Predominance of Nocturnal Sympathetic Nervous Activity in Salt-Sensitive Normotensive Subjects


To assess the relation between salt sensitivity and autonomic nervous function by power spectral analysis of heart rate variability in normotensive subjects, low and high salt diets were given to 13 normotensive men (aged 25 to 39 years) for 4 days each. Autonomic function was assessed by power spectral analysis of R-R intervals based on an autoregressive algorithm from 24-h Holter electrocardiogram. Subjects whose mean blood pressure was increased more than 3 mm Hg by high salt diet were defined as salt sensitive (SS, n = 5), and the remainder as salt resistant (SR, n = 8). Using the low frequency (LF, 0.1 Hz) and high frequency (HF, 0.25 Hz) components, the LF to total power ratio (%LF) was used as a marker of sympathetic activity, and the HF to total power ratio (%HF) as a marker of parasympathetic activity. Compared to the daytime, SR revealed a decrease in %LF and an increase in %HF during the night on both diets. In SS, these circadian changes were observed only during low-salt diet. During the night, SS showed a higher %LF and a lower %HF than SR. Plasma catecholamines tended to be decreased by the high sodium diet in SR but not in SS subjects. These results suggest that the persistent nocturnal predominance of sympathetic nervous activity in salt-sensitive men may contribute to the subsequent increase of blood pressure in these subjects. Am J Hypertens 1996;9:726-731

KEY WORDS: Autonomic nervous system, electrocardiogram, power spectral analysis, norepinephrine, salt sensitivity.

Epidemiological and intervention studies in humans as well as animal studies have revealed a close relationship between dietary salt intake and blood pressure. The response of blood pressure to salt loading is not uniform, leading to the concept of salt sensitivity. Salt sensitivity has been demonstrated not only in hypertensives but also in normotensive subjects. Of interest, salt-sensitive normotensive subjects are known to be prone to the development of hypertension in later life. Several mechanisms are reported as candidate factors in salt sensitivity including the sympathetic nervous system, the renin-angiotensin system, vascular reactivity, and renal sodium handling in essential hypertensives, although little is known regarding normotensive subjects.

The aim of this study was to assess the role of the autonomic nervous system in blood pressure regulation in salt-sensitive normotensive subjects. We used power spectral analysis of heart rate variability, which has been developed as a powerful tool for non-inva-
sive assessment of the autonomic modulation of the SA node.\textsuperscript{7-9} We used R-R intervals from Holter electrocardiogram for the power spectral analysis because this method allows assessment of the circadian changes of autonomic nervous function.\textsuperscript{10,11}

**SUBJECTS AND METHODS**

**Subjects and Blood Pressure Measurement** Thirteen normotensive healthy men (age range: 25 to 39 years, mean $\pm$ SD = 30 $\pm$ 5 years) were enrolled in this study. Routine physical and laboratory examinations showed that none of them had hypertension, hyperlipidemia, diabetes mellitus, or abnormalities of cardiac, hepatic, or renal function. The subjects received low (54 $\pm$ 5 mEq/day) and high salt (294 $\pm$ 23 mEq/day) diets in random order for 4 days each. Blood pressure was measured by an automatic sphygmomanometer using the oscillometric method (BP-103i, Colin Co., Komaki, Japan) with the subject in the supine resting position. Measurements were obtained on the fourth day of each diet period three times a day. On each occasion, at least three measurements were obtained and averaged. Salt sensitivity was defined as previously stated in the protocol by Sharma et al.\textsuperscript{12} Subjects whose mean blood pressure on the high salt diet was increased by more than 3 mm Hg compared with that on the low salt diet were defined as salt sensitive (SS), and the remainder as salt resistant (SR). Family history of hypertension was obtained by direct personal inquiry. Subjects were regarded as having a positive family history when at least one of their parents or grandparents was hypertensive.

The study protocol was approved by the ethical committee of Osaka University Medical Hospital. Informed consent was obtained from each subject, after a full explanation of the study.

**Laboratory Measurements** On the last day of each diet period, blood was collected for the measurement of serum electrolytes, biochemical parameters, plasma renin activity, and aldosterone and catecholamine concentrations after overnight fasting. Blood was obtained with the subjects in the supine position after 10 min rest. Dietary compliance was assessed by measuring the 24-h excretion of urinary sodium, chloride, potassium, and creatinine.

**Power Spectral Analysis** On the last day of each diet period, Holter ECG (Model 90205, SpaceLabs, WA) was recorded for 24 h. Artifacts and ectopic beats in the entire 24-h data were excluded manually according to the method by Lippmann et al.\textsuperscript{13} Briefly, if an ectopic beat existed, R-R intervals preceding and after it were deleted. All subsequent intervals were shifted upwards for the calculation of power spectral analysis. Furthermore, periods of poor quality recording that were obviously not suitable for spectral analysis were searched for and excluded from the analysis. Autonomic function was assessed from R-R intervals by power spectral analysis with autoregressive method using a computer program (R-R Variability Analysis Program, QP 314-D, Nihon Kohden Corporation, Tokyo, Japan). The total power was defined as the power at 0.03 Hz or greater, the low frequency power (LF) as the peak at 0.1 Hz and the high frequency power (HF) as the peak at 0.25 Hz. Using these parameters, the LF to total power ratio (%LF) was used as a marker of sympathetic activity and the HF to total power ratio (%HF) as a marker of parasympathetic activity.\textsuperscript{7} Both %LF and %HF were calculated every 10 min and averaged at intervals of 30 min. Daytime was defined as 10:00 AM to 6:00 PM and nighttime as 1:00 AM to 7:00 AM because all subjects were awake and asleep during these periods, respectively.

**Statistical Analysis** All data are expressed as mean $\pm$ SEM. We first assessed the difference by two factor analysis of variance (ANOVA). Because the values on low salt diet showed no difference by ANOVA, analysis of covariance (ANCOVA) using the computer program, Super ANOVA (Abacus Concepts, Inc., Berkeley, CA) was applied to the values on high salt diet. In ANCOVA, values on low salt diet were used as a covariant and values during high salt diet as a dependent variable. Bonferroni's test was used for multiple comparison, and t test for two-group comparison. Values for $P < .05$ were considered statistically significant.

**RESULTS**

Of 13 subjects, eight were defined as SR and five as SS. There was no significant difference between SR and SS subjects in age (29 $\pm$ 2 vs 30 $\pm$ 2 years), body mass index (22.6 $\pm$ 1.0 kg/m$^2$ vs 24.8 $\pm$ 1.6 kg/m$^2$) and the proportion of subjects with positive family history (SR: 4 of 8, SS: 4 of 5). In SS, mean blood pressure and heart rates during the high salt diet period were significantly higher than those during the low salt diet period whereas they were not different in SR (Table 1). In both groups, increased sodium excretion was observed during the high salt diet period while potassium and creatinine excretion were similar. These values did not differ between the two groups (Table 1). Plasma renin activity and aldosterone concentration were higher during the low sodium diet than those during the high sodium diet period (Table 1). They were not different between SR and SS subjects. In SR, plasma norepinephrine and epinephrine levels tended to be lower during the high salt diet period ($P = .091$ and $P = .086$, respectively) although they did not alter in SS (Table 1).

Figure 1 shows a representative recording of the power spectral analysis of R-R variability obtained in
TABLE 1. CHANGES OF HEMODYNAMICS, BLOOD AND URINARY FACTORS DURING LOW AND HIGH SALT DIETS

<table>
<thead>
<tr>
<th></th>
<th>Salt Resistant (n = 8)</th>
<th>Salt Sensitive (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Salt</td>
<td>High Salt</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>117 ± 3</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>73 ± 3</td>
<td>69 ± 3</td>
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<tr>
<td>Mean BP (mm Hg)</td>
<td>87 ± 3</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>81 ± 2</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>Blood values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>141 ± 1</td>
<td>142 ± 1</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>100 ± 1</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.4 ± 1</td>
<td>4.2 ± 1</td>
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<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>13.4 ± 0.9</td>
<td>14.5 ± 1.3</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Renin activity (ng/mL/h)</td>
<td>5.4 ± 1.4</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Aldosterone (ng/dL)</td>
<td>33.3 ± 6.5</td>
<td>10.8 ± 2.6</td>
</tr>
<tr>
<td>Norepinephrine (pg/mL)</td>
<td>210 ± 50</td>
<td>140 ± 20</td>
</tr>
<tr>
<td>Epinephrine (pg/mL)</td>
<td>27.5 ± 5.3</td>
<td>17.5 ± 2.5</td>
</tr>
<tr>
<td>Urinary excretions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (mL/24 h)</td>
<td>1363 ± 527</td>
<td>1771 ± 546</td>
</tr>
<tr>
<td>Sodium (mEq/24 h)</td>
<td>55 ± 11</td>
<td>236 ± 37</td>
</tr>
<tr>
<td>Chloride (mEq/24 h)</td>
<td>50 ± 10</td>
<td>246 ± 36</td>
</tr>
<tr>
<td>Potassium (mEq/24 h)</td>
<td>39 ± 4</td>
<td>66 ± 14</td>
</tr>
<tr>
<td>Creatine (g/24 h)</td>
<td>1.6 ± 0.1</td>
<td>1.8 ± 0.3</td>
</tr>
</tbody>
</table>

Mean ± SEM.

*P < .05, †P < .01 v low salt diet.

a 37-year-old SR subject in the daytime (2:12 PM) and at night (3:30 AM). A high peak of the LF component was noted during the daytime whereas the HF component peak was marked during the night. On high salt diet, %LF in SR showed a marked decline during the night while diurnal changes were not observed in SS (Figure 2). Similarly, %HF was higher during the night in SR whereas that in SS did not change significantly (Figure 3). In contrast, circadian variation was similar between SR and SS on low salt diet. The mean values of %LF in the daytime were similar during the two diet periods and in SR and SS subjects (Figure 4). During the night, %LF was significantly higher in SS than SR. This was observed regardless of the diet. Dietary salt intake and salt sensitivity did not affect %HF in the day. During the night, %HF was higher in SR compared to that in SS during both diets. Compared to the day, the nighttime %HF was higher in SR and in SS on the high salt diet.

**DISCUSSION**

We assessed autonomic nervous activity during ambulatory daily life in normotensive subjects in relation to salt sensitivity of blood pressure by evaluating heart rate variability by power spectral analysis from Holter electrocardiogram. As a result, salt-sensitive (SS) subjects showed an elevation of sympathetic activity and attenuated suppression of parasympathetic activity compared to salt-resistant (SR) subjects during the night.

Evaluation of heart rate variability by power spectral analysis from Holter electrocardiogram allows the noninvasive assessment of minute-to-minute rapid change as well as circadian changes of sympathetic and parasympathetic activities without disturbing daily activity. Pagani et al demonstrated that chronic oral administration of propranolol significantly reduced LF both at rest and during tilt in humans. In addition, bilateral stellectomy in dogs prevented LF increase during nitroglycerine infusion. They also reported the increase of LF by sympathetic activation due to mental stress. These reports suggest that the LF component of power spectral analysis of R-R variability reflects sympathetic activity.

Measurement of plasma norepinephrine has been used as an index of sympathetic nerve activity. In the present study, plasma catecholamines failed to differentiate the two subgroups of salt sensitivity: norepinephrine levels measured in the morning after 10 min rest tended to be decreased by high sodium diet in SR while they did not alter in SS. However, these parameters were able to differentiate the two subgroups of salt sensitivity in terms of autonomic activity by showing different diurnal changes of %LF and %HF between SR and SS subjects during high salt diet.
Several factors are reported to be involved in the mechanisms of salt sensitivity. In Dahl salt-sensitive rats, abnormalities in baroreflex control of heart rate and $\alpha_2$-adrenergic receptor regulation were reported. In humans, the pressor response to norepinephrine is augmented by salt loading in salt-sensitive subjects. Sensitive hypertensive patients show impaired suppression of plasma norepinephrine after salt loading, although this finding was not confirmed by others. In hypertensive patients, Campese et al. demonstrated different responses of plasma norepinephrine to high salt diet, namely norepinephrine level was decreased by salt loading in SR but not in SS. Combining these and our results suggests that the autonomic nervous system is one of the major determinants of salt sensitivity. The lack of difference in norepinephrine response in the present study may be explained by the fact that we enrolled normotensive subjects of relatively young age (mean: 29.5 years) whose autonomic dysregulation was not severe enough to influence the plasma catecholamine levels.

Heart rate fluctuations rhythmically under stable conditions, and is known to be affected by the autonomic nervous system. Analysis by the spectral technique of heart rate variability based on the fast Fourier or autoregressive algorithm allows the noninvasive assessment of autonomic nervous function. Using this power spectral analysis, increased vagal activation and decreased sympathetic activation were demonstrated during sleep. Because %LF decreased and %HF increased in response to tilt, the postural change rather than diurnal change may explain the circadian profile of autonomic nervous activity.
We did not obtain blood pressure measurements during sleep because cuff inflation may disturb sleep, and, hence, influence sympathetic and parasympathetic nervous activities. Therefore, the present study has limitations in that the nocturnal autonomic dysregulation in SS subjects was not directly related to the blood pressure. The absence of a difference in autonomic regulation during the daytime may be due to the various daily ambulatory activities that mask the differences between SR and SS subjects. Although we did not perform the procedure, a passive tilting test might provide more information for the differentiation of the autonomic function of the two groups. However, measurement of blood pressure during the night may provide more precise information on the relationship between blood pressure and sympathetic nervous activity.

In summary, the present study demonstrated that salt-sensitive normotensive subjects showed higher sympathetic and lower parasympathetic activities during the night compared to salt-resistant subjects, while they were similar during the day. From these results, we conclude that this nocturnal sympathetic.

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**FIGURE 2.** Diurnal changes of %LF in salt-resistant (SR, n = 8, upper) and salt-sensitive (SS, n = 5, lower) subjects on high salt diet. Data were calculated every 10 min and averaged at intervals of 30 min. Values are mean ± SEM.

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**FIGURE 3.** Diurnal changes of %HF in salt-resistant (SR, n = 8) and salt-sensitive (SS, n = 5) subjects on high salt diet. Values are mean ± SEM.
predominance may contribute to the subsequent elevation of blood pressure in salt-sensitive subjects.

REFERENCES


