Diurnal Blood Pressure Variation and Dietary Salt in Spontaneously Hypertensive Rats

David A. Calhoun, Sutao Zhu, J. Michael Wyss, Suzanne Oparil

Abstract We have previously reported that high dietary salt exposure significantly increases daytime mean arterial pressure in spontaneously hypertensive rats (SHR) but not in normotensive Wistar-Kyoto (WKY) controls. In the present study, we used a telemetry monitoring system to evaluate the effects of high dietary salt exposure on diurnal variation of mean arterial pressure and heart rate in SHR and WKY rats. After implantation of a radio frequency transducer, SHR and WKY rats were maintained on either high (8%) or basal (1%) salt diets. Hemodynamic values were then analyzed for diurnal variation with the use of a nonlinear data-fitting program. After 2 weeks of dietary exposure, high salt-fed SHR had significantly greater 24-hour mean arterial pressure (156±3 mm Hg) than SHR receiving basal (135±2 mm Hg) and WKY rats receiving high (100±2 mm Hg) or basal (100±1 mm Hg) salt diets. Rhythm analysis indicated significant increases in both daytime and nighttime mean arterial pressure during high salt exposure in SHR. In WKY rats, high salt exposure increased nighttime but not daytime mean arterial pressure, with no net effect on 24-hour mean arterial pressure. High dietary salt exposure significantly decreased heart rate in both SHR and WKY rats, and it did not significantly alter the pattern of diurnal blood pressure or heart rate variation. These results indicate that WKY rats manifest an acute sensitivity to salt ingestion but have compensatory mechanisms sufficient to prevent sustained increases in mean arterial pressure; such mechanisms are lacking in SHR. (Hypertension. 1994;24:1-7.)

Key Words • circadian rhythm • salt • telemetry • rats, inbred SHR • rats, inbred WKY

Human and animal studies demonstrate characteristic blood pressure and heart rate (HR) rhythm related to the day/night cycle. This circadian rhythm is probably important in terms of cardiovascular morbidity and mortality because sudden death, stroke, and myocardial infarction in humans tend to occur most frequently in the morning and because increased circadian variability in blood pressure is accompanied by increased end-organ damage. Therefore, factors that alter diurnal cardiovascular variation would be expected to affect cardiovascular morbidity and mortality.

Certain species and certain individuals within a species are known to be sensitive to dietary NaCl exposure; i.e., with changes in dietary NaCl ingestion, significant changes in blood pressure occur. This laboratory has demonstrated that spontaneously hypertensive rats (SHR) obtained from Taconic Farms, Germantown, NY, are NaCl sensitive. Daytime blood pressure measurements obtained from indwelling femoral artery catheters have shown that SHR manifest an increase in mean arterial pressure (MAP) of 20 to 30 mm Hg when exposed to a high (8%) NaCl diet. Normotensive Wistar-Kyoto (WKY) rats, in contrast, manifest no significant change in blood pressure regardless of dietary NaCl exposure.

New telemetric techniques permit the continuous monitoring of blood pressure and HR in freely moving, untethered animals. Such measurements can then be analyzed for circadian blood pressure and HR variation. Using this telemetric technique, we designed the following study to evaluate the effects of high dietary NaCl exposure on diurnal variation of blood pressure and HR in SHR and WKY rats.

Methods

Rat Strains and Environmental Conditions

Male SHR (IBU3 colony) and WKY rats were obtained from Taconic Farms, Germantown, NY, at 8 weeks of age. The rats were acclimated to a 12-hour light/dark cycle (light, 6 AM to 6 PM) for 1 week. During this period all animals were maintained on normal (1%) rat chow (Purina Test Diets) and allowed tap water ad libitum.

Telemetry and Data Acquisition

The Dataquest IV system (Data Sciences Inc) was used to measure telemetrically systolic blood pressure, diastolic blood pressure, MAP, and HR. The monitoring system consists of a transmitter (radio frequency transducer), receiver panel, consolidation matrix, and an IBM-compatible personal computer with accompanying software. When rats were 8 weeks old and anesthetized with phenobarbital general anesthesia, the flexible catheter of the transmitter was surgically secured in the abdominal aorta just below the renal arteries. The transmitter was sutured to the abdominal wall. Animals were housed in individual cages after operation. Each cage was placed over a receiver panel connected to the personal computer for data storage onto the hard drive. With the use of the Dataquest system, the animals are completely unrestrained and free to move within their individual cages. Hemodynamic data were sampled every 4 minutes in each animal as a wave form curve for 10 seconds. After rats regained their preoperative weights (7 to 10 days after surgery), they were maintained on either high (8%) (ISCN...
Table 1. Body Weight and Hemodynamic Characteristics at Baseline and After 2 Weeks of Dietary NaCl Exposure

<table>
<thead>
<tr>
<th>Strain and NaCl</th>
<th>Baseline</th>
<th>After 2 Weeks of Dietary Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BW, g</td>
<td>24-Hour MAP, mm Hg</td>
</tr>
<tr>
<td>SHR 8% (n=9)</td>
<td>195±9</td>
<td>140±2</td>
</tr>
<tr>
<td>SHR 1% (n=8)</td>
<td>205±10</td>
<td>139±3</td>
</tr>
<tr>
<td>WKY 8% (n=10)</td>
<td>249±34</td>
<td>104±11</td>
</tr>
<tr>
<td>WKY 1% (n=9)</td>
<td>253±30</td>
<td>105±2†</td>
</tr>
</tbody>
</table>

BW indicates body weight; MAP, mean arterial pressure; HR, heart rate; SHR, spontaneously hypertensive rats; and WKY, Wistar-Kyoto rats. Values are mean±SEM.

*P<.05 vs baseline.
†P<.05 vs respective 1% group.
‡P<.05 vs respective SHR group.

Biochemicals Purina chow with 8% NaCl) or basal (1%) NaCl rat chow for 2 weeks.

Data Analysis

Twenty-four-hour mean values, mean values of the SD of 24-hour values (used as an assessment of variation), and the mean values of the 6-hour day/night periods for MAP and HR were compared by ANOVA, with a value of P<.05 considered significant. For analysis of diurnal variation, mean values were calculated for 60-minute intervals for each diet/strain group. Rhythm analysis was performed by means of the nonlinear least-squares fitting program PHARMFIT. Twenty-four-hour cosine and all partial Fourier curves including up to the fifth harmonic (4.8-hour period) were fitted as models to the data. The following equation was used:

\[ y = \text{MESOR} + \sum \{\text{amplitude}(i) \times \cos(x - \text{acrophase}(i) \times 2\pi/\text{period}(i))\} \]

with \( i \) being the number of overlapping cosine functions. The program calculates estimates of the MESOR (midline estimating statistic of rhythm, ie, the rhythm-adjusted 24-hour mean), the amplitude (half of peak to trough of rhythmic change), and the acrophase (peak time of each component cosine function) of the harmonics together with the percentage of rhythm. The F test was used to test for zero amplitude.

Model comparison statistics over all fit models was done with the PHARMFIT subprogram SYNOPS according to Wald as described in detail by McIntosh and McIntosh. Briefly, likelihood was calculated for each fit model and transformed into a confidence statistic of rhythm, ie, the rhythm-adjusted 24-hour mean), the F test was used to test for zero amplitude.

Results

SHR were lighter and had higher baseline MAP than WKY rats (Table 1). High dietary NaCl exposure did not significantly affect weight gain in either strain. By day 4 of dietary NaCl exposure and thereafter, 24-hour MAP was significantly greater in SHR receiving the 8% NaCl diet than in the other three diet/strain groups (Table 1, Fig 1). Twenty-four-hour MAP values for WKY rats receiving the 8% NaCl or 1% NaCl diet were not different. MAP in WKY rats receiving either the 8% or 1% NaCl diet tended to be slightly lower than baseline, but the differences were significant only for the WKY 1% group (Table 1). By day 2 for WKY rats and day 5 for SHR, exposure to the high NaCl diet significantly decreased 24-hour mean HR compared with the 1% NaCl diet groups (Table 1, Fig 1).

High dietary NaCl exposure significantly increased nighttime and daytime MAP in SHR (Table 2). The increase was greatest during the 6-hour interval from midnight to 6 AM (164±1 versus 138±1 mm Hg, SHR 8% versus SHR 1%). The NaCl-induced increase in daytime pressure in SHR was not as great as the increase in nighttime MAP but was significant (149±2 versus 131±1 mm Hg, SHR 8% versus SHR 1% during the noon to 6 PM period). NaCl-induced increases in nighttime MAP were much smaller in WKY rats than SHR but were significant (105±1 versus 102±1 mm Hg, WKY 8% versus WKY 1% during the 6 PM to midnight period). NaCl-related differences in daytime MAP in WKY rats were not statistically significant (95±1 versus
TABLE 2. Six-Hour Hemodynamic Values at End of 2-Week Dietary Exposure Period

<table>
<thead>
<tr>
<th>Strain and NaCl</th>
<th>6 AM to Noon</th>
<th>Noon to 6 PM</th>
<th>6 PM to Midnight</th>
<th>Midnight to 6 AM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP, mm Hg</td>
<td>HR, bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR 8%</td>
<td>143±2</td>
<td>149±2</td>
<td>159±2</td>
<td>164±1</td>
</tr>
<tr>
<td>SHR 1%</td>
<td>128±1*</td>
<td>131±1*</td>
<td>134±1*</td>
<td>138±1*</td>
</tr>
<tr>
<td>WKY 8%</td>
<td>95±1†</td>
<td>98±1†</td>
<td>105±1*</td>
<td>104±1*</td>
</tr>
<tr>
<td>WKY 1%</td>
<td>96±1†</td>
<td>98±1†</td>
<td>102±1*</td>
<td>100±1*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; and HR, heart rate.

96±1 mm Hg, WKY 8% versus WKY 1%). High dietary NaCl exposure significantly decreased HR in SHR during nighttime and daytime periods except for the 6 PM to midnight period (Table 2). High dietary NaCl exposure significantly decreased HR in WKY rats during all nighttime and daytime periods.

Nonlinear rhythm analysis revealed significant daily variations of MAP and HR for each diet/strain group (Table 3, Figs 2 and 3). For both MAP and HR, the 24-hour period was the predominant harmonic (Table 3). Significant improvement of fit (13% to 29%) in each parameter was obtained by including additional harmonics in the fitting procedure. In SHR, for both MAP and HR, the second most predominant harmonic was the 12-hour period. In WKY rats, the second most predominant rhythm for MAP and HR was the 8-hour period. Thus, the harmonic pattern of MAP and HR variation was different for SHR and WKY rats regardless of dietary NaCl ingestion (Figs 3 and 4). However, in neither strain was the harmonic pattern significantly altered by dietary NaCl exposure. That is, in the case of SHR, despite changes in MESOR and amplitude, the pattern or time course of diurnal variation in MAP and HR was the same during high or basal dietary NaCl exposure.

SHR receiving the high NaCl diet had a rhythm-adjusted 24-hour mean MAP (MESOR) of 154±0.5

TABLE 3. Mean Arterial Pressure and Heart Rate Rhythms in SHR and WKY Rats Maintained on 8% and 1% NaCl Diets

<table>
<thead>
<tr>
<th>24-Hour Component</th>
<th>MESOR, mm Hg, bpm</th>
<th>Amplitude, mm Hg, bpm</th>
<th>Acrophase (24-Hour Clock)</th>
<th>Rhythm, %</th>
<th>Rhythm, Best Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR 8% NaCl (n=7)</td>
<td>154±0.5</td>
<td>12.5±0.6</td>
<td>0256±13 min</td>
<td>70.6</td>
<td>91.7*</td>
</tr>
<tr>
<td>MAP</td>
<td>317±0.6</td>
<td>31.9±0.9</td>
<td>0018±07</td>
<td>72.4</td>
<td>95.7*</td>
</tr>
<tr>
<td>SHR 1% NaCl (n=9)</td>
<td>131±0.2</td>
<td>5.8±0.3</td>
<td>0212±12</td>
<td>59.0</td>
<td>87.8*</td>
</tr>
<tr>
<td>MAP</td>
<td>330±0.8</td>
<td>26.6±1.1</td>
<td>0018±07</td>
<td>72.4</td>
<td>95.7*</td>
</tr>
<tr>
<td>SHR 8% NaCl (n=10)</td>
<td>101±0.2</td>
<td>7.2±0.3</td>
<td>0214±08</td>
<td>78.3</td>
<td>90.8*</td>
</tr>
<tr>
<td>WKY 8% NaCl (n=10)</td>
<td>356±0.7</td>
<td>37.0±1.0</td>
<td>0021±07</td>
<td>80.6</td>
<td>94.7t</td>
</tr>
<tr>
<td>WKY 1% NaCl (n=9)</td>
<td>99±0.2</td>
<td>4.1±0.2</td>
<td>0122±13</td>
<td>55.0</td>
<td>70.9t</td>
</tr>
<tr>
<td>MAP</td>
<td>382±1.2</td>
<td>48.2±1.7</td>
<td>0022±08</td>
<td>78.1</td>
<td>92.1†</td>
</tr>
</tbody>
</table>

MESOR indicates midline estimating statistic of rhythm; SHR, spontaneously hypertensive rats; MAP, mean arterial pressure; HR, heart rate; and WKY, Wistar-Kyoto rats. MAP was monitored by telemetry for 3 consecutive days. A partial Fourier curve, consisting of 24+12+8+6 (†), 24+8+4.8 (‡), or 24+12+8+6+4.8 (t) hour components was fitted to the data. Significance of improvement of fit by adding additional harmonics to the dominant 24-hour period was tested by multiple model comparison. Shown are rhythm-adjusted mean (MESOR), amplitude, acrophase (peak time of rhythm), and percentage of rhythm of the dominant 24-hour period together with improvement by adding harmonics. Values are mean±SD.
mm Hg and HR of 317±0.6 beats per minute (Table 3). In SHR receiving the basal NaCl diet, the MESOR for MAP was lower (131±0.2 mm Hg), and the MESOR for HR was greater (330±0.8 beats per minute). In contrast, dietary NaCl did not alter the MESOR for MAP in WKY rats. In WKY rats receiving the high NaCl diet, the MESOR for MAP was 101±0.2 mm Hg, and in WKY rats receiving the basal NaCl diet the MESOR was 99±0.2 mm Hg. The MESOR for heart rate was less in WKY rats fed the high NaCl diet than WKY rats fed the basal NaCl diet (356±0.7 versus 382±1.2 beats per minute, respectively).

Acrophases of the dominant 24-hour period for MAP occurred between 1:15 AM and 3 AM for all diet/strain groups (Table 3). For HR, the acrophases for all diet/strain groups occurred around midnight.

The variability (SD) of MAP was greater in SHR 1% than WKY 1% (Table 4). In both strains, however, high NaCl exposure significantly increased MAP variability. Variability of HR was not different in SHR and WKY rats receiving the basal NaCl diet and was significantly reduced in both strains by the high NaCl diet (Table 4).

Discussion

This laboratory has previously demonstrated that high NaCl exposure significantly increases daytime MAP in SHR but not in normotensive WKY controls. In these previous studies, blood pressure and HR were recorded during the morning or early afternoon from indwelling femoral artery cannulas in conscious, unrestrained rats that were resting quietly. When exposed to an 8% NaCl diet for 2 to 3 weeks, SHR manifested an increase in daytime MAP of 20 to 30 mm Hg. A significant change in MAP was not observed until day 7 through 10 of high NaCl exposure. Nocturnal blood pressure, when the animals are most active, was not measured in these earlier studies.

In the present study, use of the Dataquest monitoring system allowed for continuous blood pressure and HR measurement in completely unrestrained animals. Analysis of the data for patterns of diurnal variation with the use of the nonlinear fitting program PHARMFIT revealed several important observations not previously noted. First, the increase in MAP in SHR during high dietary NaCl exposure was most pronounced at night when the animals were most active (Table 2, Figs 2 and 4). Lemmer et al. using the Dataquest monitoring system to measure blood pressure, recently reported that the 24-hour blood pressure profile in SHR and WKY rats is related to the activity level of the animals, with peak blood pressure values occurring at night during the normal active period of the rats. Rats feed at night, so the acute effects of NaCl exposure would be expected to be most pronounced during that period.

The second novel observation of the current study was that WKY rats manifested small NaCl-induced increases in nocturnal MAP while active and feeding. During the daytime when the animals were resting, the difference in MAP was no longer present (Table 2, Figs 2 and 4). The increase in nocturnal MAP had not been
Detection of this small increase in nighttime MAP in WKY rats in response to dietary NaCl supplementation is probably attributable to the improved sensitivity of the telemetry monitoring system. Previously, measuring only daytime blood pressure in awake, tethered animals excluded the possibility of identifying nighttime differences.

The above data demonstrate that WKY rats manifest acute sensitivity to NaCl ingestion but have sufficient compensatory mechanisms to prevent sustained increases in MAP. Previous investigations from this and other laboratories have identified differences in renal function, neural transmitter activity, baroreflex activity, and cerebrospinal fluid NaCl concentrations between SHR and WKY rats that may be related to these compensatory mechanisms. Specifically, evidence suggests that high dietary NaCl exposure increases peripheral sympathetic nerve activity in SHR, thus increasing peripheral vascular resistance and thereby MAP. In addition, differences between SHR and WKY rats in response to acute volume loading may contribute to the differences in MAP observed in the present study. Recent studies from our laboratory have demonstrated that NaCl-resistant WKY rats adapt rapidly to a high NaCl diet, developing increased natriuretic and diuretic responses to acute volume loading. In contrast, NaCl-sensitive SHR fed a high NaCl diet fail to develop natriuretic and diuretic responses to acute volume expansion, probably resulting in volume loading and a second-
In the present study, high NaCl exposure significantly decreased 24-hour HR by day 2 in WKY rats and by day 5 in SHR (Fig 1). The NaCl-induced decrease in HR in both rat strains may be due to baroreflex activation secondary to the increase in MAP and/or intravascular volume expansion. Additional studies are necessary to test this hypothesis. Like MAP, NaCl exposure did not alter the diurnal pattern of HR variation in either SHR or WKY rats (Table 3, Figs 3 and 4). The predominant harmonic periods were the same during high or basal NaCl exposure in both rat strains.

Telemetry monitoring systems have been used to evaluate cardiovascular function in rats. Lemmer et al. used the Datquest system in combination with the PHARMFIT nonlinear data-fitting program to demonstrate that the circadian blood pressure pattern reverses in rats made hypertensive by implantation of the mouse salivary gland renin gene; ie, the transgenic rats were most hypertensive during the day while resting, as opposed to SHR and WKY rats that have peak blood pressures at night when most active.1 Mattes and Lemmer,23 using the Dataquest system to monitor blood pressure, determined that amlodipine-induced decreases in blood pressure in WKY rats are related to the circadian rhythm, with the greatest reduction in blood pressure occurring at night. Other investigators have evaluated the effects of high NaCl exposure on 24-hour blood pressure in NaCl-sensitive rat strains. Brown et al.34 recorded 24-hour blood pressure in tethered Dahl NaCl-resistant (DR) and NaCl-sensitive (DS) rats. They found that a high (8%) NaCl diet significantly increased MAP in DS rats, with the greater increase in MAP occurring at night, compared with DS rats receiving a low (0.4%) NaCl diet and DR rats receiving high or low NaCl diets. High dietary NaCl exposure did not significantly alter daytime or nighttime MAP in DR rats. High dietary NaCl exposure significantly increased blood pressure variability, as suggested by increases in the SD of MAP in DS but not DR rats.

The present study demonstrated that SHR, like DR rats, manifest both daytime and nighttime NaCl-induced increases in MAP. Unlike the DR rats in the above-mentioned study, however, WKY rats in the present study did manifest small but significant NaCl-induced increases in nighttime MAP. NaCl exposure significantly increased the variability of 24-hour MAP, as suggested by the SD of MAP, in both SHR and WKY rats (Table 4). The absence of NaCl-induced changes in MAP and in variability in DR control rats in the previous study compared with WKY controls in the present study may be secondary to strain differences between Dahl and WKY rats and/or differences in experimental technique. Blood pressure in the above studies was recorded in tethered animals and then compared during 4-hour day or night intervals. In the present study, blood pressure was recorded in untethered animals, and the entire 24-hour period was analyzed.

In conclusion, the present study demonstrates that high dietary NaCl exposure increases both daytime and nighttime MAP in SHR. In contrast, high dietary NaCl exposure significantly increases nocturnal but not daytime MAP in WKY rats, with no overall change in 24-hour MAP. Previous studies from this and other laboratories suggest that the sustained NaCl-induced increase in MAP may be related to differences in volume handling and/or differences in sympathetic nervous system activation in SHR and WKY rats. High dietary exposure decreased mean 24-hour HR in SHR and WKY rats. In neither strain did high dietary NaCl ingestion alter the pattern or time course of diurnal blood pressure or HR variation.

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