Effects of Incremental Infusions of Atrial Natriuretic Factor on Aldosterone, Renin, and Blood Pressure in Humans

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SUMMARY To evaluate the physiological effects of human atrial natriuretic factor-(99-126) (ANF), we infused ANF, 0.1, 0.3, and 1.0 μg/min, or placebo for 125 minutes on different days into six sodium-deprived normal men. During the last 45 minutes of infusion, angiotensin II, 6 ng/kg/min, was infused. Blood pressure, heart rate, plasma concentrations of ANF, aldosterone, and cortisol, and plasma renin activity (PRA) were measured before and during infusion. Steady state mean plasma ANF levels during infusion were 26.2 (placebo), 68.8 (0.1 μg ANF/min), 221 (0.3 μg ANF/min), and 648 pg/ml (1.0 μg ANF/min). Systolic blood pressure fell significantly (with 1.0 μg ANF/min), and diastolic pressure tended to rise in a dose-dependent manner, while heart rate was unchanged. PRA and plasma aldosterone fell during ANF infusion in a dose-dependent manner (significant with 0.3 and 1.0 μg ANF/min infused). The blood pressure—raising and aldosterone-stimulating effects of angiotensin II were blunted by ANF (significant only with 1.0 μg ANF/min). It is concluded that effects of ANF on blood pressure and the renin-aldosterone system occur with plasma ANF levels close to the physiological range, as well as with slightly elevated ANF levels, as observed in congestive heart failure and renal insufficiency. (Hypertension 12: 462-467, 1988)

KEY WORDS • atrial natriuretic factor • aldosterone • plasma renin activity • blood pressure

ATRIAL natriuretic factor (ANF) may be physiologically and pathophysiologically involved in ridding the body of a sodium load and in preventing central venous pressure from rising above a certain limit. 1 Most of the ANF activity in human plasma seems to be due to human ANF-(99-126).2-3 The synthetic peptide is available for clinical studies. It has an immediate natriuretic effect,4-5 but it also inhibits aldosterone synthesis in vitro6-7 and in vivo.3-5,8 However, it is controversial whether the effect of ANF on aldosterone secretion occurs with near-normal plasma concentrations of the cardiac peptide and whether the effect in vivo is more or less mediated by changes in plasma renin levels.9-11 In the present study, we attempted to find a threshold dose of infused ANF that significantly affects aldosterone and renin secretion in sodium-deprived normal men.

Subjects and Methods
Six healthy men (age range, 20–29 years; body weight range, 63–87 kg; mean body weight, 73.5 ± 8.5 kg) consented to participate in the study, the protocol of which had been approved by the ethical committee of Klinikum Steglitz (Berlin, West Germany). They were studied on 4 different days (three doses of ANF, one placebo infusion) within two periods of sodium depletion, during which the men collected their urine for the calculation of sodium and potassium balances. Sodium losses were induced to facilitate studying a possible suppressive effect of ANF on elevated basal renin and aldosterone levels. A period of sodium depletion consisted of 3 days on a low sodium diet (15 mmol sodium, 70 mmol potassium per day) plus a single 40-mg dose of furosemide (Lasix, Hoechst) on the morning of the first day. Therefore, most of the sodium loss occurred during the first 24 hours of the sodium depletion period, and the cumulative negative sodium balance at the end of Day 3 was not much different from that on the morning of Day 2. Infusion of one dose of ANF or placebo was given in the afternoon on Days 2 and 3. After an interval of 3 weeks on a free diet, the procedure of sodium depletion was
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repeated and the other two infusion studies were performed on Days 2 and 3.

The placebo infusion consisted of 5% glucose in water, 12 ml/hr, given by an infusion pump (Perfusor, Braun, Melsungen, West Germany). Human ANF-(99–126) (Bissendorf Peptide, Wedemark, West Germany) was given in the same volume of 5% glucose in doses of 0.1, 0.3, and 1.0 μg/min. The lowest ANF dose and the placebo infusion were given on Days 2 and 3 of the first sodium depletion period in a randomized order. The two higher doses were randomly administered on Days 2 and 3 of the second experimental period. As shown in Figure 1, the experimental subjects assumed the supine position at 1400, when intravenous cannulas were inserted into antecubital veins of both forearms. Blood pressure and heart rate measurements with a semiautomatic apparatus (Boso Digital II, Bosch and Sohn, Jungingen, West Germany) were started at 1500 and repeated every 10 minutes. ANF or placebo infusions were started at 1600 and lasted until 1805. Between 1720 and 1805, an infusion of angiotensin II amide (Hypertensin, CIBA), 6 ng/kg/min, was given on top of the ANF or placebo infusion to test the reactivity of blood pressure and the adrenal cortex toward exogenous angiotensin II.

Urine collection during the relatively short period of infusion experiment amounted to 150 to 170 ml.

Plasma concentrations of ANF (EDTA-treated plasma) were taken before the start of infusion and 30 minutes, 1 hour, and 2 hours after the start of infusion. ANF was measured in EDTA-treated plasma using a direct radioimmunoassay. The sensitivity of the direct assay was 0.07 nmol/L, measurements with the kit were 5 to 15% higher than those obtained with a more laborious procedure including paper chromatography prior to the radioimmunoassay. The coefficient of correlation between the two methods was 0.967 (p < 0.001). The sensitivity of the direct assay was 0.07 nmol/L. The intra-assay variability at 0.85 nmol/L was 8.2% (n = 10). The interassay variability at 0.578 nmol/L was 10.4% (n = 10).

Plasma cortisol was measured in EDTA-treated plasma using CEA Sorin kits (Saluggia, Vercelli, Italy) with antibody-coated tubes. The sensitivity of the assay was 15 to 20 nmol/L. The intra-assay variability at different cortisol levels (100, 350, and 1000 nmol/L; n = 15 each) was 5.4, 3.9, and 4.5%, respectively. The interassay variability at the same concentrations was 9.6, 5.7, and 5.5%, respectively. Serum and urinary electrolytes were measured using a flame photometer (IL 743, Instrumentation Laboratory, Paderno-Duniano, Italy).

All samples from one subject were measured on the same day within one assay to minimize the variability. For plasma cortisol and aldosterone, means of duplicate measurements were used for calculations. Statistical calculations (Wilcoxon test for correlated samples) were performed using a STATS 2 program (Statsoft, Tulsa, OK, USA) and an IBM computer (Armonk, NY, USA). Values are presented as means ± SEM unless otherwise indicated.

Results

No subjective or objective adverse effects occurred during the infusions of ANF, angiotensin II, or placebo.

Plasma ANF Levels

Before and during placebo infusion, mean plasma ANF levels were rather constant, as shown in
Figure 2. Plasma levels of ANF during the four sets of infusions. Means ± SEM are shown. If the SEM was smaller than the size of the symbol, it is not shown. At any time during the ANF infusion, plasma ANF levels were significantly higher than those obtained during placebo infusion (p < 0.01–0.0001).

Figure 2. They varied between 23.8 ± 4 and 27.0 ± 0.6 pg/ml (average of all means, 26.2 pg/ml). At the end of the angiotensin II infusion, plasma ANF (28.8 ± 2.0 pg/ml) had not changed significantly. With the ANF infusions of 0.1 and 0.3 μg/min, steady state plasma concentrations were reached at least 20 minutes after the onset of infusion. Mean ANF levels during the infusion of 0.1 μg/min ranged between 55.7 ± 4.6 and 74.6 ± 3.8 pg/ml (average of all means, 68.8 pg/ml; mean increment, 42.6 pg/ml). During ANF infusion at 0.3 μg/min, ANF levels were between 204 ± 27 and 248 ± 20 pg/ml (average, 221 pg/ml; mean increment, 152 pg/ml). With ANF infusion at 1.0 μg/min, it took about 40 minutes before a rather stable level of ANF was reached. The average plasma ANF concentration during infusion was 648 pg/ml (mean increment, 427 pg/ml). Increments in ANF levels were closely proportional to increments of the dose infused.

Blood Pressure and Heart Rate

For statistical evaluations, individual measurements of blood pressure and heart rate were averaged for the following periods: 1520 to 1550 (pre-infusion), 1610 to 1640 (early infusion), 1650 to 1720 (late infusion), and 1740 to 1800 (angiotensin II). Results are shown in Table 1. There were no significant changes in heart rate during the infusions of placebo, ANF, or angiotensin II. Systolic and diastolic blood pressure changed in a different manner. During ANF infusion, systolic blood pressure tended to fall with increasing doses of ANF. The fall was significant during the second half of the 1.0 μg/min infusion of ANF. The effect of angiotensin II on systolic BP was highly significant during placebo and the 0.1 μg/min infusion of ANF (p < 0.001), less so with the infusion of 0.3 μg ANF/min, but absent with the highest dose of ANF.

Diastolic blood pressure tended to rise with the ANF infusions of 0.3 and 1.0 μg/min (but not significantly), and the effect of angiotensin II on diastolic blood pressure was not blunted by the higher doses of ANF.

Electrolytes, PRA, Plasma Aldosterone, and Cortisol

Plasma sodium and potassium levels were in the normal range in all subjects and did not change significantly during placebo, ANF, or angiotensin II infusion (data not shown). Mean sodium excretion rates on Days 2 and 3 of the two successive sodium depletion periods were as follows: Period 1, Day 2: 11.9 ± 6.5 (SD) mmol/day; Day 3: 6.4 ± 4.9 mmol/day. Period 2, Day 2: 10.1 ± 10.5 mmol/day; Day 3: 4.1 ± 3.8 mmol/day.

Table 1. Hemodynamic Effects of ANF and Angiotensin II

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Systolic BP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Inf 1</td>
<td>Inf 2</td>
</tr>
<tr>
<td>Placebo</td>
<td>113.4±7.1</td>
<td>113.6±8.8</td>
<td>112.9±8.0</td>
</tr>
<tr>
<td>ANF, 0.1</td>
<td>114.9±5.9</td>
<td>115.1±5.1</td>
<td>112.7±5.9</td>
</tr>
<tr>
<td>ANF, 0.3</td>
<td>115.6±8.9</td>
<td>111.9±10.7</td>
<td>109.9±8.7</td>
</tr>
<tr>
<td>ANF, 1.0</td>
<td>114.7±5.8</td>
<td>108.8±8.0</td>
<td>107.3±7.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. BP = blood pressure; Ang II = angiotensin II; Inf 1 and 2 = early and later phase of placebo or ANF infusion, respectively.

* p < 0.01, † p < 0.05, compared with Inf 2 values.
‡ p < 0.05, compared with control values.
Cumulative sodium and potassium balances and basal PRA and plasma aldosterone levels for the days on which infusions were performed are shown in Table 2. For unknown reasons, cumulative sodium balances were more negative during the second period of sodium depletion (when the two higher doses of ANF were tested) than during the first period. Although this difference did not reach a level of significance, basal PRA and plasma aldosterone levels during the second period of sodium depletion were higher than those of the first period. For this reason, all changes in PRA and plasma aldosterone possibly caused by ANF infusion will be reported as a percentage of basal levels (means of measurements at 1545 and 1555). Figure 3 shows these changes. PRA fell during placebo infusion alone by 26%. It fell further during the superimposed angiotensin II infusion (negative effect of angiotensin II on renin secretion). After 80 minutes of ANF infusion at 0.1 \( \mu \)g/min, PRA had fallen by 33% (\( p \) = NS compared with placebo infusion value). With the higher doses of ANF, PRA fell by 50 and 53%, respectively (\( p < 0.05 \) vs placebo). The further fall of PRA during the angiotensin II infusion was similar with all doses of ANF infused and was not different from that seen during placebo infusion.

Plasma aldosterone fell slightly during placebo infusion. Increasing the dosage of ANF enhanced the fall of aldosterone in a dose-dependent manner (significantly greater than placebo with the two higher doses of ANF). The percent fall of PRA and plasma aldosterone below control levels in all experiments taken together was positively correlated (\( r = 0.38, p = 0.06 \)).

The superimposed angiotensin II infusion led to a sharp rise of plasma aldosterone in the placebo experiment (\( p < 0.001 \)). The effect of angiotensin II was blunted in the experiments with the two higher doses of ANF, but significantly so only during the administration of ANF at 1 \( \mu \)g/min (see Figure 3).

Mean basal plasma cortisol levels ranged between 199 ± 14 and 209 ± 10 nmol/L on the 4 experimental days (data not shown). During the 80-minute infusions of placebo or ANF, 0.1, 0.3, or 1.0 \( \mu \)g/min, mean plasma cortisol levels fell by 12, 7, 6, and 17%, respectively. The slight fall of plasma cortisol was not significantly different between ANF experiments and placebo.

**Discussion**

With this study we attempted to elucidate the dose-related short-term effects of ANF infusion in men. Most investigators have reported on effects of

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

**Figure 3.** Mean percent changes (±SEM) in PRA and plasma aldosterone during infusion of placebo (sham) or ANF, later supplemented by an angiotensin II (Ang II) infusion. Single (\( p < 0.05 \)) and double (\( p < 0.01 \)) asterisks indicate that percent falls in PRA and plasma aldosterone were significantly greater than those seen during sham infusion. Dagger (\( p < 0.05 \)) indicates that the absolute increment of plasma aldosterone between 1720 and 1750 or 1805 (Ang II infusion) was significantly smaller than that seen during sham infusion.)
single doses, either very high\textsuperscript{4, 5} or close to the physiological range.\textsuperscript{10, 11} Our studies were primarily performed to assess the relationship between ANF doses infused and plasma levels obtained using a new method of ANF extraction from plasma,\textsuperscript{12} to facilitate the design of further infusion experiments. We conducted the study in sodium-deficient subjects, whose renin-aldosterone system was stimulated, to get an idea at which infusion rates spontaneous and angiotensin-stimulated aldosterone secretion may be inhibited by ANF.

For unknown reasons, the cumulative sodium balances during the second period of sodium depletion were more negative than those obtained during the first period. The four sets of infusions were not completely randomized among the 4 infusion days within the two sodium depletion periods. However, we do not believe that this small difference in conditions for the two pairs of infusion (placebo and 0.1 \mu g ANF/min on the one hand; 0.3 and 1.0 \mu g/ANF/min on the other) markedly affected the dose-response aspect of the study. When infusions began, the subjects had been supine for 2 hours, and any difference in the orthostatic response of PRA and aldosterone due to differences in sodium deficiency would have been eliminated.

Plasma ANF levels obtained during ANF infusion at 0.1 \mu g/min were similar to those measured during bicycle exercise in normal men by Anderson et al.\textsuperscript{16} and during ANF infusion at about 0.2 \mu g/min by the same authors.\textsuperscript{10} Baseline plasma ANF levels in normal men were slightly lower in the laboratory of Anderson et al.\textsuperscript{16} than in ours. Infusing ANF at 0.3 \mu g/min, in our study led to plasma levels observed in patients with congestive heart failure and chronic renal failure.\textsuperscript{17, 18} Plasma ANF levels obtained during the administration of ANF at 1 \mu g/min may sometimes be observed in patients with very severe cardiac failure\textsuperscript{17} or during paroxysmal tachycardia.\textsuperscript{19}

It is noteworthy that the most extensive studies of ANF in men\textsuperscript{4, 5} were performed with infusion doses of approximately 7 and 3.3 \mu g/min, respectively. In the present experiment, plasma ANF levels in relation to infusion rates were higher than those reported in several other studies.\textsuperscript{4, 5, 10, 11, 20} This may partly be due to the high ANF recovery rate with the method of extraction from plasma used.\textsuperscript{12} The infusion solutions were checked several times by radioimmunoassay, and the concentrations found were always within \pm 10\% of the expected level.

Effects of incremental doses of ANF on blood pressure were characterized by a slight fall in systolic pressure (significant only with 1 \mu g ANF/min), a blunting of the effect of infused angiotensin II on systolic blood pressure, and a tendency of diastolic pressure to rise. Anderson et al.\textsuperscript{21} infused normal subjects on an unrestricted diet with angiotensin II, 10 ng/kg/min. In their study, ANF (approximately 3.3 \mu g/min) blunted the diastolic blood pressure rise more effectively than the systolic pressure. This difference in comparison with our findings may be due to differences in the subjects' sodium state. The changes in blood pressure during ANF infusion alone may indicate a decrease in blood volume during ANF infusion, possibly due to a shift of fluid from the intravascular to the interstitial space, as recently reviewed by Espiner and Nichols.\textsuperscript{3} The failure of heart rate to rise does not fit this theory, but it may be due to an interference of ANF with sympathetic tone, as observed by Holtz et al.\textsuperscript{22} in the dog.

Incremental doses of ANF led to a dose-dependent fall in PRA and plasma aldosterone concentration. Compared with the percent changes from basal levels in the placebo study, the effects of ANF on PRA and plasma aldosterone were significant with ANF infusions at 0.3 and 1.0 \mu g/min, but the changes observed with 0.1 \mu g ANF/min pointed in the same direction. It is possible that the smallest infusion dose led to a real suppression of PRA and aldosterone, but that the statistical power of this study in six subjects was not sufficient to reach the level of significance. Anderson et al.,\textsuperscript{10} who infused approximately 0.2 \mu g of ANF/min into normal subjects for 3 hours, observed a significant fall in PRA, while the fall of plasma aldosterone was not significant. Cuneo et al.\textsuperscript{2} also observed a concomitant fall of PRA and plasma aldosterone during high dose ANF infusion. The rise in PRA in the presence of a small fall in plasma aldosterone observed by Weidmann et al.\textsuperscript{4} was probably due to hypotension induced by still higher doses of ANF. It is likely that the fall of plasma aldosterone in our study was caused predominantly by inhibition of renin release.\textsuperscript{23} However, the correlation between the fall in PRA and plasma aldosterone was not impressive and just failed to be significant. Therefore, it is likely that ANF exerted its effect on aldosterone secretion by combined suppression of PRA and inhibition of the effect of angiotensin II on the adrenal level. During the last 45 minutes of ANF or placebo administration, we infused a relatively large dose of angiotensin II, which markedly stimulated aldosterone secretion. This rise was blunted significantly only by the highest ANF dose infused. Cuneo et al.\textsuperscript{11} used smaller angiotensin II doses in a recently published study. They were able to inhibit the aldosterone increase significantly by infusing 0.5 \mu g of ANF/min, a dose intermediate between the two higher doses in our study. ANF effectively inhibits aldosterone stimulation by angiotensin II in vitro\textsuperscript{4, 7} and in vivo.\textsuperscript{9, 10, 20, 21} In vitro, the basal or adrenocorticotropic hormone–stimulated secretion of cortisol may also be inhibited by ANF,\textsuperscript{7} but in our infusion study and in that of Cuneo et al.,\textsuperscript{11} cortisol secretion was found to be unaffected by ANF.

In summary, incremental infusions of ANF in sodium-depleted men led to a narrowing of the blood pressure amplitude, a concomitant fall in PRA and plasma aldosterone, and, with the highest
dose, an additional significant blunting of the aldosterone-stimulating and blood pressure-raising effect of infused angiotensin II. With the smallest dose of ANF infused, which raised plasma ANF levels to the upper edge of the physiological spectrum, none of these changes was significant, although they were relatively similar to the effects obtained with higher doses. If an endogenous hormone system is intact, one tends to underestimate its physiological significance by studying so-called threshold doses and by inducing one or more significant effects by infusing additional amounts of the hormone. For example, the physiological significance of the renin-angiotensin system in the regulation of blood pressure and of aldosterone secretion was underestimated before the advent of specific inhibitors of angiotensin converting enzyme. The physiological and pathophysiological role of ANF will be better defined once specific inhibitors of its action are available.

References

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