Enhanced Natriuretic Effect of Atrial Natriuretic Factor During Mineralocorticoid Escape in Humans

CARLO A. GAILLARD, HEIN A. KOOMANS, TON J. RABELINK, BRANKO BRAAM, PETER BOER, AND EVERT J. DORHOUT MEES

SUMMARY We examined the question of whether escape from the sodium-retaining effect of mineralocorticoid involves an increased natriuretic effect of atrial natriuretic factor (ANF). Seven healthy volunteers taking a 170 mmol Na/100 mmol K diet received an intravenous bolus (25 μg) followed by a 1-hour infusion (0.02 μg/kg/min) of ANF (human ANF-[99-126]) before and after 10 days of 9-fludrocortisone acetate, 0.5 mg b.i.d. Escape was accompanied by an increase in body weight (from 72.2 ± 12.9 to 74.0 ± 12.6 kg; p < 0.05), mean arterial pressure (from 95 ± 4 to 109 ± 3 mm Hg; p < 0.01), plasma ANF (from 9 ± 2 to 24 ± 4 pmol/L; p < 0.01), and Inulin clearance (from 124 ± 9 to 137 ± 7 ml/min; p < 0.05). Indexes for renal sodium handling (lithium and free water clearance) were compatible with a decreased “proximal” and an increased “distal” tubular reabsorption fraction. ANF infusion raised inulin clearance comparably before and after escape to 138 ± 10 and 152 ± 7 ml/min, respectively, but the natriuretic effect was much larger (p < 0.05) after escape (from 366 ± 34 to 1294 ± 278 pmol/min) than before (from 248 ± 48 to 630 ± 72 pmol/min). Indexes for tubular reabsorption were consistent with greater suppression of both “proximal” and “distal” tubular sodium reabsorption by ANF after versus before mineralocorticoid expansion. These results indicate that escape is accompanied not only by a rise in plasma ANF but also by potentiation of the natriuretic effect of ANF. This effect involves enhanced suppression of tubular sodium reabsorption rather than enhanced elevation of filtered load. Elevated blood pressure, and thus renal perfusion pressure, may be responsible for this effect. (Hypertension 12: 450-456, 1988)

KEY WORDS • atrial natriuretic factor • mineralocorticoid • renal hemodynamics • renal sodium handling • human studies

SUPPRESSION of sodium retaining factors, such as the renin-angiotensin system or sympathetic activity,1,2 and activation of natriuretic mechanisms, such as increased renal perfusion pressure,3 have been implicated in the mediation of mineralocorticoid escape. Recent studies plead for an additional role of atrial natriuretic factor (ANF), plasma concentrations of which invariably increased in the course of mineralocorticoid administration.4-8 Elevated plasma ANF was also found in patients with primary hyperaldosteronism.9

The exaggerated natriuretic response to an acute sodium load during mineralocorticoid escape10,11 suggests that, in addition to these factors, renal adaptation contributes to the escape phenomenon. This may very well include increased sensitivity to ANF, since the natriuretic effect of this hormone is also enhanced during a high sodium intake.12,13 We therefore investigated the renal response to ANF in healthy humans before and during mineralocorticoid escape.

Subjects and Methods

Studies were performed in seven healthy volunteers (5 men, 2 women), aged 23.1 ± 2.1 years. Informed consent was obtained, and the study was approved by the Hospital Ethical Committee for Studies in Humans. The subjects took a diet containing 170 mmol Na and 100 mmol K daily as outpatients, and clearance studies were performed on the morning of the 5th and 16th days of this diet. After the first clearance study 9-fludrocortisone acetate, 0.5 mg twice daily, was started. The final
dose was taken on the morning of the 16th day 2 hours before the start of the second clearance study. The subjects visited the metabolic ward daily at 1200 where meals were provided and body weight was measured. Twenty-four-hour urine collections were made throughout the study.

On the eve of each clearance study 400 mg of lithium carbonate was ingested at 2200. Clearance studies were performed between 0830 and 1300 after an overnight fast and with the subjects in supine position. At 0830 the subjects took a water load of 20 ml/kg, and additional water matching urine output was supplied for the remainder of the clearance study. At 0900 a constant infusion of inulin and p-aminohippurate (PAH) into a lower arm vein was started, preceded by a priming dose. After at least 1.5 hours of equilibration, and when urine osmolality had reached a minimal value, three freely voided urine samples were collected at 15-minute intervals. Then, a 1-hour ANF infusion (0.02 

µg/kg/min) was started, preceded by a bolus injection of 25 

µg of ANF. During the infusion four urine collections were made, followed by two additional collections after termination of the infusion. 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 an automatic sphygmomanometer device (Omega 1000, Invivo Research Laboratories, Tulsa, OK, USA) at regular intervals.

Urinary and blood samples were analyzed for osmolality (freezing point depression); sodium and potassium (flame photometry); chloride, phosphate, calcium, magnesium, and uric acid (Technicon RA-autoanalyzer, Tarrytown, NY, USA); lithium (Perkin-Elmer 3030 atomic absorption spectrophotometer, Norwalk, CT, USA); inulin; and PAH. Inulin was hydrolyzed to fructose and then determined photometrically with indoleacetic acid. 

PAH was determined photometrically by a chromogenic aldehyde reaction. Plasma renin activity (PRA) and aldosterone were measured on the day before the clearance study. ANF was measured in blood samples drawn on the 4th, 10th, 13th and 15th days of the study, 15 minutes after insertion of an i.v. cannula and after at least 1 hour of supine rest. In addition, blood for PRA, aldosterone, and ANF was taken on the day of the clearance study before and during the ANF infusion. PRA (fmol angiotensin I (Ang I)/L/sec) and plasma aldosterone were determined by radioimmunoassay. ANF was extracted from 2.5 ml of plasma by reversed-phase chromatography using Baker butylsilane wide pore extraction columns (Phillipsburg, NJ, USA), followed by elution with methanol/trifluoroacetic acid 99 : 1 (vol/vol) (recovery of ANF, 62%). After evaporation, the extract was dissolved in radioimmunoassay using antibody of Peninsula Laboratories (Merseyside, UK) according to the manufacturer’s instructions (coefficients of variation: interassay, 17%; intra-assay, 11%; ED₅₀, 8 fmol; lower limit of sensitivity, 0.5 fmol). Values were corrected for percent recovery.

The ANF used for infusion was synthetic 28 amino acid compound (human ANF-[99-126]) obtained from Bissendorf Peptide GmbH (Hanover, FRG).

Mean arterial pressure was calculated as the sum of one third of the systolic pressure plus two thirds of the diastolic pressure. Clearances of inulin (Cᵢᵤ) and PAH were regarded as markers of, respectively, glomerular filtration rate (GFR) and effective renal plasma flow. Free water clearance (Cₑ₂ₒ) 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. 

The Cₑ₂ₒ plus the clearance of chloride (Cₑₒ) was regarded as an index of delivery to the diluting segment. The term (Cₑ₂ₒ/Cₑₒ + Cₒ) therefore represents an approximation of the fractional solute delivery to the diluting segment, and the term (Cₑ₂ₒ/Cₑₒ + Cₒ) an approximation of diluting segment reabsorption. The validity of these terms has been discussed by others. The fractional excretions of lithium (FEₐₜ) and of sodium were calculated according to the standard formula.

Values are given as means ± SE. Statistical analysis was performed by one-way analysis of variance (ANOVA) for repeated measures. Differences in ANF-induced changes during and before fludrocortisone 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**

Fludrocortisone and Baseline Data

Fludrocortisone induced sodium retention and a gradual rise in weight during the first 4 days (Figure 1). From the 5th day onward, body weight stabilized and sodium excretion returned to the baseline level, indicating that escape from sodium retention had taken place. The sodium retention was accompanied by a rise in plasma ANF (Figure 2) and suppression of PRA. Mean arterial pressure increased. Pertinent data given in Table 1 concern measurements on the 2 days preceding the clearance studies, except for blood pressure, which was measured during the clearance study.

The effects of fludrocortisone on renal function can be appreciated by comparison of the baseline columns in Tables 2 and 3. Marked increments occurred in GFR, urine flow, FEₐₜ, Cₑ₂ₒ, and (Cₑ₂ₒ/Cₑₒ + Cₒ). The rise in Cₑ₂ₒ/(Cₑₒ + Cₒ) did not reach significance. In these clearance studies the baseline sodium excretion rate, both absolute and fractional, appeared higher and potassium excretion rate lower during fludrocortisone treatment, although
24-hour excretions (see Table 1) were not different from control conditions. Baseline calcium and magnesium excretion were also significantly increased.

**Effects of ANF**

In the control study ANF caused a natriuretic response that lasted throughout the duration of the infusion and diminished gradually thereafter. Figure 3 presents $C_m$, PAH clearance, mean arterial pressure, plasma ANF, and sodium excretion throughout the clearance studies. Although basal plasma ANF was increased during escape, the levels obtained during the infusion of ANF were comparable to infusion levels in the control study. ANF had no consistent effects on mean arterial pressure in either study. $C_m$ rose similarly in the control study and during fludrocortisone treatment, although baseline values were different. PAH clearance changed in neither study. In spite of these similarities, the increase in sodium excretion rate was much larger during fludrocortisone.

Table 2 and 3 give mean values of the clearance data during the 45 minutes before ANF (baseline) and during the last 45 minutes of ANF infusion (ANF). Infusion of ANF was followed by a rise in $C_m$, filtration fraction, urine flow, and urine osmolality. Estimation from changes in $C_{H_2O}$ indicated that the rise in fractional sodium excretion originated from a fall in fractional sodium reabsorption proximal to and in the diluting segment. Fractional lithium reabsorption dropped as well, and the excretion of chloride, calcium, and magnesium was enhanced by ANF. During mineralocorticoid escape all these effects were stronger with the exception of the rise in $C_m$. In addition, potassium, phosphate, and uric acid excretion increased when ANF was infused during mineralocorticoid escape, whereas no such change was observed when ANF was infused in the control study.

In the control study PRA fell from 172 ± 25 to 123 ± 17 fmol Ang I/1/sec ($p < 0.05$; blood samples taken 10 minutes before and 50 minutes after initiation of the ANF infusion). Aldosterone decreased from 157 ± 22 to 87 ± 6 pmol/L ($p < 0.05$). During escape PRA and aldosterone were lower and did not change significantly after ANF (from 23 ± 4 to 30 ± 5 fmol Ang I/1/sec, and from 94 ± 15 to 78 ± 16 pmol/L, respectively).

**Discussion**

The main feature of this study in humans is that the natriuresis induced by ANF was greatly enhanced after expansion by mineralocorticoid administration. It is therefore likely that increased sensitivity of the kidney to the natriuretic action of ANF also contributes to the escape from the sodium-retaining effect of mineralocorticoid. An enhanced natriuretic effect of ANF is also found during high sodium intake. In the latter condition, however, activity of mineralocorticoid in the kidney that would oppose natriuresis is minimal, in contrast with the present conditions where this activity is strong.
Recent reports have described a twofold to threefold rise in plasma ANF during mineralocorticoid expansion in humans, and experimental animals. This observation, confirmed by our data, is compatible with the idea that volume expansion stimulates ANF release, which in turn restores sodium balance. However, the plasma concentrations achieved did not appear to be strongly natriuretic in infusion studies, and it is likely that increased plasma ANF forms part of an integrated response to volume expansion.

The effects of either fludrocortisone or ANF alone on renal function parameters were comparable to those reported previously. In short, fludrocortisone caused a rise in inulin clearance and fractional excretion of sodium, maximal urine flow, and uric acid excretion, again compatible with increased filtered sodium load and decreased proximal tubular reabsorption fraction. Such changes probably serve to overcome the elevated reabsorption rate of sodium that has taken place in the cortical collecting tubule under the influence of mineralocorticoid.

The value for tended to increase as well. This response would indicate an increased solute reabsorption fraction in the diluting segment, which during maximal water diuresis includes the collecting tubules. These interpretations of clearance data, described more extensively elsewhere, should, of course, be taken with appropriate caution.

Infusion of ANF caused a rise in GFR, filtration fraction, urinary excretion rates of electrolytes and uric acid, and aldosterone secretion, again compatible with increased filtered sodium load and decreased proximal tubular sodium reabsorption fraction. The enhanced natriuretic effect was therefore due to more pronounced suppression of tubular reabsorption. Most of the clearance data presented in Table 2 and 3 are indeed compatible with such an effect. Whereas the larger increases in (C_H2O + C_CO) tended to increase as well.

### TABLE 2. Effects of ANF and Fludrocortisone on Renal Hemodynamics and Sodium Handling

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>ANF</th>
<th>Fludrocortisone</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>ANF</td>
<td>Baseline</td>
<td>ANF</td>
</tr>
<tr>
<td>C_E (ml/min)</td>
<td>124 ± 9</td>
<td>138 ± 10</td>
<td>137 ± 7</td>
<td>152 ± 7</td>
</tr>
<tr>
<td>C_MH (ml/min)</td>
<td>670 ± 55</td>
<td>645 ± 59</td>
<td>655 ± 87</td>
<td>680 ± 80</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>18.7 ± 0.9</td>
<td>21.6 ± 0.6</td>
<td>22.3 ± 2.0</td>
<td>23.5 ± 1.9</td>
</tr>
<tr>
<td>Sodium excretion (µmol/min)</td>
<td>248 ± 48</td>
<td>630 ± 124</td>
<td>366 ± 34</td>
<td>1294 ± 278</td>
</tr>
<tr>
<td>F_Ea (%)</td>
<td>1.4 ± 0.2</td>
<td>3.2 ± 0.6</td>
<td>1.9 ± 0.2</td>
<td>5.9 ± 1.2</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>17.7 ± 1.8</td>
<td>22.7 ± 2.4</td>
<td>24.9 ± 1.4</td>
<td>38.4 ± 3.4</td>
</tr>
<tr>
<td>Urine osmolality (mosm/kg)</td>
<td>61 ± 4</td>
<td>75 ± 6</td>
<td>47 ± 4</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>C_H2O/C_E (%)</td>
<td>11.1 ± 0.7</td>
<td>11.8 ± 0.6</td>
<td>15.1 ± 0.6</td>
<td>18.1 ± 0.9</td>
</tr>
<tr>
<td>(C_H2O + C_CO)/C_E (%)</td>
<td>12.7 ± 0.7</td>
<td>15.6 ± 1.2</td>
<td>16.8 ± 0.5</td>
<td>24.8 ± 2.0</td>
</tr>
<tr>
<td>(C_H2O + C_CO)/C_CO (%)</td>
<td>86.8 ± 1.1</td>
<td>76.7 ± 2.8</td>
<td>89.6 ± 1.3</td>
<td>74.2 ± 3.6</td>
</tr>
<tr>
<td>F_EL (%)</td>
<td>28.9 ± 1.0</td>
<td>32.3 ± 1.5</td>
<td>44.3 ± 2.4</td>
<td>54.5 ± 3.0</td>
</tr>
</tbody>
</table>

All values are means ± SEM; ANF denotes mean of fifth, sixth, and seventh urine collections.

* *p < 0.05, t *p < 0.01; a = ANF compared to baseline in control study; b = ANF compared to baseline during fludrocortisone; c = baseline control study compared to baseline during fludrocortisone; d = changes induced by ANF in control study compared to during fludrocortisone.

### TABLE 3. Urinary Excretion Rates of Electrolytes and Uric Acid During Clearance Studies

<table>
<thead>
<tr>
<th>Excretion</th>
<th>Control</th>
<th>Fludrocortisone</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>ANF</td>
<td>Baseline</td>
</tr>
<tr>
<td>Chloride (µmol/min)</td>
<td>216 ± 31</td>
<td>543 ± 111</td>
<td>247 ± 27</td>
</tr>
<tr>
<td>Potassium (µmol/min)</td>
<td>123 ± 11</td>
<td>107 ± 14</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>Phosphate (µmol/min)</td>
<td>10.8 ± 2.2</td>
<td>16.7 ± 3.9</td>
<td>13.4 ± 2.1</td>
</tr>
<tr>
<td>Calcium (µmol/min)</td>
<td>2.5 ± 0.8</td>
<td>8.3 ± 1.9</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td>Magnesium (µmol/min)</td>
<td>3.6 ± 0.4</td>
<td>7.1 ± 1.3</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>Uric acid (µmol/min)</td>
<td>3.6 ± 0.3</td>
<td>4.2 ± 0.5</td>
<td>3.7 ± 0.3</td>
</tr>
</tbody>
</table>

All values are means ± SEM; ANF denotes mean of fifth, sixth, and seventh urine collections.

* *p < 0.05, t *p < 0.01; a = ANF compared to baseline in control study; b = ANF compared to baseline during fludrocortisone; c = baseline control study compared to baseline during fludrocortisone; d = changes induced by ANF in control study compared to during fludrocortisone.
FIGURE 3. Effects of ANF (25 μg bolus followed by a 0.02 μg/kg/min infusion) on inulin clearance, \( \text{p-aminohippurate (PAH) clearance, mean arterial pressure, plasma ANF concentration, and sodium excretion before (control) and during fludrocortisone treatment. The numbers 2 through 8 denote subsequent 15-minute collection periods. Values are means ± SEM.} \)

\[
\text{C}_\text{O}, \text{C}_\text{H}_2\text{O}, \text{FE}_\text{A}, \text{ and uric acid excretion each indicate a more pronounced effect on proximal reabsorption, the larger decrease in } \text{C}_{\text{H}_2\text{O}}/(\text{C}_\text{H}_2\text{O} + \text{C}_\text{O}) \text{ indicates more pronounced suppression of diluting segment reabsorption as well. The rise in excretion of magnesium, which is reabsorbed mainly in Henle’s loop, was also larger. These results suggest that the stronger suppression of tubular reabsorption occurred in various nephron segments rather than that it was limited to any particular segment.}}
\]

Uncertainty exists with regard to the factor(s) responsible for the enhanced natriuretic effect of ANF infusion. Apparently, it was not due to a higher plasma ANF concentration, since the infusion generated comparable plasma ANF levels in the studies before and after mineralocorticoid escape, despite an elevated preinfusion level in the latter condition. Down-regulation of ANF receptors, as has been found in mesenteric arteries and glomeruli of mineralocorticoid-treated rats,

3, 7, 26 would rather lead to a blunted natriuretic response. Indeed, the vasodilator response to ANF was blunted in these rats,

6, 27 which was attributed to the receptor down-regulation. In another study in rats, however, an exaggerated blood pressure response to ANF was found during mineralocorticoid escape. Since we found no consistent effect on effective renal plasma flow during ANF infusion, whether given before or during mineralocorticoid escape, an effect of escape on the vascular response to ANF was not apparent in our study.

The volume retention of fludrocortisone administration was associated with blood pressure rise and suppression of the renin-angiotensin system or, respectively, activation of a natriuretic factor and deactivation of a sodium-conserving factor. These well-known changes are considered to play separate roles in the mediation of escape,\(^1\)\(^-\)\(^3\) but they may also potentiate the effect of ANF. In this regard it is interesting to compare the present data during mineralocorticoid escape with studies during converting enzyme inhibition. In each condition angioten-

sin II formation is suppressed, but in the case of converting enzyme inhibition blood pressure is low and natriuretic response to ANF tends to be reduced,\(^22\)\(^-\)\(^29\) whereas during mineralocorticoid escape both are apparently increased. The possibility that blood pressure is a determinant of ANF-induced natriuresis therefore deserves serious consideration. This possibility is in accord with the observation of an enhanced natriuretic effect of ANF in genetic hypertension.\(^30\)\(^-\)\(^31\) Animal experiments have shown that the natriuretic effect of ANF can be enhanced or blunted greatly by directly raising or reducing the renal perfusion pressure\(^32\)\(^-\)\(^33\) and, conversely, that ANF infusion augments pressure-natriuresis.\(^34\) Importantly, the data in those studies clearly established the existence of such an effect also within the relatively small range of blood pressure changes achieved with, respectively, mineralocorticoid (present study) or converting enzyme inhibition\(^22\) in humans.

The mechanism underlying changes in renal sodium handling during chronic blood pressure elevation is uncertain, but it probably concerns a hemodynamic effect, resulting in increased GFR and greater transmission of arterial pressure to the postglomerular capillaries by renal vasodilation.\(^2\)\(^-\)\(^3\) The resultant rise in peritubular hydrostatic pressure would reduce tubular sodium reabsorption. It has been shown in rats that escape from the sodium-retaining effect of mineralocorticoid is associated with increased hydrostatic pressure in peritubular capillaries of superficial nephrons, vasa recta, and interstitium.\(^35\) These effects may be reinforced by administration of ANF, which acts as a renal vasodilator, increasing glomerular filtration pressure,\(^36\) medullary blood flow,\(^37\)\(^-\)\(^38\) and vasa recta pressure.\(^39\)

It is plausible that this results in stronger suppres-
tion of proximal as well as distal tubular reabsorption, consistent with the data from our study. The natriuretic effect of ANF may thus depend on the prevailing peritubular Starling forces favoring sodium rejection or reabsorption, in accordance with studies in rats in which the natriuretic effect of ANF was enhanced with increased blood pressure and reduced with increased plasma oncotic pressure.40

Increased natriuresis of ANF during escape may also have resulted from the fact that the rise in GFR, though not greater than before expansion, was superimposed on an already elevated GFR. This finding implies further acceleration of tubular flow, and since glomerulotubular balance is probably imperfect,41 this would cause a further drop in tubular reabsorption fraction. On the other hand, augmentation of ANF natriuresis by increased blood pressure occurred independently of changes in GFR in animal experiments.42

Increased natriuretic response to atrial extract has been found previously in mineralocorticoid-escape rats.42 Some increase in kaliuresis, not dissimilar from that observed after escape, was noticed in that study. In this respect our data differ, since we found that during mineralocorticoid escape the kaliuretic effect of ANF was increased significantly. Indeed, enhanced delivery of NaCl and fluid to the collecting tubule in combination with high mineralocorticoid action would create the ideal setting to stimulate kaliuresis.43 However, compared with the huge natriuresis, kaliuresis was modest, and it seems likely that this response expresses a specific action of ANF on the collecting tubules. Evidently, the commonly observed absence of substantial kaliuresis despite huge natriuresis after ANF is not caused by suppression of aldosterone by ANF, since our study design implied a constant mineralocorticoid action on the kidney.

In summary, escape from the sodium-retaining effect of mineralocorticoid was accompanied by increased plasma ANF concentration and an increased natriuretic effect of ANF in this study in humans. Enhanced natriuresis involved a larger rise in fractional sodium excretion rather than in filtered load. Results of clearance studies were compatible with greater suppression of sodium reabsorption in proximal as well as distal segments of the nephron.

It is conceivable that these effects of ANF are mediated hemodynamically and that they are reinforced by the elevated renal perfusion pressure in escape. Potentiation of the natriuretic effect of ANF may take part in the establishment of escape and in the exaggerated natriuresis observed after an acute sodium load during exaggerated natriuresis observed after an acute sodium load during escape. Further definition of the role of ANF in the escape phenomenon awaits studies with specific ANF blocking agents.

Acknowledgment

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