Low Dose Atrial Natriuretic Factor in Primary Aldosteronism: Renal, Hemodynamic, and Vascular Effects

Roberto Pedrinelli, Giovanni Panarace, Marzia Spessot, Stefano Taddei, Stefania Favilla, Linda Graziaedi, Alessandra Lucarini, and Antonio Salvetti

Whether atrial natriuretic factor (ANF) plays a physiological role in primary aldosteronism has yet to be determined. In the present study, the renal, hemodynamic, humoral, and vascular effects of a synthetic (WY-47663) human analogue were studied in five water-loaded (15 ml H2O/kg) patients with adenomatous primary aldosteronism, a salt-sensitive, low renin, volume-expanded syndrome. ANF was infused for 3 hours at a low rate (0.005 μg/kg/min), which approximately doubled circulating immunoreactive ANF. Glomerular filtration rate and renal blood flow (inulin and para-aminohippurate clearance) remained stable, but sodium excretion increased significantly suggesting a dissociation between renal hemodynamics and natriuresis as well as a direct inhibitory effect on tubular sodium reabsorption by ANF. Intra-arterial diastolic blood pressure, heart rate, forearm blood flow (plethysmographic method), and arterial plasma norepinephrine did not change, but systolic blood pressure declined and hematocrit rose suggesting plasma volume contraction by ANF. Plasma aldosterone levels were unchanged indicating a loss of ANF-mediated aldosterone inhibition, possibly related to qualitative or quantitative alterations of ANF receptors in tumoral adrenal tissue. Infusion of the analogue into the brachial artery was at a rate of 0.005 μg/dl forearm tissue/min × 30 minutes, which also doubled local immunoreactive venous ANF concentrations and vasodilated forearm arterioles. These data suggest a physiological role for ANF in modulating body fluid volume even in human primary aldosteronism. (Hypertension 1989; 14:156-163)

Evidence gathered on the natriuretic, vasorelaxant, and aldosterone inhibitory properties1-2 of atrial natriuretic factor (ANF) suggests an involvement of the peptide in maintenance of fluid balance or hemodynamic control even in subjects with primary aldosteronism.3,4 A salt-sensitive, low renin, volume-expanded form of hypertension.3,4 The behavior of endogenous ANF levels during dietary sodium intake changes was consistent with that possibility,4 but no data were available regarding the biological effect of ANF infusion, particularly at low concentrations. Therefore, we decided to evaluate the end-organ responses to low dose exogenous ANF administration in patients with primary aldosteronism.

Subjects and Methods

Four men and one woman with mild to moderate uncomplicated hypertension due to primary aldosteronism (mean age 50, range 42–62 years) were enrolled. All medications were withdrawn for at least 3 weeks. The diagnosis of aldosterone-secreting adenoma was established by suppressed plasma renin activity (PRA) (range 0.01–0.23 ng angiotensin I [Ang I]/hr), high urinary aldosterone excretion (range 38–77 μg/24 hr),5 hypokalemia (range 2.0–3.2 mmol/l), and evidence of unilateral adrenal mass on computerized axial tomography (CAT) scans. The diagnosis was confirmed at surgery. Blood pressure normalized in all patients after adrenalectomy. The patients were completely familiar with the experimental procedure and at ease with the medical staff. In accordance with institutional guidelines, all patients gave their informed consent.

Experimental Protocol

All patients were hospitalized on the day before the study. Studies were carried out with the patients
in the supine position. No caloric or dietary sodium restrictions were applied. Smoking and alcohol intake were not permitted from 24 hours before and during the trial.

WY-47663, a 25–amino acid peptide synthesized by solid-phase techniques at Wyeth-Ayerst Research (Philadelphia, Pennsylvania), was provided in ampules containing 100 µg peptide in 0.005 M acetic acid. This peptide differs from the amino acid sequence of human ANF-(99–126) only by its lack of three terminal amino acids, and its in vitro potency matches the naturally occurring form. New vials of the compound were reconstituted in vehicle (Haemaccel, Behringwerke AG, Marburg, FRG) and diluted to a volume of approximately 40 ml. Preliminary experiments showed practically complete recovery of the peptide from the Haemaccel solution, thereby excluding significant binding to syringe and plastic connections.

After the patients had fasted overnight, 3-hour infusions of ANF (0.005 µg/kg/min) and placebo (0.026 ml/min) were administered in random order on two successive days. A similar infusion rate was used to increase circulating ANF levels within a physiological range in normal subjects. From 7:00 to 9:00 AM, before ANF or placebo infusion, a catheter was positioned in the brachial artery for monitoring hemodynamics and taking blood samples and in the forearm vein for exogenous infusions. From 7:00 AM, patients drank 15 ml/kg tap water and a replacement volume of water equal to the urine output, and in addition, 10 ml for insensible losses was administered every 30 minutes. During the study, urine osmolality never exceeded 100 mOsm/kg, implying complete or nearly complete antidiuretic hormone suppression. The male patients voided spontaneously at 30-minute intervals; the female patient underwent bladder catheterization. Infusion of para-aminobipurate (PAH) and inulin (diluted in 5% dextrose, Jacopo Monico, Laboratorio Chimico Biologico, Mestre, Venezia, Italy) were started at 8:00 AM, at rates of 12 and 6.1 mg/kg·min, respectively, preceded by priming doses of 8.0 and 32.0 mg/kg, respectively. Steady-state plasma concentrations of both inulin and PAH and steady urine flow were reached within 1 hour. From 9:00 AM to 2:00 PM, the formal part of the study included a 1-hour preinfusion period (vehicle infusion) and a 3-hour infusion of either ANF or vehicle followed by a 1-hour recovery period (vehicle administration). Approximately 120 ml solution (30% as Haemaccel) was infused and 140 ml blood was withdrawn during each study period.

Arterial blood samples were collected at 1-hour intervals for analysis of ANF, norepinephrine, PRA, aldosterone, cortisol, PAH, inulin, sodium, potassium, and phosphate (PO₄) concentration, as well as osmolality and hematocrit. Mean basal arterial ANF (113.2±25.2 pg/ml) was higher (p<0.001, two-tailed paired t test, r=0.96 for arterial vs. venous) than the venous ANF value (41.8±10.4 pg/ml; normal venous values for our laboratory, 24.8±3.5 pg/ml, n=10), indicating a relevant vascular extraction of the peptide in patients with primary aldosteronism similar to that reported in normal subjects.

Intra-arterial systolic and diastolic blood pressure and heart rate were monitored and recorded continuously; forearm blood flow was measured bilaterally at 15-minute intervals.

Urine samples were collected every 30 minutes for measurement of sodium, potassium, and PO₄ concentrations and osmolality.

**Local atrial natriuretic factor infusion.** These studies were performed in the afternoon (between 4:00 and 6:00 PM) of the placebo study day. The brachial artery was maintained patent by infusion of heparinized saline (6 µl/min) with a portable minipump (Microjet Bolus 2, Miles, Cavenago, Milano, Italy). After a light meal, a catheter was positioned in a deep forearm vein and 30 minutes were allowed for equilibration. A 30-minute vehicle infusion (Haemaccel, 0.206 ml/min) was then started, followed by a 30-minute infusion of ANF (0.005 µg/dl forearm tissue/min). This rate was chosen to approximate the systemic one. The length of the infusion period was previously shown to be sufficient for forearm blood flow to reach a new plateau in patients with essential hypertension.

Venous samples for analysis of ANF levels were collected before and at the end of the 30-minute infusions of vehicle and ANF. Blood pressure and heart rate were continuously monitored and forearm blood flow was measured every 3 minutes.

**Laboratory Methods**

Assays. PRA, aldosterone, and cortisol levels were measured by radioimmunoassay. Norepinephrine concentrations were analyzed with high-pressure liquid chromatography. Plasma and urinary electrolyte concentrations were measured by using flame photometry with lithium as an internal standard. Inulin and PAH were analyzed by standard photometric methods. Hematocrit was measured by the micromethod, and osmolality was assessed by freezing point depression (Osmostat, OM 6020, Kagaku Co. Ltd., Kyoto, Japan). Phosphate levels were measured photometrically by autoanalyzer (ABAVP, Abbott Laboratories, Irving, Texas).

Blood for ANF determination was collected in precooled plastic syringes containing 1.5 mg potassium ethylenediaminetetraacetate (EDTA) and aprotinin (Trasylol, Bayer, Milano, Italy). 1.5 and 1,000 units/ml blood, respectively, and transferred to precooled tubes. Samples were immediately centrifuged at 4° C (4,000 rpm for 20 minutes); plasma was divided into 2.5-ml aliquots and stored at −70° C for a maximum of 8 weeks. Plasma was processed for ANF extraction immediately after thawing at room temperature. Plasma was acidified in HCl (1N, 0.25 ml/ml plasma) and run slowly...
were monitored continuously and recorded on line.

The intra-assay and interassay varia-
tively. Final ANF values were corrected for the
ences from compounds used in the steps before
were comparable, indicating the absence of
sequence-related effects.

Results

On the day preceding the study, sodium and
potassium excretion were 101±13 and 66±12 mol/
day, respectively; aldosterone excretion rate was
67.5±7.6 μg/day. Mean PRA was 0.09±0.01 ng Ang
1/10 ml/hr. Mean supine blood pressure was 182±16/
111±8 mm Hg (indirect method).

Systemic Infusion Studies

Urinary (Table 1, Figure 1), hemodynamic (Fig-
ures 2 and 3), and humoral (Table 2, Figure 4)
control values during infusion of placebo and ANF
were comparable, indicating the absence of
sequence-related effects.
TABLE 1. Absolute Urinary Excretion of Variables During Vehicle and Atrial Natriuretic Factor Infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (µmol/min)</td>
<td>400±80</td>
<td>270±41</td>
<td>216.8±26</td>
<td>223.4±45</td>
<td>263.4±90</td>
</tr>
<tr>
<td>K (µmol/min)</td>
<td>330±114</td>
<td>400±65</td>
<td>396.8±41</td>
<td>373.4±68</td>
<td>193.2±45</td>
</tr>
<tr>
<td>FEK (%)</td>
<td>82.8±8</td>
<td>73.8±5</td>
<td>72.2±12</td>
<td>81.0±11</td>
<td>86.4±6</td>
</tr>
<tr>
<td>PO₄ (µmol/min)</td>
<td>886.8±17</td>
<td>59.2±10</td>
<td>68.4±22</td>
<td>70.4±22</td>
<td>58.0±15</td>
</tr>
<tr>
<td>CH₂O (ml)</td>
<td>837.2±4</td>
<td>33.4±5</td>
<td>33.6±7</td>
<td>36.4±6</td>
<td>40.2±1</td>
</tr>
<tr>
<td>COsm (mosm/ml)</td>
<td>836.2±14</td>
<td>33.6±9</td>
<td>41.2±20</td>
<td>42.1±17</td>
<td>34.9±14</td>
</tr>
<tr>
<td>FEPO₄ (%)</td>
<td>831.2±11</td>
<td>28.6±9</td>
<td>31.2±9</td>
<td>29.6±6</td>
<td>29.4±8</td>
</tr>
<tr>
<td>PO₄ (µmol/min)</td>
<td>827.6±5</td>
<td>34.8±7</td>
<td>34.4±7</td>
<td>43.6±10</td>
<td>28.4±6</td>
</tr>
<tr>
<td>CH₂O (ml)</td>
<td>827.1±7</td>
<td>23.6±5</td>
<td>28.5±6</td>
<td>28.2±5</td>
<td>24.5±5</td>
</tr>
<tr>
<td>COsm (mosm/ml)</td>
<td>830.1±8</td>
<td>42.7±11</td>
<td>39.5±6</td>
<td>46.8±8</td>
<td>31.7±4</td>
</tr>
<tr>
<td>CH₂O (ml)</td>
<td>812.0±1</td>
<td>10.0±1.5</td>
<td>12.4±1.4</td>
<td>10.2±2</td>
<td>12.9±0.8</td>
</tr>
<tr>
<td>COsm (mosm/ml)</td>
<td>812.4±3</td>
<td>11.5±1</td>
<td>12.1±0.5</td>
<td>13.2±0.9</td>
<td>9.8±0.8</td>
</tr>
<tr>
<td>CH₂O (ml)</td>
<td>84.63±0.7</td>
<td>3.60±0.70</td>
<td>3.07±0.30</td>
<td>3.06±0.40</td>
<td>3.52±0.7</td>
</tr>
<tr>
<td>COsm (mosm/ml)</td>
<td>83.78±0.50</td>
<td>4.58±0.60</td>
<td>4.30±1.1</td>
<td>3.95±0.70</td>
<td>3.11±0.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM during vehicle (top line) and atrial natriuretic factor (bottom line) infusion. ANF, atrial natriuretic factor; FEK, fractional excretion of potassium; FEPO₄, fractional excretion of phosphate; CH₂O, free water clearance; COsm, osmolar clearance.

*Statistical significance (p<0.01) of the interaction term “times • phase.”

Circulating immunoreactive atrial natriuretic factor. Plasma immunoreactive (ir)ANF approximately doubled during infusion of exogenous ANF (Figure 1), and the increments in irANF levels from the time-corresponding vehicle values did not differ significantly (108±8.3, 80±7.0, 95±9.2 pg/ml, respectively), indicating the attainment of steady-state conditions.

Urinary parameters. Absolute (Table 1) and fractional sodium excretion (Figure 1) and osmolar clearance (Table 1) increased during infusion of ANF relative to placebo. No significant changes in fractional and absolute potassium and PO₄ excretion or free water clearance occurred.

Systemic and regional (renal and forearm) hemodynamics and arterial hematocrit. Systolic blood pressure declined significantly during ANF infusion, but approached preinfusion values during the recovery. Supine diastolic blood pressure, heart rate, forearm blood flow (Figure 2), and GFR (Figure 3) did not change, whereas RBF tended to increase, but not significantly. Hematocrit values decreased during the vehicle infusion, but increased during the ANF infusion (Figure 3), and as a result, a signifi-
significant interaction time \times treatment emerged, disclosing a consistent influence of ANF on hematocrit.

**Humoral parameter.** Aldosterone and PRA values were not significantly changed during ANF infusion (Figure 4) nor were cortisol, norepinephrine, or potassium (Table 2). Plasma sodium and osmolality decreased in time-related fashion.

**Local Infusion Studies**

The basal venous ANF levels during this part of the study were 39.8±10.4, 38.6±12.1 pg/ml at the end of the 30-minute vehicle infusion and increased to 100.2±21.5 pg/ml at the end of ANF infusion. Forearm blood flow increased from 3.7±0.23 to 4.2±0.25 ml/dl/min (+13.5%, p<0.03, Figure 5).

**Discussion**

Although it is known that ANF promotes sodium excretion, its role and mechanism of action in physiological and pathophysiological conditions is debated. In this study, natriuresis similar to that reported in normal subjects was demonstrated during low dose ANF infusion. As regards the mechanisms of that action, water loading minimized any renal effect of antidiuretic hormone, whereas the lack of changes in PRA (likely related to a suppressed renin typical of this syndrome) and norepinephrine apparently ruled out any Ang II or sympathetic mediation of these systems. As reported by others, plasma aldosterone also was unmodified, and this result contrasts with those in normal subjects infused with equivalent amounts of the

### Table 2. Baseline Arterial Plasma Variables During Vehicle and Atrial Natriuretic Factor Infusion

<table>
<thead>
<tr>
<th>Infusion</th>
<th>NE (pg/ml)</th>
<th>Cortisol (ng/ml)</th>
<th>Na (mmol/l)</th>
<th>K (mmol/l)</th>
<th>Osmolality (mosm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>246.2±54</td>
<td>145.5±32</td>
<td>140.6±0.7</td>
<td>2.88±0.21</td>
<td>289.8±5</td>
</tr>
<tr>
<td>ANF</td>
<td>218.4±47</td>
<td>119.3±33</td>
<td>141.1±1.4</td>
<td>2.98±0.15</td>
<td>295.0±10</td>
</tr>
</tbody>
</table>

Values are mean±SEM. NE, norepinephrine; ANF, atrial natriuretic factor.
agonist\textsuperscript{7,21} to suggest quantitative or qualitative tumoral adrenal receptor changes similar to those shown in vitro.\textsuperscript{22} Other possible mechanisms for the natriuresis include renal hemodynamic changes or inhibition of direct sodium reabsorption at the proximal tubules, loops of Henle, or collecting ducts. As regards the former, RBF and GFR did not change during ANF administration; thus, although neither short-lived RBF changes nor relative redistribution to deep cortical nephrons could be excluded as a possibility during the 1-hour clearance periods, ANF-induced natriuresis appeared unrelated to renal hemodynamic changes. This conclusion is in agreement with the results of previous animal studies,\textsuperscript{23,24} but it is at variance with other suggestions.\textsuperscript{25} It is possible that differences in patient populations produce different responses. Renal vasodilation during low dose ANF administration was found in patients with congestive heart failure,\textsuperscript{26} possibly related to their high basal renal vascular tone, but whatever the case, the ineffectiveness of ANF in this study did not imply generalized vascular unresponsiveness because forearm arterioles were dilated during local infusion. On the other hand, the inconsistent changes in PO\textsubscript{4} excretion (a marker of proximal tubular reabsorption) and the absence of changes in free water clearance argue against either a proximal or loop of Henle tubular site of action.\textsuperscript{8} Rather, the unchanged urinary potassium excretion rate in the presence of increased natriuresis suggests a distal

**Figure 4.** Plasma aldosterone (ALDO) (pg/ml) and plasma renin activity (PRA) (ng angiotensin I/ml/hr) during vehicle (●—●) or atrial natriuretic factor (ANF) (○—○) infusion. Mean±SEM.

**Figure 5.** Forearm blood flow (FBF) (ml/min) and local venous atrial natriuretic factor (ANF) concentrations during local infusion of vehicle (Haemaccel, 0.201 ml/min) or atrial natriuretic factor (ANF) (0.005 µg/dl forearm tissue/min) infusion. Mean±SEM. Local venous ANF (●—●); FBF (infused forearm) (○—○); FBF (contralateral forearm) (■—■).
effect of ANF, possibly at the collecting ducts, as has been shown in vitro. Previous data in normal subjects suggested a similar mechanism of action, although it is possible that kalluresis was prevented by maximally stimulated aldosterone secretion in this patient population.

It has been shown that ANF dilates forearm vessels in normal subjects and patients with essential hypertension. However, no data were available in a syndrome such as primary aldosteronism in which elevated circulating ANF levels may down-regulate receptors and impair biological responses. In this study, arteriolar dilation was observed after local infusion of ANF, and although appropriate comparisons with normal subjects and dose-response studies are required to assess its vasodilatory potency, the data from this study suggest persistently functional vascular ANF receptors in our patients. However, any further conclusion is highly speculative, especially because of the recently shown heterogeneous classes of ANF binding sites. Perhaps more importantly from a physiopathological point of view, arteriolar vasorelaxation was probably not related to the overall systemic response to ANF because diastolic blood pressure, heart rate, forearm blood flow, and RBF did not change as would be expected from a vasodilating agent. Therefore, the decrease in systolic blood pressure that we observed probably originated from other causes, such as a decrease in plasma volume, indirectly suggested by the increments in hematocrit. Similar effects, as well as decreases in venous return and central venous pressure, have been reported in normal subjects and patients with essential hypertension.

This study provided evidence for the natriuretic, vasodilating, and plasma volume contracting action of a synthetic human ANF analogue that was infused at low doses in patients with primary aldosteronism. Although the relation between the circulating ANF levels attained during exogenous administration and those produced during endogenous stimulation in a similar patient population remains to be determined, these data, in agreement with our previous results, suggest that ANF regulates body fluid volume in patients with primary aldosteronism and possibly other related physiopathological conditions, such as the so-called “escape” to the sodium retentive effect of mineralocorticoids. A specific antagonist will, however, be needed to test conclusively this possibility.

Acknowledgments

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References


KEY WORDS • atrial natriuretic factor • aldosteronism • secondary hypertension • natriuresis • vasodilation
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