Diurnal and postural variations in plasma atrial natriuretic factor, plasma guanosine 3′: 5′-cyclic monophosphate and sodium excretion

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SUMMARY

1. We studied diurnal patterns of plasma atrial natriuretic factor, plasma guanosine 3′:5′-cyclic monophosphate and urinary sodium excretion in normal subjects after 3 days on a 200 mmol of sodium/60 mmol of potassium diet. On the fourth day blood samples and urine were collected every 3 h.

2. Two studies were performed. In study 1, normal subjects (n=8) were recumbent for 23 h from 09.00 hours to 08.00 hours the next day. In study 2, normal subjects (n=10) were permitted to ambulate from 09.00 hours to 23.00 hours and then were recumbent until 08.00 hours the next day.

3. In study 1, assumption of the recumbent posture was associated with increases in plasma atrial natriuretic factor (P<0.01), plasma guanosine 3′:5′-cyclic monophosphate (P<0.05) and urinary sodium excretion (P<0.05).

4. In contrast, in study 2 there were no significant changes in plasma atrial natriuretic factor during the day; instead, plasma atrial natriuretic factor increased overnight, reaching a peak at 24.00 hours after 1 h of recumbency (P<0.01). A smaller rise in plasma guanosine 3′:5′-cyclic monophosphate (P<0.05) occurred; urinary sodium excretion decreased markedly (P<0.01) and there was no change in creatinine clearance.

5. In both studies, recumbency was associated with an initial drop, followed by a rise, in packed cell volume.

6. These data demonstrate that assumption of the supine position induces a rise in plasma atrial natriuretic factor and accounts for most of the observed variation. This is associated with natriuresis during the day, but it does not reverse the pattern of antinatriuresis that occurs at night. Instead, changes in plasma atrial natriuretic factor appear to reflect and, in turn, influence changes in intravascular volume.

Key words: atrial natriuretic factor, diurnal variation, guanosine 3′:5′-cyclic monophosphate, posture, renin, sodium excretion.

Abbreviations: ANF, atrial natriuretic factor; cyclic GMP, guanosine 3′:5′-cyclic monophosphate; MAP, mean arterial pressure; PRA, plasma renin activity.

INTRODUCTION

The urinary excretion of sodium, water and most electrolytes is normally greater during the day than at night. This pattern will persist even if identical quantities of food and water are ingested at regular intervals throughout the 24 h. As the evening approaches and during the night, the excretion of sodium, potassium, bicarbonate and chloride gradually diminishes, and the process is reversed in the morning [1]. It has been shown that there is a small nocturnal fall in glomerular filtration rate but this cannot fully explain the phenomenon [1-3], since the excretion of phosphate shows a reversed pattern (i.e. it increases at night and diminishes during the day).

In supine subjects, a diurnal variation in plasma renin activity [PRA] occurs, with a nocturnal peak at 04.00 hours, similar to the time of lowest sodium excretion [4-6]. A similar pattern is observed for plasma aldosterone, which is also influenced by the diurnal rhythm of adrenocorticotropic hormone secretion [7, 8]. In contrast, PRA and aldosterone are normally greater during the day due to the influence of upright posture [2, 7]. Nonetheless, the rhythms of urinary sodium and potassium excretion are similar in ambulatory and continuously supine subjects [9], suggesting that nocturnal antinatriuresis is not linked to a diurnal variation in aldosterone secretion.

The role of atrial natriuretic factor (ANF) in the diurnal control of urinary sodium excretion and body fluid volumes is not understood. It is becoming clear that ANF is part of a cardiovascular control mechanism that operates to regulate volume homeostasis [10], but there
are conflicting preliminary reports concerning possible diurnal changes in plasma ANF [11, 12]. The studies described here were designed to investigate:

(1) The pattern of circulating ANF levels in relation to urinary sodium and potassium excretion, creatinine clearance and renin–angiotensin–aldosterone system over a 24 h period.
(2) The role of postural changes in any observed variations in ANF secretion.
(3) The relationship between plasma ANF and plasma guanosine 3'–5'-cyclic monophosphate (cyclic GMP) over a 24 h period.
(4) The relationship of the changes in packed cell volume, as an index of intravascular volume, to alterations in both ANF and the renin–angiotensin–aldosterone system.

Subjects

Ten normal subjects (seven males and three females) consented to take part in the studies, which were approved by the Human Rights Committee of the New York Hospital–Cornell Medical Center. Their age was 31 ± 7 years (mean ± SEM) and their weight was 69 ± 4 kg. All were in good health, as determined by clinical history, examination, full blood count and biochemical screening profile. None was receiving medication.

For 3 days each subject ate only in the Clinical Research Centre of the New York Hospital–Cornell Medical Center and this diet comprised 200 mmol of sodium, 60 mmol of potassium and 80 g of protein with 2.5 litres of fluid. Food and fluid were distributed evenly throughout the daytime and taken as three meals and an evening snack. Other than eating in the Clinical Research Centre they continued with other normal activities, but were requested to refrain from moderate to severe exercise. On the third day of the diet they performed a standard outpatient 24 h urine collection for creatinine clearance and sodium and potassium excretion. On day 4 they were admitted to the Clinical Research Centre for a 24 h period, during which the diet and fluid allowance remained the same.

For the subjects performing both protocols (designated study 1 and study 2), studies were performed approximately 6 weeks apart and were not conducted in random order.

Study 1

On admission to the Clinical Research Centre an intravenous cannula, flushed with heparinized saline, was inserted. Blood pressure was measured, by using a standard mercury sphygmomanometer, after 5 min in the seated position; thereafter blood samples were taken at 09.00 hours. Then the 24 h urine collection was completed. The subjects were then requested to lie supine for 23 h. Measurement of blood pressure followed by blood sampling was repeated at 10.00 hours (after 1 h of recumbency), at 12.00 hours and at 3-hourly intervals thereafter. Free-flow urinary collections were obtained 3-hourly after the blood samples were taken. Subjects were encouraged to sleep as permitted from 23.00 hours until 08.00 hours and then were requested to sit up; the last blood sample was taken at 09.00 hours.

Study 2

During this study the subjects were ambulant or seated during the daytime hours, but before each blood pressure measurement and blood sample collection they were seated for 5 min. At 23.00 hours they became supine until 08.00 hours and during this period were encouraged to sleep as much as possible. During this 24 h period blood pressure was taken and blood samples and free-flow urine were collected at 3-hourly intervals both during the day and night.

In both studies blood samples were taken for analysis of plasma ANF, plasma cyclic GMP, PRA, plasma creatinine and packed cell volume. The urine collections were analysed for sodium, potassium, creatinine and aldosterone.

Assays

ANF was measured by radioimmunoassay after extraction on plasma on C18 Sep-Pak cartridges; extracts were prepared within 4 weeks of collection [13]. Plasma cyclic GMP was measured by direct radioimmunoassay in ethanol extracts of plasma using commercially supplied reagents (Amersham). PRA was measured as previously described [14]. Urinary sodium and potassium and plasma and urine creatinine were determined by an autoanalyzer. Urinary aldosterone was measured as the acid-labile C18 glucuronide by using a radioimmunoassay kit from Diagnostic Products.

Statistical analysis

Results are expressed as means ± SEM.

To assess the consistency of the diurnal and postural changes in the parameters studied, repeated measures analysis of variance was performed. Where data were skewed, Friedman's test, a non-parametric repeated measures analysis, was used. The analytical models, used to compare the mean values by time were limited to either the minimum and maximum values, or the minimum, midpoint and maximal values, as most of the intermediate comparisons were of limited interest.

### Table 1. Urinary parameters for the 24 h period before both studies

<table>
<thead>
<tr>
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<th>Study 1 (n = 8)</th>
<th>Study 2 (n = 10)</th>
<th>Statistical significance</th>
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</thead>
<tbody>
<tr>
<td>24 h excretion of sodium (mmol)</td>
<td>177 ± 6</td>
<td>198 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>24 h excretion of potassium (mmol)</td>
<td>58 ± 4</td>
<td>57 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>24 h creatinine clearance (ml/min)</td>
<td>136 ± 8</td>
<td>124 ± 6</td>
<td>NS</td>
</tr>
</tbody>
</table>
Atrial natriuretic factor and diurnal variation in Na⁺ excretion

Fig. 1. Plasma ANF (○—○) and plasma cyclic GMP (●—●) during daytime (study 1, a) and night-time (study 2, b) recumbency. Values are means ± SEM. Statistical significance: *P < 0.05, †P < 0.01.

RESULTS

Study 1: plasma ANF, plasma cyclic GMP and renal function

Plasma ANF increased after the first hour of recumbency during the daytime (P < 0.01, Fig. 1) and plasma cyclic GMP increased in parallel (P < 0.05, Fig. 1). Urinary sodium excretion increased also (P < 0.05, Fig. 2) but creatinine clearance only slightly increased (not significant, Fig. 2). After the peak level of plasma ANF at 10.00 hours it fell slowly during the day, reaching its lowest level at 03.00 hours.

Fig. 2. Plasma ANF, urinary sodium and potassium excretion, creatinine clearance (\(C_\text{cr}\)) and MAP during daytime (study 1, a) and night-time (study 2, b) recumbency. Values are means ± SEM. Statistical significance: *P < 0.05, †P < 0.01.

Study 2: plasma ANF, plasma cyclic GMP and renal function

With recumbency only at night, there were no significant differences in plasma ANF during the day, but again plasma ANF increased when the supine position was assumed. After 1 h of recumbency, plasma ANF peaked at 24.00 hours, increasing from the 21.00 hours value (P < 0.01, Fig. 1). This was associated with a parallel increment in plasma cyclic GMP (P < 0.05, Fig. 1). Unlike the daytime response to supine posture, urinary sodium excretion decreased (P < 0.01, Fig. 2) and creatinine clearance was unchanged (not significant, Fig. 2). In each study urinary potassium excretion changed in parallel with urine sodium excretion (Fig. 2). After the peak level of plasma ANF at 24.00 hours it fell during the night, reaching its lowest level at 09.00 hours.

Blood pressure

Mean arterial blood pressure (MAP) was higher during the daytime recumbency in study 1 with a peak MAP between 15.00 hours and 18.00 hours (Fig. 2) and a significant reduction (P < 0.05) at night at 03.00 hours and 06.00 hours. A directionally similar change was noted in study 2 with a peak MAP during daytime ambulation.
Fig. 3. Plasma ANF, PRA, urinary aldosterone excretion (UA) and packed cell volume (PCV) during daytime (study 1, a) and night-time (study 2, b) recumbency. Values are means ± SEM. Statistical significance: *P<0.05, †P<0.01. Abbreviation: ANG I, angiotensin I.

between 09.00 hours and 12.00 hours and a 5% reduction at night at 24.00 hours and 03.00 hours (not significant, Fig. 2).

PRA and urinary aldosterone

With recumbency, either at night or during the day, PRA and urine aldosterone fell. In study 2, PRA fell at night (P<0.05, Fig. 3) as did urinary aldosterone excretion (not significant, Fig. 3). In study 1, PRA fell during the day (P<0.05, Fig. 3) and urinary aldosterone fell also (P<0.05, Fig. 3).

Packed cell volume

The packed cell volume changes followed a predictable pattern with assumption of recumbency, whether at night or during the day. Packed cell volume fell after 1 h of recumbency, reaching a nadir at the time of the greatest plasma ANF response. In study 1, with recumbency during the day, packed cell volume decreased initially (P<0.05), thereafter rising again to a peak by 18.00 hours (P<0.05, Fig. 3). In study 2, with recumbency at night, packed cell volume decreased (P<0.05), thereafter rising again by 06.00 hours (P<0.05, Fig. 3).

Pattern of renin secretion

Aside from the consistent fall in PRA with recumbency, the pattern of renin secretion differed in the two studies. During daytime recumbency in study 1, PRA remained significantly below upright values until the early morning hours, when small but significant increases occurred at 03.00 hours and 06.00 hours (P<0.05 for both, Fig. 3). These increases in renin secretion were paralleled by similar increases in aldosterone excretion (P<0.05, Fig. 3). In contrast, in the ambulatory subjects PRA and urinary aldosterone, although tending to decrease as the day progressed, were both higher during the day and lower at night.

DISCUSSION

These studies demonstrate predictable and reciprocal responses of plasma ANF and the renin-angiotensin system to a change in posture from upright to the continuously supine position. Irrespective of whether the supine position was assumed in the morning or at night, the change in posture was associated with a fall in packed cell volume, a fall in PRA and urinary aldosterone and a rise in plasma ANF. After this initial response, plasma ANF remained above, and PRA and urinary aldosterone remained below, upright values, whereas packed cell volume tended to rise towards baseline. Previous studies have demonstrated that assumption of the supine position is accompanied by a filling of the vascular space as fluid passes from the extravascular into the intravascular compartment [16]. Our data are consistent with the view that the observed changes in plasma ANF are the result of postural effects and resulting shifts in central blood volume [16, 17] and atrial stretch, which is the main stimulus to ANF secretion [18]. In man, upright posture causes dependent venous pooling and reduced venous return. The resulting reduction in central blood volume [16, 17], would serve to reduce ANF secretion. Conversely, with recumbency, there is an increase in venous return and mobilization of fluid from the extravascular to the intravascular compartments resulting in increases in atrial pressures [16, 17] and ANF secretion. Thus, with recumbency, the sustained fall in PRA and urinary aldosterone and the rise in plasma ANF suggest that the body perceives an increase in central blood volume which is in part reflected by the initial fall in packed cell volume, resulting from mobilization of fluid from extravascular to intravascular compartments [19]. The subsequent tendency for packed cell volume to rise might be the result of ANF causing a fluid shift out of the intravascular compartment, as has previously been demonstrated [13].

These data also suggest that in normal subjects there is reduced renal responsiveness to circulating ANF at night. Natriuresis, diuresis and elevation of glomerular filtration rate accompanied the rise in plasma ANF during daytime recumbency, but a similar increment in plasma ANF at night failed to induce a natriuresis. From previous studies during the day these modest increases in ANF secretion would be expected to have a physiological effect [20, 21].

Cyclic GMP, which is exported from the cell after stimulation of membrane-bound guanylate cyclase, is a second messenger of ANF-receptor-mediated actions in many tissues, including kidney. The plasma levels of cyclic
GMP have been shown to reflect ANF levels during acute and chronic stimulation of ANF release [22–24]. In general, the response of plasma cyclic GMP paralleled that of plasma ANF in these studies, supporting the biological relevance of changes in ANF both during the day and at night. However, the possible dissociation between plasma cyclic GMP and plasma ANF levels during the first 12 h of study 2 is unexplained.

The natriuretic effect of ANF is thought, at least in part, to be dependent on the maintenance of a normal renal perfusion pressure [25]. It is likely, therefore, that the night-time fall in renal perfusion pressure during sleep [2] blunts the renal response to circulating ANF at night. Previous studies have drawn contrasting conclusions of the presence or absence of a diurnal rhythm of secretion of ANF. Donckier et al. [11], in their study of the ambulatory daytime and nocturnal sleep cycles in normal subjects, observed a peak in plasma levels of ANF at 04.00 hours and a subsequent fall. Richards et al. [12], studying prolonged recumbency over a 24 h period, concluded that plasma ANF levels reached a peak at 12.00 hours and a trough in the early evening. The differing conditions of these two studies may explain their contrasting results. In the present study, which compared both conditions, we were unable to obtain any evidence of a diurnal rhythm to ANF secretion independent of postural changes.

These studies provide further insight into the relationship between ANF, the renin–angiotensin system and urinary sodium excretion in normal subjects. The main intrarenal signals which elicit or suppress renin secretion from the juxtaglomerular apparatus are distal tubular sodium chloride supply and renal perfusion pressure. In addition, renin release is suppressed by ANF and increased by sympathetic (β₁-adrenergic) stimulation [26]. In the 24 h recumbency study, the fall in PRA which followed assumption of the supine position could be the result of increased distal delivery of sodium to the macula densa and of increased plasma ANF. The subsequent rise in PRA between 03.00 hours and 06.00 hours was preceded by a fall in blood pressure and a reduction in urinary sodium excretion, suggesting that reduced renal perfusion pressure and reduced distal sodium chloride delivery may stimulate renin secretion during sleep. Aldosterone secretion paralleled renin secretion in this study, suggesting that a rise in angiotensin II may contribute to the early morning increase in aldosterone secretion.

During the normal daytime ambulatory and night-time sleep pattern, postural influences predominate and PRA increases in response to a β-adrenergic stimulus. The normal daytime increase in urinary sodium excretion was unable to offset this postural stimulus to renin secretion. Again, aldosterone excretion paralleled renin secretion.

In conclusion, these studies of normal subjects showed that ANF secretion, like that of renin and aldosterone, is influenced by prolonged changes in posture and is accompanied by reciprocal changes in PRA and aldosterone and predictable changes in packed cell volume. This study did not reveal any circadian rhythm of plasma ANF per se. Our findings suggest that changes in ANF, PRA and aldosterone may contribute to the natriuresis induced by supine posture during the daytime, but that other factors, such as reduced renal perfusion pressure, overwhelm this response at night.

REFERENCES
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