Atrial natriuretic factor
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CHAPTER 5

ANTIHYPERTENSIVE EFFECT OF A 5-DAY INFUSION OF ATRIAL NATRIURETIC FACTOR IN HUMANS

Wilbert M T Janssen, Dick de Zeeuw, Gjalt K van der Hem, and Paul E de Jong

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SUMMARY

Atrial natriuretic factor was infused in a low dose (0.2 µg/min) during 5 days in six patients with essential hypertension. Atrial natriuretic factor infusion caused plasma levels of atrial natriuretic factor to increase from 49±10 to 106±19 pg/ml. Within 4 hours after the start of the atrial natriuretic factor infusion, urinary sodium excretion increased in all subjects. Sodium balance was regained after 24 hours with a net loss of 72.3±14.6 mmol. However, systolic as well as diastolic blood pressure started to decrease gradually in all subjects only after 12 hours of atrial natriuretic factor infusion, reaching a stable level after 36 hours with a decrease of 11.5±1.5 % and 10.3±0.8 %, respectively. Heart rate increased in parallel by 12.6±3.1 %. Hematocrit rose 7.1±2.3 %. After cessation of atrial natriuretic factor infusion, plasma atrial natriuretic factor levels, sodium balance, and hematocrit returned to baseline within 24 hours, whereas blood pressure slowly returned toward baseline values over 3 days. These data show that chronic atrial natriuretic factor infusion in patients with essential hypertension causes a negative sodium balance and a rise in hematocrit, followed by a smooth decrease in blood pressure with a rise in heart rate until a new equilibrium is reached after approximately 2 days. Thus, atrial natriuretic factor in low doses appears intimately involved in the regulation of sodium balance and blood pressure in humans. Moreover, these data suggest that atrial natriuretic factor-like substances will eventually become useful antihypertensive drugs.
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INTRODUCTION

Atrial natriuretic factor (ANF) has been shown in many animal and human investigations to have potent natriuretic and vasodepressor effects when administered in high doses by bolus injection or short-term infusion [1,2]. However, whether ANF at more physiological levels is involved in the chronic regulation of sodium balance and blood pressure is still unknown. In most human studies, low-dose (<0.5 µg/min) infusion of ANF during several hours has been shown to cause a clear natriuresis without lowering blood pressure [3-5]. Animal data show that low doses of ANF may well cause a decrease in blood pressure, especially in hypertensive rats [6,7]. This decrease, however, was observed mainly after several days of ANF infusion [6-9]. We therefore, investigated the effects of a 5-day low-dose (0.2 µg/min) ANF infusion on urinary sodium excretion and blood pressure in patients with essential hypertension.

SUBJECTS AND METHODS

We studied six male patients with uncomplicated essential hypertension. Diagnostic workup included careful history and physical examination. Rapid-sequence urography was carried out in all patients; renal arteriography was performed when considered appropriate. Patient age was 51±4 years (mean±SEM, range 35-65). Renal function, as measured with \(^{125}\text{I}-\text{iothalamate}\) and \(^{131}\text{I}-\text{hippuran}\) [10], was characteristic for essential hypertension [11] with a normal glomerular filtration rate (GFR) (93±3 ml/min/1.73m\(^2\)), a reduced effective renal plasma flow (ERPF) (355±8 ml/min/1.73m\(^2\)), and an increased filtration fraction (26±1 %). Normal values in our laboratory are 116±4, 508±28, and 23±1, respectively. None had signs of heart failure. Six weeks before hospitalization, patients were instructed to adhere to a diet containing a constant amount of sodium (150 mmol/day). All antihypertensive medication had been withdrawn for 11±3 weeks (median 9, range 6-26 weeks) until diastolic blood pressure was stable. Blood pressure was measured eight times after 15 minutes of supine rest with an automatic noninvasive device (Dinamap\textsuperscript{®}, Criticon Inc, Tampa, Florida, USA). This device measures blood pressure and heart rate by oscillometric determination of cuff pressure and minute pressure pulses within the incrementally deflated cuff. Mean systolic and diastolic blood pressures were 160±5 and 101±1 mm Hg, respectively. The study was carried out in a metabolic ward and lasted 14 days. During the whole study the patients had absolute bed rest except for bathroom facilities (8:00 AM to 8:30 AM), weight measurement, and urine voiding. During the 14 days, the patients received a rhythm diet consisting of equal amounts of sodium, potassium, and fluid every 4 hours (total: 156 mmol, 90 mmol, and 1,800 ml/day, respectively), and in addition a continuous intravenous infusion of 32 ml/hr 5% dextrose and 2 ml/hr solvent (with or without ANF). After a 3-day equilibration period, during which blood pressure and sodium excretion were allowed to stabilize, a 3-day baseline period was completed, followed by a 5-day ANF infusion period and a 3-day recovery period. Each of these days started at noon. During the 5-day ANF...
infusion period synthetic human ANF(101-126) (MSD-RL, Rahway, New Jersey, USA) dissolved in 5 g mannitol/100 ml was infused at a dose of 0.2 µg/min. This is a N-terminal-deleted form of the endogenous circulating peptide with probably similar or identical bioactivity [5]. During the 11 days after equilibration, several measurements were made every 4 hours: first, venous blood samples for the measurement of immunoreactive ANF (irANF) were drawn while the patient was still in the supine position. Thereafter urine was collected by spontaneous voiding for measurement of volume, sodium, and creatinine excretion. Finally, the patients received their meal. Each day at noon blood was also drawn for determination of serum biochemistry (sodium, potassium, chloride, urea, creatinine, and transaminases) and hematocrit. The equivalent amount of sodium was substituted intravenously each time blood was drawn. Blood pressure was recorded continuously every 15 minutes with the Dinamap device. The protocol was approved by the local medical ethical committee, and all patients gave their informed consent.

Serum biochemistry and urinary sodium and creatinine concentration were measured using standard autoanalyzer techniques (Technicon Instr Corp, Tarrytown, New York, USA). Hematocrit was measured by the microcrit method. Immunoreactive ANF was determined with a commercially available 125I-ANF tracer and a highly specific ANF antibody (INCSTAR, Stillwater, Minnesota, USA). Blood samples for the measurement of irANF were collected in prechilled EDTA tubes, immediately centrifuged (3,400g, 10 minutes) at 4° C, and the plasma (0.5 ml) was stored at -25° C until all samples of one patient could be processed simultaneously. The plasma was then acidified with 1.5 ml 4% acetic acid and applied to a Sep-Pak C18 cartridge (Waters Assoc, Milford, Massachusetts, USA), pretreated with 5 ml 4% acetic acid in 86% ethanol, 5 ml

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>irANF (pg/ml)</td>
<td>51±10</td>
<td>48±10</td>
<td>49±10</td>
</tr>
<tr>
<td>Urinary volume excretion (ml/24 hr)</td>
<td>2102±192</td>
<td>2493±170</td>
<td>2241±98</td>
</tr>
<tr>
<td>Urinary sodium excretion (mmol/24 hr)</td>
<td>142±13</td>
<td>164±13</td>
<td>156±10</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>136±4</td>
<td>136±5</td>
<td>134±5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>89±3</td>
<td>89±3</td>
<td>88±3</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>105±3</td>
<td>105±4</td>
<td>103±4</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>64±4</td>
<td>64±4</td>
<td>65±4</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.4±1.1</td>
<td>41.2±1.1</td>
<td>40.5±0.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.6±2.1</td>
<td>76.4±2.1</td>
<td>76.5±2.1</td>
</tr>
</tbody>
</table>

Values are the 24-hour mean±SEM. Hematocrit was measured at noon and weight at 8 AM. IrANF, immunoreactive atrial natriuretic factor.
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methanol, 5 ml distilled water, and 5 ml 4% acetic acid. After washing twice with 3 ml distilled water, ANF was eluted three times with 1 ml 4% acetic acid in 86% ethanol, evaporated to dryness, and dissolved in 250 µl radioimmunoassay (RIA) buffer. Then 100 µl plasma extract was mixed with 200 µl antibody and incubated (18 hours, 4° C). Finally, 200 µl tracer was added. After a 22-hour incubation, free and bound ANF were separated with horse anti-sheep precipitating reagent. Intra-assay and interassay variances in our laboratory are 8.5% and 9.3%, respectively.

All results are expressed as mean±SEM. Statistical analysis was performed by analysis of variance with repeated measurements, followed by Duncan’s multiple range test. The changes during ANF infusion in the various parameters were calculated against the mean of the three baseline data of the corresponding 4-hour time periods. Differences were considered significant at \( p < 0.05 \).

RESULTS

The first 3 days in the hospital were used to allow sodium balance and blood pressure to equilibrate. Table 1 shows that all parameters were indeed stable. The 24-hour mean of the urinary sodium excretion on the last baseline day was 156±10 mmol and thus equaled the dietary intake of sodium. Twenty-four-hour blood pressure was significantly lower compared with the outpatient clinic measurement, probably resulting from the bedrest protocol and inclusion of the night values. There was a marked diurnal rhythm in urinary sodium excretion and in systolic and diastolic blood pressure. The daily variation in urinary sodium excretion was similar during these three baseline days, with maximum values at noon (33.6±2.0 mmol/4 hr) and lowest values at midnight (19.4±0.6 mmol/4 hr). Figure 1 shows that systolic and diastolic blood pressure also followed a diurnal rhythm with highest values at 4:00 PM (140.3±0.8 / 93.4±0.5 mmHg) and lowest values at 4:00 AM (130.2±1.6 / 82.4±0.9 mmHg).

Infusion of synthetic ANF caused an immediate twofold increase in plasma levels of irANF from 49±10 (range 27-88) to 106±19 (range 68-168) pg/ml \((p<0.01)\), which persisted during the whole infusion period of 5 days (Figure 1). Urinary volume excretion increased immediately after the onset of the ANF infusion. In the first 4 hours of ANF infusion, excess volume excretion as compared with baseline was 158.6±54.3 ml \((p<0.01)\). After 12 hours of ANF infusion, net volume balance was negative by 365.9±100.3 ml \((p<0.05)\). For the following days of ANF infusion, volume excretion matched baseline values again. Urinary sodium excretion also increased within 4 hours in all patients (Figure 2). In the first 4 hours of ANF infusion, excess sodium excretion was 15.6±2.9 mmol \((p<0.01)\). After 12 hours of ANF infusion, net sodium balance was - 51.8±8.3 mmol \((p<0.01)\). Urinary sodium excretion continued to exceed baseline values up to 24 hours, amounting to a net negative sodium balance of 72.3±14.6 mmol (range 32.2-138.9, \( p < 0.01 \)). Urinary creatinine excretion was 13.9±0.7 mmol/24 hr during baseline and did not change during ANF infusion (13.7±0.9 mmol/24 hr, not significant [NS]). Weight was reduced in five of the six patients, resulting in a decrease of
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Figure 1. Plasma levels of immunoreactive atrial natriuretic factor (irANF) at a 4-hour interval and systolic and diastolic blood pressure (4-hour mean±SEM) before, during, and after a 5-day, low-dose (0.2 µg/min) atrial natriuretic factor infusion in six patients with essential hypertension.
Figure 2. Effects of a 5-day, low-dose (0.2 µg/min) atrial natriuretic factor infusion on percentile changes in mean arterial pressure (MAP) (■) and heart rate (●), and on the cumulative sodium balance in six patients with essential hypertension in balance on a 156 mmol sodium diet.
Figure 3. Hematocrit before, during, and after a 5-day, low-dose (0.2 µg/min) atrial natriuretic factor infusion in six patients with essential hypertension.

0.7±0.2 kg to 75.8±2.1 kg (NS). In the remaining patient, weight did not change. In contrast to the immediate response of urinary volume and sodium excretion to ANF infusion, neither systolic nor diastolic blood pressure decreased within the first 12 hours of ANF infusion in any patient (Figures 1 and 2). However, after this delay both systolic and diastolic blood pressure began to decrease gradually in all patients. Systolic blood pressure was significantly lower than baseline values 16 hours after the start of the ANF infusion (p<0.05), whereas diastolic blood pressure became significantly lower after 36 hours (p<0.01). Thereafter, systolic and diastolic pressure remained stable until the end of the ANF infusion. The decrease in systolic blood pressure was 16.2±2.3 mmHg (11.5±1.5%, range 7.8-17.2%, p<0.01) and, in diastolic blood pressure, 9.3±0.8 mmHg (10.3±0.8%, range 7.2-12.3%, p<0.01). Heart rate was significantly elevated 24 hours after the start of the ANF infusion by 8.4±1.9 beats/min (12.6±3.1%, p<0.05). Hematocrit showed an increase in all patients of 7.1±2.3% (p<0.05), which was stable during the whole ANF infusion period (Figure 3).

After cessation of ANF infusion, plasma levels of irANF returned to or below baseline during the first recovery day, to stabilize thereafter at preinfusion values (Figure 1, NS). Urinary sodium excretion was significantly (p<0.01) decreased compared with baseline values during the 24 hours after cessation of ANF infusion. Thereafter, sodium excretion was similar to baseline values again. Thus, volume and sodium balance rapidly
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**TABLE 2.** Serum biochemistry before, during, and after a 5-day infusion of atrial natriuretic factor.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>ANF</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol/l)</td>
<td>136.5±1.6</td>
<td>138.7±1.3</td>
<td>136.2±1.9</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.4±0.1</td>
<td>4.5±0.1</td>
<td>4.5±0.1</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>104.8±1.6</td>
<td>106.3±1.0</td>
<td>105.3±1.1</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>3.5±0.4</td>
<td>3.7±0.3</td>
<td>3.3±0.4</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>87.2±1.8</td>
<td>88.7±2.4</td>
<td>90.7±2.9</td>
</tr>
<tr>
<td>SGOT (units/l)</td>
<td>17.3±1.1</td>
<td>32.5±9.1*</td>
<td>32.2±7.5*</td>
</tr>
<tr>
<td>SGPT (units/l)</td>
<td>20.5±2.3</td>
<td>57.3±19.3*</td>
<td>57.3±19.2**</td>
</tr>
</tbody>
</table>

Values (mean±SEM) were measured at noon on the last day of each period. ANF, atrial natriuretic factor; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

* p<0.05, ** p<0.01 versus baseline.

returned to baseline in 24 hours. In contrast, both systolic and diastolic blood pressure and heart rate slowly returned toward baseline values, being statistically different (p<0.05) from baseline values up to 44 hours after cessation of the ANF infusion. Although these values were not statistically different from baseline values thereafter, they did not show a complete return. On the last day of the recovery period, 24-hour mean systolic and diastolic blood pressure were still 4.3±1.4 % (NS) and 5.6±1.9 % (NS) below baseline, respectively (Figure 2). Hematocrit returned to or below baseline (p<0.05), which may reflect the amount of blood sampling in this study (390 ml in 11 days).

No sudden hypotensive responses were observed. Also no adverse changes in serum biochemistry were found, except for the serum transaminases (Table 2). In five of the six patients a transient increase in transaminases was observed. Peak serum glutamic-oxaloacetic transaminase (SGOT) levels in these patients varied from 27 to 75 units/l (normal to 40 units/l), and peak serum glutamic-pyruvic transaminase (SGPT) levels from 36 to 119 units/l (normal to 30 units/l) during ANF infusion. In two patients normal values were present again at the end of the ANF infusion. In the other three patients, normal values were present 2 weeks after the study.

**DISCUSSION**

These data show that a chronic infusion of low-dose ANF in patients with essential hypertension rapidly causes a negative sodium balance followed by a slower decrease in blood pressure and a rise in heart rate.

ANF is now recognized to be a hormone of cardiac origin, whose circulating plasma levels vary with different physiological stimuli [2]. It has thus been questioned whether
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ANF within this physiological range participates in the day-to-day regulation of sodium excretion and blood pressure [2]. In the present study, we used a very low dose infusion of ANF, increasing plasma levels only twofold. Other investigators have shown that stimuli, for instance an increase in sodium intake [12] or, more acutely, volume loading [12,13], may cause approximately threefold increases in plasma ANF levels. Therefore we assume that the ANF effects on sodium excretion and blood pressure in the present study can be assumed to represent effects of ANF levels within or close to the physiological range.

The changes in urinary sodium excretion during ANF infusion were present within 4 hours of the start of the infusion period and leveled off after 24 hours. The immediate natriuretic response to ANF even before a decrease in blood pressure coincides with studies of Anderson et al [4] and Richards et al [5] in humans. These authors found that, when infused during 2-3 hours, the same or a somewhat higher dose of ANF does indeed cause natriuresis in humans without affecting blood pressure. The amount of ANF-induced excess sodium excretion (approximately 4 mmol/hr) during the first 12 hours of ANF infusion is very close to the amounts observed by these authors. However, the ANF-induced increase in urinary sodium excretion in the present study finally leveled off. The reason is not clear from the present data, but it is conceivable that a new balance between ANF and opposing sodium-retaining factors was reached. One of the counterbalancing mechanisms involved might be the decrease in blood pressure, since during the first 12 hours of ANF infusion the excess amount of sodium excreted per hour was stable and started to diminish only when blood pressure began to decrease. After 2 days of ANF infusion, urinary sodium excretion equaled baseline values (and thus sodium intake) again, while blood pressure now remained stable. In all animal and human studies thus far, an evanescent natriuretic effect of ANF has always been associated with a decline in blood pressure [14-16]. Moreover, there is extensive evidence from animal studies that ANF-induced natriuresis depends on arterial blood pressure [17-18]. Other sodium-retaining factors possibly involved in the discontinuation of the natriuretic response to ANF might be an increase in sympathetic tone (as suggested by the increase in heart rate) or an activation of the renin-angiotensin system. Alternatively, tachyphylaxis, for instance through a down-regulation or occupancy of renal tubular ANF receptors (induced by high ANF levels [19]), cannot be excluded as a factor underlying the evanescent natriuretic effect of ANF in this study.

The most important observation of the present study is the ANF-induced gradual decrease in blood pressure, starting after a delay of 12 hours and reaching a new, stable level after 36 hours of ANF infusion. This observation is consistent with available animal-study data showing that prolonged low dose infusion of ANF may indeed cause a delayed or a more pronounced decrease in blood pressure after some days [6-9]. In humans, however, such a gradual decrease during a long-term administration of a very low dose of ANF has not yet been reported. In contrast, we and others have observed that higher doses of ANF can cause a sudden symptomatic hypotensive response [3,16,20]. The data of the present study thus suggest that doses of about 0.2 µg ANF/min

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(or maybe even lower) can be given safely and effectively over a longer period to patients with essential hypertension. However, the level of sodium balance, which we chose to be relatively high in the present study, could be of importance in this respect [16,21]. The mechanism causing the decrease in blood pressure in the present study is not clear. Data from animal studies have suggested that the decrease in blood pressure induced by low-dose infusion of ANF is related to volume depletion, to a decrease in cardiac output, or to vasodilation [6,7,22]. It has been suggested that the underlying mechanism depends on the investigated animal model [6,7]. Parkes et al [22] suggested from their study of sheep that the ANF-induced blood pressure reduction during the first days of ANF infusion is related to a decrease in cardiac output associated with a fall in blood volume and after 5 days is mediated in addition by peripheral vasodilation. In the present study, the negative volume balance (although not impressive), the reduced weight, and the stable increase in hematocrit during the 5 days of ANF infusion suggest that volume depletion via urinary losses, the redistribution of extracellular fluid volume, or both, contributes to the decrease in blood pressure. Moreover, these changes preceded the decrease in blood pressure during ANF infusion. On the other hand, the increase in heart rate during ANF infusion might suggest vasodilation. A vasodilatory action of ANF could also explain the slow return toward baseline values of blood pressure and heart rate in the recovery period in contrast to the quick return to baseline of volume balance and hematocrit. Therefore, the time course of the changes in volume balance and blood pressure in the present study could well be consistent with the suggestion of Parkes et al [22] that volume depletion as well as vasodilation contribute in a time-dependent fashion to the lowering of the blood pressure.

These observations raise the question as to whether ANF eventually will become useful as an antihypertensive drug. The present data show that continuous intravenous infusion of exogenous ANF lowers blood pressure in patients with essential hypertension during continued bedrest and a rhythm diet. Therefore, a time-related effect on blood pressure independent of ANF administration cannot be completely ruled out, nor can it be concluded from the present data that ANF will lower blood pressure under typical day-to-day circumstances. Moreover, to be convenient as a drug, effective oral or nasal preparations must be available. To find another way of administration, one could think of ANF-like analogues or drugs inhibiting the degradation of endogenous ANF. Whether the long-term efficacy of such preparations is limited by an intermittent intake is still to be demonstrated. In this respect, the observed slow return toward baseline of blood pressure during recovery, consistent with the observations of others [3,20], certainly could be a valuable property of ANF, despite its fast degradation. The increase in heart rate and the possibly transient increase in transaminases may limit the usefulness of ANF-like drugs. It is not clear from the present study whether heart rate would return to normal values after a longer period of time, as has been observed in sheep [22]. The reason for the increase in transaminases is unclear. Particularly no serological or immunological evidence for the existence of one or the other form of hepatitis could be obtained. Moreover, it should be noted, that the other liver enzymes were not affected.
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during ANF infusion and that the rise was transient. Hypothetically, the rise could be explained by the involvement of exogenous ANF in the metabolism of the transaminases. Substances that inhibit the degradation of endogenous ANF [23] might not have this side effect. Thus, studies showing the effects of ANF on blood pressure during more typical day-to-day conditions must be conducted, and several difficulties have to be solved before drugs interfering with ANF levels are considered suitable.

In conclusion, long-term, continuous infusion of very low doses of ANF, which induces ANF levels within or close to the physiological range, causes a negative sodium balance and lowers blood pressure in hypertensive humans. Moreover, ANF-like analogues or substances inhibiting the degradation of endogenous ANF may eventually become useful antihypertensive drugs.

ACKNOWLEDGMENTS

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