Sympathoinhibitory Effects of Atrial Natriuretic Factor in Normal Humans

John S. Floras, MD, DPhil

With the technical assistance of John C. Fulop, MD, and Beverley L. Senn, RN

In rats, atrial natriuretic factor (ANF) reduces sympathetic nerve activity (SNA) reflexively by sensitizing cardiac mechanoreceptors with inhibitory vagal afferents. We performed three series of experiments in 26 normal young men to document whether ANF inhibits SNA in humans and if so, to determine potential mechanisms for this phenomenon. First, we recorded muscle SNA before and during brief infusions of ANF, vehicle (saline solution), and sodium nitroprusside, titrated to achieve reductions similar to those produced by ANF in diastolic pressure and central venous pressure, and we also assessed the effect of ANF on sympathetic nerve responses to a cold pressor test (CPT). Second, we determined the effect of ANF on Doppler-derived measurements of cardiac output and responses to hypotensive (−40 mm Hg) lower-body negative pressure (LBNP) and its sudden cessation. Third, we applied nonhypotensive (−15 mm Hg) LBNP to selectively unload cardiopulmonary baroreceptors, and we released LBNP to stimulate these inhibitory afferents during sequential infusions of nitroglycerin, vehicle (saline solution), and ANF. Our key findings were that 1) reductions in arterial and central venous pressures during ANF infusion were not accompanied by anticipated reflex increases in muscle SNA; 2) ANF blunted the increase in SNA with CPT; 3) ANF increased stroke volume and cardiac output; and 4) sympathoneural responses to both the application and the sudden cessation of nonhypotensive LBNP were attenuated, not augmented, by ANF. Changes in plasma norepinephrine concentrations reflected these sympathetic nerve responses to ANF. These results do not support the concept that ANF inhibits sympathetic outflow reflexively in humans by increasing discharge from cardiac mechanoreceptors with inhibitory vagal afferents but are consistent with either a central or a ganglionic sympathoinhibitory action of ANF. ANF could facilitate hypotension and natriuresis in humans by attenuating the reflex sympathetic response to baroreceptor deactivation. (Circulation 1990;81:1860–1873)

The initial observations of de Bold and his colleagues1 demonstrated the diuretic, natriuretic, and hypotensive effects of atrial natriuretic factor (ANF). Results of experiments in rats suggest that inhibition of sympathetic outflow, effected reflexively through sensitization of cardiac receptors with inhibitory vagal afferents, may contribute to these responses.2–5 Because these afferents exert their greatest inhibitory influence on efferent renal and muscle sympathetic activity,6,7 these observations suggest that some hypotensive and natriuretic effects of ANF in humans8 might be mediated by this mechanism.

The effects of this peptide on sympathetic nerve activity (SNA) in humans have not been previously documented. We undertook this study with two aims: 1) to determine the effect of ANF on efferent muscle SNA in normal humans and 2) by documenting its actions on the circulation and its baroreflex control, to study potential mechanisms responsible for the effect of ANF on SNA. The distinctive features of this study include 1) direct microneurographic recording of muscle SNA in conscious normal humans; 2) comparison of responses to human ANF (99-126) with responses to sodium nitroprusside and nitroglycerin (NTG), infused to determine whether any changes were specific to ANF or were simply nonspecific reflex responses to reductions in systemic or central venous pressure (CVP); and 3) the use of hypotensive and nonhypotensive lower-body negative pressure (LBNP) to probe the effects of ANF on cardiopulmonary and arterial baroreflexes.
Methods

Subject Selection

We studied 26 normal men, aged 21–39 years (mean±SEM 28±1 years), in three series of experiments. A medical history, physical examination, and laboratory investigations excluded hypertension, concurrent illness, and use of medication. These protocols were approved by the Human Subjects Review Committee of the University of Toronto. Informed written consent was obtained from all participants.

Procedures

Subjects were studied while in the supine position. Blood pressure was measured by an automatic cuff recorder (Physio-Control model Lifestat 200, Redmond, Washington). An intravenous catheter was placed in the left forearm vein for infusions. After administration of local anesthesia, a central venous catheter was introduced into the antecubital vein of the right arm and advanced to an intrathoracic position. CVPs and respiratory excursions were measured continuously by Statham P23ID pressure transducers (Gould Inc., Cleveland, Ohio) and recorded simultaneously with heart rate (HR), electrocardiogram, forearm blood flow (FFB), and sympathetic neurogram (Gould model 2800S ink recorder).

FFB was measured by venous occlusion plethysmography. The proximal part of the right forearm was supported approximately 10 cm above the anterior chest wall. Circulation to the hand was interrupted by a wrist cuff inflated to 180 mm Hg. A second cuff was applied above the antecubital crease. Sequential inflations of this latter cuff to 40 mm Hg and deflations were timed to give four measurements of FFB each minute. Forearm vascular resistance (FVR, expressed as resistance units [U]) was calculated by dividing mean arterial pressure (MAP; diastolic plus 1/3 of pulse pressure [mm Hg]) by the mean of four to six measurements of FFB (ml/min/100 ml of forearm volume). LBNP was used to cause reflex forearm vasoconstriction, and its sudden cessation was used to cause reflex forearm vasodilation.

Postganglionic multifiber SNA was recorded from a muscle fascicle of the right peroneal nerve posterior to the fibular head. Methods used to obtain a mean voltage neurogram, evidence that the intermittent, pulse-synchronous discharge recorded in the neurogram represented effferent postganglionic sympathetic activity, and the criteria that distinguish muscle from cutaneous sympathetic activity or electromyographic artifacts have been described in detail elsewhere. Sympathetic bursts were identified by inspection of the mean voltage neurogram and expressed as bursts per minute. The mean interobserver variability in the visual evaluation of the microneurographic record in our laboratory is 3.9%; the mean intraobserver variability is 4.5%. Since these bursts are pulse synchronous, burst frequency may vary with HR. To adjust for changes in HR, burst frequency was expressed both as bursts per minute and as bursts per 100 heartbeats.

Cardiac output (CO) and stroke volume (SV) were calculated from continuous-wave Doppler echocardiographic recordings and two-dimensional echocardiographic measurements with a commercially available Hewlett-Packard (Andover, Mass.) or Advanced Technology Laboratories (Bothell, Wash.) echocardiograph system according to the method of Quinones et al. With a standard parasternal long-axis and/or apical view, the diameter (D) of the left ventricular outflow tract was measured, and the cross-sectional area (A) was calculated as $A = \frac{\pi D^2}{4}$. From the apical window, the Doppler technique was used to obtain an instantaneous flow-velocity profile through the left ventricular outflow tract. This profile was planimetrized to give a time-velocity integral (TVI, or stroke distance). Integrals for 5–10 heartbeats were averaged to obtain a mean TVI. SV was calculated from the product of mean TVI and the left ventricular outflow tract area as $SV = TVI \times A$, where SV is in milliliters, TVI is in centimeters, and A is in square centimeters. CO was calculated from the product of SV and HR as $CO = SV \times HR$, where CO is in milliliters per minute and HR is in beats per minute. Systemic vascular resistance (SVR) was derived from CO, MAP, and CVP with the formula

$$SVR = \frac{80 \times (MAP - CVP)}{(mm\ Hg) \times CO^{-1}} (l/min)$$

CO obtained by Doppler methods correlates favorably ($r$ as high as 0.95) with conventional invasive techniques. Interobserver variabilities of Doppler CO estimates of 4–7% have been reported.

Plasma ANF concentration was determined after extraction (mean recovery, 77%) by radioimmunoassay. The interassay and intra-assay coefficients of variation of this assay are 9% and 7%, respectively. The sensitivity of this assay is 1.5 pg/ml.

Plasma norepinephrine (NE) concentrations were determined by high-pressure liquid chromatography as described by Eriksson and Persson and Weicker et al with modifications. This assay is sensitive to 20 pg/ml of NE. Both interassay and intra-assay coefficients of variation for this technique have been calculated to be 7.5%.

Protocol

Series 1: Effect of atrial natriuretic factor on muscle sympathetic nerve activity. The aim of this series of experiments was to compare the effects of human ANF, vehicle (saline solution), and sodium nitroprusside on muscle SNA (Figure 1). Our objective was to infuse ANF and nitroprusside in doses that achieved similar reductions in diastolic pressure and CVP. We hypothesized that nitroprusside would then cause greater reflex increases in SNA and HR than would ANF. Saline solution was infused as the vehicle for ANF to control for the effects of time on
SNA. Subjects maintained a daily intake of 150 mmol Na through the week before the study.

This protocol involved eight subjects and had three parts. Subjects lay quietly for 10–15 minutes to ensure a stable baseline level of SNA. Control variables were recorded for 3 minutes, and then nitroprusside was infused. Because the effects of nitroprusside and ANF could not be predicted in advance, three doses of nitroprusside were infused sequentially, each for 3 minutes: 0.2, 0.8, and 2.0 μg/kg/min. After a 10–15 minute delay to permit values to return to control levels, baseline variables were recorded for 3 minutes, and then saline solution was given as a bolus of 10 ml over 3 minutes and then infused at a rate of 0.8 ml/min for 30 minutes. After a 10–15 minute pause, control measurements were again obtained for 3 minutes. Next, human ANF (99-126) (100 μg/ml) (IAF Biochem, Laval, Quebec; 5 μg/ml saline) was administered, first as a bolus of 50 μg (10 ml) over 3 minutes and then at a rate of 50 ng/kg/min (0.8 ml/min) for 30 minutes.

Values recorded during the last minute of each dose of nitroprusside and 20 minutes into the saline and ANF infusions were compared with their respective controls. Approximately 45–60 minutes elapsed between the end of nitroprusside infusion and the beginning of ANF infusion. Blood obtained from the central venous catheter during the last 30 seconds of each infusion was assayed for ANF and NE.

In these eight subjects, plus two additional subjects who did not receive nitroprusside, responses to a cold pressor test (90-second immersion of the left hand in ice water), a nonspecific stimulus to sympathetic neural outflow, were recorded 25 minutes into saline and ANF infusions. SNA during a 2-minute baseline period before hand immersion was compared with values obtained during the last 30 seconds of this stimulus.

Series 2: Hemodynamic and baroreflex effects of atrial natriuretic factor. This series of experiments had two aims. The first was to determine whether increased cardiac mechanoreceptor stimulation might have contributed to the relative inhibition of SNA observed during ANF infusion in series 1. Our hypothesis was that ANF would reduce cardiac filling pressures, CO, and SV. These changes would diminish the tonic inhibition that cardiac baroreceptor afferents exert on efferent sympathetic outflow to muscle. Decreased mechanoreceptor stimulation would be expected to induce reflex effects on SNA opposite to those we observed in series 1.

The second aim was to determine whether ANF altered reflex forearm vasoconstrictor responses to progressive unloading of baroreceptor afferents or reflex forearm vasodilator responses to stimulation of these afferents. Reflex responses to LBNP of as much as −20 mm Hg result from unloading cardiopulmonary receptors and thus decreasing the activity of these sensory afferents. This maneuver results in forearm vasoconstriction; HR, however, is not significantly altered. An LBNP of −40 mm Hg lowers both CVP and MAP and consequently unloads both arterial and cardiopulmonary receptors. The reflex response to this stimulus includes tachycardia and further forearm vasoconstriction. Sudden cessation of LBNP (from −40 mm Hg) causes an immediate increase in CVP and arterial pressure, resulting in reflex bradycardia and forearm vasodilatation. We hypothesized that if ANF inhibited efferent sympathetic activity in humans reflexively, the relation between FVR and CVP during LBNP or on its sudden cessation would be altered.
This protocol involved 11 subjects and had three parts. Subjects lay quietly for 10–15 minutes to establish resting control values. These were recorded for 3 minutes, and then nitroprusside was infused at a rate of 0.8 μg/kg/min for 10–12 minutes. CO was obtained by Doppler techniques 10 minutes into the infusion. Fifteen to 20 minutes later, saline solution was given as a bolus of 10 ml over 3 minutes and then infused at a rate of 0.8 ml/min for 30 minutes. Doppler-derived CO values were recorded 15 minutes into the infusion. Five minutes later, FBF was measured during a 90-second baseline and then during 90–120 seconds of graded LBNP at −5, −10, −20, and −40 mm Hg, followed by its sudden cessation. Fifteen to 20 minutes later, ANF (5 μg/ml saline) was administered, first as a bolus of 50 μg (10 ml) for 3 minutes and then at a rate of 50 ng/kg/min (0.8 ml/min) for 30 minutes. Doppler CO measurements were recorded 15 minutes into the infusion. Five minutes later, FBF was measured during a 90-second baseline and then during 90–120 seconds of graded LBNP at −5, −10, −20, and −40 mm Hg, followed by its sudden cessation.

Data on CO was obtained in 10 subjects, and reflex responses to LBNP and its sudden cessation were recorded in nine.

Series 3: Effect of atrial natriuretic factor on sympathetic nerve activity during nonhypotensive lower-body negative pressure. Using a chamber constructed to permit recording of peroneal nerve muscle SNA during LBNP, we attempted in this series to selectively unload and stimulate cardiopulmonary baroreceptor afferents by application and sudden cessation of nonhypotensive LBNP at −15 mm Hg and compare the effects of ANF, vehicle (saline), and NTG infusions on SNA during these two stimuli. We hypothesized that if ANF did sensitise or stimulate inhibitory cardiac vagal afferents, it would augment the relation between muscle SNA and CVP during LBNP or its sudden cessation.

This protocol involved 10 subjects and had three parts. Subjects lay quietly for 10–15 minutes, and then NTG was infused at a rate of either 8 or 16 μg/min (mean, 11.2±1.3 μg/min) to lower CVP by 2–4 mm Hg and to lower diastolic blood pressure by 5–10 mm Hg. Fifteen minutes later, baseline values were recorded for 3 minutes, and then LBNP was applied gradually over a 6-minute interval to reach a plateau of −15 mm Hg, which was sustained for 3 minutes. LBNP was then stopped, and after 3 minutes, the infusion was terminated. After a 15–20-minute pause, this protocol was replicated twice, first infusing saline as vehicle, and then infusing ANF in the same doses and at the same infusion rates as in series 1 and 2. Infusions of NTG and vehicle were included in this series to determine whether any effect of ANF on sympathetic nerve responses were because LBNP was applied at different initial levels of arterial and CVP, or whether they were due to a specific ANF-SNA interaction.

Values recorded during the last minute before LBNP, the third minute of LBNP −15 mm Hg, and the third minute after cessation of LBNP were compared. Blood was obtained from the central venous catheter for determination of plasma NE and ANF concentrations before and immediately before cessation of LBNP.

As before, sympathetic bursts were identified by inspection of the mean voltage neurogram. Individual burst amplitudes were measured, and integrated muscle SNA was calculated as the product of burst frequency per minute and mean burst amplitude and was expressed in arbitrary units. Anticipating that sequential application and release of LBNP might result in minor alterations in microelectrode position that could influence mean burst amplitude, we identified the maximum burst amplitude during a 3-minute interval before each of the three infusions; we assigned this burst a value of 1,000 units. All bursts recorded during that particular infusion were calibrated against this reference.21

Statistics

Means and their standard errors (SEM) are reported throughout. In series 1, a two-tailed Student’s t test was used to compare the effects of each infusion against their respective baseline control values. To reduce Type I errors due to multiplicity, comparisons of responses to the two active agents and saline solution were performed only as directed by a priori hypotheses. When required, nominal p values were adjusted to account for three-way multiple comparisons by the Bonferroni method.22 We report adjusted p values. p values of less than 0.05 were required for statistical significance. In series 2, slopes of the lines relating changes in FVR to changes in CVP during saline and ANF infusions were calculated by linear-regression techniques and then compared by ANOVA. Intervention (LBNP) and infusion (NTG, saline vehicle, and ANF) effects on hemodynamic variables and muscle SNA in series 3 were assessed with a repeated-measures ANOVA, with univariate tests of hypotheses for within-subject effects and ANOVA of contrast (i.e., infusion) variables.23

Results

Series 1

Effect of atrial natriuretic factor on sympathetic nerve activity (n=8). Infusion increased plasma ANF concentrations, from 16±3 pg/ml to 495±73 pg/ml. Twenty minutes of ANF infusion reduced diastolic blood pressure by 6.6±1.6 mm Hg (p<0.01) and CVP by 2.0±0.4 mm Hg (p<0.01; Table 1). Twenty minutes of saline infusion did not alter these values. The intermediate dose of nitroprusside (0.8 μg/kg/min) lowered diastolic blood pressure by 10.4±1.5 mm Hg (p<0.01) and CVP by 2.0±0.8 mm Hg (p<0.05; Table 1). Since these responses were not significantly different from responses to ANF (Figure 2), this dose of nitroprusside was used in subsequent comparisons with ANF.
Nitroprusside increased muscle SNA by 26 ± 4 bursts/min (p < 0.001) or 23 ± 5 bursts/100 heartbeats (p < 0.05) and HR by 18 ± 3 beats/min (p < 0.01) (Table 2 and Figure 2). In contrast, and from similar baselines, 20 minutes of ANF infusion did not affect muscle SNA and only increased HR by 8 ± 3 beats/min (p < 0.05 vs. nitroprusside; Table 2 and Figure 2). Saline infusion did not affect these variables.

Nitroprusside infusion induced significantly greater increases in SNA (+119% as bursts/min; +63% as bursts/100 heartbeats) and HR (+31% as beats/min) than ANF (+27% as bursts/min, p < 0.01; +11% as bursts/100 heartbeats, p < 0.02; +14% as beats/min, p < 0.05, respectively; Figure 2).

As nitroprusside and ANF infusions were of different duration, we also compared the effect of nitroprusside infusion (reported measurements are from the sixth minute of infusion) with values obtained during the sixth minute of ANF infusion. These data also appear in Tables 1 and 2. At this point, nitroprusside and ANF had similar effects on systemic blood pressure (−7 ± 1 vs. −5 ± 1 mm Hg systolic, p=NS; −10 ± 2 vs. −6 ± 1 mm Hg diastolic, p=NS) and CVP (−2.0 ± 0.8 vs. −1.1 ± 0.5 mm Hg, p=NS), but nitroprusside infusion elicited significantly greater increases in SNA and HR than did ANF (+35% as bursts/min, p < 0.005; +13% as bursts/100 heartbeats, p < 0.02; +20% as beats/min, p < 0.025).

**TABLE 1. Series 1: Effects of Infusions on Arterial and Central Venous Pressures**

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Arterial blood pressure (systolic/diastolic, mm Hg) Difference</th>
<th>Central venous pressure (mm Hg) Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroprusside</td>
<td>119.6±3.4/72.1±2.7/80.5±1.2</td>
<td>5.4±0.8</td>
</tr>
<tr>
<td>Saline (vehicle)</td>
<td>122.9±3.9/72.8±3.8/70.6±2.8</td>
<td>4.8±0.6</td>
</tr>
<tr>
<td>ANF</td>
<td>116.0±3.9/66.5±2.4</td>
<td>4.7±0.8</td>
</tr>
</tbody>
</table>

ANF, atrial natriuretic factor.
Values reported are for eight subjects and are mean±SEM.
*Coincides with timing of nitroprusside infusion measurements.
†Coincides with timing of saline infusion measurements.
‡p <0.01, §p <0.05, compared with respective control.

**Figure 2. Bar graphs showing effects of 0.8 µg/kg/min sodium nitroprusside (NP), saline, and 50 µg + 50 ng/kg/min atrial natriuretic factor (ANF) infusions on diastolic blood pressure (DBP, mm Hg), central venous pressure (CVP, mm Hg), sympathetic nerve activity (SNA, bursts/100 heart beats [h.b.]), and heart rate (HR, bpm). Despite similar reductions in DBP and CVP, NP increased SNA and HR, but ANF did not increase SNA.**

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Nicholas J. B. Al荡 and Donald C. Poppas

Department of Anesthesiology, University of Vermont, Burlington, Vermont

Address for correspondence: Nicholas J. B. Al荡, Department of Anesthesiology, University of Vermont, College of Medicine, 120 South Prospect Street, Burlington, Vermont 05405.
Table 2. Series 1: Effects of Infusions on Muscle Sympathetic Nerve Activity and Heart Rate

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Sympathetic nerve activity (bursts/min)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infusion</td>
</tr>
</tbody>
</table>
| Nitroprusside | 22.1±3.1 | 48.4±5.0| 36.7±4.7 | 59.8±4.6| 60±3 | 78±4$^b$
| Saline (vehicle) | 23.2±3.0 | 20.8±3.4| 40.2±6.2 | 33.4±4.5| 60±4 | 60±3
| ANF         | 6th minute$^*$ | 22.7±3.0 | 30.6±2.3$^c$ | 39.3±6.0 | 44.0±4.9| 59±3 | 72±3$^c$
|             | 20th minute$^d$ | ... | 28.8±5.0 | ... | 43.7±7.0 | ... | 67±4$^c$

Values reported are for eight subjects and are mean±SEM. ANF, atrial natriuretic factor.

*Coincides with timing of nitroprusside infusion measurements.
†Coincides with timing of saline infusion measurements.
$^p<0.01$, $^c<0.05$, compared with respective control.

Plasma NE concentrations during nitroprusside, saline, and ANF infusions were 520±82, 235±43, and 365±75 pg/ml, respectively. NE concentrations tended to be greater during ANF than during saline infusions (nominal: $p=0.0275$), whereas NE concentrations during nitroprusside infusions were significantly higher than during ANF infusion ($p<0.0001$).

Effect of atrial natriuretic factor on sympathetic outflow during cold pressor test (n=10). The cold pressor test (CPT) increased SNA during saline infusion by 28±5 bursts/100 heartbeats and, from an equivalent baseline level, by 14±4 bursts/100 heartbeats during ANF infusion ($p<0.05$; Figure 3). Thus, ANF blunted the increase in sympathetic outflow during this stimulus by 50%.

Series 2

Effect of atrial natriuretic factor on systemic hemodynamics (n=10). ANF infusion increased its plasma concentration from 20±4 pg/ml to 722±46 pg/ml. When compared with saline solution (vehicle) as control, both nitroprusside and ANF achieved significant reductions in MAP ($p<0.05$), CVP ($p<0.01$), and SVR ($p<0.05$ for NP, $p<0.01$ for ANF; Table 3) and significant increases in CO (+26% and +31%, respectively; $p<0.05$). However, the latter effect was achieved through different mechanisms: ANF increased SV by 22% ($p<0.05$), whereas nitroprusside increased HR by 20% ($p<0.01$; Table 3).

Effect of atrial natriuretic factor on forearm vascular responses to lower-body negative pressure (n=9). ANF did not cause forearm vasodilatation at rest. With progressive applications of LBNP of -5, -10, -20, and -40 mm Hg, there were graded decreases in CVP and arterial pressure and graded increases in FVR (Table 4). Similar hemodynamic responses were seen during saline and ANF infusions. ANF did not change forearm vasoconstrictor response to either -20 or -40 mm Hg LBNP (Figure 4). The slope of the line relating changes in FVR (U) to changes in CVP (mm Hg) during graded LBNP from 0 to -40 mm Hg was -1.7±0.4 U/mm Hg during saline and -1.6±0.4 U/mm Hg during ANF infusions ($p=NS$).

With the sudden cessation of -40 mm Hg LBNP, there was an abrupt rise in CVP and MAP and a reflex fall in FVR. With ANF, the increase in CVP on sudden cessation of LBNP was not as great as during saline infusion, but reflex dilatation was similar during the two infusions (Table 4 and Figure 4). The slope of the line relating changes in FVR (U) to changes in CVP (mm Hg) was -1.8±0.5 U/mm Hg during saline and -1.4±0.6 U/mm Hg during ANF infusions ($p=0.62$).

Effect of atrial natriuretic factor on heart rate responses to lower-body negative pressure. Compared with saline infusions, HR during ANF infusions was quicker, both at rest and during LBNP ($p<0.05$). LBNP at -5 and -10 mm Hg lowered CVP without altering systemic arterial pressure or HR significantly. However, the reflex increase in HR from rest to LBNP -40 mm Hg was augmented during ANF infusion (+29±3 vs. +22±3 beats/min, $p<0.01$), as was the reflex bradycardia with the sudden cessation.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Bar graph showing effects of saline (□) and atrial natriuretic factor (ANF, □) infusions on sympathetic nerve activity (SNA, bursts/100 heartbeats [h.b.]) before (baseline) and during the last 30 seconds of the cold pressor test (CPT). ANF did not affect baseline SNA but blunted neural response to this stimulus.
of LBNP (−30±4 vs. −17±4 beats/min, p<0.001; Table 4 and Figure 5).

Series 3

Effect of atrial natriuretic factor on sympathetic nerve activity during and after nonhypotensive lower-body negative pressure (n=10). In this series, ANF infusion increased its plasma concentration from 22±3 to 448±52 pg/ml. Comparison of hemodynamic and reflex responses to LBNP at −15 mm Hg and its sudden cessation during sequential infusion of NTG, saline solution (vehicle), and ANF appears in Table 5. During ANF infusion, LBNP lowered CVP by 3.3±0.4 mm Hg (p<0.01) but did not elicit reflex increases in SNA (−0.4±4.0 bursts/min, p=0.93; −1.3±4.1 bursts/100 heartbeats, p=0.75; +195±110 U, p=0.11). In contrast, during this saline (vehicle) infusion, this same intervention evoked significant increases in SNA (+11.4±2.2 bursts/min, p<0.001; +13.8±3.4 bursts/100 heartbeats, p<0.01; +306±91 U, p<0.01; Figure 6). When assessed in terms of bursts/100 heartbeats, the increase in SNA with LBNP −15 mm Hg during saline infusion (±29%) was significantly greater than the response to LBNP during ANF infusion (−1%, p=0.03; Table 5). However, responses to LBNP during these two infusions are not strictly comparable, in that the initial level of CVP before LBNP was higher during saline infusion (5.5±1.0 vs. 2.3±0.9 mm Hg), and the effect of this intervention tended to be greater (−4.9±0.6 mm Hg, p<0.10). The key comparison in this series is between responses to LBNP and its release during ANF and NTG infusions.

During these two infusions, LBNP had no effect on systolic pressure (p=0.42), MAP (p=0.19), or diastolic blood pressure (p=0.19) but lowered CVP (p<0.002) and increased HR (p=0.04). Although no interactions between this infusion and the intervention in any of these variables were detected (i.e., effects of LBNP on these variables, during these two infusions, were indistinguishable), during NTG infusion, LBNP increased SNA (+9.3±4.0 bursts/min, p<0.05; +8.2±5.3 bursts/100 heartbeats, p=0.15; ±656±180 U, p=0.005; Figure 6). When expressed as U, the increase in SNA with LBNP −15 mm Hg during NTG (+80%) was significantly greater than the response to LBNP during ANF (+25%) (p=0.05; Table 5 and Figure 6).

Effects of LBNP on plasma NE paralleled these effects on muscle SNA: LBNP increased plasma NE from 221±22 to 286±24 pg/ml during NTG (p<0.0005); from 172±14 to 233±24 pg/ml during saline (p<0.005), and from 275±14 to 301±46 pg/ml during ANF (p=0.31) infusions.

Release of LBNP during ANF infusion increased CVP by +3.6±1.0 mm Hg (p<0.01) and had modest, inhibitory effects on muscle SNA (−3.7±2.4 bursts/min, p=0.16; −2.8±3.2 bursts/100 heartbeats, p=0.41; −257±85 U, p<0.01; Table 5 and Figure 6). Release of LBNP had more consistent sympathoinhibitory effects during saline infusion (−10.3±2.0 bursts/min, p<0.001; −13.5±3.7 bursts/100 heartbeats, p<0.01; −267±57 U, p<0.001), and although the inhibitory effects of this intervention on SNA were significantly greater during vehicle than during ANF infusion (p<0.01, expressed as bursts/min and as bursts/100 heartbeats; Table 5), the increase in CVP on release of SNA tended to be greater during saline infusion, and the level of CVP achieved on its cessation was higher (Table 5).

The hemodynamic effects of release of LBNP, during NTG and ANF infusions, were again indistinguishable (CVP increased by 3.7±0.6 mm Hg), but the reflex sympathetic responses to this stimulus again differed: muscle SNA fell (by 13.6±2.5 bursts/min, p<0.005; by 13.6±3.8 bursts/100 heartbeats, p<0.01; and by 694±166 U, p<0.002), and, when expressed as U, inhibition of SNA on release of LBNP −15 mm Hg during NTG infusion (−47%) was significantly enhanced when compared with the response to this stimulus during ANF infusion (−26%, p<0.03; Figure 6).

Thus, when compared with responses during NTG and saline (vehicle) infusions, sympathetic nerve responses to the application and sudden cessation of
**Table 4. Series 2: Effects of Infusions on Arterial and Central Venous Pressures and on Reflex Responses to Application and Release of -40 mm Hg LBNP**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before ANF</th>
<th>ANF (50 μg+50 ng/kg/min)</th>
<th>Release of ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>LBNP (mm Hg)</td>
<td>LBNP</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>115 ± 3</td>
<td>114 ± 3</td>
<td>113 ± 3</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>81 ± 2</td>
<td>80 ± 2</td>
<td>81 ± 2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>63 ± 2</td>
<td>63 ± 2</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>3.5 ± 1.1</td>
<td>1.8 ± 1.0</td>
<td>-0.1 ± 0.9</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>57 ± 2</td>
<td>58 ± 2</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>Forearm blood flow (ml/min/100 ml)</td>
<td>3.2 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>Forearm vascular resistance (U)</td>
<td>28 ± 3</td>
<td>31 ± 4</td>
<td>34 ± 4</td>
</tr>
</tbody>
</table>

LBNP, lower-body negative pressure; ANF, atrial natriuretic factor. Values reported are for nine subjects and are mean ± SEM.

**Discussion**

Circulatory and neurohumoral responses to infusion of ANF in young men were compared with those observed with responses to sodium nitroprusside and nitroglycerin and with responses to infusion of central and non-specific vasodilators, solution as a vehicle for ANF. Our key observations were: (1) reductions in diastolic pressure and CVP were not associated with reductions in sodium nitroprusside infusion; (2) ANF attenuates baroreceptor responses to nitroglycerin treatment; (3) ANF increased ANF in plasma NE concentrations reflected by ANF. These findings are consistent with the concept that ANF attenuates the reflex sympathetic responses to baroreceptor deactivation. Our discussion focuses on potential mechanisms to account for these findings and limitations of our methods and protocol, and implications of these observations.

**Floras Effects of ANF on Efferent Nerve Activity (Figures 1 and 5).**
We reasoned that if ANF did sensitize or stimulate cardiac mechanoreceptors and thereby increase cardiac baroreceptor afferent activity, withdrawal of this enhanced inhibitory input would result in augmented reflex sympathoexcitatory or forearm vasoconstrictor responses to application of nonhypotensive LBNP. Similarly, restoration of this enhanced inhibitory input by sudden cessation of LBNP should be accompanied by augmented reflex sympathoinhibitory and forearm vasodilatory responses to this stimulus. By analogy, arginine vasopressin, which sensitizes cardiac baroreceptors with inhibitory vagal afferents, augments the forearm vasodilation observed on sudden cessation of LBNP. However, ANF did not alter the forearm vasodilator response to the release of LBNP or to its graded application (either to −20 mm Hg, which selectively unloads cardiopulmonary baroreceptor afferents, or to −40 mm Hg, which unloads both cardiopulmonary and arterial baroreceptor afferents) in series 2. These results would suggest that ANF does not modulate baroreflex regulation of FVR. It could be argued that the absence of an effect of ANF on forearm vascular responses to LBNP in this series of experiments did not exclude this potential mechanism entirely, since reflex vasoconstriction could have been offset by the direct vasodilator effect of ANF. However, if this were the case, ANF should have augmented the reflex vasodilator response to the sudden cessation of LBNP, which it did not. Similar doses of ANF and similar experimental strategies were employed by Takeshita et al., who reported that ANF attenuated

Figure 4. Line plot showing effect of 50 μg+50 ng/kg/min infusion of atrial natriuretic factor (ANF) on the changes in forearm vascular resistance (FVR, units [U]) and central venous pressure (CVP, mm Hg) with the application of lower-body negative pressure (LBNP) at −5, −10, −20, and −40 mm Hg (left panel) and with the sudden cessation of LBNP at −40 mm Hg (right panel). Open squares indicate responses during saline infusion (p=NS) in nine subjects, and solid diamonds indicate responses during ANF infusion. CVP decreased progressively with each level of negative pressure and increased abruptly with cessation of LBNP. FVR tended to be less with ANF throughout this maneuver (p=NS), but neither reflex forearm vasoconstrictor (left) nor reflex forearm vasodilator (right) responses to this stimulus were altered by ANF.

Figure 5. Line plot showing effect of 50 μg+50 ng/kg/min infusion of atrial natriuretic factor (ANF) on changes in heart rate (HR, beats/min) and central venous pressure (CVP, mm Hg) with application of lower-body negative pressure (LBNP) at −5, −10, −20, and −40 mm Hg (left panel) and with sudden cessation of LBNP (right panel). Open squares indicate responses during saline infusion in nine subjects (p=NS), and solid diamonds indicate responses during ANF infusion. CVP decreased progressively with each level of LBNP and increased abruptly with cessation of LBNP. HR was more rapid during ANF, both before and during LBNP. Both reflex tachycardia during LBNP (left panel) (p<0.01) and reflex bradycardia on sudden cessation of LBNP (p<0.001) (right panel) were augmented by ANF.
Forearm vasoconstrictor responses to LBNP, and by Volpe et al., who reported augmented forearm vasoconstrictor responses to LBNP during ANF infusion. Our own observations fall squarely between these two discordant reports.

This conflict is resolved by our third series of experiments involving direct recordings of SNA during nonhypotensive LBNP. The key finding in these experiments was that the sympathoneural (and plasma noradrenergic) responses to both the application and the sudden cessation of nonhypotensive LBNP were attenuated by ANF. Since responses to these two stimuli were not augmented by ANF, our results do not support the concept that ANF inhibits sympathetic outflow reflexively in humans by increasing discharge from cardiac mechanoreceptors with inhibitory vagal afferents.

These results are consistent instead with either a central or a ganglionic sympathoinhibitory action of ANF. This conclusion is supported by additional data from series 1: Even though the initial (prestimulus) CVP was lower, ANF attenuated the increase in SNA from baseline during the CPT, a nonspecific (i.e., non-baroreflex-mediated) sympathoexcitatory stimulus. Certainly, the presence of immunoreactive ANF and receptors for this peptide in and near brain sites involved in cardiovascular regulation suggests the potential for a central sympathoinhibitory and possibly α2-adrenoceptor-mediated action of this peptide. ANF-like immunoreactivity has also been detected in sympathetic ganglia. Thus, ANF might also reduce postganglionic effector muscle SNA by acting as an inhibitory regulator of ganglionic neurotransmission. Our observations are consistent with either mechanism; the microneurographic technique does not permit us to discriminate between potential central and ganglionic effects of ANF on muscle SNA.

Interpretation of HR responses to LBNP and its sudden cessation is more complex. The reflex bradycardiac response to cessation of LBNP at −40 mm Hg, which increased arterial and CVP, was augmented by ANF. The incremental increase in HR with −5, −10, or −20 mm Hg LBNP in series 2 and −15 mm Hg LBNP in series 3, stimuli that selectively unload cardiopulmonary baroreceptors, was not altered by ANF, whereas the increase in HR with LBNP −40 mm Hg, which also unloads arterial baroreceptors, was augmented.

These findings initially suggested that ANF might sensitize arterial baroreceptor afferents in humans and that withdrawal of this enhanced inhibitory input would result in augmented chronotropic responses to hypotensive stimuli or their reversal. However, other investigators, using the neck collar technique, report that ANF either attenuates or has no effect on carotid baroreflex-mediated cardioacceleration in humans, and in our first series, the chronotropic response to arterial baroreceptor unloading was attenuated, not amplified, when compared with nitroprusside. Can these apparently conflicting observations in our first two series of experiments be reconciled? Although these two interventions had similar effects on systemic arterial pressure, a key distinction between these two studies is the level of CVP (and hence, the potential for interaction between these two sets of inhibitory afferents) attained. CVP fell to 3 mm Hg during ANF infusion in the first series of experiments but was reduced by −4 mm Hg by LBNP −40 mm Hg during ANF infusion. Studies by Thoren et al. in rats documented opposing effects of atriopeptins on the arterial (sympathoexcitatory) and cardiac vagal (sympathoinhibitory) baroreflex control of HR. Takeshita et al. were unable to detect any effect of changes in CVP on sinus node responses to arterial baroreflex stimulation in humans, but CVP was only varied within the range of 1–9 mm Hg in these experiments. If ANF does sensitize arterial baroreceptor afferents in humans, our observations suggest that this action may be evident only at much lower cardiac filling pressures.

Strengths and Limitations of Methods and Protocol

Several key strengths of our methods and protocol bear emphasis. First, the microneurographic technique allows direct study of the effects of ANF on central sympathetic outflow and overcomes diffi-
cultivies that arise when variables such as vascular resistance, arterial pressure, or HR are used to evaluate the effects of agents, such as ANF, that also have direct vascular effects. 45,46 In series 3, for example, we were able to resolve the controversy raised by Takeshita et al.32 and by Volpe et al.,33 who described entirely opposite effects of ANF on forearm vascular responses to LBNP.

Second, responses to ANF were compared with responses to sodium nitroprusside and NTG, infused to determine whether any changes were specific to ANF or were simply nonspecific reflex responses to reductions in systemic pressure or CVP. Vehicle (saline solution) was also infused to exclude any temporal effects on these responses. Although ANF did attenuate responses to nonhypotensive LBNP in series 3 when compared with saline, a strict comparison is not possible because the initial level of CVP before LBNP was greater during the saline infusion, and the effect of this intervention on CVP was also enhanced. In contrast, the initial level of CVP and the hemodynamic effects of LBNP were equivalent during NTG and ANF infusions, and yet, the effects of this stimulus on SNA were distinctly different. Third, protocols included hypotensive and nonhypotensive LBNP to probe the effects of ANF on cardiopulmonary and arterial baroreflexes and the CPT, which was applied to increase sympathetic outflow through nonbaroreflex mechanisms.

We recognize several potential limitations of our methods and protocol but do not believe that these detract from our key findings. It could be argued that the hemodynamic stimulus to baroreceptor unloading in series 1 was greater during nitroprusside than after 20 minutes of ANF infusion since nitroprusside also lowered systolic blood pressure. However, Sundlof and Wallin47 found that the systemic stimulus to a sympathetic burst was a reduction in diastolic, not systolic, blood pressure; the effect of these two infusions on diastolic blood pressure at this time was not significantly different. In any event, equivalent reductions in systolic and diastolic pressures were achieved by 6–7 minutes of ANF and nitroprusside administration, yet the neural and HR responses to these two infusions differed significantly. Sundlof and Wallin47 also described a positive correlation between changes in pulse pressure and sympathetic burst activity. Since pulse pressure increased from 47 to 51 mm Hg during nitroprusside infusion and from 49 to 54 mm Hg during ANF infusion, the different sympathetic nerve responses to these two infusions cannot be explained by differences in their effects on pulse pressure, or systolic and diastolic blood pressures.

Even if there were differences in the effects of ANF and nitroprusside on systemic arterial pressure, such differences would not explain the lack of reflex sympathetic activation in response to the 2 mm Hg fall in CVP during ANF infusion. Sundlof and Wallin7 reported an increase in burst frequency of 25% in response to −10 mm Hg LBNP and an increase in burst frequency of about 30% in response to −15 mm Hg LBNP. More recently, Victor and Leimbach,48 applying progressive nonhypotensive LBNP, reported that at −5 mm Hg LBNP, CVP fell by 2 mm Hg, and burst frequency rose by 35%; at −10 mm Hg, CVP fell by 2.4 mm Hg, and burst frequency increased by 40%; at −15 mm Hg, CVP fell by 3.6 mm Hg, and sympathetic burst frequency increased by 60%. Seals49 documented a 54% increase in muscle SNA burst frequency during LBNP −10 mm Hg. Scherrer et al.,50 applying −5 mm Hg, documented a reduction in CVP of 2.5 mm Hg and an increase in sympathetic burst frequency of 70%. Thus, even if ANF did not decrease diastolic pressure at all, its effects on CVP alone would be

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nitrolycerin</th>
<th>Saline (vehicle)</th>
<th>ANF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>LBNP (−15 mm Hg) of LBNP</td>
<td>Rest</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>125±2</td>
<td>121±3</td>
<td>123±3</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>88±2</td>
<td>85±2</td>
<td>88±2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>69±3</td>
<td>68±3</td>
<td>70±2</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>2.9±1.1</td>
<td>−0.6±0.6</td>
<td>3.2±0.9</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>62±3</td>
<td>69±3</td>
<td>61±3</td>
</tr>
<tr>
<td>Sympathetic nerve activity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| (bursts/min)                          | 38.9±3.8     | 48.2±2.4        | 34.6±2.9*| 28.4±2.9| 39.8±2.7| 29.5±3.1*| 39.1±4.2| 39.5±5.7| 35.8±5.8|*
| (bursts/100 heartbeats)               | 62.7±5.0     | 70.9±4.5        | 57.3±4.9| 48.0±3.6| 61.8±3.6*| 48.3±4.8*| 57.2±3.3| 55.9±4.8| 53.1±4.6|*
| (units, U)                            | 817±94       | 1,473±234*      | 779±105*| 621±73| 927±112| 660±122| 775±74| 970±88| 714±108|*

LBNP, lower-body negative pressure; ANF, atrial natriuretic factor.

Values reported are for 10 subjects and are mean±SEM.

*p<0.05, †p<0.01, compared with response to intervention (LBNP, −15 mm Hg or its release) during ANF infusion.
expected to increase SNA by 25–70%. Indeed, in series 3, LBNP –15 mm Hg increased integrated SNA by 49% during the control (saline) infusion. Therefore, the absence of sympathetic activation in series 1 provides strong evidence for a sympathoinhibitory action of ANF.

Since our hypothesis in series 1 was that reflex responses to baroreceptor unloading would be attenuated by ANF, it was critical that the dose of ANF infused would elicit reductions in both systemic pressure and CVP. This dose increased plasma ANF concentrations beyond the normal physiological range in young adults\(^5\) but within levels documented in healthy elderly subjects\(^6\) and in congestive heart failure patients.\(^7\,\,8\)

We (as did Thoren et al\(^9\)) did not randomize the order of infusions since we appreciated that the effects of ANF on CVP and any inhibitory effects of ANF on ganglionic neurotransmission\(^10\,\,11\) would be sustained. In contrast, the effects of these short infusions of nitroprusside and NTG are brief and do not require volume or other interventions for their reversal. All hemodynamic and sympathoneural variables returned to baseline promptly after these vasodilators were stopped (Tables 1, 2, and 4), and approximately 45–60 minutes elapsed between the end of their infusion and the beginning of ANF infusion. Had the order of infusions truly been important, we might have anticipated baseline effects or significant evidence of equilibration during infusion of vehicle (saline solution). Since these did not occur, the order of infusion cannot be considered a critical issue in the interpretation of these observations.

The Doppler technique of measuring SV and CO compared favorably with Fick and/or thermodilution techniques: correlations on the order of 0.90–0.95 have been reported when absolute values have been compared.\(^12\,\,13\) Measurement of relative or percent changes in Doppler CO appears even more reliable than absolute values.\(^14\,\,15\) Determination of CO by Doppler techniques is sensitive to changes in preload and afterload as well as cardiac inotropy.\(^16\) Thus, interventions that alter these variables could affect their measurement. However, the contrasting effects of nitroprusside and ANF on SV in these subjects cannot be explained simply by changes in the loading conditions of the heart specific to ANF since nitroprusside and ANF infusions caused similar reductions in systemic pressure and CVP.

Lastly, it should be emphasized that our recordings were of muscle SNA only. Since ANF has organ-specific effects on sympathetic outflow,\(^17\) these peroneal nerve recordings may not accurately reflect sympathetic discharge to other vascular beds.

**Implications**

In contrast to vasodilators such as nitroprusside, ANF appears to lower systemic arterial pressure in humans without eliciting reflex increases in plasma NE concentrations.\(^8\,\,18\,\,19\) However, since plasma NE concentrations are themselves an indirect and insensitive index of sympathetic traffic,\(^20\) such observations cannot be considered definitive evidence for sympathoinhibition by ANF and cannot be used to probe potential sympathoinhibitory mechanisms in humans.

These experiments are noteworthy in two respects. First, they document directly and consistently a sympathoinhibitory action of ANF in humans. Second, our findings indicate that ANF modulates muscle SNA in humans primarily through a central or a ganglionic sympathoinhibitory action. This could contribute to its hypotensive action in humans. A similar inhibition of efferent renal SNA in humans could facilitate natriuresis through renotubular as well as hemodynamic mechanisms.\(^21\) Also, this sympathoinhibitory effect of ANF may account for the abrupt development of hypotension during and after prolonged ANF infusions, particularly in response to changes in posture, as reported by several groups.\(^22\,\,23\,\,24\)

**Acknowledgments**

The author gratefully acknowledges the important contributions of the following colleagues. Plasma ANF concentrations were performed under the supervision of Dr. P. Y. Wong, Division of Clinical Biochemistry, Toronto General Hospital. Plasma NE concentrations were assayed in the laboratory of Dr. Andrew Baines, Department of Clinical Biochemistry, Toronto General Hospital. The author is indebted to Dr. Judith Miller for her assistance with statistical computations and to Mary Anne Beddows for her secretarial support.

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**KEY WORDS** • baroreceptor reflex • cardiac output • muscle sympathetic nerve activity • norepinephrine
Sympathoinhibitory effects of atrial natriuretic factor in normal humans.

J S Floras

Circulation. 1990;81:1860-1873
doi: 10.1161/01.CIR.81.6.1860

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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