SUMMARY We sought to demonstrate a hypotensive effect from infusions of atrial natriuretic factor (ANF) into humans and to describe the mechanism(s) of this effect. Cardiovascular and hormonal responses to human ANF-(99-126) (125 ng/kg bolus followed by a 30-minute infusion at 25 ng/kg/min) were determined in eight conscious volunteers and compared with responses of eight time-control subjects who received isotonic saline. Baseline levels of ANF (52.8 ± 5.5 pg/ml) increased 8.8-fold after 30 minutes of ANF infusion but were unchanged in the time controls. Plasma levels of renin, aldosterone, vasopressin, sodium, potassium, and osmolality did not change during infusions. A transient 5% reduction in mean arterial pressure related to a 12% reduction in peripheral resistance was observed 10 minutes after the priming bolus of ANF. This response was not sustained during the remainder of the ANF infusion period, nor did it occur in two additional subjects who received ANF infusions without the priming bolus. Steady state responses consisted of significant reductions in central venous pressure (15%), stroke volume (13%), and cardiac output (10%), but no reduction in blood pressure. Plasma norepinephrine levels and peripheral resistance increased (34% and 9%, respectively) during ANF administration. These data indicate that steady state responses to ANF in humans consist of decreases in cardiac filling pressures, which reduce cardiac output, unload cardiopulmonary baroreceptors, and activate the sympathetic nervous system. Blood pressure is well maintained despite striking increases in plasma ANF. (Hypertension 11: 537-544, 1988)

KEY WORDS • atrial natriuretic factor • blood pressure • sympathetic nervous system • central venous pressure

Atrial natriuretic factor (ANF) is a 28 amino acid peptide that circulates in human plasma. It is synthesized in secretory granules located primarily within the atrial myocytes and is released from these storage sites during atrial distention. ANF produces diuresis, natriuresis, and vasorelaxation. In addition, ANF may also inhibit renin, aldosterone, and vasopressin release and produce intravascular fluid shifts.

The cardiovascular effects, particularly the hypotension associated with ANF infusions, have been inconsistent in past reports despite the fact that ANF has well-described, potent vasodilating effects. Systemic administration of ANF into humans appears to reduce blood pressure only when it is given as a bolus or infused in pharmacological doses. Several recent studies in anesthetized and conscious experimental animal preparations indicate that ANF-induced hypotension may not be due to reductions in peripheral resistance but is more often associated with reductions of cardiac output. In the present study, we employed several infusion protocols and complete hemodynamic monitoring to evaluate ANF's cardiovascular effects in human volunteers. Both bolus and continuous infusions of the 28 amino acid human ANF-(99-126), which is the primary form of ANF that circulates in human plasma, were used, while direct arterial pressure, central venous pressure, and cardiac output were monitored and plasma norepinephrine levels were determined.

Our data indicate that bolus administration of ANF produces dynamic responses consisting of transient hypotension mediated by reductions in peripheral resistance. However, continuous infusions of ANF pro-
duce steady state effects that include reductions of cardiac filling pressures, which activate the sympathetic nervous system to maintain blood pressure at baseline levels.

**Subjects and Methods**

Sixteen healthy volunteers, ranging in age from 20 to 28 years, participated in this study after providing informed written consent. Eight subjects received ANF infusions, and eight served as time controls and received isotonic saline. Subjects were randomized to these groups and not informed of the nature of the infusate until studies were completed.

Subjects were instructed to eat a morning meal with no added salt and to abstain from substances containing caffeine, theophylline, and nicotine. Overnight fluid deficits were replaced with 750 ml of orally administered fluids at breakfast. The subjects took nothing by mouth after this meal and arrived in the laboratory at 0900.

**Instrumentation**

An 18-gauge catheter was inserted into a peripheral vein and kept patent by a slow infusion of isotonic saline at 30 ml/hr. Another catheter was inserted under sterile conditions into a jugular vein and advanced into an intrathoracic vein to monitor central venous pressure. A radial artery catheter was inserted for direct blood pressure measurements and for blood sampling. Central venous pressure, arterial pressure, and an electrocardiogram were displayed on an oscilloscope. Digital readings of pressure and heart rate were updated each cardiac cycle.

An impedance cardiograph (Surcom, Minneapolis, MN, USA) was employed to obtain continuous measurements of stroke volume and cardiac output. This method has been described and validated in our laboratory and elsewhere. Briefly, this method consists of placement of three circumferential electrodes about the neck and abdomen and a fourth electrode on the forehead. A 100-kHz, 4-mA current is transmitted to the outer two electrodes, and impedance changes within the thorax are detected from the inner two electrodes. Stroke volume is derived from standard calculations. A heart sound microphone placed on the anterior chest wall is employed to aid in the determination of ventricular ejection time.

Absolute stroke volumes derived from impedance cardiograms compare favorably with those derived from standard invasive methods. Most correlations have been greater than 0.8. Furthermore, our previous studies have demonstrated that this method is exquisitely sensitive to small changes in left ventricular stroke volume in humans when compared with simultaneous left ventriculograms (r = 0.89). This method was therefore used to monitor beat-by-beat responses to ANF infusions.

Systemic vascular resistance was expressed as the ratio of mean arterial pressure and cardiac output (in units of dyn·sec·cm⁻²). Forearm blood flow and distensibility were measured using standard venous occlusion plethysmography employing a mercury-in-Silastic, temperature-compensated strain gauge and saddle placed about the forearm. Forearm vascular resistance was calculated as the ratio of mean arterial pressure to forearm blood flow. Forearm venous distensibility was calculated from measurements of forearm girth during random intermittent inflations (20, 35, and 50 mm Hg) of a congesting cuff on the upper arm.

**Procedures**

Subjects voided and were then instrumented while supine. Thirty minutes after instrumentation, baseline hemodynamic recordings were obtained and 50 ml of arterial blood was withdrawn for determination of plasma vasopressin, aldosterone, renin activity, catecholamines, ANF, electrolytes, hematocrit, and osmolality. ANF (0.2 mg mixed in 10 ml of preservative-free 0.9 normal saline and filtered through a 0.22-μm filter) was mixed immediately before each study and refrigerated. In six volunteers, ANF was given as a 125 ng/kg intravenous bolus and was followed by a continuous 30-minute infusion at 25 ng/kg/min. In two additional subjects, the initial priming bolus of ANF was not given before the 30-minute infusion period. Equal volumes of isotonic saline were given to eight volunteers.

Continuous hemodynamic data were recorded on a strip chart recorder (Model 7, Grass, Quincy, MA, USA) and simultaneous digital displays were observed. Forearm blood flow was obtained at 30-second intervals for the first 10 minutes of infusion and during the 28th to 30th minute of infusion. Blood sampling was repeated in all subjects after completion of the 30-minute infusion period. This procedure was followed by measurements of forearm distensibility while ANF infusions continued. Hemodynamic data obtained during the control period and during the 10th and 30th minute of infusions were statistically analyzed, as were biochemical data obtained during control and at 30 minutes of infusion. Results were compared with Student's paired and unpaired t tests and analysis of variance (ANOVA).

**Biochemical Analyses**

Plasma arginine vasopressin concentration was determined using antisera and radioimmunoassay procedures described previously. Plasma renin activity was analyzed by a modification of the method of Sealey and Laragh using angiotensin I antisera kindly provided by Dr. Jean Sealey. Plasma aldosterone was extracted with dichloromethane and measured using highly specific antisera (Diagnostic Products, London, Ontario, Canada).

Norepinephrine was determined from plasma samples that were first adsorbed onto alumina, then eluted, filtered, and applied to a 5-μm, 4.5 x 250-mm octadecyl reverse-phase column (IBM, Wallingford, CT, USA), and separated under isocratic conditions using a 0.1 M phosphate buffer containing Na₂EDTA, NaOH, and Pic B8 (Waters, Milford, MA, USA; pH = 4.8;
The internal standard used was 3,4-dihydroxy-benzylamine (Sigma Chemical, St. Louis, MO, USA). Catecholamines were calculated from the peak heights using an integrator. The sensitivity of the system was 20 to 25 pg/200 μl injectate, with a 98.5% recovery of norepinephrine.

Plasma ANF was extracted (1 ml) and reconstituted for analysis from plasma collected in tubes containing EDTA, aprotinin, and soybean trypsin inhibitor as described previously. The assay procedure was modified for the measurement of ANF in human plasma by using antisera raised against human ANF-(99–126) (Peninsula Laboratories, Belmont, CA, USA). The tracer used was [125I]human ANF-(99–126) obtained from Amersham (Arlington Heights, IL, USA). Recovery of increasing amounts of this standard added to human plasma was 82%, while recovery of [123I]ANF was 92%. The sensitivity of the assay was 3 pg/tube, and the concentration of peptide that produced 50% binding was 17 to 20 pg/tube. Interassay and intrasay coefficients of variation averaged less than 10%.

Plasma sodium and potassium were determined using ion sensitive electrodes (Nova-1; Nova Biochemical, Boston, MA, USA). Plasma osmolality was determined using freezing point depression osmometry (Osmette A; Precision Systems, Boston, MA, USA).

**Results**

In the present study, eight volunteers received ANF infusions while eight served as time controls and received isotonic saline infusions. Baseline hemodynamics (Table 1) and plasma levels of ANF, renin activity, vasopressin, and norepinephrine (Table 2) did not differ between groups.

Thirty-minute infusions of ANF increased plasma levels of ANF 8.8-fold, as shown in Table 2. Neither 30-minute ANF nor placebo infusions altered hemato-

crit, electrolytes, and osmolality, nor did these infusions influence plasma renin activity, aldosterone, or vasopressin (see Table 2). There was a significant 34% increase in plasma norepinephrine during ANF infusions.

Figure 1 demonstrates the progressive reduction in central venous pressure produced by ANF infusion. This reduction was significantly different from the time-control group response within 10 minutes of initiating infusions.

Figure 2 summarizes the 10- and 30-minute responses of the other cardiovascular variables during infusions. Mean arterial pressure and systemic vascular resistance decreased by 4.8 ± 1.2 mm Hg and 201 ± 60 dyn·sec·cm⁻¹, respectively, at 10 minutes into ANF infusions. However, these responses were not sustained over the remaining 20 minutes of infusion; mean pressure returned to baseline, while systemic vascular resistance was increased significantly (9%) above baseline. Stroke volume and cardiac output declined by 11.3 ± 4 ml/beat (13%) and 0.5 ± 0.18 L/min (10%) after 30 minutes of ANF infusion.

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**Table 1. Baseline Cardiovascular Data Obtained from Supine Volunteers**

<table>
<thead>
<tr>
<th>Cardiovascular variable</th>
<th>Time control (n = 8)</th>
<th>ANF (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>59.9 ± 3.7</td>
<td>60.1 ± 4.7</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>95.2 ± 10.1</td>
<td>85.5 ± 4.3</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>5.4 ± 0.4</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td>143.1 ± 2.1</td>
<td>142.5 ± 6.2</td>
</tr>
<tr>
<td>Diastolic pressure (mm Hg)</td>
<td>73.8 ± 1.6</td>
<td>74.2 ± 2.5</td>
</tr>
<tr>
<td>Systemic vascular resistance (dyn·sec·cm⁻²)</td>
<td>1473.0 ± 121.0</td>
<td>1650.0 ± 232.0</td>
</tr>
<tr>
<td>Forearm vascular resistance (mm Hg·ml⁻¹·min⁻¹·100 ml)</td>
<td>27.3 ± 6.9</td>
<td>34.1 ± 8.1</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>6.1 ± 0.7</td>
<td>7.2 ± 1.0</td>
</tr>
</tbody>
</table>

Data are means ± SEM.

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**Table 2. Plasma Values from Supine Volunteers**

<table>
<thead>
<tr>
<th>Plasma concentrations</th>
<th>Time control</th>
<th>ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Δ30 min</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.3 ± 1.0</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>139.0 ± 1.0</td>
<td>1.1 ± 2.2</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>3.8 ± 0.1</td>
<td>-0.1 ± 0.1</td>
</tr>
<tr>
<td>Osmolarity (mosm/kg)</td>
<td>286.9 ± 1.1</td>
<td>-1.0 ± 0.9</td>
</tr>
<tr>
<td>Renin activity (ng Ang I/ml/hr)</td>
<td>1.9 ± 0.6</td>
<td>-0.5 ± 0.3</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>2.6 ± 0.4</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td>Vasopressin (pg/ml)</td>
<td>3.5 ± 0.4</td>
<td>-0.1 ± 0.3</td>
</tr>
<tr>
<td>ANF (pg/ml)</td>
<td>37.2 ± 4.6</td>
<td>3.1 ± 2.4</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>107.5 ± 23.0</td>
<td>-14.5 ± 5.7</td>
</tr>
</tbody>
</table>

Data are means ± SEM. Δ30 min = average change from respective baseline after 30-minute infusions; Ang I = angiotensin I.

*p < 0.05, compared with respective baseline value.
†p < 0.05, compared with time control.
Figure 1. Central venous pressure response to 30-minute infusions of ANF or placebo (time control). Single asterisk indicates response significantly different between groups \( (p < 0.05) \). Double asterisk indicates significant reduction from baseline and significantly different response from that of the time-control group \( (p < 0.01) \).

Forearm venous distensibility curves are depicted in Figure 3. These were virtually identical in the two groups during baseline conditions. ANF infusions resulted in a slight increase and placebo infusions resulted in a slight decrease in forearm distensibility. The net statistical effect was an apparent augmentation of forearm distensibility during ANF infusions \( (p < 0.05) \).

The cardiovascular responses of two subjects to continuous 30-minute infusions of ANF \( (25 \text{ ng/kg/min}) \) are shown in Figure 4. In these volunteers, a priming bolus was not given before initiating infusions and there were no initial reductions in blood pressure or peripheral resistance. Steady state responses and plasma ANF levels after 30-minute infusions were similar to those of the volunteers who had received an initial priming bolus of ANF. This similarity consisted of a 30% increase in plasma norepinephrine and a significant increase in peripheral resistance (see Figure 4).

**Discussion**

Studies in experimental animals and the few reports in humans have revealed inconsistent blood pressure responses to ANF infusions despite its well-known potent vasodilating effects. In the present study, we recorded dynamic responses to ANF infusions when priming boluses preceded infusions. These responses consisted of a transient hypotension mediated by reductions in peripheral resistance. However, steady state effects of ANF appear to activate the sympathetic nervous system (independently of arterial baroreceptor reflexes); plasma norepinephrine and peripheral resistance were elevated within 30 minutes of infusion. These responses may have been mediated by low-pressure baroreceptor reflexes: ANF reduced cardiac filling pressures, which decreased afferent signals from cardiopulmonary baroreceptors and elicited reflex increases of sympathetic outflow.

**Infusions**

In six volunteers, ANF was administered as an initial priming bolus of 125 ng/kg followed immediately by a continuous infusion at 25 ng/kg/min. This protocol was derived with knowledge of the pharmacokinetic principles of this short-lived peptide. We sought to initiate immediate elevations of plasma ANF and to maintain relatively constant levels for 30 minutes by continuous infusion of this peptide. Steady state plasma levels of ANF after 30 minutes of continuous infu-
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Figure 3. Forearm distensibility curves were virtually identical between groups at baseline (top graph) but were significantly greater ($p < 0.05$, by ANOVA) after 30 minutes of ANF infusions compared with the time-control (placebo) group (bottom graph).

sion of ANF were $519 \pm 56$ pg/ml. This value is an eightfold increase from basal levels and is similar to that seen in pathophysiological states such as congestive heart failure, renal failure, and severe liver cirrhosis.29-32 Similar plasma levels of ANF were attained without a priming dose of ANF in two subjects. However, these levels likely were established more gradually than in those receiving priming boluses of ANF.

Early Transient Responses to ANF

Ten minutes ($\pm 0.6$ minute) after initiating infusions with a priming bolus of 125 ng/kg, arterial pressure had declined to its minimum. Significant reductions of systolic (3%), diastolic (7%), and mean pressure (5%) occurred. This response was secondary to a decrease in systemic vascular resistance. Forearm vascular resistance tended to decline as well, but this response was not significant. Continuous infusions of ANF that were not preceded by a priming bolus did not elicit hypotension or reduce peripheral resistance. These findings suggest that the blood pressure-lowering effect of ANF is dependent on either the rate of change in plasma ANF levels or the absolute plasma concentration of this peptide (or both). Support for the latter possibility is derived from the data of Ohashi et al.14: Continuous infusions (without a priming bolus) of human ANF-(99–126) (100 ng/kg/min; four times the dose used in the study) into supine volunteers lowered mean blood pressure by 10 mm Hg. This decrease was associated with significant elevations of plasma cyclic guanosine 3',5'-monophosphate (which may induce vasodilation). Of interest, 100 ng/kg/min infusions of the synthetic rat (25 amino acid sequence) ANF peptide did not lower blood pressure in the human volunteers studied by Cody et al.33 Thus, the molecular sequence may be an important parameter in determining the vascular responsiveness in humans.

In the present study, the reduction in peripheral resistance after bolus administration of ANF was not due to decreases in forearm resistance. However, potent forearm vasodilator responses have been demonstrated when ANF is infused directly into the brachial artery of humans.11 Furthermore, continuous incremental systemic infusions of ANF into humans produce progressive increases in forearm skin blood flow.28 We cannot exclude the possibility that other vascular beds (e.g., renal and splanchnic) may have been involved in this transient reduction in resistance.34-36 Furthermore, pathophysiological boluses of ANF into humans may

Figure 4. Cardiovascular responses of two subjects to 30-minute infusions of ANF (25 ng/kg/min). These infusions were initiated without a priming bolus. TPR = total peripheral resistance.
have transiently reduced sympathetic neural outflow and vascular resistance, thereby promoting an initial hypotension.37

Steady State Effects of ANF

The initial decreases in systemic vascular resistance and blood pressure produced by ANF were not sustained despite continuous infusions of the peptide. The major effects of steady state infusions of ANF (either with or without the priming bolus) were on central venous pressure and cardiac output. Central venous pressure declined progressively (to 15% below baseline) during each 30-minute infusion period. Several earlier studies in conscious experimental animals15-18 and humans33 also demonstrated reductions in central venous pressure, right atrial pressure, and pulmonary capillary wedge pressure during ANF infusions. These reductions in cardiac filling pressures may have been due to increases in venous capacitance or to enhanced renal excretion of fluid volume. However, support for these two possibilities is weak, since ANF is not a powerful venodilator4 and ANF infusions provoke marked reductions in circulating blood volume in anephric animals.38, 39 A more likely explanation for the consistent reductions in central venous pressure noted during ANF infusions is that intravascular fluids shift to extravenous sites through alterations in Starling forces (particularly hydraulic conductivity) within the capillary beds.40, 41 In the present study, measurements of forearm volume changes during venous occlusion revealed greater volume increases during ANF infusions. This effect may be due to compliance or permeability changes in the forearm. If this isolated response occurs throughout the entire vascular tree, it is not difficult to reconcile the significant reductions in central venous pressure produced by ANF.

The reduction in cardiac output noted during ANF infusions has been an inconsistent finding in previous reports. This response has been demonstrated in several conscious animal preparations,15-18 while studies in humans33 have failed to demonstrate such an effect. None of these reports employed the 28 amino acid circulating form of this peptide in human plasma.1

Other ANF analogues may have subtle differences in biological activities.32, 43 Our findings of reduced stroke volume and cardiac output during ANF infusions are strengthened by the demonstration of the absence of change in these parameters in the time-control population. The reductions in stroke volume and cardiac output during ANF infusions are most likely due to Frank-Starling mechanisms involved by reductions in cardiac filling pressures, since ANF does not appear to reduce cardiac inotropy directly.44

Sympathetic Nervous System Activation

We were unable to demonstrate a sustained reduction in blood pressure or peripheral resistance in human volunteers receiving ANF infusions, despite the well-described vascular smooth muscle relaxing effect of this peptide. In contrast, ANF infusions resulted in an elevation of peripheral resistance. Several recent studies in conscious rats, sheep, and dogs have also demonstrated that peripheral resistance does not decrease and may increase markedly during ANF infusions.15-18 We believe this response is predictable based on our understanding of cardiopulmonary baroreceptor physiology. ANF-induced reductions in central venous pressure unload low pressure cardiopulmonary baroreceptors and elicit vasconstriction.55, 45

In support of this explanation (i.e., a reflex neural mechanism eliciting resistance increases), we and others10, 47, 48 have documented elevations of plasma norepinephrine during ANF infusions. These elevations in norepinephrine do not appear to be related to arterial baroreceptor reflex activation in response to the initial transient drop in blood pressure produced by ANF because plasma norepinephrine increased similarly during infusions of ANF that were not preceded by boluses and therefore did not provoke hypotension.

We reason that reductions in central venous pressure did not elicit significantly measurable reflex forearm vasconstriction in the present study because direct vascular effects of ANF that increase forearm skin and muscle blood flow11, 28 may have offset measurable reflex increases in forearm vascular resistance.

Hormonal Responses to ANF Infusions

We were unable to demonstrate significant reductions in renin, aldosterone, and vasopressin in our investigation despite in vitro evidence that ANF inhibits the release of these hormones.7, 8 We speculate that the pronounced reductions in central venous pressure produced by ANF in the present study may have offset potential ANF-induced reductions in these hormones. Furthermore, the short infusion protocol, which was sufficient to demonstrate profound cardiovascular effects of ANF, may have been insufficient to provoke more gradual hormonal responses. Larger doses of ANF infused into seated or salt-depleted human volunteers over longer periods appear to reduce plasma concentrations of these hormones.33, 47, 48

Our results indicate that infusions of human ANF-(99-126) that produce pathophysiological elevations of plasma ANF do not produce sustained reductions in blood pressure in humans. A 125 ng/kg priming bolus of human ANF-(99-126) provoked only transient reductions in peripheral resistance and blood pressure. These responses were not sustained during continuous infusions of this peptide, nor did they occur in subjects who received continuous infusions of ANF without a preceding bolus. Steady state responses to ANF infusions of 25 ng/kg/min consisted of reductions in cardiac filling pressures and concomitant declines in stroke volume and cardiac output. These responses produced reductions of cardiopulmonary baroreceptor stimulation, which elicited reflex sympathetic nervous system activation; peripheral resistance and plasma norepinephrine levels were elevated above baseline levels during steady state elevations of ANF.
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