Circadian Rhythm of Catecholamine Excretion in Rats after Phase Shift of Light-Dark Cycle

Ayako SUDO and Keiichi MIKI

Department of Industrial Physiology
National Institute of Industrial Health
21-1, Nagao 6-chome, Tama-ku, Kawasaki 214, Japan

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Abstract: To clarify the time course of circadian rhythm adaptation to a phase shift of the light-dark (LD) cycle, urinary excretion of catecholamines was measured in rats before and after a 12-hour or 6-hour phase delay of a 12-hour light and 12-hour dark schedule. In rats under a basal condition, distinct circadian rhythms in catecholamine excretion were observed, especially in adrenaline excretion. During the 1st and 2nd days after a 12-hour phase delay, the acrophase and amplitude of adrenaline rhythm remained almost unchanged, but thereafter the acrophase was retarded and the amplitude was reduced. The acrophase once again became constant after 5 or 6 days, but the ratio of amplitude to mesor in the circadian rhythm of adrenaline excretion and the ratio of light-period to 24-hour noradrenaline excretion were readjusted to the new LD schedule on 11th or 12th day. In the 6-hour phase delay of the LD cycle, similar findings were observed, and the results suggested adaptation on the 5-6th day. It is considered that the circadian rhythms of the sympathetic adrenomedullary function are restored, at the latest, 12 days after a 12-hour delay of the LD cycle, and 6 days after a 6-hour delay, suggesting that rats need approximately 1 day to adapt to a 1-hour phase shift.

Keywords: Adrenaline — Noradrenaline — Dopamine — Urine — Circadian rhythm — Light-dark cycle — Phase shift — Rats

INTRODUCTION

Some physiological functions fluctuate according to the time of day. Synchronization of the circadian rhythms of these functions and daily physical and mental activities is assumed to be necessary to ensure healthy daily lives. When the subject abruptly finds himself in a different activity and rest cycle after a
transmeridian flight, the circadian rhythms of physiological functions have been demonstrated to adapt only slowly, a fact which is probably associated with subjective complaints during the first several days. In the cases of night work and shift work in industry, workers are also forced to follow an unusual time schedule. To evaluate the effects of night or shift work on a worker's health, the process of adaptation of circadian rhythms should be clarified. Experimental studies using rodents would provide basic information about changes and recoveries of circadian rhythms due to a phase shift, because the physiological parameters of rats are known to exhibit distinct circadian rhythmicity similar to that of humans.

Laboratory animals are usually maintained under a 12-hour light and 12-hour dark cycle in an artificially illuminated room. Our previous study revealed that urinary excretion of catecholamines in rats kept under these conditions is higher in the dark period than in the light period, suggesting existence of circadian rhythms of catecholamine excretion in the rat. Observation of circadian catecholamine rhythms in rats after alteration of the light-dark schedule would present precise information about the adaptation of sympathetic adrenomedullary activity to a phase shift. To our knowledge, little is known about the time course of changes in catecholamine excretion rhythms in rats after a phase shift of the lighting schedule. In this report, we describe circadian variations of urinary catecholamine excretion in rats before and after a 6- or 12-hour phase delay of the light-dark cycle.

**METHODS**

Six male 4-week-old Sprague-Dawley rats purchased from Clea Japan, initial body weight 79–83 g, were housed individually in metabolic cages, and kept under a 12-hour light and 12-hour dark condition with lights on between 1:00 and 13:00 for 2.6 weeks. Then, the duration of the light period was prolonged by 12 hours and the lights were turned off at 1:00, resulting in a 12-hour phase shift of the light-dark cycle. Successive 4-hour urine samples were collected from each rat for 3 days before and for 7 days after the phase shift, in the manner described below. Urine was also collected on the 11th, 12th, 14th and 15th days.

Three other male Sprague-Dawley rats kept as described above were used to study the effect of a 6-hour phase delay on the catecholamine rhythms. The initial lighting schedule for these rats called for lights on between 8:00 and 20:00. Thereafter, the light period was extended by 6 hours, and lights were turned off at 2:00 the next morning. Urine was collected at 3-hour intervals for 3 days before and for 7 days after the 6-hour phase shift.

Throughout the experiments, the temperature and humidity were controlled at 25 ± 1°C and 55 ± 5%, respectively, and animal care, including cleaning the room and taking body weight measurements, was carried out at around 10 a.m. The intensity of illumination inside the cage was 50–100 lx, and food and water were
given *ad libitum*. Urine samples of each rat were collected in glass tubes containing 6N H$_2$SO$_4$, by means of a fraction collector. Urine sampling was also performed in the same manner at least 1 day before each of the consecutive urine collections, to habituate the rats to the urine collection procedure. Catecholamines in rat urine were analyzed by the HPLC-THI method after purification with alumina$^9$, and the creatinine content was measured using an AutoAnalyzer$^5$. Catecholamine excretion was expressed as pmol per mg of creatinine. Mesor, amplitude, acrophase and probability of circadian catecholamine rhythms were estimated by least squares fitting to cosine function$^6$ of observations for each day, regarding the periodicity as 24 hours unless otherwise stated.

Statistical significance of the differences in the values before and after the phase shift was determined by the paired t-test, and a difference of $p < 0.05$ was considered significant.

RESULTS

Circadian rhythm of adrenaline excretion before and after a phase delay of the light-dark cycle:

The circadian rhythm of urinary adrenaline excretion in rats before and after a 12-hour or 6-hour phase delay of the light-dark cycle is illustrated in Figure 1. A distinct circadian rhythm of adrenaline excretion was observed in the rats before the phase shift. Adrenaline output during the dark period was about 5 times higher than that during the light period. The mean of the probabilities for adrenaline circadian periodicity, which was calculated by the above-mentioned method based on observations over the 3-day period before phase shift, was $p = 0.13\%$, indicating significant circadian rhythms for the urinary excretion of adrenaline. The probabilities obtained from one day's observations ranged between 3 and 26\% during the first 4 days after the phase shift, and in a range from 2 to 14\% with a mean of 4.9\% during the other period.

For about 2 days after a 12-hour phase shift of the lighting schedule, the adrenaline circadian rhythm was still obvious; however, thereafter, the rhythm was disturbed—the acrophase was retarded (Fig. 2) and the amplitude was reduced (Fig. 3). On 5th or 6th day, the acrophase seemed to return to a constant value, but the amplitude tended to remain reduced. The ratio of amplitude to mesor of the adrenaline rhythm became constant on 11th or 12th day, indicating readjustment to a new lighting schedule.

Similar findings were observed in a 6-hour phase delay of the LD cycle of rats. The changes were less pronounced, and the amplitude-mesor ratio became constant 5–6 days after, indicating rapid adaptation to a 6-hour phase delay compared with a 12-hour phase delay.
Fig. 1. Circadian rhythm of adrenaline excretion in rats before and after a 6-hour or 12-hour phase delay of the light-dark cycle.
Each point represents the arithmetic mean value from 6 observations in the 12-hour phase shift (lower part) and 3 observations in the 6-hour phase shift (upper part). The vertical bars indicate the standard errors of the means (s.e.m.). Each shaded area indicates the dark phase of the light-dark cycle.

Fig. 2. Changes in the acrophase of circadian adrenaline rhythm in rats before and after a phase delay of the light-dark cycle.
Data represent mean ± s.e.m. Open circles indicate the acrophase (hour) of adrenaline rhythm before and after a 6-hour phase shift of the light-dark cycle, and solid circles indicate the values for a 12-hour phase shift. The acrophase was determined by the least squares spectrum analysis from 1-day's observations, regarding the periodicity as 24 hours, and expressed as hours after lights off in the delayed light-dark cycle.
Noradrenaline and dopamine outputs during light and dark periods before and after a phase delay of the light-dark cycle:

During the pre-shift 3 days, urinary excretion of noradrenaline and dopamine was found to be higher in the dark period than in the light period. Probability of circadian periodicity of noradrenaline and dopamine excretion, calculated with 3-day observations, was 2.7% for noradrenaline and 0.8% for dopamine, indicating significant circadian rhythms. After the phase shift, however, the periodicity of these 2 catecholamines was not so remarkable, compared with the adrenaline circadian rhythm, and the probability became 30–40% 3–4 days after the shift. Therefore, statistical analysis was performed using the data during 12-hr light periods and during 12-hr dark periods, but the indices obtained from the cosine fitting method were not used.

The ratio of noradrenaline outputs during 12-hour light periods to the outputs during 24 hours is shown in Figure 4. Before the LD phase shift, the ratio was 36%, indicating a greater noradrenaline output during the dark periods than during the light periods (p < 0.01). After a 12-hour phase delay, the ratio significantly increased compared to the pre-shift ratio. After the 11th day, the ratio returned to its pre-shift level. In a 6-hour phase delay, the ratio of noradrenaline excretion tended to be higher until the 5th day.

Circadian dopamine rhythm was also disturbed by the treatment of light-dark reversal, but seemed to be restored by the 6th day after the phase shift (Fig. 5).
The noradrenaline and dopamine rhythms also showed more rapid adaptation to the 6-hour phase delay than to the 12-hour phase shift.
DISCUSSION

The present experiment indicated that urinary output of catecholamines, especially adrenaline, in rats was significantly higher during the dark periods than during the light periods in the two light-dark schedules (lights on during 1:00-13:00 or 8:00-20:00), confirming the existence of circadian rhythms of urinary catecholamine excretion in rats kept under a 12-hour light and 12-hour dark cycle. Table 1 summarizes the present results and our previous study4, showing that the ratios of the light period values to total 24-hour values of catecholamine excretion are almost the same regardless of when lights go on. Therefore, it seems safe to conclude that catecholamine excretion depends mainly on the light-dark cycle, rather than on the time of day.

It has been demonstrated by polygraphic studies that rats spend most of the light period sleeping and most of the dark period awake7. Spontaneous locomotor activity has been reported to be greater during the dark period than during the light period8. It is also known that sympathetic adrenomedullary functions are greatly influenced by various physical and mental activities9. Thus, the circadian catecholamine rhythm in rats could be partially explained by the enhanced activity of the sympatho-adrenomedullary system, which is associated with longer period during which rats are awake and their higher locomotor activity in the dark phase10.

It is likely, however, that the circadian rhythm of plasma level of adrenaline described by others is less pronounced than the circadian rhythm of urinary adrenaline. McCarty et al.11 reported that the plasma adrenaline level in rats did not change according to the time of day, although the noradrenaline level was slightly higher in the dark period than in the light period. De Bore and van der Gugten12 recognized that the mean levels of circulating adrenaline and noradrenaline during the night-time activity period were higher than during the daytime resting period, although the amplitude of the plasma adrenaline rhythm was definitely small.

Table 1. Urinary excretion of catecholamines in rats under 12-hr light, 12-hr dark cycles

<table>
<thead>
<tr>
<th>Light period</th>
<th>Age</th>
<th>N</th>
<th>Adrenaline</th>
<th>Noradrenaline</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Light Dark</td>
<td>%L Light Dark</td>
<td>%L Light Dark</td>
</tr>
<tr>
<td>1:00-13:00</td>
<td>6</td>
<td>6</td>
<td>23±3 75±7</td>
<td>23 697±28 886±39</td>
<td>44 4.5±0.2 6.0±0.3</td>
</tr>
<tr>
<td>2:00-14:00</td>
<td>7</td>
<td>6</td>
<td>22±2 79±9</td>
<td>22 430±14 509±23</td>
<td>46 3.9±0.3 5.9±0.4</td>
</tr>
<tr>
<td>8:00-20:00</td>
<td>6</td>
<td>4</td>
<td>21±3 85±2</td>
<td>20 618±20 719±45</td>
<td>46 4.5±0.2 6.5±0.5</td>
</tr>
<tr>
<td>14:00-2:00</td>
<td>9</td>
<td>6</td>
<td>23±3 78±10</td>
<td>23 367±17 464±37</td>
<td>44 3.8±0.2 5.6±0.3</td>
</tr>
</tbody>
</table>

a: no. of rats examined. b: mean and s.e.m. of catecholamine excretion during the light period (pmol/mg creatinine for adrenaline and noradrenaline, nmol/mg creatinine for dopamine). c: catecholamine excretion during the dark period. d: % of the value during the light period to the sum of the values during the light period and dark period.
compared with that of urinary adrenaline rhythm. The reason for the difference between the blood and urine levels is not clear. It might be assumed, however, that blood sampling often involves invasive manipulation even in the case of catheterized animals, which means that sympathetic adrenomedullary system is activated in the animals. The effect of an indwelling tube or needle and blood loss on physiological functions might cause an indistinct circadian rhythm of plasma catecholamine levels.

The present study examined circadian rhythms of catecholamine excretion after an abrupt phase shift of the light-dark cycle in rats, and elucidated the time course of adaptation of the catecholamine rhythm. Based on changes in the acrophase and amplitude of the rhythm, and the light period/24-hour period ratio of catecholamine excretion, it is thought that it took the rats about 12 days to adapt to a reversed light-dark schedule. The results of the 6-hour phase delay experiment demonstrated that adaptation took place 5–6 days after the phase shift. As a result, it might be considered that rats require approximately 1 day to adapt to a 1-hour phase delay of the LD cycle.

It has been reported that the circadian pattern of locomotor activity in rats re-adjusts to a reversed light-dark cycle 6 days after light reversal. Fillenz and O'Neil described that the difference in the level of motor activity in rats between original 12-hour lights on period and the new lights on period (12-hour) showed a complete inversion by day 6 after light reversal. Morimoto et al. reported that the diurnal rhythms of food intake and plasma 11-OHCS were completely reversed after 7 days on a reversed light-dark regimen. The circadian rhythms of locomotor activity, food intake and plasma 11-OHCS level seem to adapt more quickly to a light-dark reversal than circadian variations of catecholamine excretion. Although the reason for the discrepancy is not clear, it should be noted that the circadian rhythm of the sympathetic adrenomedullary system might be still disturbed after re-establishment of the circadian rhythms of other indices, such as adrenocortical activity, under reversed lighting conditions.

Flinck and Doe reported that when a human subject flew from Minneapolis to Seoul, a 9-hour time lag, the circadian rhythms of urinary 17-OHCS, sodium and potassium excretion were resynchronized with the new time schedules after 9 days. Aschoff et al. also investigated the effects of 7 transmeridian flights on circadian rhythms and computed re-entrainment speeds in human subjects. They estimated that the mean shift rate is 57 min day\(^{-1}\) for eastbound flights (phase delay), and 92 min day\(^{-1}\) for westbound flights (phase advance). The results of the present study agree with their estimate, suggesting that some of the circadian rhythms of humans and rodents require approximately 1 day to adapt to a 1-hour phase delay.

In three-shift work schedules, workers did their job about 8 hours earlier or later on the shift-exchange day. Until adaptation to the new shift work, many of circadian rhythms of the physiological functions are not synchronized, and such
circadian rhythms are probably out of phase with wakefulness-sleep or work-rest cycles of the workers. Desynchronization caused by night and shift work is assumed to be more serious than that caused by jet flights, because the adaptation to the phase shift never takes place due to an incomplete phase shift of environmental conditions.

As described above, the influence of a phase shift in the light-dark cycle appeared rather slowly, so that original circadian rhythms remained almost unchanged for about 2 days after the phase delay. This finding is interesting from the viewpoint of work physiology. If the light-dark schedule returns to normal after a 2- or 3-day shift, circadian rhythms would be modified only slightly, which means that quick recoveries can be expected. However, if the light-dark cycle returns about 2 weeks later, the circadian rhythm would be continuously disturbed throughout about 4 weeks. Although how to extrapolate the effects on rat circadian rhythms to effects on human ones should be carefully considered, the present findings suggest that the effect of a phase shift in the work-rest cycle on circadian rhythms is rather small if the duration of each shift is short. That is, physiological functions in shift workers will be less disturbed by a quick rotation in the shift work schedule, as pointed out by Knauth and Rutenfranz. Further investigation, however, will be necessary to elucidate the mechanism of adaptation of the circadian rhythms of physiological functions in humans and animals.

REFERENCES


