Atrial Natriuretic Factor Influences Renal Diurnal Rhythm in Essential Hypertension

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We investigated in six patients with essential hypertension the effect of a low dose atrial natriuretic factor infusion for 5 days on the diurnal rhythm of renal electrolyte excretion. Atrial natriuretic factor infusion increased the net excretion of sodium and caused a delay in its time of maximal diurnal urinary excretion. Similarly, atrial natriuretic factor caused an increase in the net excretion of chloride, calcium, and magnesium and also changed the diurnal rhythms of these electrolytes. In contrast, atrial natriuretic factor did not change the net excretion of potassium, phosphate, and uric acid, nor did atrial natriuretic factor change the diurnal rhythms of these solutes. During baseline, the time points of maximal urinary excretion of sodium and potassium overlapped, whereas atrial natriuretic factor infusion caused sodium excretion to peak 2.2±0.3 hours (p<0.02) after the potassium excretion peak. During baseline, the time of maximal urinary excretion of sodium did not correlate with the time of highest blood pressure, whereas it correlated negatively with mean plasma aldosterone concentration. In contrast, during atrial natriuretic factor infusion the time of maximal urinary excretion of sodium correlated positively with the time of highest blood pressure, whereas it did not correlate with mean plasma aldosterone concentration. These data suggest that atrial natriuretic factor is involved with the diurnal rhythm of the urinary excretion of sodium and that atrial natriuretic factor-induced natriuresis is mediated in part by blood pressure and plasma aldosterone. (Hypertension 1992;20:80–84)

Key Words • atrial natriuretic factor • circadian rhythm • electrolytes • glomerular filtration rate • essential hypertension

Renal sodium handling has been found to be abnormal in patients with essential hypertension (EH). One example is the exaggerated natriuresis in EH after volume loading. Also the diurnal time of maximal urinary sodium excretion has been reported to be different in patients with EH compared with normotensive subjects. In normotensive subjects maximal urinary sodium excretion occurs later than maximal potassium excretion. In patients with EH this relation has not yet been investigated. Since atrial natriuretic factor (ANF) is known to be released during volume expansion and may give rise to an enhanced natriuresis in EH, we investigated in patients with EH the relation between the time of maximal diurnal urinary sodium and potassium excretion as well as the effects of low (physiological) dose ANF infusion on this relation.

Methods
Six male patients (mean age, 51±4 years) with uncomplicated EH (blood pressure, 160±5/101±1 mm Hg; mean±SEM) were enrolled in the study. The study protocol was approved by the hospital's Medical Ethical Committee, and all patients gave informed consent. Patients were placed on a diet that contained 150 mmol sodium per day and were asked to discontinue any medication for at least 6 weeks before the study. The patients were then admitted to our metabolic ward for a 14-day period, where they held absolute bed rest except for urine voiding and bathroom facilities (8 to 8:30 AM). They received a rhythm diet consisting of equal amounts of sodium (156 mmol/day), potassium (90 mmol/day), and fluids (1,800 ml/day) every 4 hours during the whole study. In addition, they were continuously infused with 32 ml/hr dextrose 5% (to ensure adequate diuresis) and 2 ml/hr solvent (with or without ANF). The first 3 days were used for equilibration. The next 3 days were used to measure baseline values. Baseline sodium excretion was 154±10 mmol/24 hr, equal to the daily sodium intake. The baseline period was followed by a 5-day ANF infusion period, during which synthetic human ANF (101–126, MSD-RL, Rahway, N.J.) dissolved in 5 g mannitol per 100 ml was infused at a dose of 0.2 μg/min. Recovery measurements were performed during the last 3 days. During all periods blood pressure was recorded every 15 minutes with an automatic noninvasive device (Dinamap, Critikon Inc., Tampa, Fla.). Mean arterial pressure (MAP) was calculated as two thirds diastolic plus one third systolic blood pressure. Urine collection was performed every 4 hours for analysis of the urinary excretion of sodium (U_{NaV}), chloride (U_{ClV}), potassium (U_{KV}), calcium (U_{CaV}), phosphate (U_{P_{Na}V}), magnesium (U_{MgV}), and uric acid (U_{UA}V). Blood was sampled every 4 hours during all study days before urine voiding for

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measurement of immunoreactive ANF (irANF). To minimize the amount of blood withdrawal, blood samples for determination of the diurnal rhythms of glomerular filtration rate (GFR), plasma renin activity (PRA), and plasma aldosterone concentration (PAC) were only taken every 4 hours during the last 24 hours of each period. Each time that blood was drawn, an equivalent amount of sodium and chloride was administered intravenously.

Urinary electrolyte concentrations were measured using standard autoanalyzer techniques (Technicon Instr. Corp., Tarrytown, N.Y.). Blood samples for measurement of irANF, PRA, and PAC were collected in prechilled EDTA tubes and immediately centrifuged, and the plasma was stored at -25°C to process all samples of one patient simultaneously. irANF was determined after extraction on a Sep-Pak C18 cartridge (Waters, Milford, Mass.) using a commercially available 125I-ANF tracer and a highly specific ANF antibody (INCSTAR, Stillwater, Minn.).

PRA and PAC were measured by radioimmunoassay. Within 4 hours after the start of ANF infusion U\text{N,V}, U\text{C,V}, and U\text{P,V} increased compared with baseline. MAP started to decrease that time, the urinary excretion of all solutes matched baseline values again. After cessation of ANF infusion, plasma irANF returned to preinfusion values, whereas baseline values again. No changes were observed in the mean values of GFR, PRA, or PAC during ANF infusion. All data are given as mean±SEM. Statistical analysis for the effects of ANF infusion was done by analysis of variance with a repeated measurement design followed by Duncan's multiple range test and by Pearson's correlation coefficient for correlation between variables. Differences were considered statistically significant at $p<0.05$.

**Results**

ANF infusion resulted in an immediate increase in plasma irANF from 49±10 to 110±20 pg/ml (Table 1). Within 4 hours after the start of ANF infusion $U_{\text{N,V}}$, $U_{\text{C,V}}$, and $U_{\text{P,V}}$ increased compared with baseline. Net cumulative negative balances for these urinary electrolytes after 48 hours of ANF infusion were: 72±15 mmol for sodium ($p<0.01$), 60±12 mmol for chloride ($p<0.01$), 1.42±0.39 mmol for calcium ($p<0.01$), and 0.42±0.17 mmol for magnesium ($p<0.05$). The increase in absolute amounts of $U_{\text{N,V}}$ during the first 4 hours of ANF infusion tended to correlate with preinfusion values of MAP ($r=0.67$, $p<0.01$). In contrast, ANF infusion did not induce changes in $U_{\text{N,V}}$, $U_{\text{P,V}}$, and $U_{\text{P,V}}$ compared with baseline. MAP started to decrease gradually 12 hours after the onset of the ANF infusion. After 40 hours, MAP had decreased by 11.8±1.4 mm Hg ($p<0.01$) and did not decrease any further. At that time, the urinary excretion of all solutes matched baseline values again. No changes were observed in the mean values of GFR, PRA, or PAC during ANF infusion. Thus, during the last 3 days of ANF infusion all variables except plasma irANF and MAP equaled baseline values again. After cessation of ANF infusion, plasma irANF returned to preinfusion values, whereas the negative cumulative balances for all electrolytes were compensated for by retention of these electrolytes. MAP only recovered slowly.

Significant circadian rhythms were found for the urinary excretion of sodium (at least $p<0.025$, $p<0.005$, and $p<0.025$ in each individual during baseline, ANF infusion, and recovery, respectively), chloride

| Table 1. Immunoreactive Atrial Natriuretic Factor, Urinary Solute Excretion, and Mean Arterial Pressure Calculated From Data of Three Baseline Days, First Two and Last Three Days of Atrial Natriuretic Factor Infusion, and the Last Two Recovery Days |
|-----------------|--------------|--------------|--------------|--------------|
| Parameter       | Baseline     | ANF (0–48 hr) | ANF (48–120 hr) | Recovery     |
| irANF (pg/ml)   | 49±10        | 110±20*      | 106±19*       | 41±11        |
| Sodium (mmol/4 hr) | 26.2±1.6    | 31.6±1.4*    | 25.3±1.2      | 26.2±2.4     |
| Chloride (mmol/4 hr) | 25.0±1.4    | 29.5±1.0*    | 24.2±1.1      | 25.2±1.9     |
| Potassium (mmol/4 hr) | 11.8±0.6    | 10.5±0.4     | 11.0±0.4      | 12.3±0.3     |
| Calcium (mmol/4 hr) | 0.97±0.18   | 1.09±0.19*   | 0.98±0.17     | 0.93±0.17    |
| Phosphate (mmol/4 hr) | 3.78±0.20   | 3.64±0.17    | 3.62±0.1      | 3.83±0.11    |
| Magnesium (mmol/4 hr) | 0.68±0.05   | 0.72±0.05*   | 0.67±0.08     | 0.63±0.07    |
| Uric acid (mmol/4 hr) | 0.70±0.03   | 0.70±0.01    | 0.74±0.02     | 0.77±0.02    |
| MAP (mm Hg)     | 105±3       | 98±3*        | 93±2*         | 97±4*        |
| GFR (ml/min/1.73 m²) | 90±3        | ...          | 89±2          | 87±3         |
| PRA (nmol/l/hr) | 0.52±0.07   | ...          | 0.64±0.08     | 0.63±0.11    |
| PAC (nmol/l)    | 0.21±0.02   | ...          | 0.23±0.03     | 0.23±0.02    |

Values are mean±SEM. Mean of glomerular filtration rate (GFR), plasma renin activity (PRA), and plasma aldosterone concentration (PAC) were each calculated over the last day of each period. ANF, atrial natriuretic factor; irANF, immunoreactive ANF; MAP, mean arterial pressure.

*p<0.01 versus baseline, ’p<0.025, ’p<0.05.
(p<0.025, p<0.025, and p<0.01), potassium (p<0.001, p<0.001, and p<0.001), calcium (p<0.01, p<0.05, and p<0.05), phosphate (p<0.05, p<0.001, and p<0.005), magnesium (p<0.025, p<0.05, and p<0.05), and uric acid (p<0.01, p<0.05, and p<0.025). Figure 1 shows a "typical" example of the fitting of the sinusoid for diurnal Un,V and Ua,V. MAP was also found to have a significant circadian rhythm (at least p<0.025, p<0.005, and p<0.05 in each individual during baseline, ANF infusion, and recovery, respectively). A significant circadian rhythm of plasma irANF in the individual patients was present only in two of the six patients during baseline and in one (other) patient during ANF infusion. A significant rhythm for GFR was found in all individual patients (at least p<0.05 in each individual during baseline, ANF infusion, and recovery), whereas no rhythm was found for individual values of PRA and PAC.

Interestingly, during ANF infusion only the rhythm characteristics were changed significantly in those electrolytes in which the excretion had increased during the first days of ANF infusion, i.e., sodium, chloride, calcium, and magnesium (Table 2). During ANF infusion a time shift was found in the acrophases of Ua,N (p<0.025), Ua,V (p<0.025), and Ua,V (p<0.01) and a decrease in the amplitude of Ua,N (p<0.05) in all individual patients. In contrast, the rhythm characteristics of Ua,V, Ua,N,V, and Ua,V did not change during ANF infusion. Interestingly, this resulted in a significant change in the relation between the acrophases of Ua,N and Ua,V during baseline the acrophases of Ua,N and Ua,V coincided, but during ANF infusion the acrophase of Ua,V preceded that of Ua,N in all individual patients (Figure 2, r<0.02). Remarkably, the shift was greatest in those patients in whom baseline acrophase was earliest (r=-0.91, p<0.02). The shift in the difference between the acrophases of Ua,N and Ua,V correlated with mean plasma irANF reached during ANF infusion (Figure 3, r=+0.92, p<0.01). Omitting the subject with the highest irANF level during ANF infusion, the correlation persisted (r=+0.79, p<0.05). For Ua,V a similar correlation was found (r=+0.87, p<0.02). During ANF infusion the acrophase of Ua,N correlated with the acrophase of MAP (r=+0.83, p<0.05), whereas such a relation was absent during baseline (r=0.42, p>0.1). Thus, MAP may have had a greater influence on urinary sodium excretion during ANF infusion than during baseline. In contrast, mean PAC correlated negatively with the acrophase of Ua,N before ANF infusion (r=-0.82, p<0.05), whereas this relation was absent during ANF infusion (r=-0.065, p>0.1). During recovery, the acrophases of Ua,N and Ua,V and the amplitude of Ua,N,V returned to baseline (Table 2, Figure 2).

Discussion

The present baseline data show that in our EH subjects, the acrophases of Ua,N and Ua,V coincide,
whereas during low dose ANF infusion these acrophases dissociate. Data in the literature on diurnal rhythms are scarce. However, it is known that in normotensive subjects, the acrophase of $U_{NaV}$ precedes that of $U_{KRV}$. Moreover, Kawasaki et al. showed that the acrophase of $U_{NaV}$ rhythm in patients with EH occurs earlier than in normotensive subjects, whereas the acrophase of $U_{KRV}$ is similar in these groups.2 As such, our baseline data are comparable with those in the literature. We observed a wide variation in these diurnal rhythms despite careful standardization. We found that the ANF-induced shift was greatest when baseline acrophase was earliest. Thus, an underlying difference in individual diurnal rhythm may contribute to the reported wide variation in effects of ANF between individuals.11

The intriguing result of the present study is that we found that the acrophase of sodium rhythm shifted during continuous low dose ANF infusion (which only doubled irANF levels), whereas potassium rhythm was unaffected. Thus, during ANF infusion $U_{NaV}$ acrophase preceded that of $U_{KRV}$ in all EH patients, whereas it did not during baseline. Although this study was not placebo controlled, we interpret the shift in sodium acrophase to be due to the effect of ANF infusion. We observed changes in the rhythm characteristics of other electrolytes besides sodium (chloride, calcium, and magnesium). All of these parameters were affected in their net cumulative excretion during the first 48 hours of ANF infusion. In contrast, no rhythm changes were observed in those solutes that were unaflectected in their excretion by ANF infusion, such as $U_{CaV}$. Our results on net cumulative excretion are in agreement with studies showing that short-term ANF infusion also enhances the absolute urinary excretion of sodium, chloride, calcium, and magnesium, whereas the excretion of potassium is not affected.12 Moreover, all the changes in rhythms were reversed during the recovery period, and the changes in irANF levels and the shifts in the acrophases of $U_{NaV}$ and $U_{KRV}$ correlated.

It is difficult to explain how ANF shifted the acrophase of $U_{NaV}$ in our patients with EH. Although the effect of ANF on the many mechanisms suggested to underly diurnal excretory rhythms cannot be evaluated from this study, the present data are compatible with an important role for the influence of blood pressure and aldosterone (PAC) on ANF-induced sodium excretion. Richards et al. showed the influence of blood pressure on the short-term natriuretic effect of ANF. Although in the present study this relation only reached borderline significance, the present data on rhythmic parameters show this influence of blood pressure on ANF-induced changes in $U_{NaV}$ in another way: during ANF infusion the acrophases of blood pressure and $U_{NaV}$ correlated significantly, whereas this relation was absent during baseline. Moreover, we showed that net sodium excretion returned to baseline levels during ANF infusion as blood pressure decreased. This is in agreement with data on short-term ANF infusion showing a decrease in ANF-induced sodium excretion as blood pressure decreases.13,14 Animal studies have shown that ANF may decrease preglomerular vessel tone.15 ANF infusion thus could render the (vasoconstricted) hypertensive kidney more sensitive to systemic blood pressure rhythm through preglomerular vasodilation, thereby shifting the acrophase of $U_{NaV}$ toward that of blood pressure. However, this hypothesized mechanism cannot explain the absence of a shift in the rhythm of $U_{KRV}$. The observed negative correlation between $U_{NaV}$ and PAC during baseline and the absence of this relation during ANF infusion conditions is in favor of a role for PAC in the modulation of ANF-induced natriuresis, as suggested by others,16 and may contribute to the dissociation of the effects of ANF on $U_{NaV}$ and $U_{KRV}$. Also a direct (tubular) effect of ANF may undery the observed correlation of irANF levels and shift in the acrophase of...
sodium excretion. The present data do not support a significant role for changes in GFR in ANF-induced natriuresis.

In summary, we showed that low dose ANF infusion significantly changes the absolute amounts and diurnal rhythms of \( U_{\text{m}} V \), \( U_{\text{c}} V \), \( U_{\text{a}} V \), and \( U_{\text{m}} V \) in patients with essential hypertension. Moreover, the present data suggest that ANF-induced natriuresis in humans is mediated in part by blood pressure and aldosterone. ANF may at physiological levels be involved in the (diurnal) regulation of sodium handling, at least in essential hypertension.

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References

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