DISCRIMINATION AND SPECTRAL RESOLUTION OF ULTRADIAN AND CIRCADIAN PERIODICITIES FOR 24-HOUR BLOOD PRESSURE PATTERNS ON SODIUM MANIPULATION IN NORMOTENSIVE HUMANS

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The effects of a severe (10–20 mEq/24-h) but short-term (7-days) restriction in dietary sodium on 24-h blood pressure (BP) patterns were investigated in 20 normotensive volunteers (10 men, 10 women; 20–25 years old) by means of non-invasive automatic sphygmomanometric monitoring (recordings at 1-h interval) at home, with subjects in bedrest. Statistical methods used include bivariate Gaussian distribution analysis for systolic and diastolic BP bivariate discriminant analysis, periodic regression analysis with a 24-h period (cosinor method) and spectral analysis of time series. Casual BP measurements were unable to detect the tensinogenic effects, while the bivariate Gaussian analysis documented a total rearrangement in the pattern distribution to show discriminated values of BP after sodium restriction. A significant decrease (p < 0.01) in the 24-h mean level (mesor) for systolic BP was observed. An increase in the extent of fluctuation (amplitude) for the circadian components was documented by the spectral analysis, mainly in diastolic BP patterns. Such a phenomenon of 'amplitude magnification' was not accompanied by a change in the reproduction of the ultradian and circadian harmonic components. The extensive changes in the time structure support the hypothesis of an interaction of sodium regimen with the centrally-located oscillators which physiologically organize the circadian rhythmicity of BP.

AN extensive literature deals with the inter-relationship of salt intake and blood pressure. The majority of these studies have been performed by means of sodium manipulation in hypertensives,1–6 fewer in normotensives.7–9 One of the authors of the present paper has suggested that human hypertensives can be divided into two or three subgroups in terms of salt sensitivity, i.e., 1. salt sensitive; 2. non-salt sensitive,10 and 3. paradoxically salt sensitive.11 Non-salt sensitive Type II, described recently12 may be included in the 'paradoxically salt-sensitive' subgroup.11 It is important to stress that these conclusions were drawn by considering blood pressure a time-qualified variable to be dynamically monitored along the 24-h span.10–12

The importance of temporal monitoring for investigating the relationship between salt and blood pressure is emphasized in this study which is aimed to explore the tensinogenic effects of a severe salt restriction in normotensives.

Key words:
Biorhythm
Blood pressure
Data series analysis
Normotensive subjects
Salt intake

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1296 Japanese Circulation Journal Vol. 51, November 1987
**TABLE 1** MOST PROMINENT CHARACTERISTICS OF SUBJECTS INVESTIGATED

<table>
<thead>
<tr>
<th>Dietary sodium</th>
<th>Casual BP (mmHg)</th>
<th>Plasma concentration (mEq/L)</th>
<th>Urinary excretion (mEq/24-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BW (Kg)</td>
<td>SBP</td>
<td>K⁺</td>
</tr>
<tr>
<td><strong>Habitual</strong></td>
<td>59–72</td>
<td>110–120 70–75</td>
<td>139–143</td>
</tr>
<tr>
<td>(P)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values given as range. Statistical analysis of the equality of the mean of Student’s t test. BW = Body Weight; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; NS = Not Significant.

**Protocol of Study on Individual Subjects**

**HABITUAL SODIUM**

<table>
<thead>
<tr>
<th>Intake (mEq/24h)</th>
<th>Na⁺</th>
<th>K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>120–140</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SODIUM DEPRIVATION**

<table>
<thead>
<tr>
<th>Rest-activity schedule</th>
<th>DanR*</th>
<th>R⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>50–70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Meals:**

<table>
<thead>
<tr>
<th>Light</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

**Time (days):**

<table>
<thead>
<tr>
<th>Test:</th>
<th>Sample:</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:00</td>
<td>06:00</td>
</tr>
<tr>
<td>12:00</td>
<td>18:00</td>
</tr>
<tr>
<td>24:00</td>
<td>00:00</td>
</tr>
</tbody>
</table>

*DANR = Diurnal Activity-Nocturnal Rest; R = Recumbency

Fig.1. The protocol adopted in this investigation.

**MATERIALS AND METHODS**

1. **Subjects and protocol**

   Twenty clinically healthy subjects (10 men and 10 women; 20–25 years old) volunteered with informed consent for this study. They were selected as normotensive individuals by checking that their systolic (S) and diastolic (D) blood pressure (BP) was below 140/85 mmHg on at least 3 occasions (casual BP). The normality of BP was confirmed by other casual measurements during the course of the study.

   The clinical characteristics of the subjects investigated are summarized in Table I, remembering that 8 out of 20 subjects had a positive history of essential hypertension in their families.

2. **Protocol**

   The protocol of this study is illustrated in Fig.1.

   The subjects were monitored for BP and heart

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Fig. 2. 24-h patterns of systolic (S) and diastolic (D) blood pressure (BP) and heart rate (HR) on habitual and restricted sodium intake in 20 normotensives.

rate (HR), at home, by means of a non-invasive, automated device (Model Omega 1000, manufactured by Invivo Research Laboratories, Tulsa, Oklahoma, USA), programmed for a sphygmomanometric measurement (oscillometric technique) every 60 min, with the inflatable cuff attached to the upper non-dominant arm. The device was equipped with a printer for a print-out of data and time. The dynamic BP monitoring was made the subjects in bed-rest. During their stay in bed, they were permitted to sit, watch television, listen to the radio and talk to other people. At least 15 min prior to the BP measurement, they had to keep a supine posture. The recumbent period was initiated at least 8 hours prior to the monitoring. Usually the recumbency started on the evening preceding the BP 24-h recording (first measurement at 07:00).

The subjects received 3 meals at a scheduled time (breakfast at 08:00, lunch at 12:30 and dinner at 18:30) for at least 7 days prior to the study. The subjects were asked to take the dietary regimen on normal sodium (120–140 mEq/day) and potassium (50–70 mEq/day) intake for at least 7 days, and then, on restricted sodium (10–20 mEq/day) and normal potassium intake for 7 days. The two conditions of sodium intake have been conventionally called habitual sodium and restricted sodium intakes, respectively. In both sodium intakes, the 24-h BP monitoring was programmed on the last day of each dietary regimen. The calorie intake was 25 Kcal/Kg of ideal weight supplied by 3 g/Kg of carbohydrates, 0.8 g/Kg of lipids and 1 g/Kg of proteins throughout both dietary regimens. No significant changes in body weight were observed for any of the subjects on the 2 different diets.

The protocol was planned to fix the length of day with the light on at 07:00 and the light off at 23:00.

3. Data series analysis

The data were statistically processed by means of a complex procedure. The time-qualified data were averaged to obtain the hourly mean (± standard deviation; SD) for each dietary condition. The statistical analysis of the difference in SBP, DBP and HR before and after the sodium restriction was made by means of Student's paired t-test.

In a second statistical method the frequency distribution of the data were analysed by means of bivariate Gaussian analysis.

The statistical analysis of difference in frequency distribution has been made by means of a computerized bivariate discriminant analysis for microcomputers.

The third biostatistical approach was made by treating the time-qualified data series of each individual by means of the single-cosinor analysis to construct a sinusoidal wave, and to estimate the waveform parameters in terms of mesor (rhythm-adjusted mean; M), amplitude (one half of the total extent in the sinusoidal fluctuation; A), and acrophase (timing of the sinusoidal crest related to local midnight; Φ).

The rhythmmetric estimates were summarized by means of the population-mean cosinor to describe the biorhythmic phenomenon for

Japanese Circulation Journal Vol. 51, November 1987
the group.  

The statistical difference between the rhythmometric properties was made by means of Hotelling’s T2 test, a multivariate analysis for vectorial units and directional data.

In the final statistical method the time-qualified data were analysed in their harmonic components by means of spectral analysis, a procedure for resolving cyclic periodicities in a temporal profile of raw data.

The spectral resolution was made by incrementally fitting a periodic regression analysis with prefixed periods ranging from 1 cycle every 2 hours to 1 cycle every 28 hours. With this domain of periodicities, the spectral analysis enables us to explore the ultradian (periodicities less than 20 hours) as well as the circadian (periodicities from 20 to 28 hours) spectrum of the biorhythmic functions in nature.

RESULTS

The 24-h profiles for SBP, DBP, and HR in 20 normotensives on habitual as well as restricted sodium intake are displayed in Fig. 2. The time-qualified values (mean ± SD) of SBP, DBP and HR showed a certain variability along the 24-h span in both conditions of sodium intake. A statistically significant difference of the mean was found in SBP (p < 0.01), but not in DBP, even though the mean value decreased from 70.1 ± 9.1 to 68.3 ± 8.0 mmHg, whereas HR did not change at all.

Figure 3 tridimensionally displays the bivariate Gaussian distribution for SBP and DBP in 20 normotensives on habitual as well as restricted sodium intake. The configuration of the bivariate region indicates a different area for the SBP and DBP distribution in subjects non-restricted as well as restricted in their sodium intake.

A highly statistically significant difference in these distributions was found by using the bivariate discriminant analysis (F = 114.84; degrees of freedom = 2 and 957; p < 0.01), as shown in Fig. 4.

Figure 4 illustrates the sinusoidal waves and polar plots resulting from the periodic regression analysis of cosinor using the 24-h period. Circadian rhythms were statistically validated for SBP, DBP and HR in both the conditions of sodium intake as indicated by the P values for the rhythm detection level.

A statistically significant difference was found by the multivariate analysis of the rhythmometric parameters in the mesor for SBP (Table II). The decrease in DBP mesor, and the increase in SBP and DBP amplitude were found to be of border-
Fig. 5. Best-fitting cosine curves and polar plots representing the outcomes by the cosinor analysis approaching the 24-h patterns of systolic (S) and diastolic (D) blood pressure (BP) and heart rate (HR) on habitual and restricted sodium intake in 20 normotensives.

TABLE II MULTIVARIATE ANALYSIS BY HOTELLING'S T2 TEST OF COSINOR-DERIVED ESTIMATES FOR THE CIRCADIAN RHYTHMS OF SYSTOLIC (S) AND DIASTOLIC (D) BLOOD PRESSURE (BP) AND HEART RATE (HR). THE STATISTICAL CONTRASTS RELATE TO THE COMPARISON OF RHYTHMOMETRIC PARAMETERS QUANTIFIED IN NORMOTENSIVE SUBJECTS BEFORE AND AFTER A RESTRICTION IN DIETARY SODIUM INTAKE

<table>
<thead>
<tr>
<th>Rhythmometric parameters compared</th>
<th>Variables compared</th>
<th>SBP</th>
<th>DBP</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>A + \Phi</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>M</td>
<td>p &lt; 0.01</td>
<td>BS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>A</td>
<td>BS</td>
<td>BS</td>
<td>BS</td>
<td>NS</td>
</tr>
<tr>
<td>A as % of M</td>
<td>BS</td>
<td>BS</td>
<td>BS</td>
<td>NS</td>
</tr>
</tbody>
</table>

A = Amplitude; \Phi = Acrophase; M = Mesor; NS = Not significant; BS = Borderline significant (0.05 < p < 0.1)

Japanese Circulation Journal Vol. 51, November 1987
Fig. 6. Spectral analysis resolving cycles with ultradian (from 2-h to 19-h) and circadian (from 20-h to 28-h) period (TAU) in the 24-h patterns of systolic (S) and diastolic (D) blood pressure (BP) and heart rate (HR) on habitual (left side) and restricted sodium intake (right side) in 20 normotensives. The periodicities validated as fundamental components can be identified by their “percent rhythm” above the line which divides the significant and not significant harmonics.

Fig. 7. Sodium restriction exerts differential changes on mesor, amplitude and acrophase of systolic blood pressure in normotensives with or without a familial predisposition to hypertension.

line significance (0.05 < p < 0.1). No significant P value was detected by the statistical comparison of the rhythmic parameters of the HR circadian rhythm. No statistically relevant difference was estimated for the acrophase timings in all the rhythms calculated.

The spectral analysis of the 24-h patterns for SBP, DBP and HR on habitual and restricted sodium diet is illustrated in Fig. 6. The statistically significant periodicities were found in the spectral resolution from a 17- or 18-h cycle for SBP, a 16- or 19-h cycle for DBP and a 15-h cycle for HR, respectively. Despite the negligible dissimilarities in the low-frequency ultradian components, the circadian periodicities were invariably detected in all the rhythmic spectra. After the sodium restriction, however, the circadian waves showed an increase in their amplitude of fluctuation, in particular, for DBP, and a decrease for HR.

Figure 7 displays the most prominent differences for BP rhythmmometry in the two groups, categorized in relation to family history for hypertension. The magnification of amplitude for SBP is not detectable in sodium-restricted subjects with a familial predisposition to hypertension.

DISCUSSION

The anecdotal observation that dietary sodium restriction acts to decrease BP has been confirmed in this study. The lowering effect has been demonstrated in "non-hypertensive" subjects, extending the plethora of reports dealing with hypertensive patients.1-7,11,12 The hypertensive effect of sodium restriction in normotensives has been detected by comparing the 24-h BP patterns, while the casual sphygmomanometric measurements have not shown such a phenomenon (see Table I). In our opinion, the discordance between the homeostatic (not chronobiologic) and chronobiologic assessment in this study draws our attention to the unquestionable importance of biometric methodology as a reliable tool for investigating the sodium/BP relationship in humans.

Basically, a casual measurement may be misleading when dealing with a variable characterized by a rhythmicity in its temporal patterns. Conceivably, the influence of an external or environmental factor on such a biological oscillating function may cause changes in the mean level (alias, mesor), and/or in the extent (amplitude), and/or in the wave timing (acrophase). If this complex interaction occurs, a casual measurement may be inadequate to describe the rearrangement in the 24-h patterns of the variable investigated. This weakness is well illustrated in the present study. Despite the insignificant difference in the casual pressures, the bivariate analysis of the Gaussian distribution has shown that the 24-h BP patterns change their entire numerical density and arrangement after the restriction of dietary sodium. The bivariate discriminant analysis has demonstrated that the rearrangement after sodium restriction causes the argumental classes for the SBP and DBP distribution to be highly capable of discrimination.

The cosinor analysis has defined the rhythmic properties in the 24-h BP patterns which are influenced by the sodium restriction. The periodic regression analysis has demonstrated that the reduction in salt intake is not associated with the disruption of the circadian rhythm of BP. The rhythmicity, not only remains, but also preserves its phasic cadence with the acrophase timing located in the afternoon. Interestingly, the cosinor-derived estimates have shown that the mesor decrease is the most sensitive change induced by salt deprivation in 24-h BP patterns of the normotensive individuals. The mesor change is, however, accompanied by little increment in the extent of the oscillation for the 24-h period. This phenomenon of amplitude magnification was found to be of borderline significance, but to be wide enough for recording by the monitoring system and better analyzed by the spectral analysis.

In dealing with the oscillating BP function, a major question is whether or not the manipulation of dietary salt can interfere with the spectral structure, considering that the naturally occurring circadian rhythms may have periodic components in the ultradian domain as well. The spectral analysis of the 24-h BP patterns has demonstrated that the spectrum of the ultradian and circadian components is not modified by the sodium restriction. In detail, no significant ultradian wave was seen to be added, and no significant circadian component was seen to be removed. A prominent phenomenon that was seen, however, was the increase in amplitude for the 20 ~ 28-h circadian components sustaining the 24-h DBP patterns. Reconstructed circadian rhythms of BP approximated by the fit of the 24-h cosine curve, revealed an amplitude increase in the face of a mesor decrease, in association

Japanese Circulation Journal Vol. 51, November 1987
Biostatistics, 24-h BP patterns and Na

with the reduction in salt intake in hypertensive subjects\textsuperscript{12}

Taking this confirmatory reference into consideration, it seems that the binomium ‘mesor decrease/amplitude increase’ is the stereotypic response of the 24-h BP patterns to sodium restriction in humans. To explain the higher response in hypertensives, it can be argued that both the mesor decrement and the amplitude enhancement assume a graduation depending on the BP values before the sodium manipulation. However, the genetic background should be ascertained in a larger study taking into account the fact that our results, even though limited to a small number of subjects, indicate a different response in relation to the parental history of hypertension.

Suggestions aside, it must be stressed that the sodium-induced changes in the spectral periodicities should lead us to consider dietary salt as a ‘modulatory agent’ for the chronoorganization of the 24-h BP patterns in humans. Furthermore, the characteristic quality of rhythm modulator raises the question of the mechanism(s) underlying the interaction of dietary sodium with BP. Much research has shown that the electrolyte can interact with the vascular apparatus at different levels. The cation is able to influence the arterial vascular resistance and reactivity\textsuperscript{19–21} and the activity of the sympathetic nervous system\textsuperscript{22–25} A central action has also been described with the identification of sodium-sensitive pontine structures\textsuperscript{26}.

In our opinion, the modification of the BP time structure focuses our attention on a centrally-located interaction with the ‘rhythm donors’ (biological clocks) which physiologically regulate the time of the BP circadian rhythmicity\textsuperscript{27,28} A peripheral mechanism could not adequately explain the phenomenon in terms of reorganization for the entire oscillatory structure of BP chronophysiolog in sodium-restricted humans.

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Japanese Circulation Journal Vol. 51, November 1987


