Aging But Not Sodium Loading Significantly Attenuated Diurnal Change in Blood Pressure in Stroke-Prone Spontaneously Hypertensive Rats

Bin Zhao, Katsuhiko Kohara, and Kunio Hiwada

In the present study, we evaluated the effect of aging on diurnal change in blood pressure in stroke-prone spontaneously hypertensive rats (SHRSP/Izm) using a telemetry system. Diurnal changes in blood pressure, heart rate, and locomotor activity were determined in unrestrained, freely moving condition in 24-week-old male SHRSP/Izm (n = 6) and 40-week-old male SHRSP/Izm (n = 6). Diurnal change in blood pressure was also investigated in 40-week-old male spontaneously hypertensive rats (SHR/Izm, n = 6) and Wistar-Kyoto rats (WKY/Izm, n = 5) as age-matched controls of the strain. All rats were kept in a 12-h light/dark cycle (light period from 06:00 to 18:00, and dark period from 18:00 to 06:00). Rats were active in dark phase and inactive in light phase. Mean blood pressures (MBP) were significantly higher in light phase as well as dark phase in old SHRSP/Izm compared with the other three groups. Light/dark phase ratio of MBP was significantly higher in old SHRSP/Izm compared with the other three groups. We observed a significant positive relationship between light/dark phase ratio of MBP and left ventricular mass index in a studied population of rats (r = 0.547, P < .01).

Because SHRSP is a salt-sensitive model, the effect of high salt loading on the circadian pattern of blood pressure was also investigated. Male SHRSP/Izm, at the age of 22 weeks, were maintained on high salt (8%) for 2 weeks. High salt exposure significantly increased dark phase as well as light phase MBP in SHRSP/Izm. However, light/dark phase ratio of MBP was not significantly different from normal salt-fed (0.6%) SHRSP/Izm. These results indicate that aging and end-organ damage were associated with the alteration of diurnal change in blood pressure in SHRSP/Izm.


KEY WORDS: Diurnal change, nondipper, aging, salt, telemetry, Wistar-Kyoto rat (WKY/Izm), spontaneously hypertensive rat (SHR/Izm), stroke-prone spontaneously hypertensive rat (SHRSP/Izm).

The nondipper phenomenon has been reported to be relevant to several pathologic conditions in human, including certain forms of secondary hypertension, diabetes mellitus, aging, autonomic nervous dysfunction, and advanced end-organ damages. On the other hand, the pathologic meaning of the alteration of diurnal change in blood pressure has not been fully understood in animals. Although an early study, using indwelling catheters, demonstrated the inverted
diurnal change in blood pressure in old spontaneously hypertensive rats (SHR), recent studies using a telemetry system demonstrated that SHR⁹,¹⁰ and stroke-prone spontaneously hypertensive rat (SHRSP)¹¹ had normal diurnal change in blood pressure. Various manipulations¹²⁻¹⁵ have also failed to attain the non-dipper phenomenon in animal models.

Several underlying mechanisms has been postulated for the alteration of circadian variation of blood pressure in humans.¹ Recently, Uzu et al¹⁶ reported that salt sensitivity was related to the non-dipper phenomenon in sodium loading hypertensive patients, and this finding has been confirmed by other investigators.¹⁷ In animal models, high salt loading failed to change the diurnal change in blood pressure in SHR, although their blood pressure increased in response to high sodium.¹²

In the present study, we evaluated the effect of aging on diurnal change in blood pressure by telemetry system in stroke-prone SHR (SHRSP/Izm). Because SHRSP shows more severe forms of hypertension than SHR¹⁸ and has more advanced end-organ damage than SHR,¹⁹ that model would be more susceptible to the alteration of diurnal change in blood pressure than SHR. Because SHRSP is a salt-sensitive model,²⁰ we also investigated whether a high salt loading changes the diurnal pattern of blood pressure in SHRSP/Izm.

**MATERIALS AND METHODS**

**Rat and Environmental Conditions** Male SHRSP/Izm, SHR/Izm, and Wistar-Kyoto rats (WKY/Izm) were obtained from the Disease Model Cooperative Research Association (Kyoto, Japan).²¹ All rats were kept in a 12-h light/dark cycle (light on from 06:00 to 18:00, alternating with darkness from 18:00 to 06:00). Rats were active during the dark period (Figure 1). Room temperature was maintained at 22°C and humidity at 50%. They were fed on a standard rat diet (0.6% of NaCl) and allowed free access to the tap water. All experiments were conducted in accordance with the Guide for Animal Care and Experiment at Ehime University School of Medicine.

**Experimental Protocol** Experiments consisted of two parts directed to investigate 1) the effect of aging, and 2) the effect of sodium loading on the circadian change in blood pressure. The diurnal changes in blood pressure were determined in 24-week-old male SHRSP/Izm (n = 6), and 40-week-old male SHRSP/Izm (n = 6). The diurnal changes in blood pressure were also determined in 40-week-old male SHR/Izm (n = 6) and WKY/Izm (n = 5) as age-matched controls of old SHRSP/Izm. To assess the effect of sodium loading, 22-week-old male SHR/Izm (n = 6) were placed on a high sodium rat diet (8% of NaCl) and kept for 2 weeks. At the age of 24 weeks, their diurnal change in blood pressure were determined.
Surgical Procedures Under ether anesthesia, the cannula of the sensor (TA11PA-C40, Data Sciences International Inc., St. Paul, MN) was inserted in the abdominal aorta through the left femoral artery. The tip of the cannula was placed against the blood flow at the level below the renal arteries. The sensor was fixed subcutaneously in the back of the rat. This procedure was less invasive than an abdominal incision. The accuracy of the telemetry system with this procedure has been reported. Animals were housed individually in plastic cages on the receiver panel connected to the recording system. They were allowed free access to the tap water and rat food.

Telemetry and Data Acquisition The Dataquest System (Data Sciences International Inc.) was used to measure telemetrically systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), heart rate (HR), and locomotive activity. Hemodynamic data were obtained every 5 min as a waveform for 10 sec for 96 h after the implantation of the sensor. Locomotive activity was monitored as changes in signal strength due to locomotion at intervals of 5 min. For data analysis, hemodynamic and behavioral parameters were averaged every 1 h. Data from day 2 to day 4 (72 h) were used for analysis. At the end of the experiments, rats were killed by an overdose of pentobarbital sodium and their left ventricles were weighed. Left ventricular mass index (LVMI) was calculated by the formula: LVMI = left ventricular weight / body weight.

Statistical Analysis All values are expressed as mean ± SD if not specified. The differences among groups on the diurnal changes in MBP, HR, and locomotor activity were evaluated by ANOVA with repeated measurement. The difference among the groups was evaluated by analysis of variance followed by Duncan’s multiple range test. A probability less than 0.05 was defined as statistically significant.

RESULTS

Effect of Aging on Diurnal Changes in Blood Pressure Figure 1 depicts the diurnal changes in MBP, HR, and locomotor activity in old WKY/Izm, old SHR/Izm, and young and old SHRSP/Izm on day 3 after implantation of the sensor. There were clear circadian variations in MBP, HR, and locomotive activity in synchrony with a dark/light cycle. However, the absence of diurnal changes in blood pressure and attenuation of diurnal changes in HR were observed in old SHRSP/Izm compared with the other three groups. There was no difference in locomotor activity among the four groups.

Table 1 summarizes the diurnal change in MBP from day 2 to day 4 after the implantation of the sensor. The light phase and dark phase MBP were significantly higher in old SHRSP/Izm than WKY/Izm, SHR/Izm, and 24-week-old SHRSP/Izm. Figure 2 summarizes mean light/dark phase ratio of MBP of 3 days, from day 2 to day 4 after implantation of the sensor. Light/dark phase ratio of MBP in old SHRSP/Izm was significantly higher compared with age-matched WKY/Izm and SHR/Izm as well as 24-week-old SHRSP/Izm.

Effect of Sodium Loading on Diurnal Change in Blood Pressure Salt loading significantly increased blood pressure of 24-week-old SHRSP/Izm in both light phase and dark phase (Table 1). However, the light/dark phase ratio of MBP was not significantly different from that of young SHRSP/Izm fed a normal diet (Figure 2).

Left Ventricular Mass Index and Light/Dark Phase Ratio of Mean Blood Pressure To evaluate further the relationship between end-organ damage and diurnal changes in blood pressure, relations between LVMI and 24-h mean MBP as well as light/dark phase ratio of MBP were investigated (Figure 3). Left ventricular mass was significantly related to 24-h mean MBP in all animals used in experiment 1. There was also a significant positive relationship between LVMI and light/dark phase ratio of MBP in total rats used in experiment 1. Analysis including 24-week-old SHRSP/Izm with a high NaCl loading also shows significant positive correlation between LVMI and 24-h MBP (r = 0.88, P < .0001), and light/dark phase ratio of MBP (r = −0.49, P < .01).

DISCUSSION
Diurnal change in blood pressure in animal models of hypertension has been the focus of research. Advancement in telemetry urges researchers to study in animals the underlying mechanisms of diurnal changes in blood pressure. However, demonstration of the nondipper phenomenon in the rat model of hypertension seems difficult. Early study using the indwelling catheters to measure blood pressure demonstrated that adult (17- to 20-week-old) and old (30- to 32-week-old) SHR showed the inverted circadian variation of blood pressure. In contrast, recent studies using the telemetry system failed to demonstrate an attenuation of circadian change in blood pressure in adult SHR. Even SHRSP at the age of 20 weeks has been shown to have clear diurnal changes in blood pressure. To date, the TGR(mRen-2)27 rat, a genetic model of hypertension generated by introducing the mouse Ren-2 salivary gland renin gene into the genome of the Sprague-Dawley rat, is the only model that consistently shows reverse pattern of diurnal change in blood pressure.
The high prevalence of the nondipper pattern of diurnal change in blood pressure is one of the features of hypertension in the elderly. An international study with a large database demonstrated that there was a decrease in blood pressure at night and that the night/day ratio of blood pressure increased with age. In the present study, we demonstrated that aging significantly attenuated diurnal change in blood pressure in SHRSP/Izm. Because we observed a diurnal change in age-matched SHR/Izm and WKY/Izm, aging itself was not sufficient to eliminate circadian change in blood pressure in rats. Several mechanisms are proposed to explain the nondipper phenomenon. The baroreceptor reflex dysfunction has been shown to be involved in mechanisms for the increased fluctuation of blood pressure. Recently, Makino et al demonstrated that sinoaortic denervation disrupted the circadian rhythm of MBP in rats. Because the baroreceptor reflex function has also been shown to be attenuated in SHR, baroreceptor reflex dysfunction alone may be insufficient to explain the nondipper phenomenon in old SHRSP/Izm. As an end-organ damage, we evaluated LVMI. We observed that LVMI was the highest in old SHRSP/Izm. Furthermore, we observed a significant positive correlation between LVMI and light/dark phase ratio of MBP, as well as 24-h MBP. These findings may indicate that end-organ damage explains at least a part of the attenuation of diurnal change in old SHRSP/Izm. However, as the relationship between LVMI and light/dark phase ratio of MBP was evaluated in rats from different strains, a study with SHRSP alone with different strains is needed.

### TABLE 1. MEAN BLOOD PRESSURE, BODY WEIGHT, AND LEFT VENTRICULAR WEIGHT IN RATS

<table>
<thead>
<tr>
<th></th>
<th>40-week old WKY/Izm</th>
<th>40-week old SHR/Izm</th>
<th>40-week old SHRSP/Izm</th>
<th>24-week old SHRSP/Izm</th>
<th>24-week old SHRSP/Izm + NaCl</th>
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<td>(5)</td>
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<td>Second day</td>
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<tr>
<td>24-h MBP (mm Hg)</td>
<td>109.1 ± 8.4</td>
<td>161.5 ± 13.2*</td>
<td>217.1 ± 17.1*†‡</td>
<td>192.2 ± 6.0*†</td>
<td>218.2 ± 18.0*†‡</td>
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<tr>
<td>Light phase MBP (mm Hg)</td>
<td>105.1 ± 7.9</td>
<td>157.8 ± 15.1*</td>
<td>215.7 ± 19.5*†‡</td>
<td>188.6 ± 6.9*†</td>
<td>188.6 ± 6.9*†‡</td>
</tr>
<tr>
<td>Dark phase MBP (mm Hg)</td>
<td>113.0 ± 9.1</td>
<td>165.1 ± 11.7*</td>
<td>218.6 ± 17.5*†‡</td>
<td>195.6 ± 6.1*†</td>
<td>223.6 ± 19.7*†‡</td>
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<td>Third day</td>
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<tr>
<td>24-h MBP (mm Hg)</td>
<td>109.0 ± 7.0</td>
<td>160.2 ± 14.5*</td>
<td>217.8 ± 14.9*†‡</td>
<td>191.6 ± 12.0*†</td>
<td>221.2 ± 20.5*†‡</td>
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<tr>
<td>Light phase MBP (mm Hg)</td>
<td>105.8 ± 7.3</td>
<td>154.8 ± 15.4*</td>
<td>216.2 ± 12.8*†‡</td>
<td>186.5 ± 11.4*†</td>
<td>217.8 ± 19.9*†‡</td>
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<tr>
<td>Dark phase MBP (mm Hg)</td>
<td>112.1 ± 6.7</td>
<td>165.6 ± 13.8*</td>
<td>219.4 ± 17.4*†‡</td>
<td>196.7 ± 13.1*†</td>
<td>224.5 ± 21.6*†‡</td>
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<td>Fourth day</td>
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<tr>
<td>24-h MBP (mm Hg)</td>
<td>105.8 ± 5.6</td>
<td>153.1 ± 20.0*</td>
<td>225.1 ± 17.7*†‡</td>
<td>192.6 ± 10.7*†</td>
<td>220.3 ± 20.3*†‡</td>
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<tr>
<td>Light phase MBP (mm Hg)</td>
<td>102.9 ± 5.5</td>
<td>147.5 ± 24.5*</td>
<td>223.5 ± 17.9*†‡</td>
<td>187.9 ± 9.2*†</td>
<td>215.4 ± 20.5*†‡</td>
</tr>
<tr>
<td>Dark phase MBP (mm Hg)</td>
<td>108.7 ± 5.7</td>
<td>138.7 ± 16.4*</td>
<td>197.8 ± 8.9*†‡</td>
<td>226.9 ± 18.0*†</td>
<td>225.2 ± 20.2*†‡</td>
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<tr>
<td>Body weight (g)</td>
<td>448.8 ± 22.3</td>
<td>403.5 ± 11.6*</td>
<td>310.7 ± 25.3*†‡</td>
<td>323.7 ± 8.0*†</td>
<td>255.8 ± 12.8*†‡</td>
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<td>Left ventricular weight (mg)</td>
<td>820 ± 57</td>
<td>1093 ± 31*</td>
<td>1185 ± 153*†‡</td>
<td>962 ± 123*†‡</td>
<td>978 ± 74*†‡</td>
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<tr>
<td>LVMI (mg/g body weight)</td>
<td>1.67 ± 0.07</td>
<td>2.71 ± 0.08*</td>
<td>3.83 ± 0.50*†‡</td>
<td>2.97 ± 0.08*†</td>
<td>3.82 ± 0.17*†‡</td>
</tr>
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</table>

Values are mean ± SD. Number in the parenthesis indicates number of rats. WKY/Izm, Wistar-Kyoto rat; SHR/Izm, spontaneously hypertensive rat; SHRSP/Izm, stroke-prone spontaneously hypertensive rat; NaCl, high salt loading; MBP, mean blood pressure; LVMI, left ventricular mass index.
* P < .05 v 40-week old WKY.
† P < .05 v 40-week old SHR.
‡ P < .05 v 24-week old SHRSP.

![FIGURE 2](image-url)
different stages of end-organ damage would be more appropriate to reach a conclusion.

Recently, several studies have clearly shown that salt sensitivity is significantly related to the nondipper phenomenon in humans. In patients with salt sensitivity, a high salt loading diet did not change the dipper pattern. In an animal model, however, a high salt diet has failed to change the diurnal pattern of blood pressure in SHR. The finding in the present study that SHRSP/Izm fed an 8% NaCl diet showed an increase in their blood pressure by more than 25 mm Hg, further support the salt sensitivity of SHRSP/Izm. However, high salt did not change the diurnal change in MBP in SHRSP/Izm. These findings were consistent with a study using Dahl salt-sensitive rats. Hashimoto et al demonstrated that a high salt diet significantly increased blood pressure in Dahl salt-sensitive rats; however, circadian rhythm of blood pressure was not changed when assessed as a percent amplitude. These findings may suggest that a high salt diet alone is not sufficient to change the circadian pattern of blood pressure even in a salt-sensitive rat model. These findings may also indicate that the underlying mechanisms of the nondipper phenomenon are different between rats and humans.

In summary, we demonstrated that old SHRSP/Izm had an alteration of diurnal change in blood pressure. Aging, in combination with an advancement of end-organ damage, may contribute to the alteration of diurnal change in blood pressure in SHRSP/Izm. Old SHRSP/Izm may be a useful animal model to study the mechanisms of the nondipper phenomenon.

ACKNOWLEDGMENT

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REFERENCES

6. Pickering TG, James GD: Determinants and conse-
quences of the diurnal rhythm of blood pressure. Am J Hypertens 1993;6:166S–169S.


