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ORIGINAL ARTICLE

A critical role of sympathetic nerve regulation for the treatment of impaired daily rhythm in hypertensive Dahl rats

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There is a deep relationship between impaired circadian rhythm and hypertension. However, the detailed mechanisms between the daily sleep-wake rhythm and cardiovascular disorders have not yet been elucidated. To clarify the mechanism, we examined salt-sensitive Dahl rats that were fed normal chow (n=10), high-salt chow (n=10) and high-salt chow with bisoprolol (n=10). Simultaneous electroencephalogram, electromyogram and locomotor activity were examined to analyze the sleep–wake state. We also examined heart rate, blood pressure and echocardiographic findings to verify the presence of hypertension. Hypertension with impaired ventricular contraction was observed in the rats with high-salt-chow consumption whereas normal-chow rats did not show these disorders. Although rats with the normal diet showed a standard daily rhythm with normal rapid eye movement (REM) sleep duration and locomotor activity, the high-salt-diet group exhibited an impaired daily rhythm with suppressed REM sleep and significant abnormal locomotor activity. Bisoprolol significantly improved the daily sleep–wake rhythm and locomotor activity. We showed that an impaired daily rhythm was closely related to the development of hypertension. Regulation of sympathetic nerve alterations may have a key role in the treatment of hypertension and circadian rhythm disorder.
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INTRODUCTION

It is well known that there is a deep relationship between sleep disorders and cardiovascular diseases such as hypertension and congestive heart failure (CHF). The fact that patients with advanced CHF induced by hypertension frequently suffer from central breathing disorders[1] suggests that CHF is causally related to alterations in the central control of breathing, resulting in Cheyne–Stokes respiration during sleep.[2] Disorders in the central control of breathing are a key factor in altered daily sleep–wake rhythm and are also an independent predictor for premature death.[3,4] Although it is known that cardiovascular diseases are closely related to circadian rhythm,[5] the relationship among changes in circadian rhythm, sleep disorders and cardiovascular diseases has not yet been elucidated.

It has been reported that long-term treatment with β-receptor-blocking substances (β-blockers) improves hypertension and CHF. β-Blockers also reduce the inappropriate increase of ventilation during exercise and alleviate central breathing disorders during wakefulness.[6,7] These positive effects result in improved exercise capacity and slower progression of underlying hypertension and CHF. However, little is known about the effects of β-blocker treatment on sleep disorders with cardiovascular diseases. Thus, we hypothesized that sympathetic nerve activation has a pivotal role in the development of hypertension and alteration of the daily rhythm. To clarify the mechanisms, we analyzed the daily rhythm in salt-sensitive Dahl rats with hypertension and CHF. In this study, we clearly showed that sympathetic nerve activation was closely related to an impaired daily sleep–wake rhythm and the development of hypertension. β-Blocker treatment significantly improved the daily rhythm and hypertension by the suppression of sympathetic nerve activation.

METHODS

Animals

Male Dahl rats (8- to 10-week-old, 330–360 g) were obtained from Crea Japan (Tokyo, Japan) and were housed in isolated examination chambers for 1 week before the examination. The animals were maintained at a temperature of 24 ± 1 °C, at a relative humidity of 54 ± 6% and in a light–dark cycle of 12:12 h (lights were turned on at 0900 hours and turned off at 2100 hours).[8–10] All experimental protocols were performed in accordance with the Guidelines for Animal Experimentation as stipulated by Tokyo Medical and Dental University.

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Treatment protocols

A β-blocker, bisoprolol, was kindly provided by Mitsubishi Tanabe Pharma Corporation (Chuo-ku, Osaka, Japan). The animals were randomly assigned to one of three treatment groups: (1) administration of a vehicle (1 mg kg^{-1}) with normal chow (native group, n=10), (2) administration of a vehicle (1 mg kg^{-1}) with high-salt chow (nontreated group, n=10) and (3) administration of bisoprolol (1 mg kg^{-1}) with high-salt chow (β-blocker group, n=10). The rats received water and were supplemented with commercial feed with either high (8.0%, wt/wt) or normal (0.3%, wt/wt) levels of sodium chloride (Oriental Yeast, Tokyo, Japan). We administered bisoprolol using the intragastric intubation method. A standard tube (KN-348, ϕ1.2×L80 mm; Natsume, Tokyo, Japan) was used. We administered the compound or vehicle at 0900 and 2100 hours.

Body weight, heart rate and blood pressure in conscious rats

The body weight, heart rate (HR) and blood pressure (BP) of all rats were measured on days 7, 14, 21, 28 and 56 (n=6 each day). HR and BP (systolic blood pressure, diastolic blood pressure and mean blood pressure) were measured in conscious rats using a tail-cuff system (BP-98A; Softron, Tokyo, Japan).[11]

Echocardiogram

Transthoracic echocardiography was performed on animals anesthetized by intraperitoneal administration of pentobarbital sodium (0.25 mg kg^{-1}; Dainihon Chemical, Osaka, Japan) on days 7, 14, 21, 28 and 56 (n=6 each day). An echocardiography machine with a 7.5 MHz transducer (Toshiba, Tokyo, Japan) was used for left ventricular echocardiographic recordings. A two-dimensional targeted M- and B-mode echocardiogram was obtained along the short-axis view of the left ventricle at the papillary muscles. Left ventricular fractional shortening, end-diastolic dimension and other factors were calculated from the M-mode echocardiograms over three consecutive cardiac cycles according to the leading-edge method by American Society for Echocardiography.[12,13] Measurements were made offline by two independent investigators.

Locomotor activity

Locomotor activity was detected as described.[14] Briefly, spontaneous locomotor activity was detected by a pyroelectric infrared sensor (NS-AS01; Neuroscience, Tokyo, Japan) that sensed the movement of the rats in their individual cages (36×36×25 cm). The infrared sensor was placed on the top of the cage through a hole. The sensor was connected to a personal computer with an analog-to-digital converter and software (SleepSign; Kissei Comtec, Nagano, Japan) for acquiring and processing data.[14]

Electroencephalogram and electromyogram

Simultaneous electroencephalogram and electromyogram recordings were performed as described.[10] Briefly, three cortical gold-plated screw electrodes and paired stainless-steel electrodes were fixed to the skull with dental acrylic resin to record the electroencephalogram and electromyogram. For measurements, lead wires of the electrodes were connected to an electroencephalogram/electromyogram amplifier (MEG-6116; Nihon Kohden, Shinjuku-ku, Tokyo, Japan) through a six-strand cable with a slip ring that allowed the rats to move freely. The amplifier was connected to a personal computer with an AD converter and software (SleepSign; Kissei Comtec) for acquiring and processing data. On the basis of the electroencephalogram and electromyogram recordings, we classified sleep–waking states as wakefulness, rapid eye movement (REM) sleep and non-REM sleep. The results were verified visually according to the standard criteria.

Statistical analysis

Statistical analyses were performed using repeated measures of analyses of variance followed by *post hoc* analysis. Probability (P) values <0.05 were considered to indicate statistical significance.

RESULTS

Measurement of BP and HR in awake and free-moving rats

On day 56, the HR in rats fed the high-salt diet with the bisoprolol treatment decreased as compared with the nontreated (high-salt chow) group. Although the high-salt diet caused a marked increase in BP (systolic blood pressure, diastolic blood pressure and mean blood pressure) compared with the native (normal chow) group, the bisoprolol treatment did not significantly decrease BP (Figure 1).

Echocardiography

Echocardiographic data on day 56 indicated that the wall was thicker in the nontreated (high-salt chow) group compared with the native (normal diet) group, although there were no differences in the diameter and contraction of the left ventricular chamber. Bisoprolol treatment restored the left ventricular posterior wall thickness to the levels of the native group rats, but it did not affect ventricular contraction (Figure 2).

Electroencephalogram

Although rats with the normal diet exhibited the standard daily sleep–wake rhythm with normal REM sleep, the nontreated (high-salt chow) group exhibited an impaired daily rhythm with suppressed REM sleep. During the light phase, the high-salt-chow group experienced a significant increase in the duration of wakefulness and a decrease in

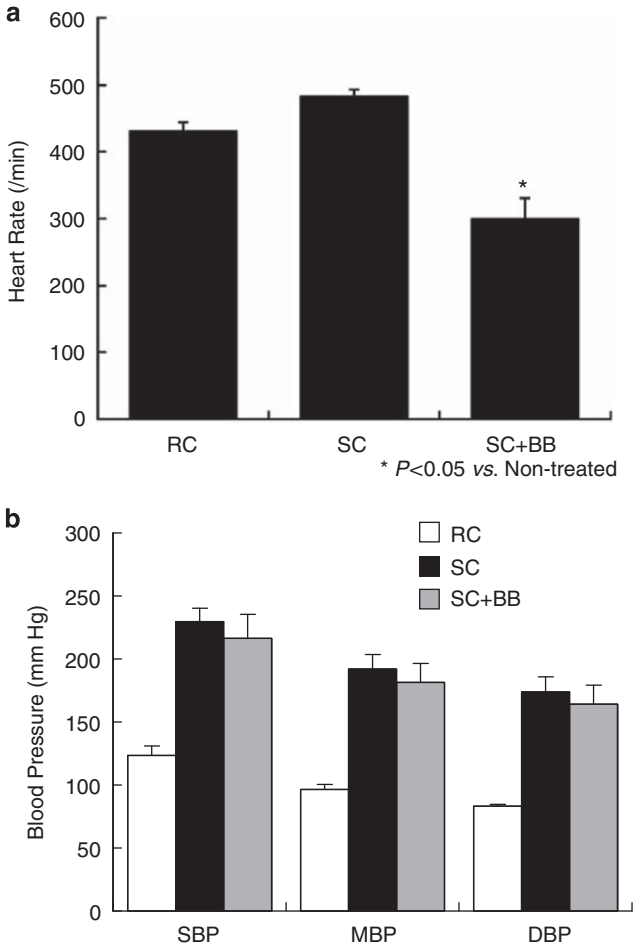

Figure 1 Heart rate and blood pressure. Panel **a** shows heart rate (HR) and panel **b** shows blood pressure (BP) on day 56. The high-salt-diet group experienced a marked increase in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MDP) compared with the normal-diet group. β-Blocker treatment decreased HR without a significant change in SBP, DBP and MBP compared with the nontreated, high-salt group. BB, β-blocker; RC, regular chow; SC, salt chow.

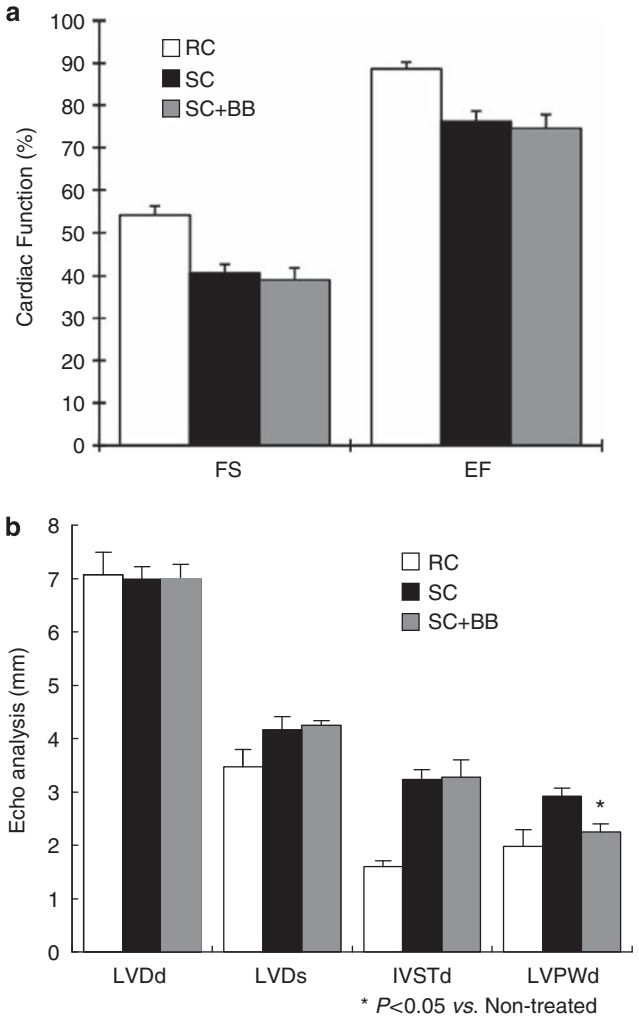

Figure 2 Echocardiography. Echocardiographic data on day 56 indicated that wall thickness was greater (**b**) and fractional shortening was impaired (**a**) in the high-salt group compared with the normal group, although there was no difference in the diameter of the left ventricular chamber. β-Blocker treatment recovered the left ventricular posterior wall thickness (LVPWd) to the levels of native-group rats. BB, β-blocker; EF, ejection fraction; FS, fractional shortening; IVSTd, end diastolic intraventricular septal thickness; LVDd, end diastolic left ventricular dimension; LVDs, end systolic left ventricular dimension; LVPWd, end diastolic left ventricular posterior wall thickness; RC, regular chow; SC, salt chow. *$P<0.05$ *vs.* nontreated.

non-REM sleep compared with those in the native (normal chow) group. Nontreated, high-salt-chow rats experienced significantly decreased wakefulness and increased non-REM sleep during the dark phase. REM sleep in the nontreated, high-salt-fed rats was significantly suppressed in both the light and dark periods. It is noteworthy that bisoprolol treatment significantly recovered the daily sleep–wake rhythm in both the light and dark periods, but it did not affect BP (Figure 3).

Locomotor activity

Although rats with the normal diet showed standard daily locomotor activity, there was significantly abnormal daily locomotor activity in the nontreated (high-salt) group on day 56. The movement of the nontreated (high-salt) rats during the light phase was similar to the movement during the dark phase. However, bisoprolol treatment dramatically recovered the activity to normal levels; the rats showed less movement during the light phase and more active movement during the dark phase (Figure 4). However, the treatment did not affect BP.

Body and heart weight

The heart weighed more in the nontreated (high-salt chow) group compared with the native (normal chow) group on day 56. However, no significant difference was detected in either body or heart weight between the nontreated (high-salt-chow) group and the bisoprolol-treated group during the observation period (Figure 5).

DISCUSSION

In this paper, we clearly showed that an impaired daily sleep–wake rhythm and the development of hypertension were closely related to sympathetic nerve activation in Dahl rats. Bisoprolol treatment significantly improved the daily sleep–wake rhythm through the suppression of sympathetic nerve activation, but the treatment did not affect BP.

The analysis of HR and BP variability revealed the importance of the autonomic control of circulation, such as in hypertension, acute myocardial infarction and heart failure.[15] Thus, circadian rhythm is an important modulator of cardiovascular function not only in physiological conditions but also in disease states. It is well known that sleep exerts significant effects on the autonomic nervous system, systemic hemodynamics, cardiac function, endothelial function and coagulation. Epidemiological and pathophysiological studies indicate that there is a causal relationship between primary circadian rhythm abnormalities and cardiovascular disease.[16] Sleep disturbances may occur as a result of several medical conditions, including obesity and heart failure. Therefore, linking daily sleep–wake rhythm disorders to cardiovascular disease is important for revealing pathophysiology and developing therapeutic strategies.[16]

It has been reported that circadian rhythm is altered in various animal models of cardiovascular disorders. In rat hearts with pressure-overload hypertrophy,[17] rhythmic expression of clock-controlled genes was reduced. In rats with streptozotocin-induced diabetes, the circadian rhythms of the hearts were shifted because of altered expression of clock genes.[18] However, no report has elucidated the significance of the circadian system in the development of hypertension and heart failure. In this study, we clarified that daily sleep–wake rhythm and locomotor activity were altered in the pathological condition of hypertension. An impaired daily rhythm was closely associated with the development of hypertension, sleep–wake disorders and locomotor activity.

We found that administration of a low-dose β-adrenergic-receptor blocker negated the daily-rhythm changes consequent to hypertension and CHF, but the treatment did not significantly affect BP. In clinical settings, it has been reported that long-term treatment of patients with advanced CHF with sufficient doses of β-blockers reduced the prevalence and severity of central sleep apnea.[19] During the development of CHF, upregulated systemic norepinephrine levels alter clock genes in the cardiovascular system. Although the causal relationship between β-blockers and the clock system has not yet been clinically elucidated, it has been reported that norepinephrine influences the timing of the circadian clock within cardiomyocytes.[20] This means that the regulation of sympathetic neurotransmitters using β-blockers affects the clock system in cardiovascular cells. The β-blocker treatment affects not only physiological conditions but also transcriptional conditions, such as peripheral clock genes. In this study, we used a β1-selective blocker, bisoprolol, which reduces the frequency and intensity of

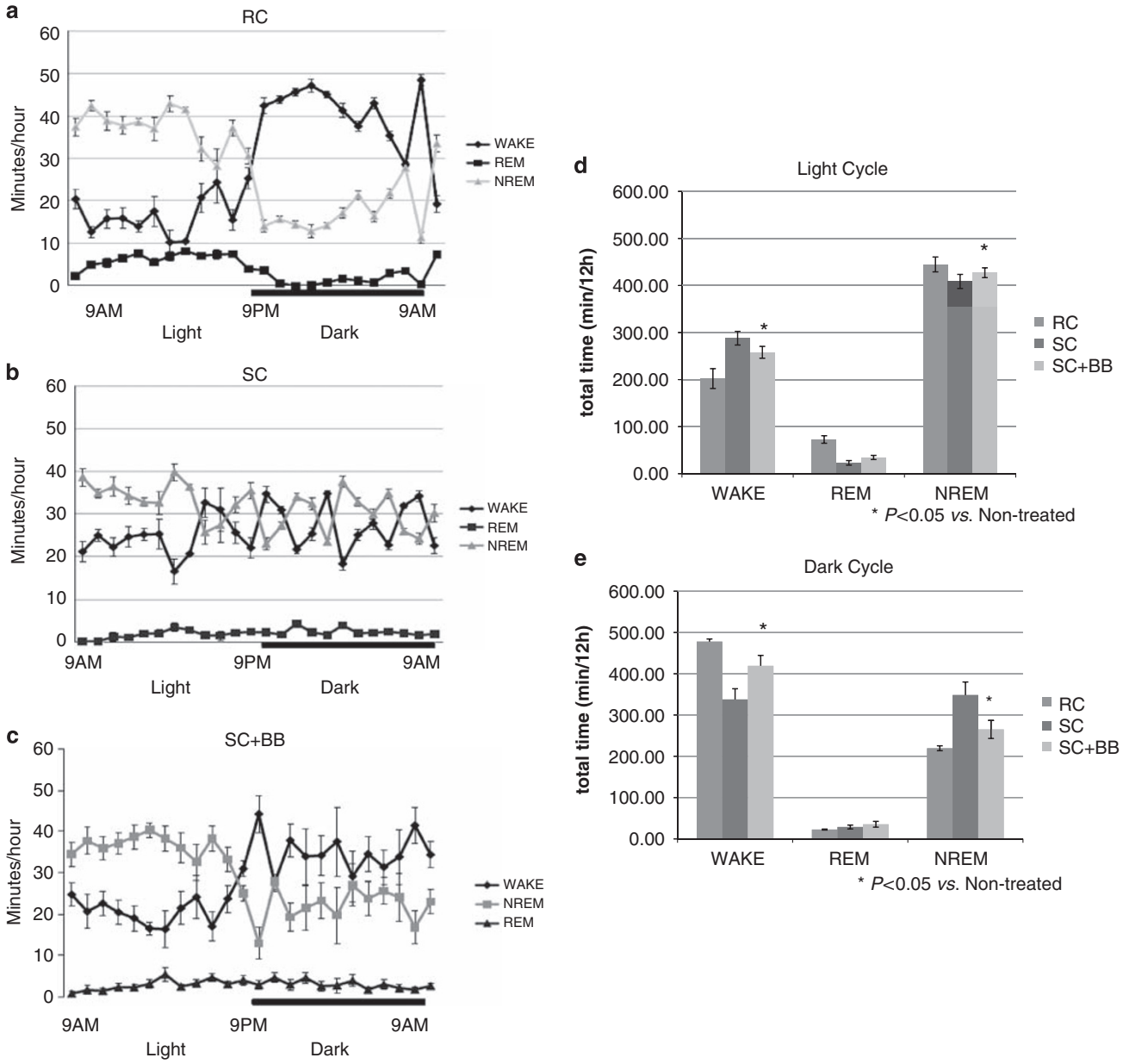

Figure 3 Electroencephalogram (EEG). EEG results for rats at week 8 were characterized for the three groups: (**a**) rats given normal chow, (**b**), nontreated rats fed high-salt chow and (**c**) rats treated with a β-blocker and fed high-salt chow. Quantitative data for the sleep conditions during (**d**) the light cycle and (**e**) the dark cycle. Although rats fed the normal diet showed a standard daily rhythm with normal REM sleep, the nontreated high-salt group exhibited an impaired daily rhythm with suppressed REM sleep. β-Blocker treatment significantly recovered the sleep–wake rhythm. During the light cycle (**d**), the high-salt-chow group experienced a significant increase in the duration of wakefulness and a decrease in non-REM sleep compared with the normal group. High-salt-chow rats experienced significantly decreased wakefulness and increased non-REM sleep during the dark cycle (**e**). β-Blocker treatment improved abnormal wakefulness and non-REM sleep in both the light and dark cycles. BB, β-blocker; NREM, non-REM sleep; RC, regular chow; SC, salt chow.

ischemic episodes through the regulation of circadian rhythm.[21] Sayer *et al.* reported that bisoprolol modifies fibrinolytic activity through alteration of the clock system and the sympathoadrenal system.[22] The TIBBS group also revealed that bisoprolol had an effect that was prognostically favorable.[23] These results indicate that modulation of sympathovagal balance is one of the favorable effects of β-blockade. Because different β-blockers may exert different effects, these β-blocking effects should be compared with one another in future studies.

In this study, we did not consider the possible role of the renin–angiotensin–aldosterone system in hypertension and circadian rhythm disturbances. It is known that upregulation of the renin–angiotensin–aldosterone system in transgenic TGR(mREN-2)27 rats results in serious alterations of the clock system and an inverted 'non-dipping' BP profile.[24] The sympathetic system and the renin–angiotensin–aldosterone system are also closely related, and inhibition of the sympathetic system may diminish renin–angiotensin–aldosterone system activity and vice versa. Expression of the clock genes in the brain areas involved in BP control[25] as well as expression of genes encoding enzymes from the catecholamine biosynthetic pathway were disturbed in TGR rats.[26] Thus, direct measurement of tyrosine hydroxylase activity would be useful to directly clarify the mechanism; further investigation is needed.

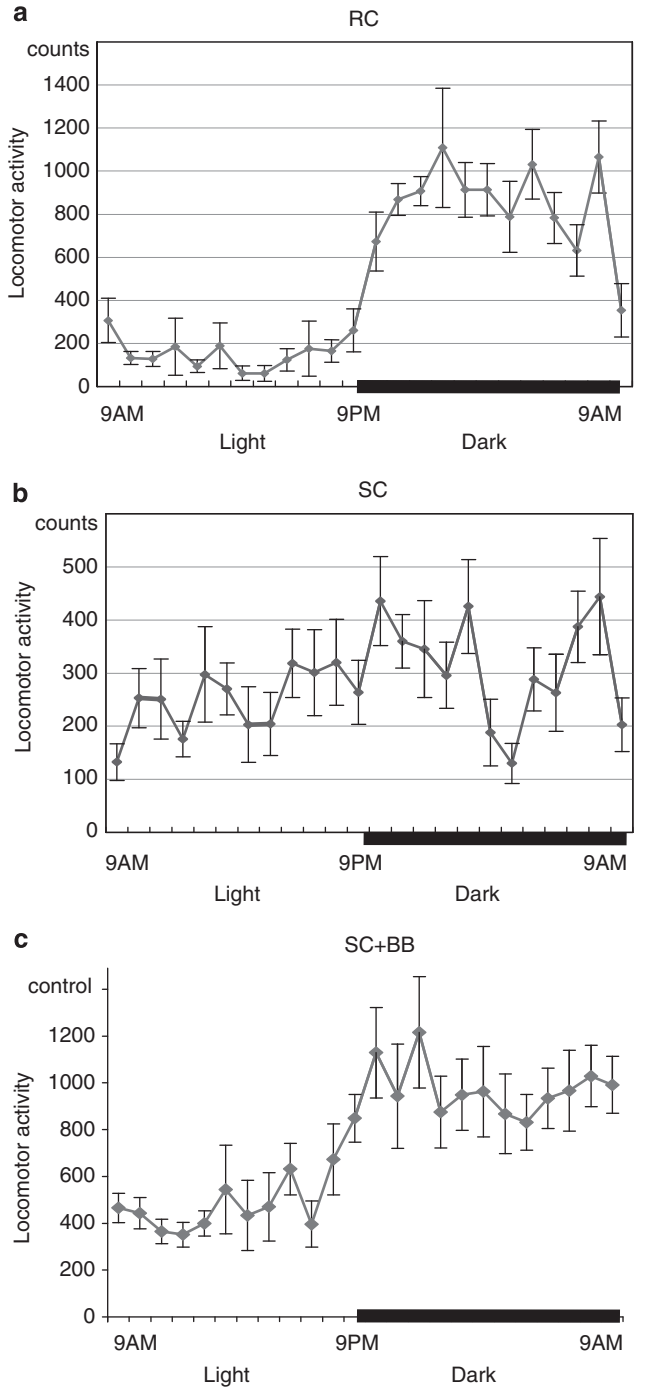

Figure 4 Locomotor activity. Locomotor activity of rats on day 56 was characterized for the three groups: (**a**) rats given normal chow, (**b**) nontreated rats fed high-salt chow and (**c**) rats treated with a β-blocker and fed high-salt chow. Although rats fed the normal diet showed standard locomotor activity, the nontreated, high-salt group exhibited significantly abnormal locomotor activity. However, β-blocker treatment with the high-salt chow dramatically recovered the activity to normal levels. BB, β-blocker; RC, regular chow; SC, salt chow.

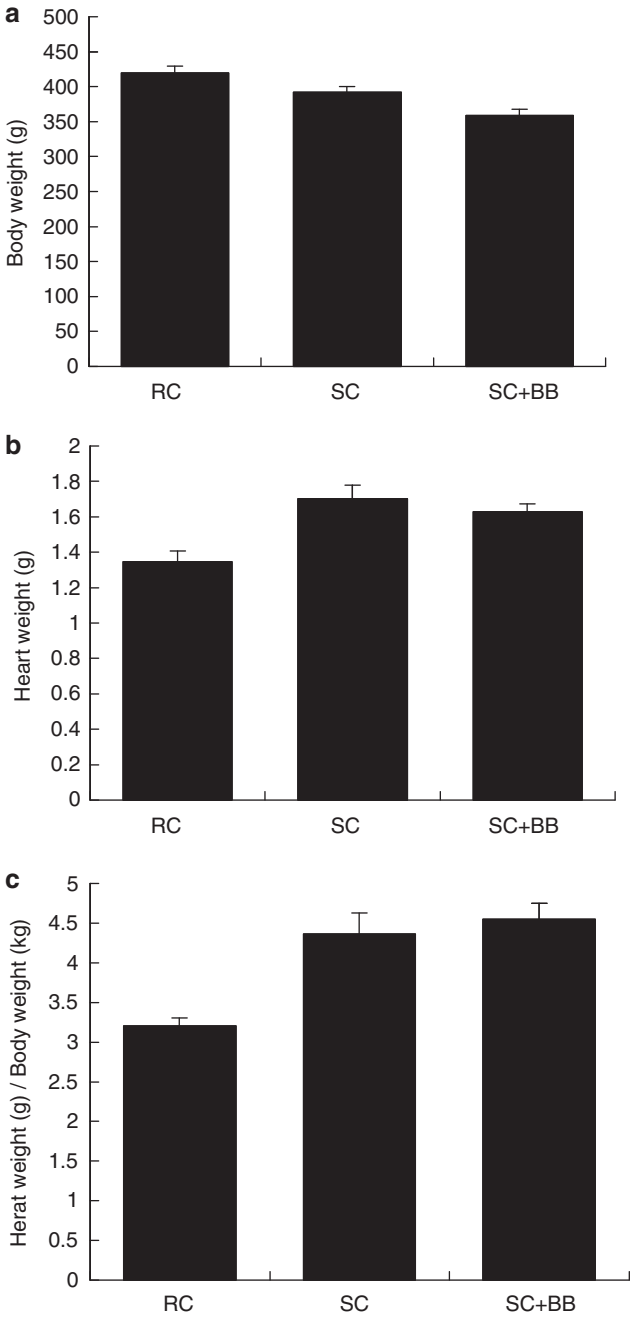

Figure 5 Body and heart weight. Body (**a**) and heart (**b**) weight on day 56 were measured, and the ratios (**c**) were calculated. No significant difference was detected in body weight among the groups. Although the ratio of heart weight to body weight was greater in the high-salt group than in the normal group, β-blocker treatment did not affect the ratio. BB, β-blocker; RC, regular chow; SC, salt chow.

This study had some limitations. We should consider the fact that salt treatment leads not only to arrhythmias and sympathetic nerve system disturbances but also to physical inactivity based on 'global sickness.' We cannot exclude the possibility that the high-salt supplement affected the rats globally; however, it does not specifically interact with the clock system. Although it is difficult to identify the detailed global and specific mechanisms *in vivo*, this conclusion could be confirmed if we checked for clock gene expression in the suprachiasmatic nuclei because daily locomotor rhythms rely on the suprachiasmatic nuclei pacemaker function. It is also possible that abolishment of the daily REM/non-REM rhythms may be simply a consequence of the observed inactivity, which is based on a 'globally sick' phenotype without specific clock involvement. To clarify the

pathophysiology, it would be helpful to have constant darkness data to differentiate between 'global sickness' and the 'specific action' of salt on the clock system.

In conclusion, we showed that an impaired daily sleep–wake rhythm and behavioral responses are closely related to the development of hypertension. Regulation of sympathetic nerve alterations may have a key role in the treatment of cardiovascular diseases. Further study is needed to elucidate the pathophysiology of this connection for the development of new treatment in clinical settings.

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