Cardiovascular Effects of Atrial Natriuretic Factor in Anesthetized and Conscious Dogs

Hollis D. Kleinert, Massimo Volpe, Geoffrey Odell, Donald Marion, Steven A. Atlas, Maria J. Camargo, John H. Laragh, and Thomas Maack

SUMMARY Atrial natriuretic factor lowers blood pressure in normotensive and hypertensive animal models. The present study examined the mechanism of the blood pressure-lowering effect in 10 normotensive dogs. Four awake dogs previously instrumented with electromagnetic flow probes for measurement of cardiac output and catheters for systemic hemodynamic and cardiac dynamic measurements were studied. After a 30-minute control period, a 3 μg/kg bolus followed by 0.3 μg/min/kg of a 24-residue synthetic atrial natriuretic factor was infused for 30 minutes, followed by a 1-hour recovery period. Mean arterial pressure fell significantly during infusion (control, 125 ± 4; infusion, 108 ± 5; recovery, 125 ± 9 mm Hg; p < 0.05) and was accompanied by a slight but significant bradycardia (control, 144 ± 7; infusion, 134 ± 5; recovery, 145 ± 7 beats/min; p < 0.05). Significant reductions in cardiac output (control, 2.66 ± 0.60; infusion, 2.18 ± 0.60; recovery, 2.74 ± 0.60 L/min; p < 0.05), stroke volume (control, 18.4 ± 3.9; infusion, 16.0 ± 4.2; recovery, 19.0 ± 3.7 ml/beat; p < 0.05), and maximum increase in rate of change of left ventricular systolic pressure (control, 2475 ± 200; infusion, 2088 ± 216; recovery, 2487 ± 243 mm Hg/sec; p < 0.05) were also observed during infusion. No significant changes in total peripheral resistance or central venous pressure were noted, although the latter tended to fall during infusion. A similar pattern was observed in six pentobarbital-anesthetized dogs, except that infusion of atrial natriuretic factor did not induce bradycardia. These data indicate that mean arterial pressure is lowered in these models by a mechanism other than reduction in total peripheral resistance, namely a reduction in cardiac output. (Hypertension 8: 312-316, 1986)

Key Words • synthetic auriculin A • cardiac output • total peripheral resistance • normotensive dogs • cardiac dynamics • blood pressure regulation

Atrial natriuretic factor (ANF) is a peptide or group of peptides with both natriuretic and vascular actions. The natriuretic effect of ANF is likely due to its renal hemodynamic actions, which include an increase in efferent arteriolar tone and glomerular filtration rate (GFR) and may be accompanied by a redistribution of flow to the inner medulla.1,2

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Atrial natriuretic factor inhibits both receptor- and nonreceptor-mediated vasoconstriction of isolated rabbit aorta,4 as well as carbachol-induced contraction of chick intestinal smooth muscle.6 It appears that the initial degree of vascular tone can dictate whether ANF will elicit a vasoconstrictive or vasodilatory effect, as well as influence the magnitude of that effect. For example, in the functioning isolated kidney, perfused without vasoconstrictors, ANF causes a gradual, consistent rise in renal vascular resistance.4 On the other hand, in isolated kidneys preconstricted by hormonal (angiotensin II, norepinephrine, vasopressin) or nonhormonal (ouabain, tetracaine) agents, addition of ANF leads to a prompt decrease in renal vascular resistance.4,7 Further, ANF lowers blood pressure modestly in normotensive animals but profoundly in hypertensive animals.8-12

The present study examined, in normal, anesthetized, and untrained conscious dogs the question of whether the consistent blood pressure-lowering effect
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of ANF during steady state is due to a decrease in peripheral vascular resistance or cardiac output, or both.

Materials and Methods

Six female mongrel dogs (average weight, 18 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated, and placed on a Harvard respirator (Millis, MA, USA). A third intercostal thoracotomy was performed, and an electromagnetic flow probe (Howell Instruments, Camarillo, CA, USA) placed on the aorta for the measurement of cardiac output (CO) using an SP2202 Blood Flowmeter (Gould/Statham, Oxnard, CA, USA). Catheters were placed in the left ventricle through the aorta to measure the maximum rate of change of pressure (dP/dt), in the pulmonary capillary bed through the jugular vein using a Swan-Ganz F7 catheter for the measurement of pulmonary wedge pressure, and in the aorta through the femoral artery for the continuous measurement of mean arterial pressure (MAP) and heart rate. All pressures were measured with Statham transducers (model P23Db) and recorded on a Gould recorder 2400S (Saddlebrook, NJ, USA). An intravenous line was connected to a Harvard infusion pump (model 975) for the infusion of saline and synthetic ANF (California Biotechnology, Mountain View, CA, USA). Urine was collected through a Foley catheter inserted in the bladder for urine volume measurements, and electrolytes determined by potentiometry (NOVA, Newton, MA, USA).

Four untrained female mongrel dogs were studied awake. The dogs were instrumented 3 days before experimentation began. Catheters were filled with heparin, tunneled under the skin, and exteriorized at the nape of the neck. The same general procedures as those described for the anesthetized dogs were used, except that central venous pressure, from a femoral catheter advanced deep into the thoracic vena cava, was monitored instead of pulmonary wedge pressure.

In the anesthetized dogs, three 10-minute control periods were performed, followed by a bolus of synthetic ANF (auriculin A), 3.0 μg/kg body weight, and a 30-minute constant infusion of 0.3 μg/min/kg body weight. A 1-hour recovery period followed the infusion. The blood pressure was monitored continuously, and all other parameters were measured at 1-minute intervals during control and experimental periods and at 15-minute intervals during recovery periods. Saline was infused during surgery (150 ml as a prime followed by a 2.5 ml/min) and continued throughout the experiment. The same protocol was used in conscious dogs.

Auriculin A is a 24 amino acid peptide and was provided by California Biotechnology or acquired from Peninsula Labs (Belmont, CA, USA).

Total peripheral resistance (TPR) was calculated as the quotient of MAP and CO, multiplied by 80 to derive the units (dyn·sec·cm⁻²). Stroke volume was calculated as the quotient of CO divided by heart rate. The absolute maximum dP/dt was determined from the first derivative of the left ventricular systolic pressure curve and corrected for changes in afterload by dividing by diastolic pressure (dP/dt/P). Data are presented as means ± SE and were compared by paired t test, using each dog as its own control. Significance was accepted if p was less than 0.05.

Results

The average values of the hemodynamic as well as urinary excretory functions in anesthetized dogs during control, infusion, and recovery are shown in Table 1. The MAP fell significantly (12 ± 1%) after 10 minutes of infusion and further after 30 minutes of infusion (18 ± 3%) but returned to control levels during recovery. This change was not associated with a change in heart rate. During the first 10 minutes of infusion TPR tended to fall (5 ± 3%); however, TPR rose slightly at 30 minutes of infusion (4 ± 7%) and continued to rise during recovery (27 ± 15%). None of these changes in TPR were statistically significant. Conversely, CO fell significantly by 8 ± 3% and further by 20 ± 4% of control at 10 minutes and 30 minutes of infusion, respectively, but did not completely reverse during recovery (10 ± 3%). The fall in CO was associated with a significant decrease in maximum dP/dt (12 ± 3%) after 30 minutes of infusion that was not completely reversed during recovery (8 ± 2%). When corrected for the reduction in afterload, as shown by dP/dt/P values, no change or a slight increase in dP/dt was noted. No significant change in pulmonary wedge pressure was noted, although the values showed a slight tendency to fall during infusion. As shown in Table 1, stroke volume was reduced significantly (19 ± 6%) at 30 minutes of infusion. These hemodynamic and cardiac dynamic changes were accompanied by a significant diuresis (144 ± 36%) and natriuresis (166 ± 83%), while kaliuresis was not significantly altered.

The effect of synthetic ANF infusion on MAP, heart rate, TPR, CO, maximum dP/dt, corrected dP/dt (dP/dt/P), central venous pressure, and stroke volume in conscious dogs is shown in Table 2. The MAP began to fall immediately, was significantly reduced by 10 minutes (12 ± 1%), and continued to fall throughout the infusion period (16 ± 2% at 30 minutes). Unlike the results in the anesthetized dogs, a slight (7 ± 2%) but significant bradycardia was observed after 30 minutes of infusion in conscious dogs. As in the anesthetized dogs, TPR was not altered significantly and CO was reduced significantly at 30 minutes (21 ± 5%). A significant reduction in absolute maximum dP/dt was observed (8 ± 2% at 10 minutes; 15 ± 6% at 30 minutes) and was attributable, at least in part, to a reduction in afterload. Central venous pressure was not altered significantly. All values returned to control levels during the recovery period. The calculated stroke volume was significantly reduced by 15 ± 5% at 30 minutes but returned to control level during recovery. Auriculin infusion caused a slight increase in urine output with only an average twofold increase in urinary sodium excretion.
and no change in urinary potassium excretion (measurements taken in 2 of 4 dogs; data not shown).

**Discussion**

The present study confirms that synthetic ANF lowers blood pressure and demonstrates that in normal anesthetized and untrained conscious dogs this effect is due mainly to a decrease in CO rather than a decrease in TPR. During the first 10 minutes of ANF infusion, calculated TPR tended to fall. It then returned to or above control levels in the remainder of the infusion periods and in the recovery period. Thus, ANF had only a transient vasodilatory effect, and MAP continued to fall while peripheral resistance was not different.

### Table 1. Effects of a 30-Minute Infusion (3 µg/kg Bolus, 0.3 µg/min/kg Constant Infusion) of Synthetic Auriculin on Cardiovascular and Urinary Excretory Parameters in Six Anesthetized Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control*</th>
<th>At 10 min</th>
<th>At 30 min</th>
<th>Recovery†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>126±11</td>
<td>111±10†</td>
<td>105±12‡</td>
<td>128±11</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>152±12</td>
<td>136±14‡</td>
<td>129±16‡</td>
<td>155±12</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>109±11</td>
<td>98±11‡</td>
<td>94±12‡</td>
<td>114±11</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>132±9</td>
<td>135±10</td>
<td>131±11</td>
<td>132±11</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>1.56±0.11</td>
<td>1.43±0.12‡</td>
<td>1.25±0.13‡</td>
<td>1.39±0.10‡</td>
</tr>
<tr>
<td>Total peripheral resistance (dyn·sec·cm⁻⁵)</td>
<td>6569±545</td>
<td>6310±606</td>
<td>6910±879</td>
<td>7495±746</td>
</tr>
<tr>
<td>Maximum +dP/dt (mm Hg/sec)</td>
<td>2087±111</td>
<td>2048±101</td>
<td>1834±118‡</td>
<td>1930±141‡</td>
</tr>
<tr>
<td>Corrected dP/dt§</td>
<td>20±6</td>
<td>23±8‡</td>
<td>21±8</td>
<td>18±7</td>
</tr>
<tr>
<td>Pulmonary wedge pressure (mm Hg)</td>
<td>9.4±1.2</td>
<td>8.7±1.8</td>
<td>9.0±1.4</td>
<td>7.5±1.8</td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>12.1±1.2</td>
<td>11.1±1.4</td>
<td>10.0±1.4</td>
<td>10.5±1.2</td>
</tr>
<tr>
<td>Urinary volume (ml/min)</td>
<td>0.53±0.18</td>
<td>1.15±0.38‡</td>
<td>0.96±0.35</td>
<td>0.16±0.04</td>
</tr>
<tr>
<td>U₉ NaV (µEq/min)</td>
<td>131±46</td>
<td>242±82‡</td>
<td>228±76‡</td>
<td>20±5‡</td>
</tr>
<tr>
<td>U₉ KV (µEq/min)</td>
<td>41±5</td>
<td>75±20</td>
<td>56±13</td>
<td>32±10</td>
</tr>
</tbody>
</table>

Values are means ± SE. dP/dt = rate of change of left ventricular systolic pressure; U₉ NaV = urinary sodium excretion, U₉ KV = urinary potassium excretion.

*Control = mean of three 10-minute control periods.
†Recovery = 1 hour.
‡p < 0.05, compared with control values.
§( + dP/dt)/P.

### Table 2. Effects of a 30-Minute Infusion (3 µg/kg Bolus, 0.3 µg/min/kg Constant Infusion) of Synthetic Auriculin on Systemic Hemodynamics and Cardiac Dynamics in Four Conscious Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control*</th>
<th>At 10 min</th>
<th>At 30 min</th>
<th>Recovery†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>129±4</td>
<td>113±3‡</td>
<td>108±5‡</td>
<td>125±9</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>173±6</td>
<td>161±4‡</td>
<td>155±10‡</td>
<td>173±8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>101±4</td>
<td>90±8</td>
<td>89±7‡</td>
<td>101±8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>144±6</td>
<td>140±7</td>
<td>134±5‡</td>
<td>145±7</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>2.66±0.6</td>
<td>2.48±0.6</td>
<td>2.18±0.6</td>
<td>2.74±0.6</td>
</tr>
<tr>
<td>Total peripheral resistance (dyn·sec·cm⁻⁵)</td>
<td>4129±658</td>
<td>3982±694</td>
<td>4464±787</td>
<td>4071±756</td>
</tr>
<tr>
<td>Maximum +dP/dt (mm Hg/sec)</td>
<td>2475±200</td>
<td>2288±187‡</td>
<td>2088±216‡</td>
<td>2487±243</td>
</tr>
<tr>
<td>Corrected dP/dt§</td>
<td>24±3</td>
<td>24±2</td>
<td>23±3</td>
<td>25±4</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>7.4±1.4</td>
<td>7.3±1.6</td>
<td>6.5±1.6</td>
<td>10±1.7</td>
</tr>
<tr>
<td>Stroke volume (ml/beat)</td>
<td>18.4±3.9</td>
<td>17.4±3.4</td>
<td>16.0±4.2‡</td>
<td>19.0±3.7‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. dP/dt = rate of change of left ventricular systolic pressure.
*Control = mean of three 10-minute control periods.
†Recovery = 1 hour.
‡p < 0.05, compared with control values.
§( + dP/dt)/P.
from control levels. The initial response is consistent with the vascular response to a slow bolus of ANF. The reason for the bimodal effect of ANF on TPR is not entirely clear. A similar effect was noted in the renal vasculature of anesthetized and conscious dogs. In isolated kidneys perfused in the absence of vasoconstrictors, ANF causes a modest but sustained increase in renal vascular resistance. It is possible that ANF is a primary vasodilator, which would account for the initial fall in TPR, but that it secondarily releases a vasoconstrictor substance or elicits a reflex compensation that nullifies or even overcomes its vasorelaxant effect.

A fall in vascular resistance may contribute to the ANF-induced reduction in blood pressure in other experimental circumstances. This mechanism would be especially likely when baseline resistance is elevated by high circulating levels of vasoconstrictors, such as angiotensin II and norepinephrine, which could be antagonized in vivo by ANF, as has been demonstrated in vitro. We have shown that ANF antagonizes angiotensin II–induced contraction in isolated aorta to a greater extent than that induced by other vasoconstrictors. This finding may explain the greater sensitivity to the blood pressure–lowering effect of ANF in renin-dependent hypertension. In addition, it cannot be excluded that the net effect on TPR will depend on the dose of administered ANF. Conceivably, much higher doses than those used in the present study could lead to a net decrease in TPR. Whatever the mechanism, the present data clearly indicate that ANF is able to decrease MAP without a significant change in TPR. These results are consistent with the findings of Lappe et al., who noted a reduction of blood pressure but were unable to demonstrate systemic vasodilation in response to synthetic atriopeptin II in the conscious rat.

The exact mechanism by which ANF decreased CO cannot be decided by the present experiments. Heart rate was unchanged in anesthetized dogs, and, therefore, did not contribute to the fall in CO. Although the dose of synthetic peptide employed in this study induced natriuresis, the fall in CO was not secondary to external fluid losses, since these were replaced with saline. With respect to blood volume, the venous return was assessed using either pulmonary wedge pressure (anesthetized dogs) or central venous pressure (conscious dogs). Venous pressure, which was relatively high during control due to saline-induced volume expansion, fell slightly but not significantly during ANF infusion according to both methods. Since neither measurement can accurately assess venous pooling unless pooling is marked, the possibility that venodilation leads to a reduction in venous return and thereby in stroke volume cannot be ruled out. Indeed, stroke volume did fall significantly. Although stroke volume may have been reduced by a possible decrease in contractility, as suggested by the reduction in maximum dP/dt, this appears to be unlikely since, when corrected for a reduction in afterload, dP/dt was unchanged or slightly improved during ANF infusion. Any effect on cardiac contractility may have been indirect (e.g., altered Starling forces due to decreased left ventricular filling, or enhanced vagal activity offsetting reflex tachycardia with no net change in heart rate). The present data do not allow for a distinction among the possibilities.

It is unlikely that the ANF effects observed herein were due to anesthesia, since similar patterns of response were noted in anesthetized and conscious dogs. An important exception was the slight but significant bradycardia observed in conscious dogs during ANF infusion, which may have further contributed to the decrease in CO and MAP. This bradycardia may have resulted from enhanced vagal activity, a response that could have been depressed by anesthesia. In this regard, Ackermann et al. have reported that crude atrial extracts reduce blood pressure and heart rate in normal rats only when the vagi are intact. In their experiments, as in the present ones, the hypotensive response was due to a reduction in CO and not in systemic vascular resistance. The mean heart rate of our conscious dogs was higher than values reported by some but similar to those reported by others. However, the baseline heart rates in our study did not influence the cardiovascular response, since individual dogs with heart rates in the lower end of the range displayed a response that was identical to those with higher values.

From the present data as well as previous studies, it appears that the effects of ANF on blood pressure, blood flow, and vascular resistance depend, both in magnitude and direction, on the particular experimental model employed. In the present models, anesthetized and untrained conscious dogs, the steady state ANF-induced decrease in MAP apparently was due predominantly to a decrease in CO rather than a decrease in TPR. Whether this decrease represents a physiological mechanism of blood pressure control cannot be answered until information is available regarding the normal circulating levels of ANF and the factors affecting its secretion. Our findings indicate, however, that in addition to its established vascular and renal effects, ANF has other cardiovascular actions that may play an important role in overall homeostasis of the cardiovascular system.

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References

4. Borenstein HB, Cupples WA, Sonnenberg H, Veress AT. The effects of a natriuretic atrial extract on renal hemodynamics
and urinary excretion in anesthetized rat. J Physiol (Lond) 1983;334:133–140
5. Kleinert HD, Maack T, Atlas SA, Januszewicz A, Sealey JE, Laragh JH. Atrial natriuretic factor inhibits angiotensin-, nor-
epinephrine-, and potassium-induced vascular contractility. Hypertension 1984;6(suppl I):I-143–1-I-147
8. Volpe M, Odell G, Kleinert HD, et al. Effect of atrial natriuretic factor on blood pressure, renin, and aldosterone in Gold-
12. Pegrum BL, Tnppodo NC, Cole FE, MacPhee AA. Hypotensive effect of atrial natriuretic factor (rANF) in conscious Wis-
tar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. [Abstract]. Fed Proc 1984;43:453
621–627
19. Olsen CO, Tyson GS, Maier GW, Spratt JA, Davis JW, Ran-
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H D Kleinert, M Volpe, G Odell, D Marion, S A Atlas, M J Camargo, J H Laragh and T Maack

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