Plasma and Urinary Norepinephrine Values at Extremes of Sodium Intake in Normal Man

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SUMMARY To examine the effects of a wide range of sodium intake on plasma and urinary norepinephrine values in normal man, we studied 14 men at six levels of sodium intake from 10 to 1500 mEq/24 hrs. Mean blood pressure increased from 83.8 ± 1 (SEM) to 100.3 ± 3 mm Hg, while cardiac index increased from 2.6 ± 0.1 to 3.6 ± 0.3 liters/min/m² (p < 0.001). Upright venous plasma norepinephrine concentration decreased from 467 ± 63 to 67 ± 24 pg/ml, while urinary norepinephrine excretion decreased from 543 ± 3.4 to 23.4 ± 2.9 μg/24 hrs. There was no effect of sodium intake on blood pressure responses to isometric hand-grip contraction. The urinary sodium excretion was inversely correlated with urinary norepinephrine excretion (r = —0.46, p < 0.001). There was a significant inverse multiple correlation of mean blood pressure and plasma and urinary norepinephrine values (correlation coefficient = 0.72, p < 0.001).

These results indicate that sodium homeostasis has a significant effect on plasma and urinary norepinephrine values. Sympathetic nervous system activity appears to decrease with sodium loading in normal subjects. These responses may have facilitated the excretion of massive salt loads in normal subjects and may have modulated the increases in blood pressure. (Hypertension 1: 261-266, 1979)

KEY WORDS • catecholamines • urinary norepinephrine • plasma norepinephrine • sodium loading • sympathetic nervous system • autonomic nervous system

INCREASES in arterial blood pressure have been observed with increases in salt intake in subjects with diminished renal function since the report of Ambard and Beaujard in 1904.1 Subsequently, similar blood pressure observations have been made in normal persons subjected to large increases in salt intake.2-4 We recently reported that increases in blood pressure occurred in normotensive human volunteers when they were subjected to a sodium intake in excess of 800 mEq per day.5 The sympathetic nervous system plays a major role in the blood pressure regulation of normal persons,6 and has also been implicated in the development of experimental hypertension,7 as well as in certain forms of human essential hypertension.8-10 We therefore observed indices of sympathetic and adrenal medullary activity during the course of these studies. The purpose of our study was to delineate the responses of normal subjects and to provide a basis of comparison for subsequent studies in hypertensive individuals. Our data suggest that the state of sodium homeostasis affected the upright venous plasma norepinephrine concentration and urinary norepinephrine excretion in normal subjects. Sympathetic nervous system activity as reflected by our measurements appeared to decrease with salt loading. A decrease in sympathetic nervous system activity may have facilitated the excretion of massive salt loads.

Methods

Fourteen normotensive, healthy male volunteers (mean age 32 years, range 18-40) were obtained by advertisement and were studied at the Indiana University Clinical Research Center. The protocol was approved by the Indiana University Medical Center Human Use and Clinical Research Center Committees and informed consent was obtained from each volunteer after detailed explanation of the procedures to be performed. None had a family history of hypertension.

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Sodium Intake Levels

Observations were made after at least 3 days at six levels of sodium intake, namely: 10, 300, 600, 800, 1200, and 1500 mEq/24 hrs. All 14 subjects were studied at the 10 and 300 mEq/24 hr levels. Eight subjects then received 800 and 1500 mEq/24 hrs sodium, while six subjects received 600 and 1200 mEq/24 hrs sodium. The subjects were given a constant diet containing 10 mEq sodium, 80 mEq potassium, 65 g protein, 50 g fat, 279 g carbohydrate, 400 mg calcium, and 1000 mg phosphorous daily. All meals were eaten at the Clinical Research Center. Dietary sodium intake was maintained at 10 mEq/24 hrs for 7 days. For 3 days 290 mEq sodium, in the form of sodium chloride, was added to the diet (300 mEq sodium diet). Either 590 or 790 mEq sodium (600 and 800 mEq sodium diet) was added to the 10-mEq diet for the next 3 days. In order to achieve these high levels of intake, sodium was given with bouillon between meals and at bed time. For the final 3 days, the subjects were hospitalized and received the 600 mEq or 800 mEq sodium diet. Throughout the night they received 600 or 700 mEq sodium, respectively (1200 and 1500 mEq/24 hr sodium intake) in the form of I.V. normal saline. Fluid intake (distilled water) was allowed ad libitum.

Procedures

The subjects were weighed every morning before breakfast after voiding. Blood pressures were obtained daily before meals by the indirect auscultatory technique. The same mercury manometers (Baum, Inc., New York, NY) and cuffs were employed throughout the study. The subjects rested supine in a darkened room for 5 minutes after which blood pressure and measurements of heart rate were obtained in the nondominant arm each minute for 5 minutes. The same observers were responsible for these measurements throughout the study.

Daily 24-hour urine specimens were obtained for the determination of sodium, potassium, creatinine, and norepinephrine concentrations. Acetic acid, which protects against urinary norepinephrine loss during storage, was used as a preservative. At 8:00 a.m. on the morning of the final day at each level of sodium intake, venous blood specimens were obtained from the basilic vein, following 2 hours of ambulation, for hematocrit, creatinine, sodium, potassium, plasma renin activity, plasma aldosterone and plasma norepinephrine concentrations.

Blood Pressure Responses to Hand-Grip

On the morning of the final day at each level of sodium intake, blood pressure responses to isometric hand-grip were determined. The static muscular effort consisted of sustained hand-grip on a hydraulic, adjustable dynamometer (J. A. Preston, New York, NY). The subjects were trained in the full experimental procedure before the actual experiments. All subjects were studied in the supine position after breakfast, without sedation. Maximum voluntary contraction (MVC) was determined 30 minutes before the start of the actual experiment. Hand-grip was performed in the dominant hand. During hand-grip the subjects were urged to breathe as normally as possible and to relax all muscles not involved in the contraction. After a basal 15-minute relaxation period, blood pressure measurements were obtained each minute for 5 minutes in the nondominant arm. Thereafter the subjects were studied during a 30% MVC hand-grip sustained for 3 minutes. Blood pressure was measured every 30 seconds during the contraction period. The change in mean blood pressure in response to hand-grip in each subject was calculated by subtracting the mean value obtained during the 5-minute period before contraction from the mean value of measurements obtained during the 3-minute contraction period.

Echocardiographic Findings

Cardiac index, stroke index, end-systolic left ventricular volume, and end-diastolic left ventricular volume were measured noninvasively on the final day at each level of sodium intake by means of echocardiography. This technique has been intensively studied and carefully validated in our institution. Rasmussen et al. compared Fick stroke volume to echocardiographically calculated mitral valve stroke volume in 16 patients with normal left ventricular and no angiographic evidence for coronary artery disease. The results of the two techniques were highly correlated ($r = 0.95$).

Initially, left ventricular diastolic and systolic internal dimensions (LVIDd and LVIDs) were determined. The left ventricular end-diastolic dimension (LVIDd) was measured from the leading edge of the left septal surface to the leading edge of the posterior wall endocardium at the peak of the R wave. The end-systolic dimension (LVIDs) was measured from similar points at the peak of anterior position of the posterior left ventricular wall. End-systolic and diastolic volumes were determined by cubing the respective internal dimensions. The cube method was utilized since the subject group could be assumed to have symmetrically contracting, normally shaped left ventricles. Stroke index was estimated from the formula:

\[
\text{Stroke index} = \frac{(LVIDd)^3 - (LVIDs)^3}{BSA (M^2)},
\]

and cardiac index was estimated with the formula:

\[
\text{Cardiac index} = \frac{(LVIDd)^3 - (LVIDs)^3 \times HR}{BSA (M^2)},
\]

where HR = heart rate and BSA = body surface area. All echocardiograms were recorded by the same technician and were evaluated independently by two observers.

Laboratory Methods

The concentration of norepinephrine in plasma and urine was measured using a radioenzymatic assay.
Norepinephrine was converted to radiolabeled epinephrine using partially purified bovine adrenal phenylethanolamine-N-methyl-transferase and tritiated S-adenosylmethionine. The epinephrine formed was isolated by batch alumina adsorption chromatography. Residual unreacted S-adenosylmethionine was precipitated with phosphotungstic acid and the epinephrine was finally extracted by bis-ethylhexylphosphoric acid in toluene and quantified using liquid scintillation spectrophotometry.

Sodium and potassium concentrations in plasma and urine were measured by flame photometer (Instrumentation Laboratories, Boston, MA). Creatinine was measured by an automated technique (Technicon, Chauncey, NY). Plasma renin activity and plasma aldosterone were measured by previously reported radioimmunoassay methods. The data were analyzed statistically by analysis of variance (repeated measures analysis, where appropriate). The relationship between urinary sodium excretion and blood pressure was also subjected to quadratic regression analysis. The 95% limits of probability were accepted as significant.

**Results**

Table 1 outlines the effect of sodium intake on variables which by repeated measures analysis of variance interacted significantly with sodium intake ($p < 0.05$). The observations were obtained on the final day at each level of sodium intake. Weight increased in significant increments from the 10 mEq/24 hr level. The urinary sodium excretion ($U_{Na}V$) approached the total sodium intake at each level. A significant kaliuresis ($U_KV$) occurred above a sodium intake of 300 mEq/24 hrs. Increases were observed between both the 300 and 600 mEq/24 hrs, and between the 1200 and 1500 mEq/24 hr levels of sodium intake ($p < 0.05$). No consistent effects on plasma sodium and potassium were observed. Mean blood pressure (MABP) increased significantly between the 10 and 800 mEq/24 hr levels and again between the 1200 and 1500 mEq/24 hr levels of sodium intake ($p < 0.05$). The relationship between $U_{Na}V$ and mean blood pressure is depicted in figure 1. Quadratic regression analysis revealed a significant relationship with a correlation coefficient of 0.50 ($p < 0.001$). The relationship is defined by the expression: $y = 84.6 + 0.000398 X + 0.0000387 X^2$.

**Table 1. The Effects of Sodium Loading on Variables Interacting Significantly with Sodium Intake (mean ± SEM).**

<table>
<thead>
<tr>
<th>Subjects (no.)</th>
<th>Sodium intake (mEq/24 hrs)</th>
<th>Sodium intake (mEq/24 hrs)</th>
<th>Sodium intake (mEq/24 hrs)</th>
<th>Sodium intake (mEq/24 hrs)</th>
<th>Sodium intake (mEq/24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>14</td>
<td>14</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Δ Weight (kg)</td>
<td>-</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>$U_{Na}V$ (mEq/24 hrs)</td>
<td>15 ± 4</td>
<td>278 ± 18</td>
<td>543 ± 61</td>
<td>706 ± 24</td>
<td>1122 ± 68</td>
</tr>
<tr>
<td>$U_KV$ (mEq/24 hrs)</td>
<td>63 ± 5</td>
<td>71 ± 4</td>
<td>132 ± 13</td>
<td>142 ± 7</td>
<td>150 ± 11</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>83.8 ± 1.3</td>
<td>85.8 ± 1.5</td>
<td>86.8 ± 1.0</td>
<td>91.1 ± 2.8</td>
<td>93.1 ± 1.5</td>
</tr>
<tr>
<td>Cardiac index (liter/min/m$^2$)</td>
<td>2.6 ± 0.1</td>
<td>2.8 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>2.9 ± 0.1</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>PNA (pg/ml)</td>
<td>467 ± 63</td>
<td>272 ± 41</td>
<td>260 ± 24</td>
<td>136 ± 64</td>
<td>160 ± 19</td>
</tr>
<tr>
<td>$U_Na$ (ng/24 hrs)</td>
<td>54.3 ± 3.4</td>
<td>39.1 ± 2.6</td>
<td>40.1 ± 4.1</td>
<td>31.6 ± 6.7</td>
<td>42.5 ± 5.0</td>
</tr>
<tr>
<td>PRA (ng AI/ml/3 hrs)</td>
<td>11.9 ± 2.3</td>
<td>2.6 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>PA (ng/100 ml)</td>
<td>43.9 ± 6.9</td>
<td>8.7 ± 1.8</td>
<td>6.3 ± 1.5</td>
<td>4.6 ± 1.6</td>
<td>2.8 ± 1.0</td>
</tr>
</tbody>
</table>

Abbreviations: $U_{Na}V$ = urinary sodium excretion; $U_KV$ = kaliuresis; MABP = mean arterial blood pressure, $P_{Na}$ = plasma norepinephrine concentration; $U_Na$ = urinary norepinephrine excretion; PRA = plasma renin activity; PA = plasma aldosterone concentrations.
inversely correlated with the change in mean blood pressure \((r = -0.61, p < 0.05)\) and with the cumulative increase in total body sodium as calculated from urinary values \((r = -0.75, p < 0.05)\).

Urinary norepinephrine excretion \((U_{Ne})\) decreased \((p < 0.05)\) between the 10 and 300, and 1200 and 1500 mEq/24 hr levels of sodium intake. Urinary norepinephrine excretion, which was measured daily, was inversely correlated \((r = -0.46, p < 0.001)\) with urinary sodium excretion (fig. 3). The relationship is defined by the expression \(y = -0.0174 X + 48.4\). Urinary norepinephrine excretion was also inversely correlated with mean blood pressure \((r = -0.21, p < 0.01)\). No correlation between the change in urinary norepinephrine excretion and cumulative increase in total body sodium was identified \((r = -0.46, p > 0.05)\).

Plasma renin activity (PRA) decreased significantly between the 10 and 300 mEq/24 hr levels of sodium intake \((p < 0.05)\), and again between the 300 and 800 mEq/24 hr levels \((p < 0.05)\). Plasma aldosterone (PA) decreased between the 10 and 300, and 300 and 800 mEq/24 hr levels of sodium intake \((p < 0.05)\). Mean blood pressure was inversely correlated with PRA \((r = -0.38, p < 0.001)\) and plasma norepinephrine concentration \((r = -0.40, p < 0.001)\). Plasma norepinephrine concentration and PRA were correlated \((r = 0.52, p < 0.001)\), as were urinary norepinephrine excretion and PRA \((r = 0.41, p < 0.001)\).

A multiple regression analysis was performed with mean blood pressure as the dependent variable and cardiac index, renin, aldosterone, norepinephrine values in plasma, and urinary norepinephrine excretion as independent variables. Plasma norepinephrine concentration and urinary norepinephrine excretion entered the equation. The addition of the remaining independent variables failed to improve the correlation. The expression defining the relationship among mean blood pressure, plasma norepinephrine concentration and urinary norepinephrine excretion is as follows: \(\text{MABP} = 105.1 - 0.03 \ (P_{Ne}) - 0.24 \ (U_{Ne})\) (multiple correlation coefficient = 0.72, \(p < 0.001\)).
Discussion

The autonomic nervous system plays a major role in the regulation of arterial blood pressure. Short-term autonomic control of blood pressure is vested primarily in baroreceptor reflexes, chemoreceptors within the walls of great vessels, and receptors within the vasomotor center. In addition, a number of studies suggest that the autonomic nervous system influences long-term arterial blood pressure regulation at least in part by modulating the excretion of salt and water by the kidney. The autonomic nervous system also participates in the control of renin release. In addition, direct adrenergic innervation of glomerular arterioles and proximal tubules has been demonstrated by histochemical techniques and by electron microscopy. In the dog, baroreceptor reflex stimulation of renal sympathetic nerves produces an increase in renal tubular sympathetic reabsorption without changes in glomerular filtration rate, renal blood flow, or intrarenal distribution of blood flow. Furthermore, it has been shown that cardiopulmonary receptors exert a tonic inhibition on both renal nerve activity and renin release. The magnitude of the inhibition was directly related to blood volume.

In the present study, a sodium intake of 800 mEq/24 hrs, which is twofold greater than that consumed by even the most salt-gluttonous societies, resulted in increases in weight and cardiac index, and a modest increase in blood pressure. The increase in blood pressure is most readily explained by the increase in cardiac index resulting from an increase in extracellular fluid volume. Increasing the sodium intake still further to 1500 mEq/24 hrs amplified the effects; however, the increase in blood pressure was still relatively modest. Obviously, important adjustments accompanied the increases in sodium intake which facilitated the renal excretion of the massive sodium load.

The upright plasma norepinephrine concentration, which provided a means for assessing sympathoadrenal medullary function, decreased with sodium loading and was directly correlated with plasma renin activity. Both were inversely correlated with urinary sodium excretion and blood pressure. Changes in both were correlated with calculated changes in total body sodium. These relationships provide evidence that not only were these two pressor substances not directly responsible for the increases in blood pressure, but also that their prompt suppression may have ameliorated the effects of sodium loading.

Urinary norepinephrine excretion decreased in our subjects with sodium loading. The urinary norepinephrine excretion was inversely correlated with the urinary sodium excretion, but it was not correlated with the calculated increase in total body sodium. Although the urinary norepinephrine excretion may represent in part norepinephrine released into the cardiovascular system, it is likely that a considerable contribution comes from the kidneys themselves. A previous study from our laboratory has shown that the clearance of norepinephrine increases with standing and exceeds the clearance of creatinine in that position. Since with upright posture the amount of norepinephrine excreted in the urine exceeded the amount filtered, it is likely that the urinary norepinephrine excretion is at least in part related to the activity of the adrenergic renal nerves.

In addition to the relationship between mean blood pressure and urinary sodium excretion in the present study, mean blood pressure was also directly correlated with cardiac index, and inversely correlated with plasma renin activity, plasma aldosterone concentration, plasma norepinephrine concentration, and urinary norepinephrine excretion. Multiple regression analysis revealed a close correlation of mean blood pressure with plasma and urinary norepinephrine values, which was not improved by the addition of other variables. This regression, which in no way implies cause and effect, provides an additional demonstration of the close relationship between mean blood pressure following sodium loading and norepinephrine in plasma and urine of normal subjects.

A number of previous studies has raised the possibility that sodium may alter vascular reactivity to humoral vasoactive substances. Since hemodynamic responses to static effort are related to a powerful activation of the sympathetic nervous system, and occur even in the face of beta-blockade, isometric hand-grip contraction might be expected to result in augmented responses under the condition of a high sodium intake. In the present study, no interaction between the blood pressure responses to hand-grip contraction and the level of sodium intake were identified. Thus, the present study fails to show evidence of an enhanced pressor response to stimuli known to increase adrenergic activity.

Our subjects were faced with excreting massive salt loads. Even at the highest level of sodium intake, homeostasis was achieved in a relatively short period...
of time. The urinary sodium excretion approached the sodium intake after 72 hours. Presumably our subjects were able to excrete such large salt loads not only by an increase in glomerular filtration rate and blood pressure, and by decreases in plasma renin and aldosterone, but also by tonic inhibition of the sympathetic nervous system, either via baroreceptor responses or via some other influence upon the renal nerves. Although these acute studies were performed at levels of sodium intake well outside those at which sodium is considered a necessary constituent of the diet, they may nevertheless have relevance to the study of hypertension. Subtle aberrations in autonomically mediated sodium excretory mechanisms may contribute to elevation of blood pressure in some individuals when they indulge in the high salt intake characteristic of our society.

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