeys at p<0.05.
the monkeys, in which a decrease in calculated total peripheral resistance with ANF was observed, cardiac output remained constant, although arterial pressure fell slightly. In view of these results, the concept of metabolic autoregulation must be considered, which would act to reduce total peripheral resistance by local mechanisms in the face of reduced arterial pressure and might be expressed more readily in the presence of ganglionic blockade, during which perfusion pressure was very low in the monkeys. It is also conceivable that in the monkeys a component of direct peripheral vasoconstriction still persisted, which was counteracted by local autoregulatory and metabolic mechanisms.

Another puzzling aspect of the integrated response to ANF is the lack of tachycardia in response to the hypotension in the conscious dogs and conscious rats. This phenomenon has been noted by others as well,10,55,56 although not uniformly,7,11,21,27,29 and is consistent with the concept that the hypotensive effect of ANF is not due simply to peripheral vasodilation, which would unload arterial baroreflexes and increase heart rate reflexly. In contrast to the bradycardiac effect observed in dogs, ANF elicited modest tachycardia in the conscious monkey. Modest tachycardia has also been noted in normal subjects.52,57 The increase in heart rate was no longer observed after ganglionic blockade in the present study, which is consistent with the concept that ANF elicits baroreflex-mediated effects on peripheral resistance in monkeys but not in conscious dogs.

Another major difference between the conscious dogs and monkeys was the response to ganglionic blockade, which did not reduce arterial pressure significantly in dogs but did so in monkeys. It is likely that sympathetic tone was highest in the conscious monkey, at an intermediate level in the conscious rat, and lowest in the conscious dog. With this in mind, it is possible to speculate that ANF induces reflex peripheral vasoconstriction more readily in the presence of enhanced sympathetic tone.

Clearly, ANF is a complex hormone with multiple mechanisms of action. Although ANF induces peripheral arteriolar vasoconstriction at the doses employed in this investigation, it may induce peripheral arteriolar vasodilation at massive doses.5,9,38,39 ANF has also been suggested to induce venodilation,11,21 venoconstriction,15 or no effect58 on the venous system. In conscious dogs, these are likely to be direct vasoconstrictor effects; in rats and monkeys, a combination of direct and reflex vasoconstrictor mechanisms appears to operate. In this connection, two other observations require mentioning. First, a major component of the reduction in cardiac output and increase in peripheral resistance occurred during the recovery period after cessation of the infusion of ANF and at a time when ANF levels had returned to baseline. Second, ANF increased hematocrit in conscious dogs, rats, and monkeys; this increase has been observed previously in nephrectomized rats59,60 and in intact humans.50 These data might suggest that fluid shifts resulted in a fall in circulating blood volume, as has been noted by others,59,60 which is an additional factor acting to increase peripheral resistance. These fluid shifts were not reflexly mediated; they were observed in all species studied in the presence of ganglionic blockade and in the conscious dogs with arterial baroreceptor and cardiac receptor denervation and in prior studies in humans without attendant increases in plasma catecholamines.50 Furthermore, the changes in hematocrit were not due to splenic contraction, since the current experiments were conducted in splenectomized dogs.

In summary, the present investigation demonstrates that in the conscious splenectomized dog, ANF, even at doses that increase circulating levels 50-fold, increases total peripheral resistance via a direct peripheral arteriolar vasoconstrictor mechanism, while it reduces cardiac output and increases hematocrit. These mechanisms are also apparent in conscious rats. However, in rats, a component of
the ANF-induced vasoconstrictor response is reflexly mediated. This component appears to be even greater in conscious monkeys. Thus, ANF is a complicated hormone eliciting a combination of direct and reflex effects on resistance vessels and also, potentially, on capacitance vessels, which are species dependent.

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References


KEY WORDS • atrial natriuretic peptide • cardiac output • total peripheral resistance • vasoconstriction • cardiac reflex • species difference
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Y T Shen, M A Young, J Ohanian, R M Graham and S F Vatner

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