Sodium Loading and Posture Modulate Human Atrial Natriuretic Factor Plasma Levels

ALAN S. HOLLISTER, ISSEI TANAKA, TERUAKI IMADA, JACK ONROT, ITALO BIAGGIONI, DAVID ROBERTSON, AND TADASHI INAGAMI

SUMMARY Atrial natriuretic factor is postulated to act through atrial stretch receptors as a volume regulatory hormone that stimulates diuresis and natriuresis in response to increased atrial pressure. To characterize the stimuli associated with the release of atrial natriuretic factor in humans, we studied 14 normal subjects, both in the supine position and after 10 minutes in an upright posture, while they were on a regular diet (Day 0) and during 3 days of supplemental sodium chloride intake (8 g/day). Radioimmunoassay of plasma atrial natriuretic factor was performed with rabbit antibody to the human hormone amino acids (102–126). Urinary sodium excretion increased from 111 ± 13 mEq/day (mean ± SEM) on Day 0 to 275 ± 15 mEq/day by the third day (Day 3) of high sodium intake. The level of atrial natriuretic factor in the supine position rose from 17 ± 4 pg/ml (Day 0) to 76 ± 13 pg/ml on Day 3 (p < 0.001) and after 10 minutes in an upright posture on Day 3, the level fell to 32 ± 10 (p < 0.005). Plasma concentrations of atrial natriuretic factor correlated positively with spot and 24-hour urinary sodium excretion and weight gain, and correlated negatively with plasma aldosterone and renin activity. We conclude that the response of atrial natriuretic factor to sodium loading and posture change in humans is appropriate for a volume regulatory hormone.

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KEY WORDS • volume regulation • renin • aldosterone • atriopeptin

T he theoretical concept of a hormone responsible for regulating plasma volume and sodium excretion was advanced 50 years ago.1,2 In 19563 granules in atrial myocytes were identified, and in 1979 the density of these myocyte granules was found to depend on alterations in fluid and electrolyte balance.4 Subsequently, extracts of atria were shown to have potent diuretic and natriuretic effects in animals,5–7 to relax smooth muscle,8 and to antagonize vasoconstriction induced by angiotensin II and norepinephrine.9 Isolation and purification of these atrial extracts led to the identification, sequencing, and synthesis of rat10–13 and human atrial natriuretic factor (ANF).14 Administration of synthetic ANF has led to marked increases in the excretion of free water, sodium, chloride, and potassium and to modest increases in phosphate, calcium, and magnesium in animal15–17 and human subjects.18,19 Further animal studies have demonstrated that mechanical stretching of the atria results in increased plasma natriuretic activity.20 These findings suggest that ANF may function as a volume regulatory hormone.21

Sodium loading in human subjects increases extracellular fluid volume and plasma volume and results in an increase in atrial pressure.2,22,23 Atrial pressures are also higher in the supine position, as compared with upright posture.24 If increased atrial pressure is responsible for ANF release, then sodium loading and supine posture should increase plasma ANF concentrations. Using a newly developed radioimmunoassay for human plasma ANF,25 we tested the hypothesis that ANF is a volume regulatory hormone. We report here that sodium loading and supine posture are associated with a higher plasma ANF concentration and increased sodium excretion.
Methods

Eight male and six female normotensive subjects on an ad libitum diet participated in the study; their average age was 33 ± 2 years (mean ± SEM). All subjects were drug-free, and all voluntarily agreed to participate in the study. The protocol was reviewed and approved by the Vanderbilt University Committee for the Protection of Human Subjects. Each subject presented to the Elliot V. Newman Clinical Research Center at 0700 a.m. on Day 0, completed a 24-hour urine collection, then remained supine for 1 hour. Vital signs were taken, and blood was drawn for measurement of plasma immunoreactive atrial natriuretic factor and renin, packed-cell volume, aldosterone, serum sodium, potassium, chloride, calcium, magnesium, creatinine, and phosphorus. The subjects then stood upright for 10 minutes; vital signs were recorded again; blood was drawn for plasma aldosterone, ANF, and renin measurements; and a urine sample was collected (spot urine) for measurement of sodium and creatinine. The 24-hour urine samples were analyzed for sodium, potassium, chloride, calcium, magnesium, creatinine, and phosphorus. The subjects then began taking 2 g of sodium chloride by mouth four times daily and continued the ad libitum diet. Nine subjects repeated the Day 0 protocol after 1 and 2 days of sodium loading, and all 14 repeated the protocol on Day 3.

Plasma ANF levels were measured by radioimmunoassay with a modified method for the measurement of rat ANF.25,26 Nine-milliliter blood samples were mixed with 1 ml of an ice-cold protease inhibitor solution containing ethylenediaminetetraacetic acid (10 mg/ml), aprotinin (500 kallikrein inhibitor units per milliliter; Sigma Chemical, St. Louis, MO, USA), and soybean trypsin inhibitor (500 BAEE units per milliliter; Sigma), and promptly centrifuged at 4°C. Plasma (2 ml) was applied to a Sep-Pak C-18 cartridge (Waters Associates, Milford, MA, USA), which had been equilibrated with 0.1 M acetic acid. After being washed with 20 ml of 0.1 M acetic acid, ANF was eluted in 3 ml of 80% methanol, lyophilized, and redissolved in radioimmunoassay buffer. The recovery of human ANF (99–126) (Peninsula Laboratories, Belmont, CA, USA) by this extraction method was 77%. The radioimmunoassay was performed with synthetic human ANF (102–126)27 radioiodinated by the chloramine-T method25 and anti–human ANF (102–126) antiserum raised in rabbits. This antiserum cross-reacted 100% with human ANF (99–126) and 33% with rat ANF (99–126). Antibody-bound ligand and free ligand were separated by centrifugation after addition of 12.5% polyethylene glycol (molecular weight, 8000) and 0.2% bovine β-globulin.26 The intraassay and interassay coefficients of variation were 6.1% and 10.0%, respectively. Synthetic human ANF (99–126) was used as the standard, and ANF values are expressed as equivalents of this peptide. The limit of sensitivity of this assay was 10 pg/ml for the first five subjects and 4 pg/ml for the other nine. Plasma ANF concentrations that were at or below the limit of assay sensitivity are reported as 10 or 4 pg/ml, respectively.

Serum and urinary electrolytes, creatinine, aldosterone, and plasma renin were assayed in the Core Laboratory facilities of the Clinical Research Center.

Data were compared by paired t tests within subjects and by t tests between subject groups. Probabilities less than 0.05 were considered significant.

Results

Effect of Sodium Loading on Blood Pressure, Heart Rate, Renin, and Aldosterone

An intake of 8 g of sodium chloride per day for 3 days, in addition to the usual dietary sodium intake, produced no change in the supine or upright blood pressure and heart rate or in the heart rate response to orthostatic stress (Table 1). For the group as a whole, body weight increased from 75.0 ± 2.1 kg (mean ± SEM) to 75.7 ± 2.4 kg (p < 0.02 by paired t test). Before initiation of sodium loading (Day 0), both renin and aldosterone levels increased appropriately with upright posture, whereas the absolute levels and the change induced by upright posture were reduced after 3 days of sodium loading.

Effect of Sodium Loading on Blood and Urinary Values

The red blood cell packed volume was reduced slightly by sodium loading (Table 2). This could not be accounted for by the amount of blood drawn during the study. Serum creatinine and electrolytes were unchanged except for a small increment in calcium.

Urinary electrolyte values reflected the increased sodium chloride intake, with increased excretion of potassium, calcium, magnesium, and phosphorus (see

<table>
<thead>
<tr>
<th>TABLE 1. Effect of Sodium Loading on Blood Pressure, Heart Rate, Plasma Renin, and Aldosterone in 14 Normal Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pastarame</td>
</tr>
<tr>
<td>Blood pressure (torr)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td>Renin (ng ANG I/ml/hr)</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. ANG I = angiotensin I.
* p < 0.05 vs supine value; † p < 0.05 vs Day 0 upright value.
TABLE 2. Effect of Sodium Loading on Blood and Urine Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of Subjects</th>
<th>Day 0</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed-cell volume</td>
<td>6</td>
<td>44.8 ± 1.2</td>
<td>41.7 ± 0.3*</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>14</td>
<td>140 ± 0.3</td>
<td>140 ± 0.4</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>6</td>
<td>4.1 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Chloride (mEq/l)</td>
<td>6</td>
<td>103 ± 0.6</td>
<td>104 ± 0.6</td>
</tr>
<tr>
<td>Calcium (mEq/l)</td>
<td>6</td>
<td>4.5 ± 0.1</td>
<td>4.8 ± 0.1*</td>
</tr>
<tr>
<td>Magnesium (mEq/l)</td>
<td>6</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>6</td>
<td>3.3 ± 0.1</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>14</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mEq/24 hr)</td>
<td>14</td>
<td>111 ± 13</td>
<td>275 ± 15*</td>
</tr>
<tr>
<td>Potassium (mEq/24 hr)</td>
<td>6</td>
<td>47 ± 8</td>
<td>69 ± 6*</td>
</tr>
<tr>
<td>Chloride (mEq/24 hr)</td>
<td>6</td>
<td>74 ± 10</td>
<td>265 ± 29†</td>
</tr>
<tr>
<td>Calcium (mEq/24 hr)</td>
<td>6</td>
<td>5.9 ± 1.1</td>
<td>11.7 ± 1.5†</td>
</tr>
<tr>
<td>Magnesium (mEq/24 hr)</td>
<td>6</td>
<td>6.5 ± 0.8</td>
<td>9.3 ± 0.8*</td>
</tr>
<tr>
<td>Phosphorus (g/24 hr)</td>
<td>6</td>
<td>0.74 ± 0.08</td>
<td>0.87 ± 0.07</td>
</tr>
<tr>
<td>Creatinine (mg/24 hr)</td>
<td>14</td>
<td>1459 ± 87</td>
<td>1453 ± 105</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.05 vs Day 0; †p<0.01 vs Day 0.

Table 2). Creatinine excretion was unchanged. Urinary clearance of creatinine did not vary with the high sodium intake (Table 3), but sodium clearance more than doubled. Similarly, the fractional excretion of sodium was increased, whether calculated from the 24-hour or spot urine collection.

Effect of Sodium Loading on Plasma Atrial Natriuretic Factor

Plasma concentrations of ANF were 17 ± 4 pg/ml when the subjects were in the supine position and consuming a mean of 111 mEq of sodium per day (Day 0, Figure 1). Ten minutes of upright posture was associated with a modest fall in the plasma ANF concentration on Day 0, but the limits of assay sensitivity preclude a statement regarding the extent or implications of this fall. Three days of high sodium intake resulted in a significant elevation of plasma ANF concentrations. Under these conditions, upright posture caused a significant fall in plasma ANF.

During the 4 days of the study, supine plasma ANF concentrations correlated significantly, by linear regression, with 24-hour urinary sodium excretion ($r=0.59$, $p<0.001$; Figure 2), urinary sodium clearance ($r=0.68$, $p<0.001$), and the fractional excretion of sodium both over 24 hours ($r=0.56$, $p<0.001$) and in the spot samples obtained at the same time that blood was drawn to measure plasma ANF concentrations ($r=0.53$, $p<0.01$).

The increase in supine ANF between Day 0 and Day 3 also correlated with the change in body weight ($r=0.94$, $p<0.001$; Figure 3), and this association remained significant by multivariate analysis that controlled for the influence of 24-hour urinary sodium, sodium clearance, and fractional excretions of sodium. There were significant negative correlations between the changes in supine ANF from Day 0 to Day 3 and the reduction in supine renin activity ($r=-0.61$, $p<0.05$) or the reduction in supine aldosterone ($r=-0.88$, $p<0.05$; Figure 4, A and B, respectively).

Discussion

Studies of endogenous plasma ANF in animals indicate that sodium and volume loading and direct atrial distention induce a release of ANF and a disappearance of secretory granules from atrial myocytes. Human studies have identified elevated concentrations of ANF, detectable by radioimmunoassay, in paroxysmal atrial tachycardia and in congestive heart failure. Rapid saline infusion has also been shown to elicit modest increases in human plasma ANF concentrations. We have recently reported that the plasma ANF concentration is positively associated with atrial pressure and right ventricular end-diastolic pres-
Figure 2. Supine plasma atrial natriuretic factor (ANF, pg/ml), as compared with 24-hour urine sodium excretion. Blood was drawn after 8 hours of fasting and 1 hour in the supine posture, while subjects were on a normal diet and after 1, 2, and 3 days of dietary supplementation with sodium chloride (8 g/day). The line was calculated by least-squares linear regression; \( n = 38, r = 0.69, p<0.001 \).

Figure 3. Change in supine plasma atrial natriuretic factor (ANF) between Day 0 and Day 3 of sodium loading, plotted against weight (WT) change induced by this protocol. The line was determined by least-squares linear regression \( (n = 13, \ r = 0.94, \ p<0.001) \).

Sodium loading causes an increase in extracellular fluid volume, weight gain, and natriuresis. In our study the addition of 8 g of sodium chloride per day to an ad libitum diet caused an increase in body weight in normal subjects, reaching a maximum at 2 or 3 days, and was associated with an increase in the fractional excretion of sodium and elevated plasma concentrations of ANF. Suppression of plasma renin activity and aldosterone was also demonstrated with sodium loading, as we and others have noted previously in human subjects \cite{31,34} and with ANF infusion in dogs. The association between the plasma ANF concentration and fractional excretion of sodium in our study was strengthened when the changes in body weight were controlled. In general, those subjects with the largest weight gain had the greatest increment in plasma ANF and the greatest increase in fractional excretion of sodium, and those with little or no weight gain had little change in plasma ANF. These findings support the concept that ANF is a volume regulatory hormone in normal human subjects and that plasma levels of ANF, renin, and aldosterone reflect effective plasma volume.

Our sodium-loading protocol produced no change in serum sodium concentrations, reducing the possibility that plasma osmolality or sodium concentrations controlled ANF release. Antidiuretic hormone (ADH) is stimulated by short-term sodium loading and upright posture \cite{36,37} and recent animal studies suggest that ADH stimulates ANF release \cite{38}. Despite stimuli that increase plasma ADH activity, however, our findings demonstrate a fall in plasma ANF with upright posture. In the absence of ADH measurements, we cannot differentiate between volume alone and ADH alone as the stimulus responsible for the increased ANF plasma levels found in sodium-loaded supine subjects.

Upright posture has been shown to reduce atrial filling pressures and urine output. \cite{39} This stimulus reliably increases sympathetic nervous activity, resulting in an increase in circulating catecholamines, redistribution of blood flow, increases in plasma renin activity and aldosterone concentration, and an increased heart rate. \cite{34,40} Our findings indicate that upright posture results in a fall in plasma ANF levels, most noticeably in the sodium-loaded state. This suggests that the fall in atrial pressures accompanying upright posture may decrease ANF release, and despite reports that vasopressor agents induce ANF release in rats, \cite{38} the presence of elevated plasma catecholamines and ADH in upright human subjects is not sufficient to maintain ANF release. Our previous work, in which we used a tilt table while measuring...
atrial pressure, also indicates that the change in the ANF concentration and secretion rate is directly proportional to the change in atrial pressure induced by a position change.33

In summary, we have demonstrated that 8 g of sodium chloride per day in addition to a regular diet results in an increase in supine and upright plasma immunoreactive ANF in normal human subjects. The increase in plasma ANF is positively correlated with weight gain and total and fractional sodium excretion and is negatively correlated with plasma renin and aldosterone. Upright posture is associated with a fall in plasma ANF, possibly because of a reduction in atrial pressure. Our findings are consistent with the hypothesis that in normal humans ANF is a volume regulatory hormone that stimulates increases in urinary excretion of sodium.

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