Four-Hour Infusions of Synthetic Atrial Natriuretic Peptide in Normal Volunteers


SUMMARY Two doses of synthetic atrial natriuretic peptide (0.5 and 5.0 μg/min) and its vehicle were infused intravenously for 4 hours in eight salt-loaded normal volunteers, and the effect on blood pressure, heart rate, renal hemodynamics, solute excretion, and secretion of vasoactive hormones was studied. The 0.5 μg/min infusion did not alter blood pressure or heart rate, whereas the 5.0 μg/min infusion significantly reduced the mean pressure by 20/9 mm Hg after 2.5 to 3 hours and increased the heart rate slightly. Inulin clearance was not significantly changed, but the mean p-aminohippurate clearance fell by 13 and 32% with the lower and higher doses, respectively. Urinary excretion of sodium and chloride increased slightly with the lower dose. With the higher dose, a marked increase in urinary excretion of sodium, chloride, and calcium was observed, reaching a peak during the second hour of the infusion. Potassium and phosphate excretion did not change significantly. A brisk increase in urine flow rate and fractional water excretion was seen only during the first hour of the high-dose infusion. Signs and symptoms of hypotension were observed in two subjects. No change in plasma renin activity, angiotensin II, or aldosterone was observed during either infusion, but a marked increase occurred after discontinuation of the high-dose infusion. In conclusion, the 5 μg/min infusion induced a transient diuretic effect, delayed maximal natriuretic activity, and a late fall in blood pressure, with no change in inulin clearance but a dose-related decrease in p-aminohippurate clearance. Despite large amounts of sodium excreted and blood pressure reduction, no counterregulatory changes were observed in the renin-angiotensin-aldosterone system or plasma vasopressin levels during the infusion. (Hypertension 8 [Suppl II]: II-96-II-105, 1986)

KEY WORDS • glomerular filtration rate • effective renal plasma flow • filtration fraction • natriuresis • blood pressure • renin-angiotensin-aldosterone system • arginine vasopressin

Atrial natriuretic peptides (ANPs) have recently been added to the list of circulating hormones that seem to play a part in cardiovascular, volume, and sodium homeostasis. These peptides are synthesized and secreted by the atria\(^1,2\) and can be found in the blood stream.\(^3-7\) The availability of synthetic ANPs has made it possible to study their effects in normal humans, providing a basis for the rational use of these substances to treat various diseases.

Atrial natriuretic peptides have been administered to normal volunteers as bolus injections only\(^8,9\) or bolus injections followed by a short infusion.\(^4\) In these studies, the blood pressure effects as well as solute excretion were monitored. The peptides elicited natriuresis and diuresis\(^8,9\) and induced a transient increase in creatinine clearance,\(^4\) while slightly decreasing blood pressure.\(^5\)

Although the triggering mechanisms and the daily profile of ANP secretion in humans are not yet known, it seems likely that a continuous infusion would yield more information on its physiological role in blood pressure regulation and renal handling of solutes than a bolus injection. We have recently evaluated the dose-response relationship for blood pressure, heart rate, and skin blood flow during an ANP infusion lasting several hours.\(^10\) The aim of the present study was to assess the pharmacological effect of a 4-hour infusion of synthetic ANP, at two different doses, or its vehicle on renal hemodynamics, urinary solute excretion, and plasma levels of various hormones in healthy volunteers under sodium-loaded conditions.
Subjects and Methods

Eight male volunteers, aged 23 to 28 years (mean, 25.4 years) and weighing between 62 and 79 kg (mean, 68.8 kg), participated in the study. Each volunteer gave a medical history and underwent a complete physical examination. Routine laboratory tests were done before and after the administration of the drug. The nature and the purpose of the study had previously been explained, and written informed consent obtained. The protocol was approved by the Hospital Ethics Committee.

Design of the Study

The subjects were allowed free dietary sodium intake and given 5 g/day of NaCl in the form of salt capsules during the 5 days preceding each study day. Urine samples were collected during the 24 hours of the fifth day to estimate electrolyte excretion. On the study day, the volunteers came to the hospital metabolic unit at 0700 after an overnight fast. They had been instructed to drink 250 ml of tap water after rising. Upon arrival, they were weighed and placed on a bed. They remained supine, except for voiding, and fasted throughout the study procedure. Three intravenous catheters were inserted into antecubital veins: two in the same forearm for infusions of inulin, p-aminohippurate (PAH), and the experimental drug, and the third catheter in the contralateral forearm for blood drawing.

Two doses of synthetic atrial natriuretic Ile12- (3-28)exohexapeptide and its vehicle were infused and compared at weekly intervals in a single blind three-way crossover study. The study period consisted of a 2-hour baseline period and a 4-hour treatment period, followed by a 2-hour recovery phase. After a priming dose, infusion of inulin and PAH in 0.9% saline in amounts calculated to provide plasma concentrations of approximately 400 and 20 /g/ml, respectively, were started at a rate of 2 ml/min, to measure the glomerular filtration rate (GFR) as the clearance of inulin and the effective renal plasma flow (ERPF) as the clearance of PAH. Two hours later, an infusion of the vehicle (0.9% saline) or of ANP was started and set to deliver 0, 0.5, or 5.0 /g/min of ANP.

Blood pressure and heart rate were measured at 30-minute intervals. Twelve-lead electrocardiograms were recorded before and 0.5, 4, and 6 hours after the start of the experimental infusions. Blood samples for the measurement of electrolytes, inulin, and PAH were drawn at hourly intervals. Blood samples for the measurement of osmolality, plasma renin activity, immunoreactive angiotensin II, aldosterone, arginine vasopressin, venous pH, and bicarbonate content were drawn at -2, -0.5, 0.5, 1, 4, and 6 hours from the start of the infusion. Urine samples were collected at hourly intervals. To ensure adequate urine flow, the subjects were asked to drink 200 ml of water per hour.

Drug

ANP L-364,343 is a synthetic polypeptide of 26 amino acids. It has been synthesized at Merck Sharp and Dohme Research Laboratories (Rahway, NJ, USA).11, 12 The sequence corresponds to α-human ANP,13, 14 except for the substitution of isoleucine for methionine at position 12 and deletion of the two N-terminal amino acids serine and leucine. Its biological activity is equivalent to the naturally occurring hormone.

Analytical Methods

The methods used to measure plasma renin activity15 (Nussberger et al., unpublished data), plasma angiotensin II,16 aldosterone,17 and arginine vasopressin16 have been reported previously.

Inulin and PAH concentrations in plasma and urine were measured by a microadaptation of a diphenylamine procedure19 and by a microadaptation of a Bratton-Marshall procedure,20 respectively. Osmolality was determined as the freezing point depression with a Roehling Automatik Mikroosmometer (Berlin, Germany). Sodium and potassium were analyzed by direct flame photometry; calcium, phosphate, and plasma chloride were quantified photometrically (Greiner Electronics Selective Analyser G300; Lengenthal, Switzerland); and urinary chloride was measured with a Corning chloride meter (Halstead, England). A 105 hand-held digital pH-meter in automatic temperature compensation mode (Corning, Halstead, Essex, England) was used for the determination of urinary pH.

Constant infusions were carried out with a syringe pump (Perfusor ED 1-300; B. Braun, Melsungen, Germany) for ANP and a volumetric infusion and transfusion pump (Doltron PIM 717; Uster, Switzerland) for inulin and PAH.

Renal Parameters and Statistical Evaluation

Clearances were calculated by the traditional method, using the formula 

\[ C_x = \frac{U_x \times V}{P_x} \]

with \( C \) representing clearance of substance \( x \), \( U_x \) and \( r_x \) urine and plasma concentrations of substance \( x \), and \( V \) the rate of urine flow in milliliters per minute. Fractional excretion was calculated as the clearance of substance \( x \) divided by the clearance of inulin (Cinulin/GFR). Plasma concentrations used for the clearance calculations were the average of the values obtained at the beginning and at the end of each clearance period. The filtration fraction was calculated as the ratio between inulin and PAH clearances (GFR/ERPF).

Free water clearance (C\(_{\text{H}2\text{O}}\)) was calculated as the urinary flow rate (V) minus osmolar clearance (C\(_{\text{osm}}\)).

\[ C_{\text{osm}} = V - C_{\text{H}2\text{O}} \]

The statistical significance of differences was evaluated by a two-way analysis of variance, with replications followed by the Fisher least significant difference test; a p value of less than 0.05 was considered the minimum level of significance.19 Of the eight subjects enrolled in the study, only six received all three scheduled treatments, and only data for these six subjects have been used for the statistical evaluation. Results are expressed as means ± SEM.

Results

Blood Pressure and Heart Rate

Figure 1 depicts the time course of blood pressure and heart rate. No change was observed with the vehi-
Figure 1. Time course of blood pressure and heart rate before, during, and after 4-hour infusions of 0 (vehicle), 0.5, and 5 μg/min of atrial natriuretic peptide of the rat sequence (ANPr) in sodium-loaded normal volunteers.

Renal Function

The effect of ANP infusion on renal function is summarized in Table 1. GFR was not affected by ANP, although a trend toward an increase in GFR during the first hour (120% of the baseline value) was apparent with the 5.0 μg/min infusion (Figure 2, upper panel). ERPF was slightly reduced by the 0.5 μg/min infusion (p < 0.05 vs vehicle at 3 hours) but was markedly reduced from 2 to 4 hours by the 5.0 μg/min infusion (78.4% [p < 0.02], 71.6% [p < 0.001], and 61.8% [p < 0.001] of the baseline value at 2, 3, and 4 hours, respectively), slowly returning toward control values during the recovery phase (see Figure 2, middle panel). The filtration fraction therefore rose from the preinfusion value of 20.9% to 25.1% during the 0.5 μg/min infusion (p < 0.05 vs vehicle) and from 19.6 to 29.9% during the 5.0 μg/min infusion (p < 0.001) (see Figure 2, lower panel).

Electrolytes and Water Excretion

Data on electrolyte excretion are summarized in Table 2.

Sodium and Chloride

Mean sodium excretion during the 24 hours preceding the experimental days was 269, 281, and 207 mmol for the vehicle, 0.5 μg/min, and 5.0 μg/min infusion phases, respectively. This corresponds to a daily salt intake of 12 to 16 g. The amount of sodium administered as the result of the infusion of inulin and PAH was approximately 250 μmol/min, to which the infusion of ANP or its vehicle added 77 μmol/min. Sodium removed by the blood sampling process has not been considered.

At time 0, mean sodium excretion was around 265 μmol/min. During and after the vehicle infusion, mean sodium excretion remained stable at around 350 μmol/min. During the infusion of 0.5 μg/min of ANP, the maximal sodium excretion rate increased to 472 μmol/min during the second hour and remained above control values during the third and fourth hours (p < 0.05 vs vehicle); during the first hour of the recovery period it returned to the value during the vehicle infusion and dropped below that value during the second hour of recovery (Figure 3, upper panel). During the infusion of 5.0 μg/min of ANP, marked natriuresis was observed, with a peak during the second hour of the infusion (968 μmol/min, p < 0.001). From the third hour on, the natriuresis, although still twice the value during the vehicle infusion (732 μmol/min), began to fall, reaching 488 μmol/min during the fourth hour. Recovery phase values were well below those observed during the vehicle infusion (187 and 108 μmol/min at 5 and 6 hours, respectively). The chloride excretion rate was similar to that of sodium (see Figure 3, middle panel).

Mean fractional sodium excretion was 1.8% at time 0 and remained between 2.2 and 2.4% during the saline infusion. After 2 hours of infusion of 0.5 and 5.0 μg/min of ANP, it had increased to 3.0 and 5.6%, respectively. At the same time, mean fractional chloride excretion, which was stable at around 2.6 to 3.0% with the vehicle infusion, increased to 4.5 and 7.5% with the low- and high-dose infusion of ANP, respectively (see Table 1). Plasma sodium and chloride concentrations did not change significantly in response to ANP.

Potassium

Potassium excretion did not increase but actually decreased during the three study periods, including the period of vehicle administration. The largest reduction occurred during the high-dose infusion of ANP. Mean
TABLE 1. Effect of a 4-Hour Infusion of Atrial Natriuretic Peptide on Renal Function in Salt-Loaded Volunteers

<table>
<thead>
<tr>
<th>Renal function</th>
<th>ANP (µg/min)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>GFR (ml/min)</td>
<td>0</td>
<td>113 ± 3</td>
<td>113 ± 4</td>
<td>113 ± 4</td>
<td>115 ± 6</td>
<td>105 ± 3</td>
<td>109 ± 3</td>
<td>110 ± 5</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>111 ± 5</td>
<td>113 ± 7</td>
<td>113 ± 5</td>
<td>114 ± 4</td>
<td>114 ± 5</td>
<td>110 ± 2</td>
<td>109 ± 3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>108 ± 6</td>
<td>130 ± 4</td>
<td>118 ± 8</td>
<td>118 ± 4</td>
<td>124 ± 6</td>
<td>104 ± 6</td>
<td>107 ± 8</td>
</tr>
<tr>
<td>ERPF (ml/min)</td>
<td>0</td>
<td>570 ± 8</td>
<td>569 ± 13</td>
<td>569 ± 17</td>
<td>570 ± 29</td>
<td>538 ± 16</td>
<td>536 ± 20</td>
<td>553 ± 16</td>
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<td></td>
<td>0.5</td>
<td>531 ± 22</td>
<td>494 ± 31</td>
<td>468 ± 28</td>
<td>462 ± 26 $</td>
<td>461 ± 26 $</td>
<td>484 ± 19</td>
<td>504 ± 27</td>
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<td></td>
<td>5.0</td>
<td>554 ± 32</td>
<td>533 ± 21</td>
<td>434 ± 26 $</td>
<td>397 ± 23 $</td>
<td>342 ± 65 $</td>
<td>468 ± 43 $</td>
<td>494 ± 32</td>
</tr>
<tr>
<td>FF (%)</td>
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<td>19.8 ± 0.5</td>
<td>20.0 ± 0.5</td>
<td>20.5 ± 0.5</td>
<td>19.6 ± 0.4</td>
<td>20.2 ± 0.4</td>
<td>19.9 ± 0.5</td>
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<tr>
<td></td>
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<td>20.9 ± 0.4</td>
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<td>25.1 ± 1.1 $</td>
<td>22.8 ± 0.7</td>
<td>21.8 ± 0.6</td>
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<td>5.0</td>
<td>19.6 ± 0.6</td>
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<td>29.9 ± 1.85 $</td>
<td>29.7 ± 1.95 $</td>
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<td>21.6 ± 1.4 $</td>
</tr>
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<td>FEH (%)</td>
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<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.4 ± 0.2 $</td>
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<td></td>
<td>0.5</td>
<td>1.6 ± 0.1</td>
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<td>5.0</td>
<td>1.9 ± 0.2</td>
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<tr>
<td>FEc (%)</td>
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<td>3.0 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>2.6 ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.1 ± 0.1</td>
<td>2.7 ± 0.2</td>
<td>3.7 ± 0.44 $</td>
<td>4.5 ± 1.15 $</td>
<td>3.4 ± 0.4 $</td>
<td>2.8 ± 0.4</td>
<td>2.2 ± 0.3</td>
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<tr>
<td></td>
<td>5.0</td>
<td>2.1 ± 0.3</td>
<td>4.9 ± 0.74 $</td>
<td>7.5 ± 0.99 $</td>
<td>5.6 ± 0.64 $</td>
<td>4.1 ± 0.55 $</td>
<td>1.3 ± 0.2 $</td>
<td>0.8 ± 0.31 $</td>
</tr>
<tr>
<td>FEn (%)</td>
<td>0</td>
<td>21.6 ± 2.9</td>
<td>20.9 ± 1.9</td>
<td>20.2 ± 2.7</td>
<td>16.4 ± 2.5</td>
<td>15.9 ± 2.3 $</td>
<td>13.5 ± 2.3 $</td>
<td>11.5 ± 1.8 $</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>19.2 ± 2.2</td>
<td>18.2 ± 2.7</td>
<td>17.7 ± 2.6</td>
<td>15.7 ± 3.0</td>
<td>12.9 ± 1.9</td>
<td>11.4 ± 2.3</td>
<td>9.7 ± 0.9 $</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>18.2 ± 2.4</td>
<td>18.4 ± 1.9</td>
<td>13.7 ± 2.1 $</td>
<td>9.0 ± 1.3 $</td>
<td>9.0 ± 1.7 $</td>
<td>12.8 ± 4.2 $</td>
<td>10.8 ± 0.8 $</td>
</tr>
</tbody>
</table>

Values are means ± SEM. ANP = atrial natriuretic peptide; GFR = glomerular filtration rate; ERPF = effective renal plasma flow; FF = filtration fraction; FE = fractional excretion.

$ p < 0.05 vs time 0. $ p < 0.01 vs time 0. $ p < 0.001 vs vehicle.

Fractional potassium excretion fell from 21.6 to 11.5%, from 19.2 to 9.7%, and from 18.2 to a low of 9% after the vehicle, low-dose, and high-dose infusions, respectively (see Table 2).

Calcium

Urine calcium excretion followed a pattern similar to that of sodium (see Figure 3, lower panel). It did not change appreciably during the vehicle infusion and increased only slightly with the 0.5 µg/min infusion, from 4.6 to a maximum of 6.3 µmol/min at 2 hours. It increased significantly with the 5.0 µg/min infusion, from 4.3 to 11.5 µmol/min at 1 hour and to 15.3 µmol/min at 2 hours; it decreased to 11.5 and 7.9 µmol/min during the third and fourth hours of the infusion, respectively, and fell below control values to 2.8 and 1.4 µmol/min (p < 0.05 vs vehicle), during the 2-hour recovery phase.

Phosphates

Mean phosphate excretion remained between 18.7 and 20.5 µmol/min during the vehicle phase. No significant change was observed with the low-dose infusion, but a lower excretion tended to occur during the recovery phase. Although mean phosphate excretion increased somewhat with the high-dose ANP infusion (from 18.5 to 27.0 µmol/min at 1 hour) and tended to decrease during the recovery phase, these changes were not statistically significant.

Diuresis, Fractional Excretion of Water, and Free Water Clearance

All relevant data are summarized in Table 3. Urine flow (Figure 4, upper panel) and the fraction of filtered water excreted (V/GFR) remained stable during the vehicle infusion and the low-dose ANP infusion, although during the recovery phase of the latter infusion a significant decrease could be observed. A brisk increase in mean urine flow, from 7.3 to 15.1 ml/min, occurred during the first hour of the 5.0 µg/min infusion, but by the fourth hour, urine flow had fallen below values observed during the vehicle infusion. The mean fractional excretion of water almost doubled during the first hour of the high-dose ANP infusion, from 6.9 to 11.6%, then immediately returned to values that were not statistically different from those obtained with the vehicle infusion.

The tonicity of the urine remained stable during the entire study period with the vehicle and low-dose ANP infusions. During the first hour of the high-dose infusion, the urine became slightly more hypotonic, and the free water clearance reached a high positive value (see Figure 4, middle panel). Urine became hypertonic during the recovery phase. The high-dose ANP infla-
sion produced a twofold increase in osmolar clearance at 2 hours (see Figure 4, lower panel), and a 2.5-fold increase in mean free water clearance occurred at 1 hour.

Urinary pH was the same during the vehicle and low-dose infusions. It did not change at peak diuresis during the high-dose infusion but fell significantly below vehicle infusion values (−0.7 and −0.8 pH units, p < 0.01) during the recovery period. The plasma bicarbonate concentration was not significantly altered during any of the study periods.

**Plasma Hormones**

As a result of high sodium intake during the five days preceding each experimental phase, baseline values of plasma renin activity, angiotensin II, and aldosterone were very low.

**Figure 2.** Effects of 4-hour infusions of atrial natriuretic peptide of the rat sequence (ANP) at 0.5 µg/min (dark gray columns) and 5 µg/min (light gray columns) and its vehicle (blank columns) on inulin clearance (upper panel), p-aminohippurate (PAH) clearance (middle panel), and filtration fraction (lower panel) in sodium-loaded normal volunteers. The significance of differences is represented by open circles, for comparisons with time 0, and by asterisks, for comparisons with the vehicle infusion, as follows: p < 0.05 (1 sign), p < 0.01 (2 signs), and p < 0.001 (3 signs).

**Figure 3.** Influence of 4-hour infusions of atrial natriuretic peptide (ANP) at two doses and its vehicle on the urinary excretion rate of sodium (upper panel), chloride (middle panel), and calcium (lower panel) in sodium-loaded normal volunteers. Symbols are explained in the legend for Figure 2.
**Table 2. Effect of a 4-Hour Infusion of Atrial Natriuretic Peptide on Electrolyte Excretion in Salt-Loaded Volunteers**

<table>
<thead>
<tr>
<th>Electrolyte excretion (µmol/min)</th>
<th>ANP (µg/min)</th>
<th>Hours</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
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<td>U_{Na} × V</td>
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<td>0.5</td>
<td>0.5</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>276 ± 18</td>
<td>357 ± 21</td>
<td>351 ± 32</td>
<td>354 ± 30</td>
<td>338 ± 26</td>
<td>343 ± 34</td>
<td>368 ± 33</td>
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<tr>
<td></td>
<td>253 ± 14</td>
<td>351 ± 35</td>
<td>472 ± 54†</td>
<td>474 ± 39†§</td>
<td>447 ± 42*§</td>
<td>360 ± 43</td>
<td>255 ± 41‡</td>
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<tr>
<td></td>
<td>276 ± 28</td>
<td>729 ± 102†</td>
<td>968 ± 155‡</td>
<td>732 ± 91‡</td>
<td>488 ± 116*</td>
<td>187 ± 35§</td>
<td>108 ± 33.4</td>
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<td>327 ± 33</td>
<td>443 ± 55*</td>
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<td>391 ± 42</td>
<td>315 ± 30</td>
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<td>665 ± 101†</td>
<td>942 ± 158‡</td>
<td>688 ± 85‡</td>
<td>452 ± 109*</td>
<td>161 ± 37</td>
<td>79 ± 28§</td>
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<td>U_{K} × V</td>
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<td>4.9 ± 0.5</td>
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<td>4.6 ± 1.4</td>
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<td>U_{p} × V</td>
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<td>19.1 ± 2.1</td>
<td>19.3 ± 2.0</td>
<td>17.9 ± 2.5</td>
<td>16.0 ± 0.9</td>
<td>13.7 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.5 ± 2.1</td>
<td>27.0 ± 3.2</td>
<td>25.1 ± 3.5</td>
<td>24.0 ± 2.8</td>
<td>21.8 ± 5.0</td>
<td>16.5 ± 1.1</td>
<td>13.6 ± 2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. ANP = atrial natriuretic peptide. Other abbreviations denote urinary excretion of sodium (U_{Na} × V), chloride (U_{Cl} × V), potassium (U_{K} × V), calcium (U_{Ca} × V), phosphate (U_{P} × V).<sup>p < 0.05 vs time 0. \ p < 0.001 vs time 0. \ p < 0.01 vs vehicle. \ p < 0.001 vs vehicle.</sup>

**Table 3. Effect of a 4-Hour Infusion of Atrial Natriuretic Peptide on Water and Osmolality in Salt-Loaded Volunteers**

<table>
<thead>
<tr>
<th>Water/osmolality</th>
<th>ANP (µg/min)</th>
<th>Hours</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (ml/min)</td>
<td>0</td>
<td>7.3 ± 1.5</td>
<td>5.9 ± 0.8</td>
<td>6.5 ± 0.9</td>
<td>6.6 ± 1.2</td>
<td>6.8 ± 1.0</td>
<td>6.0 ± 0.9</td>
<td>6.5 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7.8 ± 1.2</td>
<td>6.9 ± 0.9</td>
<td>7.7 ± 1.0</td>
<td>6.5 ± 0.9</td>
<td>6.3 ± 0.8</td>
<td>3.5 ± 0.7*</td>
<td>5.1 ± 1.0*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>7.3 ± 1.0</td>
<td>15.1 ± 1.4**§</td>
<td>9.7 ± 1.3</td>
<td>8.0 ± 1.0</td>
<td>4.5 ± 1.1</td>
<td>1.8 ± 0.3*§</td>
<td>1.8 ± 0.4†‡</td>
<td></td>
</tr>
<tr>
<td>F/E_{H2O} (%)</td>
<td>0</td>
<td>6.6 ± 1.4</td>
<td>5.4 ± 0.8</td>
<td>5.8 ± 0.8</td>
<td>5.9 ± 1.1</td>
<td>6.4 ± 0.9</td>
<td>5.7 ± 0.9</td>
<td>6.1 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7.2 ± 1.2</td>
<td>5.9 ± 0.8</td>
<td>7.1 ± 0.9</td>
<td>5.8 ± 0.9</td>
<td>5.5 ± 0.8</td>
<td>3.3 ± 0.7†</td>
<td>4.7 ± 0.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>6.9 ± 1.1</td>
<td>11.6 ± 0.9‡</td>
<td>8.2 ± 0.8</td>
<td>6.8 ± 0.8</td>
<td>4.3 ± 0.6§</td>
<td>1.6 ± 0.2‡</td>
<td>1.6 ± 0.4‡‡</td>
<td></td>
</tr>
<tr>
<td>U_{osm} × V</td>
<td>0</td>
<td>1236 ± 76</td>
<td>1308 ± 41</td>
<td>1275 ± 85</td>
<td>1183 ± 64</td>
<td>1161 ± 56</td>
<td>1183 ± 107</td>
<td>1175 ± 63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1126 ± 50</td>
<td>1262 ± 101</td>
<td>1493 ± 125*</td>
<td>1382 ± 136</td>
<td>1346 ± 88</td>
<td>990 ± 115</td>
<td>977 ± 40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>1146 ± 67</td>
<td>1893 ± 245†</td>
<td>2349 ± 341†§</td>
<td>1794 ± 258†§</td>
<td>1342 ± 363</td>
<td>704 ± 80*§</td>
<td>586 ± 77†‡</td>
<td></td>
</tr>
<tr>
<td>C_{osm} (ml/min)</td>
<td>0</td>
<td>4.3 ± 0.3</td>
<td>4.6 ± 0.1</td>
<td>4.5 ± 0.3</td>
<td>4.2 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>4.2 ± 0.4</td>
<td>4.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.0 ± 0.2</td>
<td>4.5 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>4.9 ± 0.5</td>
<td>4.8 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td>3.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.0 ± 0.2</td>
<td>6.6 ± 0.9†§</td>
<td>8.2 ± 1.2†§</td>
<td>6.3 ± 0.9†</td>
<td>4.8 ± 1.3</td>
<td>2.0 ± 0.4</td>
<td>2.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>C_{H2O} (ml/min)</td>
<td>0</td>
<td>3.0 ± 1.4</td>
<td>1.3 ± 0.8</td>
<td>2.0 ± 0.8</td>
<td>2.4 ± 1.0</td>
<td>2.7 ± 0.9</td>
<td>1.9 ± 0.8</td>
<td>2.4 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>3.8 ± 1.2</td>
<td>2.2 ± 0.8</td>
<td>2.7 ± 0.9</td>
<td>1.6 ± 0.8</td>
<td>1.5 ± 0.7*</td>
<td>0.0 ± 0.6†</td>
<td>1.6 ± 0.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>3.7 ± 1.1</td>
<td>9.1 ± 0.6†</td>
<td>2.7 ± 0.7</td>
<td>2.4 ± 0.9</td>
<td>1.6 ± 1.0</td>
<td>0.4 ± 0.2±†</td>
<td>0.3 ± 0.4±‡</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. ANP = atrial natriuretic peptide; V = urinary flow rate; F/E_{H2O} = fractional excretion of water; U_{osm} × V = osmolar load; C_{osm} = osmolar clearance; C_{H2O} = free water clearance.<sup>p < 0.05 vs time 0. \ p < 0.001 vs time 0. \ p < 0.01 vs vehicle. \ p < 0.001 vs vehicle.</sup>
Figure 4. Urine flow rate, clearance of free water (C_H2O), and osmolar clearance as influenced by 4-hour infusions of atrial natriuretic peptide (ANP) at two doses and its vehicle in sodium-loaded normal volunteers. Symbols are explained in the legend for Figure 2.

Figure 5 depicts the angiotensin II and aldosterone values measured during the three experimental infusions. Angiotensin II remained at very low levels until the end of the infusions at 4 hours. Two hours after discontinuation of the high-dose ANP infusion, angiotensin II concentrations were markedly increased (p < 0.001 vs vehicle). A tendency toward enhanced angiotensin II levels was already apparent after 4 hours, but this was mostly due to changes observed in the two volunteers who developed signs and symptoms of marked hypotension (see below). Aldosterone followed the same pattern.

The results of plasma renin activity and arginine vasopressin measurements are shown in Figure 6. Plasma renin activity exhibited the same pattern as that described for angiotensin II and aldosterone, but arginine vasopressin did not change with either of the ANP doses.

Tolerance

During the fourth hour of the high-dose infusion of ANP, one volunteer felt lightheaded when he got up to void. After 2 hours of the high-dose infusions, another volunteer had a sudden fall in blood pressure in the supine position, accompanied by bradycardia, nausea, and diaphoresis. The infusion was immediately stopped, and all the signs and symptoms disappeared within 5 minutes. After this incident, the 5.0 μg/min dose was no longer administered. No significant changes in electrocardiographic tracings occurred during any of the studies.

Discussion

In these studies it was found that ANP had various blood pressure, renal, and hormonal effects in normal volunteers. At the high dose, it decreased blood pressure and increased the heart rate. It also reduced ERPF
in a dose-dependent manner, without changing GFR, thus raising the filtration fraction. It enhanced the urinary excretion of sodium, chloride, and calcium but altered neither kaliuresis nor phosphaturia. At the high dose, it induced a marked but transient diuresis with an increase in the free water clearance. It did not change the already low levels of plasma renin activity, angiotensin II, and aldosterone, but after discontinuation of ANP, all these hormones increased.

The effects of ANP on blood pressure and heart rate were seen only at an infusion rate of 5.0 μg/min and mostly with a lag time of more than 2 hours (i.e., after infusion of a cumulative dose of more than 600 μg). This confirms our previous observations. A puzzling feature of ANP pharmacology is the dissociation between the short half-life and the relatively long lag-time and duration of action on blood pressure after either a bolus injection or short infusions. The direct vasodilator action of the compound is not sufficient to reduce blood pressure, and other factors, such as natriuresis, intracellular shifts of electrolytes, the lack of response of the renin-angiotensin-aldosterone system, or the effect of ANP on the autonomic nervous system, may have played a part.

ANP has been shown in experimental studies either not to change GFR or to markedly increase it. Although a trend toward an increase in GFR was transiently observed during the high-dose infusion, no statistically significant change in GFR was noted in our experiments. Clearance periods of 1 hour may be too long to reflect short-lived variations in GFR. Nevertheless, GFR alterations, if they occur, must be transient, and they do not seem to explain fully the renal effects of ANP infusions. Besides a too-long integration time, other factors might explain the absence of GFR changes in our study, such as species differences, use of synthetic instead of natural atrial extracts, use of infusions instead of bolus injections, or differences in doses administered (we used small doses, compared with those used in experiments showing increases in GFR).

ANP has been shown to decrease, to increase, or not to alter renal plasma flow, depending on the experimental conditions. However, it appears that ANP does not increase ERPF in a sustained manner in normal intact animals. The dose-dependent fall in PAH clearance observed with ANP infusions in our study may reflect either a fall in ERPF or diminished tubular PAH secretion. The present data do not permit us to distinguish between the two possibilities. The distinction between an alteration in ERPF and a change in PAH extraction is relative, since it has been shown that the extraction of PAH is reduced during acute vasodilation and increased during vasoconstriction. Furthermore, a direct effect of ANP on PAH secretion cannot be ruled out, since it has been shown that a low-molecular-weight substance in the plasma of salt-loaded animals inhibits the uptake of PAH by rabbit kidney cortical slices. Finally, the decrease in renal plasma flow may partially be explained by the increased hematocrit.

The decrease in ERPF observed during infusion of ANP has two possible explanations. One is the vasodilating effect of ANP, which has been shown to be comparable in nature to that of sodium nitroprusside and may reduce the venous return and consequently the cardiac output, a determinant of renal blood flow. Another possibility is a shift of renal blood flow away from the cortex. Since PAH clearance reflects only cortical plasma flow, this shift could alter the extraction of PAH, therefore affecting the accuracy of an ERPF estimation based on PAH clearance. However, such a shift in renal blood flow between cortex and medulla due to any vasoactive agent is still controversial. The ensuing rise in the filtration fraction has important consequences for renal solute excretion. Since a rise in the fraction increases the efferent arteriolar protein concentration and hence oncotic pressure, an increase in proximal solute reabsorption will result. This mechanism would tend to counteract the diuretic effect of ANP and could possibly explain the bell-shaped natriuresis curve observed in the present study, as well as in animal studies with the same synthetic ANP (E. H. Blaine, personal communication, 1985). The ANP-induced increase in the filtration fraction is a general finding, since it has been observed in isolated
perfused rat kidney,25, 29 in intact dogs,16, 27 and in rats.28

That ANP promotes sodium and calcium excretion with chloride as the principal anion is accepted by all investigators. However, the effect of ANP on potassium and phosphate excretion is still debated, though in healthy volunteers, no marked change in phosphate excretion has so far been reported. The qualitative nature of solute excretion is important, since it points to the mechanism (or mechanisms) of action underlying ANP. Experimental studies have suggested either that the increase in renal solute excretion is due primarily to hemodynamic changes25, 26, 28, 29 or that this effect is mediated not by changes in GFR22, 23, 32, 40-45 but rather by redistribution of blood flow,31 by inhibition of proximal tubular reabsorption,44, 45 by loop reabsorption,32, 43 or by altered collecting duct reabsorption.22, 40-45 Our study was not designed to examine this issue. Nevertheless, the absence of parallel changes in GFR and natriuresis and the absence of marked changes in phosphate excretion, a known marker of proximal tubular reabsorption, in association with unmodified potassium excretion, are indirect arguments against a primary hemodynamic mechanism and a proximal site of action of ANP under the present experimental conditions. Indeed, increased distal delivery of sodium due either to an increase in the filtered load or to an inhibition of proximal reabsorption would increase potassium excretion, unless distal reabsorption of sodium were simultaneously impaired. In any case, a distal tubular effect must be present to explain our findings.

The high-dose ANP infusion induced transient water diuresis (positive free water clearance) in the absence of osmotic hemodilution. Since no change was observed in arginine vasopressin levels and since ANP does not seem to interact with arginine vasopressin receptors (M. A. Napier, personal communication, 1985), the transient nature of the increased water diuresis cannot be attributed to arginine vasopressin. A more likely explanation is either increased delivery of filtrate out of the proximal tubule (see above discussion of the trend toward an increase in GFR at the high dose) or diminished free water loss secondary to sodium transport inhibition.

In the absence of substantial changes in plasma bicarbonate concentrations, the changes in urinary pH seen after discontinuation of the high-dose ANP infusion suggest no disturbance in buffer capacity but rather an increase in acid secretion. Since the rate of hydrogen-ion secretion is modulated by aldosterone, it is not surprising to find this pH modification coupled with an increase in potassium excretion and paralleling the surge in the plasma aldosterone level, once the inhibitory effect of ANP has vanished.

In spite of marked natriuresis and a fall in blood pressure, we observed no significant effect of ANP on levels of plasma renin activity, angiotensin II, aldosterone, or arginine vasopressin — observations similar to those of Richards et al.8 and Tikkanen et al.4 This may conflict with reports of a depressor effect of ANP on these hormones.26, 27, 46, 47 However, there is probably no contradiction, since in the present study, plasma renin activity, angiotensin II, and aldosterone levels were extremely low to start with, because of salt loading, and a depressor effect of ANP could not have been manifested. Interestingly, a significant increase in these hormones was observed during the recovery phase, suggesting that ANP nevertheless had an inhibitory effect on their secretion.

Since ANP is expected to have a short half-life and therefore steady state conditions should be achieved rapidly with infusion, the relatively slow development of the renal response, which was maximal only during the second hour of continuous infusion, raises some interesting questions about its mechanism of action. The in vitro inhibition of basal and stimulated aldosterone release by atrial extracts49 suggests that a decrease in aldosterone levels could contribute to the natriuresis. The time course of the natriuresis observed in our study could support this view. However, the absence of any significant change in aldosterone during our study does not support such a view, though the absence of kaliuresis in the face of marked natriuresis points to a similar site of action for ANP. Alternatively, ANP may have to induce protein synthesis in the tubular cells to become active.

In summary, 4-hour infusions of two doses of synthetic ANP were administered to normal volunteers, and their effects were compared with those of vehicle administration. The high dose (5 µg/min) induced a transient diuretic effect, delayed maximal natriuretic activity, and a late fall in blood pressure. No change in GFR occurred, but a dose-dependent decrease in ERPF and a consequential rise in the filtration fraction were observed. Despite large amounts of sodium excreted and blood pressure reduction, ANP administration was largely unaccompanied by counterregulatory changes in the renin-angiotensin-aldosterone system and in arginine vasopressin levels.

Note added in proof: The unpublished data of Nussberger et al. mentioned in the text (see Analytical Methods) will be published in 1986.45

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