Enalapril Attenuates Natriuresis of Atrial Natriuretic Factor in Humans

CARLO A. GAILLARD, HEIN A. KOOMANS, AND EVERT J. DORHOUT MEES

SUMMARY We studied the effect of converting enzyme inhibition with enalapril on the natriuresis observed after administration of atrial natriuretic factor (human ANF-[99–126], given as a 100-μg bolus i.v. injection) in eight healthy humans consuming a 100 mmol sodium diet. Without enalapril, sodium excretion rose from 127 ± 19 (mean ± SE) to 437 ± 103 μmol/min in the first 20 minutes after ANF was administered. Clearance studies performed during maximal water diuresis indicated a rise in glomerular filtration rate (inulin clearance), free water clearance, phosphate, lithium, uric acid, and magnesium excretion. Four days of enalapril (20 mg b.i.d.) increased effective renal plasma flow (p-aminohippurate clearance) and reduced blood pressure (from 114/71 ± 2/2 to 105/60 ± 2/1 mm Hg). Under these conditions baseline sodium excretion was not different from the control study, but it rose less after ANF (from 117 ± 22 to 242 ± 63 μmol/min), and the increments in glomerular filtration rate, free water clearance, phosphate, lithium, uric acid, and magnesium were all blunted and nonsignificant. In addition, effective renal plasma flow tended to fall; this effect was not observed when ANF was given without enalapril. These results support the notion that the effects of ANF on renal hemodynamics and on tubular sodium handling depend on renal angiotensin II and that blood pressure reduction may interfere with the ANF-induced natriuresis. (Hypertension 11: 160–165, 1988)

KEY WORDS • atrial natriuretic factor • enalapril • renal sodium handling • angiotensin II • renal hemodynamics

RENAL vasodilator effects, leading to a rise in glomerular filtration rate (GFR) and medullary blood flow, have been proposed to play a major role in the natriuresis evoked by atrial natriuretic factor (ANF).1–3 Glomerular hemodynamics and filtration4, 5 and medullary blood flow6, 7 are normally under the regulating influence of angiotensin II. Therefore, an interaction is suspected between ANF and angiotensin II. In the isolated kidney preconstriction with angiotensin II was shown to enhance the vasodilator and natriuretic effects of ANF.2 In contrast, the natriuretic response to ANF is attenuated in conditions accompanied by a stimulated renin-angiotensin system, such as low sodium intake10, 11 or heart failure.12, 13 This emphasizes the importance of other circulatory factors, such as arterial pressure.

At present it is unknown whether the natriuretic effect of ANF requires an intact renin-angiotensin sys-

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lithium carbonate was ingested at 2200. Clearance studies were performed between 0830 and 1300 after an overnight fast. At 0830 the subjects took a water load of 20 ml/kg. Throughout the remainder of the clearance study, additional water, matching urine output, was supplied and the subjects remained supine. At 0900 a constant infusion of inulin and p-aminobenzoate (PAH) into a lower arm vein was started, preceded by a priming dose. After at least a 1.5-hour equilibration period, and when urine osmolality had reached a minimal value, three freely voided urine samples were collected at 20-minute intervals. Then a bolus injection of 100 μg of ANF was given intravenously and four additional urine collections were made. Blood samples were taken halfway through each collection period through an intravenous cannula placed on the lower arm contralateral to the infusion arm. During the clearance study blood pressure was recorded with a word automatic sphygmomanometer device (Omega 1000, In Vivo Research Laboratories, Tulsa, OK, USA) at 5-minute intervals. This frequency was increased to 2-minute intervals during the period extending from 10 minutes before to 10 minutes after the administration of ANF.

Urine and blood samples were analyzed for osmolality (freezing point depression), sodium and potassium (flame photometry), chloride, phosphate, calcium, magnesium, and uric acid (Technicon RA-1000 autoanalyzer, Tarrytown, NY, USA), lithium (Perkin-Elmer 3030 atomic absorption spectrophotometer, Norwalk, CT, USA), inulin, and PAH. Inulin was hydrolyzed to fructose and determined photometrically with indoleacetic acid.14 PAH was determined photometrically by a chromogenic aldehyde reaction. Plasma renin activity (PRA), aldosterone, and ANF were measured in blood samples drawn before and 10 (ANF only), 30, and 90 minutes after injection of ANF. PRA (expressed as femtomoles of angiotensin I [Ang I] per liter per second) and plasma aldosterone were determined by radioimmunoassay. ANF was extracted from 2.5 ml of plasma by reverse phase chromatography using Baker butyl wide-pore extraction columns (Phillipsburg, NJ, USA), followed by elution with methanol/glacial acetic acid (49:1 vol/vol; recovery of ANF, 62%). After evaporation, the extract was dissolved in radioimmunoassay buffer and aliquots were used for determination by radioimmunoassay using an antibody of Peninsula Laboratories (Merseyside, UK) according to the manufacturer's instructions. Angiotensin converting enzyme activity was measured in blood obtained before the second clearance study by a colorimetric method.15 Plasma renin activity (PRA), aldosterone, and ANF were measured in blood samples drawn before and 10 (ANF only), 30, and 90 minutes after injection of ANF. PRA (expressed as femtomoles of angiotensin I [Ang I] per liter per second) and plasma aldosterone were determined by radioimmunoassay. ANF was extracted from 2.5 ml of plasma by reverse phase chromatography using Baker butyl wide-pore extraction columns (Phillipsburg, NJ, USA), followed by elution with methanol/glacial acetic acid (49:1 vol/vol; recovery of ANF, 62%). After evaporation, the extract was dissolved in radioimmunoassay buffer and aliquots were used for determination by radioimmunoassay using an antibody of Peninsula Laboratories (Merseyside, UK) according to the manufacturer's instructions. Angiotensin converting enzyme activity was measured in blood obtained before the second clearance study by a colorimetric method.15

The ANF used for intravenous injection was the synthetic 28 amino acid compound (human ANF [99–126]), obtained from Bachem (Bubendorf, Switzerland). It was prepared for injection by dissolving 1 mg of the peptide in 2 ml of sterilized acid saline and the solution was then diluted to 10 ml with Haemacel (Behring, Marburg, FRG). After passing through a 0.22 μm filter, the solution was stored in 1-ml volumes at −70°C. These volumes, each containing 100 μg of ANF, were defrosted 30 minutes before use.

Mean arterial pressure was calculated as the sum of one third of the systolic pressure plus two thirds of the diastolic pressure. Inulin clearance (C_{in}) and PAH clearance were regarded as markers of GFR and effective renal plasma flow, respectively. Free water clearance (C_{H2O}) during maximal water diuresis was taken as an index of sodium reabsorption in the diluting segment, which is defined as the nephron beyond the point of isotonicity in the thick ascending limb of Henle's loop. Free water clearance plus the clearance of chloride (C_{Cl}) was regarded as an index of solute delivery to the diluting segment. The equation (C_{H2O} + C_{Cl})/C_{in} therefore represents an approximation of fractional solute delivery to the diluting segment, and the equation C_{H2O}/(C_{H2O} + C_{Cl}) is an approximation of diluting segment reabsorption. The validity of these equations has been discussed by others. The fractional reabsorption of lithium, which has been advanced as a marker of sodium reabsorption in the proximal tubule, was calculated as 1 − lithium clearance (C_{in})/C_{in}.

Values are given as means ± SE. PRA and aldosterone were analyzed after logarithmic transformation. Statistical analysis was performed by one-way analysis of variance (ANOVA) for repeated measures. Differences in ANF-induced changes during and before enalapril treatment were analyzed by two-way ANOVA for a randomized block design. The statistical significance of the differences was tested by Student's t test for paired observations using Bonferroni's protection.

Results

Enalapril and Baseline Data

There were no significant differences in body weight and 24-hour sodium excretion before and after 4 days of enalapril treatment (Table 1). Sodium excretion (mean values of the day before the clearance studies are presented in Table 1) matched sodium intake during both studies. Paired comparison of the blood pressure measurements obtained at 1200 yielded significantly lower values for systolic and diastolic pressures during enalapril treatment. Mean values averaged over 3 days before enalapril and 3 days during enalapril treatment are presented in Table 1. Adherence to the medication was apparent from the marked rise in PRA and from the low converting enzyme activity in the blood obtained during the second clearance study (0.046 ± 0.008 μmol/L/sec; normal range, 0.3–1.2 μmol/L/sec). The fall in mean aldosterone was not significant because a slight rise was measured in two subjects. Large or slight decrements in plasma ANF were found in seven subjects (p = 0.13).

Enalapril caused a profound fall in filtration fraction (Table 2) owing to an increase in renal plasma flow. There was a rise in minimal urine osmolality and in fractional lithium reabsorption, but no significant change in fractional free water clearance, diluting segment solute delivery, or diluting segment reabsorption. Electrolyte and uric acid excretion were not different after 4 days of enalapril treatment (Table 3).
Effects of ANF

Without enalapril treatment, injection of ANF caused a brief natriuresis, peaking in the first 20-minute collection period, and a rise in inulin clearance, which continued in the second collection period (Figure 1). PAH clearance did not change. Although baseline values did not differ, no significant rise in inulin clearance and a smaller increase in sodium excretion rate were observed when ANF was given during enalapril treatment. PAH clearance, clearly elevated at the outset, tended to drop.

As the natriuretic effect was most noticeable in the first 20-minute collection period after ANF injection, only a slight, nonsignificant rise in inulin clearance was found. Nevertheless, filtration fraction rose significantly, mainly because of the coincident fall in PAH clearance, which, however, did not become significant until the second 20-minute collection period (see Figure 1). Most striking was the blunted rise in absolute and fractional sodium excretion rate. Neither a significant increase in free water clearance and diluting segment delivery nor a significant drop in lithium clearance was observed. The fall in fractional reabsorption in the diluting segment was less than that observed in the study without enalapril treatment. The effect on chloride and calcium excretion was also significantly less during enalapril treatment. A similar tendency existed for the excretion of the other electrolytes and uric acid, but the responses did not differ significantly.

Both PRA and plasma aldosterone decreased during the clearance studies (Figure 2). The fall in PRA was proportionately similar without and with enalapril treatment, although during enalapril treatment baseline PRA was obviously elevated. No control studies were performed to establish the normal diurnal variation. Plasma ANF rose comparably during the two studies. Blood pressure showed no consistent change, although a transient drop (one or two measurements) of less than 5% was generally observed anywhere between 2 and 8 minutes after ANF administration. This response was not different during enalapril treatment. Pulse rate, not altered after 4 days of enalapril, also showed no consistent change after ANF administration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Enalapril treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium excretion (mmol/day)</td>
<td>90 ± 11</td>
<td>112 ± 8</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>114 ± 2</td>
<td>105 ± 2*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>71 ± 2</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>86 ± 1</td>
<td>79 ± 1†</td>
</tr>
<tr>
<td>Plasma sodium (mmol/L)</td>
<td>143 ± 1</td>
<td>139 ± 1†</td>
</tr>
<tr>
<td>Plasma potassium (mmol/L)</td>
<td>4.0 ± 0.2</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td>Plasma aldosterone (pmol/L)†</td>
<td>179 ± 47</td>
<td>158 ± 22</td>
</tr>
<tr>
<td>Plasma renin activity (fmol Ang I/L/sec)†</td>
<td>255 ± 49</td>
<td>3149 ± 827*</td>
</tr>
<tr>
<td>Plasma ANF (pmol/L)</td>
<td>16.4 ± 4.8</td>
<td>10.7 ± 2.2</td>
</tr>
</tbody>
</table>

All values are means ± SEM. ANF values obtained during first 20 minutes after ANF administration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>ANF</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{in} (ml/min)</td>
<td>109 ± 7</td>
<td>111 ± 4</td>
<td>*</td>
</tr>
<tr>
<td>C_{PAH} (ml/min)</td>
<td>569 ± 48</td>
<td>696 ± 35</td>
<td>NS</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>19.7 ± 1.2</td>
<td>16.1 ± 0.7</td>
<td>*</td>
</tr>
<tr>
<td>Na excretion (μmol/min)</td>
<td>127 ± 19</td>
<td>117 ± 22</td>
<td>*</td>
</tr>
<tr>
<td>FE_{Na} (%)</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>†</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>15.5 ± 1.4</td>
<td>13.9 ± 1.1</td>
<td>*</td>
</tr>
<tr>
<td>Urine osmolality (mosm/kg)</td>
<td>51 ± 3</td>
<td>58 ± 2</td>
<td>*</td>
</tr>
<tr>
<td>C_{H2O}/C_{in} (%)</td>
<td>11.9 ± 1.2</td>
<td>10.4 ± 0.9</td>
<td>*</td>
</tr>
<tr>
<td>(C_{H2O} + C_{Cl})/C_{in} (%)</td>
<td>12.7 ± 1.3</td>
<td>11.3 ± 1.1</td>
<td>†</td>
</tr>
<tr>
<td>C_{H2O}/(C_{H2O} + C_{Cl}) (%)</td>
<td>93.8 ± 0.7</td>
<td>92.6 ± 0.7</td>
<td>*</td>
</tr>
<tr>
<td>FR_{Li} (%)</td>
<td>66.2 ± 2.1</td>
<td>71.9 ± 1.5</td>
<td>*</td>
</tr>
</tbody>
</table>

All values are means ± SEM. ANF values obtained during first 20 minutes after ANF administration.

* p < 0.01, † p < 0.05: a, ANF compared with baseline before enalapril; b, ANF compared with baseline during enalapril; c, baseline before enalapril compared with baseline during enalapril; d, changes induced by ANF before enalapril compared with during enalapril.
TABLE 3. Urinary Excretion Rates of Electrolytes and Uric Acid During the Clearance Studies

<table>
<thead>
<tr>
<th>Excretion (μmol/min)</th>
<th>Before enalapril</th>
<th>Enalapril treatment</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>ANF</td>
<td>Baseline</td>
</tr>
<tr>
<td>Chloride</td>
<td>90 ± 15</td>
<td>338 ± 84</td>
<td>92 ± 14</td>
</tr>
<tr>
<td>Potassium</td>
<td>85 ± 8</td>
<td>88 ± 8</td>
<td>93 ± 12</td>
</tr>
<tr>
<td>Phosphate</td>
<td>11.3 ± 1.1</td>
<td>16.8 ± 1.7</td>
<td>12.6 ± 2.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.4 ± 0.8</td>
<td>10.9 ± 2.2</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>Magnesium</td>
<td>4.6 ± 1.6</td>
<td>7.9 ± 1.6</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>Uric acid</td>
<td>3.1 ± 0.2</td>
<td>3.9 ± 0.4</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

All values are means ± SEM. ANF values obtained during first 20 minutes after ANF administration.

Discussion

Without enalapril treatment, ANF induced changes in renal performance that were comparable with those seen previously: an immediate transient rise in GFR, filtration fraction, and water and electrolyte excretion.22 The rise in free water clearance suggests increased solute delivery to the diluting segment, defined as the nephron beyond the point of isotonicity in the thick ascending limb of Henle's loop.18\( \Delta V_{\text{H2O}}/\Delta (C_{\text{H2O}} + C_{\text{Cl}}), \) an index of diluting segment reabsorption, was decreased. The excretion of phosphate, uric acid, and lithium, which are mainly reabsorbed in the proximal tubules,20,21 and of magnesium, which is mainly reabsorbed in the thick ascending limb of Henle's loop,23 was increased. Similar changes in renal function and electrolyte excretion after ANF have been found in human studies by others.10,11,24 An increase in maximal free water clearance and lithium clearance has also been observed in dogs.25,26 Notwithstanding the many uncertainties inherent in these clearance techniques, these results suggest that the increased sodium excretion observed after ANF involved a rise in the filtered load and a fall in fractional reabsorption in both proximal and distal nephron segments. Yet, due to the marked rise in GFR, absolute sodium reabsorption may have been increased, in conformity with animal studies.27

The main feature of this study is that the natriuretic effect of ANF was attenuated during enalapril treatment. In addition, the rise in GFR, solute free water clearance, phosphate, uric acid, and magnesium excretion and the fall in lithium reabsorption were blunted and nonsignificant. Fractional reabsorption in the diluting segment was suppressed to a lesser extent than that observed without enalapril treatment. Thus, all changes in renal function seen after ANF administration, in GFR as well as in renal sodium handling, were attenuated during enalapril treatment. One exception was the rise in filtration fraction, which was approximately the same as that observed without enalapril treatment but mainly caused in this instance by a fall in effective renal plasma flow.

Inhibition of angiotensin II formation causes renal

Figure 1. Effects of administration of human ANF, 100 μg i.v., on inulin clearance, PAH clearance, and sodium excretion before (−) and during (+) enalapril treatment. Values are means ± SEM. Single (p<0.05) and double asterisks (p<0.01) indicate a significant difference from the pre-ANF value.

Figure 2. Changes in PRA, aldosterone concentration (both semilogarithmic scale), and plasma immunoreactive ANF during the clearance studies before (•) and during (○) enalapril treatment. Values are means ± SEM. Asterisk (p<0.05) indicates a significant difference from the pre-ANF value.
vasodilation, which was marked in our study by the rise in effective renal plasma flow coincident with a slight decrease in blood pressure. Renal vasodilation seems of prime importance for the natriuretic action of ANF. In particular, preglomerular vasodilation, leading to increased GFR and filtered sodium load, and increased medullary blood flow have been found. The latter probably causes medullary washout and reduced sodium reabsorption in the medullary ascending limb of Henle's loop, which would explain at least partly the fall in fractional free water clearance and the rise in fractional sodium excretion observed after ANF administration. Studies in perfused, isolated kidneys have shown that the vasodilator and natriuretic actions of ANF are enhanced by preconstriction with substances such as norepinephrine and angiotensin II and reduced when vasoconstriction is impaired with a calcium entry blocker. This finding led to the idea that the vasorelaxant effect of ANF concerns antagonism of the prevalent vasoconstrictive activity. Accordingly, renal vasodilation through interference with renal angiotensin II formation would attenuate the natriuretic effect of ANF. It has also been suggested that, in the absence of vasoconstrictors, ANF has mild vasoconstrictive properties, with preferential influence on the efferent arteriole. This would account for the present observation that the ANF-induced rise in filtration fraction was associated with a drop in PAH clearance only during enalapril treatment.

Until recently, a direct effect of ANF on proximal tubular reabsorption has been denied. This would leave unexplained the increased excretion after administration of ANF of lithium, phosphate, and uric acid, alleged indicators of proximal tubular sodium reabsorption. However, the micropuncture study in rats by Harris et al. has shown that ANF may directly impair sodium reabsorption in the proximal tubule, but only when this reabsorption is enhanced by the addition of angiotensin II to the peritubular fluid. Clearly, this interesting finding would provide an explanation for many of our findings, such as the blunted effects of ANF during enalapril treatment on fractional sodium excretion, free water clearance, and lithium reabsorption.

Other indications that angiotensin II may enhance the response to ANF are that the hypotensive effect of ANF in anesthetized rats was absent after pretreatment with captopril and that infusion of ANF in rats with experimental renovascular hypertension caused a natriuresis and a fall in blood pressure only when the blood pressure was renin-dependent. Also, Trippodo et al. found that the natriuretic effect of ANF in rats is enhanced by infusion of pressor doses of angiotensin II, vasopressin, or a combination of epinephrine and norepinephrine. However, saralasin did not attenuate the natriuresis seen after ANF administration in that study. In other studies in rats the natriuresis following ANF was either unaffected or potentiated when ANF was given directly after captopril. During unilateral intrarenal infusion of a subpressor dose of angiotensin II in dogs, the natriuretic effect of systemically administered ANF was less in the infused kidney than in the contralateral kidney. These conflicting observations emphasize that the intensity of the natriuresis after ANF depends on other factors besides the renin-angiotensin system.

Under conditions of low salt intake, the renin-angiotensin system is stimulated while the natriuretic response to ANF (i.e., the absolute and not the percent increase in sodium excretion) is blunted compared with the effect during normal or high salt intake. Similarly, in the sodium-retaining condition of heart failure, the natriuretic effect of ANF is blunted. If stimulation of angiotensin tends to enhance the natriuretic effect, this blunted response must be determined by other factors, such as low blood pressure or high aldosterone concentration. Indeed, lowering of renal perfusion pressure in dogs has been shown to impair the increase of natriuresis and GFR after ANF administration. In this respect it is important to note that enalapril, not unexpectedly, caused a fall in blood pressure, which may have played at least an additional role in the attenuation of the ANF-induced natriuresis.

Our previous observation of unaltered natriuretic effect during prostaglandin synthesis inhibition with indomethacin presents a situation where a suppressed renin-angiotensin system may exist without a blunted response to ANF. The concentration of renal prostaglandins, considered to be low during inhibition of prostaglandin synthesis or a high sodium diet may form another factor determining the response to ANF.

Enalapril treatment tended to lower the plasma ANF concentration, but this effect cannot explain the blunted natriuretic response, since after ANF injection the plasma concentration reached comparable levels with and without enalapril treatment (see Figure 2). Interestingly, the PRA, which was markedly elevated during enalapril treatment, decreased to proportionately similar degrees during the clearance studies with and without enalapril treatment. How this concerns diurnal rhythm or an effect of ANF cannot be determined from these data, however.

In summary, converting enzyme inhibition with enalapril attenuated the natriuretic effect of ANF in humans taking a 100 mmol sodium diet. Clearance studies were compatible with a rise in GFR and a fall in fractional sodium reabsorption in the proximal tubule and in the diluting segment after ANF administration, but these effects were all blunted during enalapril treatment. ANF caused a fall in effective renal plasma flow only during enalapril treatment. If it is true that ANF causes natriuresis mainly through renal vasodilation, these results support the idea that this action depends on renal angiotensin II and that in its absence even a mild vasoconstrictive effect can be observed. Also, the lower blood pressure during enalapril treatment may participate in the attenuation of the ANF-induced natriuresis. Finally, reduced angiotensin II formation may have interfered with the action of ANF on the proximal tubule. The present results were obtained in studies using a rather high dose of ANF. Clearly, evaluation of the meaning of our findings in terms of nor-
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