The Daily Pattern of Cardiovascular Parameters in Kurosawa and Kusanagi-Hypercholesterolemic (KHC) Rabbits

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Abstract: We studied characteristics of the daily pattern of heart rate (HR), blood pressure (BP), body temperature (BT), and locomotor activity (LA) in conscious and unrestrained Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbits and age-matched normal Japanese white (JW) rabbits, using a telemetry system. In all JW rabbits, nocturnal patterns were observed in HR, BT and LA. In the 5 months group of KHC rabbits, however, diurnal rhythm was observed in HR, and in the 10 months group of KHC rabbits, it was also shown in LA. The nocturnal pattern was observed only in BT in 10 months KHC rabbits. Mean blood pressure (MBP) in JW and KHC rabbits showed no clear daily pattern. The mean daily values of HR and BT were not altered between the 5 months and 10 months groups in KHC rabbits, although those in JW were lower in the 10 months group than in the 5 months group. Moreover, the daily values of HR and MBP in KHC rabbits tended to be higher than those in the age-matched JW rabbits. The pulse pressure in the 10 months group of KHC rabbits tended to be greater than the 5 months groups of KHC and JW rabbits. Furthermore, short-term variabilities in BP in the 5 months KHC rabbits were significantly lower than those in the other groups. From these results, it is suggested that the cardiovascular function, including the autonomic nervous function is altered with the development of atherosclerosis in KHC rabbits.

Key words: atherosclerosis, hypercholesterolemia, circadian rhythm, heart rate, blood pressure, radiotelemetry

Introduction

The Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbit is a strain with spontaneous hypercholesterolemia produced by serial breeding of mated pairs of mutant Japanese white rabbits first noted to have hypercholesterolemia at Japan Laboratory Animals, Inc., in 1985. The serum total cholesterol and triglyceride levels in this strain (500–700 and 200–300 mg/dl, respectively) are far higher than those in the normal rabbit [15]. It has been clarified that the hypercholesterolemia in the KHC rabbit, similar to the condition in the

(Received 13 November 2001 / Accepted 29 January 2002)
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Watanabe heritable hyperlipidemic (WHHL) rabbit [2, 28], is attributable to a deficiency in low-density lipoprotein (LDL)-receptors [15]. Atherosclerosis develops in the aortic arch and around the orifices of the intercostal, mesenteric and renal arteries within 4 months after birth and then spreads to the peripheral arteries with aging [15].

It is expected that atherosclerosis alters vascular properties and characteristics of hemodynamics, as it has been shown that atherosclerosis is an important risk factor in severe cardiovascular diseases such as myocardial infarction [9, 24]. Several epidemiologic studies have indicated a circadian pattern of adverse cardiac events, with incidence of myocardial infarction and sudden cardiac death peaking in the morning [18, 29]. Therefore, a morning surge in sympathetic nervous activity has been hypothesized to underlie the circadian pattern of cardiac risk [16, 18, 19, 29].

Thus, it is meaningful and useful to investigate the characteristics of cardiovascular function in KHC rabbit which is thought to be an ideal animal model for studying cardiovascular hemodynamics as well as the biochemical and pathological characteristics of atherosclerosis and hypercholesterolemia. Accordingly, we observed the daily patterns of heart rate (HR), blood pressure (BP), body temperature (BT) and locomotor activity (LA) in conscious and unrestrained KHC rabbits using a telemetry system. Moreover, the characteristics of the cardiovascular function in KHC rabbits were compared with those in age-matched normal Japanese white (JW) rabbits.

**Materials and Methods**

**Animals and housing**

Nineteen KHC rabbits (age: 5 to 12 months, Japan Laboratory Animals Inc., Tokyo, Japan) and 19 JW rabbits (age: 5 to 12 months, Saitama Experimental Animals Supply Co. Ltd., Saitama, Japan) were divided into 4 groups (Table 1). They were housed in individual cages (height 35 cm, width 40 cm, and depth 50 cm) within a room where a light-dark cycle (LD 12:12; lights-on at 8:00 and off at 20:00) was maintained. Standard rabbit pellets (RC4; Oriental Yeast Co., Tokyo, Japan) and water were supplied ad libitum.

<table>
<thead>
<tr>
<th>Table 1. The groups of JW and KHC rabbits</th>
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<tr>
<td><strong>Group</strong></td>
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<td><strong>n</strong></td>
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<tr>
<td><strong>Age (months)</strong></td>
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<tr>
<td><strong>Body weight (kg)</strong></td>
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<tr>
<td>JW</td>
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<tr>
<td>10 months</td>
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<td>KHC</td>
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<td>10 months</td>
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**Implanting the transmitter**

A telemetric transmitter for electrocardiogram (ECG) and BT (TA10CTA-D70, Data Sciences, St. Paul, MN, USA) or ECG and BP (TA11PA-D70, Data Sciences, St. Paul, MN, USA) was implanted under pentobarbital sodium anesthesia (35 mg/kg, i.v.). Eight KHC and 8 JW rabbits were implanted with ECG and BT transmitter. The paired wire electrodes of the transmitter were placed at the cervical subcutaneous region over the scapula and femur (standard limb lead II) and the skin was closed by suture. Another 11 KHC and 11 JW rabbits were implanted with BP transmitter. The catheter of the transmitter was implanted into the abdominal aorta through the arteria saphena. Both types of transmitter also detected LA.

**Data recording**

A signal receiving board (RL-2000, Data Sciences, St. Paul, MN, USA) was attached to one side of the rabbit cages. The signals of HR, BT and LA from the ECG transmitter were continuously recorded every 5 min by a Data Quest analyzing system (Data Sciences, St. Paul, MN, USA) for 20–30 days. Similarly, the signals of HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) and LA from the BP transmitter were also recorded. These values were recorded 2–3 weeks after implantation in order to exclude the effects of implantation.

**Data analysis**

The hourly values for each rabbit in each period were taken and then were summarized for groups to get a mean ± SEM. Statistical analyses were made using Student’s paired t-test to compare the mean values in the light phase to those in the dark phase within each group. Student’s t-test was also used to compare between groups.
**Fig. 1.** Changes in hourly averaged values for 10 days of heart rate, body temperature and locomotor activity during 24 h in JW and KHC rabbits. Values are mean ± SEM. A: JW (HR, LA: n=11, BT: n=4), KHC (HR, LA: n=9, BT: n=4); B: JW (HR, LA: n=8, BT: n=4). KHC (HR, LA: n=10, BT: n=4).

**Results**

The changes in a 24 h plot of the hourly HR, BT and LA in JW and KHC rabbits are shown in Fig. 1. The 5 months and 10 months groups are shown in Figs. 1A and B, respectively. In the 5 months and 10 months groups of JW rabbits, nocturnal patterns were observed in HR, BT and LA. Particularly in 5 months JW rabbits, there were two clear peaks, before the lights on and after the lights off, in LA. In KHC rabbits, however, nocturnal rhythms were observed only in BT. HR in the light phase was relatively higher than that in the dark phase. There were two peaks in LA in the 5 months KHC rabbits, after the lights on and lights off, similar to observations in the age-matched JW rabbits. LA in the light phase was higher than that in the dark phase in the 10 months KHC rabbits, as same as HR changes.

Figure 2 shows the summarized values of HR, BT and LA in the light and dark phase. In JW rabbits, the values of HR and BT in the 10 months group were lower than that in the 5 months group, although LA in both groups of JW rabbits were similar. In the 5 months JW rabbits, the values of HR, BT and LA in the dark phase were significantly higher than those in the light phase. Similarly in the 10 months JW rabbits, the value of HR in the dark phase was higher than that in the light phase. In KHC rabbits, however, the values of HR and BT were little different between the 5 months and 10 months groups. Moreover, in the 5 months KHC rabbits, the value of HR in the dark phase was significantly lower than that in the light phase. There
was the same tendency in the value of LA in the 10 months KHC rabbits, though the value of BT in the dark phase was higher than that in the light phase. The values of HR in each group of KHC rabbits, tended to be higher than those in the age-matched JW rabbits.

The changes in the 24 h plots of the hourly SBP, MBP and DBP in the 5 months and 10 months groups of JW and KHC rabbits are shown in Figs. 3A and B, respectively. In all groups, the SBP, MBP and DBP showed no clear daily pattern. However, SBP in the 10 months KHC rabbits tended to rise slightly in the light phase as compared with the other groups. The summarized values of SBP, MBP, DBP and the pulse pressure derived from hourly SBP and DBP in the light and dark phases are shown in Fig. 4. The light and dark phases values of SBP, MBP and DBP in both KHC groups tended to be slightly higher than those in the age-matched JW rabbits. The pulse pressure in the 10 months KHC rabbits tended to be higher than the other groups, especially it was significantly higher than that of the 5 months KHC rabbits. No significant differences were observed among the 3 other groups.

Short-term variabilities in SBP, MBP and DBP in the light and dark phases were compared between KHC and JW rabbits (Table 2). The results obtained in the dark phase showed the same tendencies in the light phase among the 4 groups, but SD values of SBP, MBP and DBP in the 5 months KHC rabbits were significantly lower than those in the other groups.

Discussion

This study has demonstrated the characteristics of the daily pattern of HR, BP, BT and LA in KHC and JW rabbits. The daily pattern in those parameters in KHC rabbits were different from the age-matched JW rabbits. HR in KHC rabbits had a diurnal pattern, although JW rabbits showed a nocturnal pattern. BP, especially pulse pressure, in the 10 months KHC rabbits was higher than that in age-matched JW rabbits throughout the day. Moreover, short-term variabilities in BP in the 5 months KHC rabbits were significantly lower than those in other groups. These results suggest that the development of atherosclerosis may have relevance to the alteration of the rhythmicity in biobehavioral parameters.

In rabbits, many biobehavioral parameters including LA, BT, food and water intake, and urine and feces excretion show nocturnal patterns in their circadian rhythms [6, 7, 13, 25, 26]. However, only a little information is available for circadian rhythms in HR and BP [6, 7, 23, 27]. Sato et al. [23] reported that HR and BP in rabbits showed nocturnal patterns using telemetry measurements. They also suggested the difficulty of
the analysis of the circadian rhythm in BP in rabbits, because short-term variabilities in BP were very large.

Nocturnal patterns were obtained in HR, BT and LA in JW rabbits. These results are consistent with earlier studies. However, there was no clear rhythmicity in BP in both JW and KHC rabbits. It seems that these results are dependent on the unique characteristics of the short-term variabilities in BP in rabbits.

In KHC rabbits, atherosclerotic lesions spread over the aorta to the peripheral arteries with aging from 5 months to 10 months [15]. We observed that the pulse pressure in the 10 months KHC rabbits became significantly higher than in the 5 months KHC rabbits and age-matched JW rabbits. Because atherosclerosis alters vascular properties, these changes were probably related to the alteration of vasoelastic properties with growing atherosclerotic lesions in the aorta. Moreover, it has been reported that the baroreceptor activity is closely related to vasoelastic properties [3, 4]. Our recent observations also support these facts, because baroreflex control of BP in KHC rabbits was impaired [1] and underlying aortic nerve activity was altered [20]. Those impaired baroreflex control functions in KHC rabbits might be responsible for the daily changes of BP or HR.

Moreover, impaired baroreflex control function in KHC rabbits could be associated with BP in KHC rabbits which was higher than that in age-matched JW
rabbits. On the other hand, it has recently been reported that serum lipids have a direct effect on endothelial function [5, 8, 10], and that normal vascular tone requires a functioning endothelial lining [17]. Infusion of inhibitors of endothelium-derived nitric oxide can raise BP [11, 12], and hypercholesterolemia is associated with disorders of endothelium-dependent dilation. Therefore, high serum cholesterol in KHC rabbits also could be one of the causes of hypertension in KHC rabbits.

Although BT showed a nocturnal rhythm in KHC rabbits, HR and LA showed a diurnal rhythm. One of the possible explanations is that the oscillator for BT rhythm differs from that for HR or LA in rabbits. This
**BIOBEHAVIORAL RHYTHM IN KHC RABBITS**

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<th>10 months</th>
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<tr>
<td></td>
<td>JW</td>
<td>KHC</td>
<td>JW</td>
<td>KHC</td>
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<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
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<tr>
<td>light</td>
<td>11.2 ± 1.4</td>
<td>7.6 ± 0.5</td>
<td>11.6 ± 0.5</td>
<td>10.7 ± 0.5</td>
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<tr>
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<td>8.4 ± 0.7</td>
<td>12.2 ± 0.7</td>
<td>11.4 ± 0.7</td>
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<tr>
<td>total</td>
<td>11.5 ± 1.2</td>
<td>8.0 ± 0.6</td>
<td>12.0 ± 0.6</td>
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<td><strong>MBP</strong></td>
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<tr>
<td>light</td>
<td>10.5 ± 1.2</td>
<td>7.7 ± 0.8</td>
<td>10.1 ± 0.5</td>
<td>9.0 ± 0.2</td>
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<tr>
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<td><strong>DBP</strong></td>
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<tr>
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<td>9.9 ± 1.1</td>
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<tr>
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<td>9.6 ± 0.9</td>
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<tr>
<td>total</td>
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<td>6.7 ± 0.3</td>
<td>9.4 ± 0.7</td>
<td>8.4 ± 0.2</td>
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SBP: systolic blood pressure, MBP: mean blood pressure, DBP: diastolic blood pressure. *: p<0.05, significantly different from 5 months KHC rabbits. †: p<0.05, significantly different from the age matched JW rabbits. a: Twelve hourly standard deviation values of blood pressure for each rabbit in each period were taken and averaged to get an average per rabbit and then summarized for seven rabbits to get a mean ± SEM for each of the periods.

Possibility is supported by the fact that suprachiasmatic nucleus was not the oscillator for BT rhythm in rats and the BT rhythm did not always correspond to the LA rhythm [14, 21, 22]. However, further studies will be needed to clarify the difference of rhythmicity between BT and HR or LA in KHC rabbits.

In this study, the daily rhythm of HR in KHC rabbits was different from that in JW rabbits. Because the difference in the daily pattern of HR seemed not to be derived from the difference of LA in KHC rabbits, these differences might be related to different rhythmicities of the underlying autonomic nervous functions between KHC and JW rabbits.

In conclusion, we have shown that the cardiovascular function in KHC rabbits was different from that in JW rabbits. It seems that these differences are due to the development of atherosclerosis. These changes of cardiovascular function in KHC rabbits may play a role in the pathogenesis of cardiovascular diseases.

**References**