Circadian Rhythm in the Cerebral Resistance to Hypoxia in Mice

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ABSTRACT—The cerebral resistance to hypoxia in mice was investigated by measuring the survival time under both hypobaric and normobaric hypoxic conditions. In the ad libitum fed mice, there was a circadian variation in the survival time that was longer during the light period than during the dark period under hypobaric hypoxic conditions. The survival time under normobaric hypoxic conditions also exhibited a similar circadian variation in the ad libitum fed mice, whereas the rhythm of the survival time was completely reversed by the restriction of food presentation (9:00–15:00). These findings suggest that there is a circadian rhythm in the cerebral resistance of mice to hypoxia, which can be shifted by the time of food presentation. Furthermore, regression analyses revealed a negative correlation between the survival time of mice exposed to hypoxia and body temperature, and blood glucose levels. These indicate that the cerebral resistance to hypoxia was intimately associated with body temperature and blood glucose that both show a circadian rhythm in mice.

Keywords: Circadian rhythm, Resistance to hypoxia, Body temperature, Blood glucose, Survival time

The hypoxic mouse model, in which mice are subjected to either hypobaric or normobaric hypoxic gas, has been widely used for screening cerebral protective drugs by measuring their ability to prolong survival time in mice exposed to hypoxia (1–3). Although the hypoxic mouse model is based on a simple concept, the results are influenced by several variables, including the following: oxygen concentration, dose and injection route of drug, interval between drug injection and hypoxic exposure, and ambient temperature (4–6). Furthermore, time-to-time variations are thought to be one of the possible causes of considerable variations and low reproducibility of the results. In this regard, Kwarecki et al. (7) have reported that in rats exposed to hypobaric hypoxia, rats with exposure during the light period (10:00) had a survival time twice as long as that of animals with exposure during the dark period (22:00). The circadian variations in the resistance to hypoxia are, however, not characterized in detail in the above report, since it is based on 2 sets of data determined at a 12-hr interval.

On the other hand, there has been several reports that the prolonged survival time of mice exposed to hypoxia may be due to the decrease of body temperature (8, 9). Additionally, injection of glucose was reported to prolong the survival time of hypoxic mice (10, 11). The body temperature and blood glucose level, as variables that in-

fluenced the results in the hypoxic mouse model, are well known to exhibit evident circadian rhythms (12–15). Thus, circadian rhythms in body temperature and blood glucose may possibly be related to circadian variation of the resistance to hypoxia in mice. These led us to examine the circadian variation of the resistance to hypoxia at 3-hr intervals in mice and its relationship to the circadian rhythms of body temperature and blood glucose under both ad libitum feeding and reversed feeding by restriction of food presentation.

MATERIALS AND METHODS

Animals

Male ICR mice weighing about 30 g (5- to 6-week-old) at the time of the experiments were obtained from Japan SLC, Inc., Hamamatsu; and they were housed on a light-dark cycle (light on 7–19 hr) at a constant temperature (23–24 °C) and humidity for two weeks before the experiments.

The mice were divided into two groups. In one group, food and water were freely available; and in the other group, access to food was restricted to 6 hr (9–15 hr) daily for two weeks, but water was freely available. All experiments were carried out in late winter and late spring.

Experimental hypoxia

In the test of normobaric hypoxia, mice were placed in a chamber (plastic desiccator, 2.5 l) through which a gas mixture of nitrogen and oxygen (96:4 v/v) flowed at the rate of 5 l/min at the ambient temperature of 23°C. The survival time was determined by the time interval between the start of introduction of mice into the hypoxic gas chamber and the cessation of respiration.

In the test of hypobaric hypoxia, mice were placed in a chamber (glass desiccator, 12 l), equipped with a vacuum pump and manometer. The inside pressure of the chamber was lowered to 200 mmHg within 25 sec by initiating air suction. The survival time was determined by the time interval between the start of air suction and the cessation of respiration.

Assay of blood glucose

Blood samples (0.05 ml) were each mixed with 0.25 ml of 1.8% Ba(OH)₂ and 0.25 ml of 2% ZnSO₄. After centrifugation, glucose in the supernatant was determined by a glucose assay kit (Blood Sugar GOD Perid test, Boehringer Mannheim Yamanouchi, Tokyo) using the glucose oxidase-peroxidase system.

Assay of body temperature

Body temperature was measured with a thermistor probe (Terumo Co., Tokyo), the tip of which was inserted to a depth of 2 cm into the anus of a mouse.

Statistical analysis

Data are shown as the mean $\pm$ S.E.M. Comparisons of the mean values were made by analysis of variance followed by Duncan's multiple range test.

RESULTS

The survival time of mice exposed to hypoxia was determined every 3 hr over a 24-hr period. As shown in Fig. 1, a circadian variation was found in the survival time of mice that were subjected to hypobaric hypoxia under ad libitum feeding conditions. The survival time was longer during the light period than during the dark period. In mice that were exposed to normobaric hypoxic gas, the survival time exhibited a similar circadian rhythm under ad libitum feeding conditions (Fig. 2). When the access of mice to food was restricted to 9–15 hr, the survival time was inversely shorter during the light period than during the dark period. Such food restriction caused about a 12-hr shift in the peak of the survival time. Furthermore, under every experimental condition, the values of the survival time during the rest period were found to vary greatly as compared to those during the active period. The mice with weak locomotor activity in spite of being in the

Fig. 1. Circadian rhythm of the survival time of ad libitum fed mice under hypobaric hypoxia. $N=10$. Significant differences were as follows: $P<0.01$: 24 hr vs. 15 and 18 hr, 3 hr vs. 18 hr; $P<0.05$: 12 hr vs. 3 and 24 hr, 15 hr vs. 3, 6 and 21 hr, 18 hr vs. 6 and 21 hr, 9 hr vs. 3 and 24 hr (Duncan's multiple range test).

Fig. 2. Circadian rhythm of the survival time of mice under normobaric hypoxia. $\circ$: Ad libitum fed mice ($N=12$), $\bullet$: feeding-restricted mice ($N=12$). Significant differences were as follows: [ad libitum fed mice] $P<0.01$: 12 hr vs. 3, 21 and 24 hr, 15 hr vs. 21 hr, 18 hr vs. 3, 21 and 24 hr; $P<0.05$: 15 hr vs. 3 and 24 hr, 9 hr vs. 12 and 18 hr, [feeding-restricted mice] $P<0.01$: 6 hr vs. 9 and 21 hr, 3 hr vs. 9 and 21 hr; $P<0.05$: 9 hr vs. 12 and 24 hr, 15 hr vs. 3 and 6 hr, 18 hr vs. 3 and 6 hr (Duncan's multiple range test).

rest period probably cause such variations.

Under the same conditions, body temperature exhibited an evident circadian rhythm. The rhythmicity of body temperature also shifted in the mice subjected to restricted feeding (12). These circadian rhythms of body tempera-

Fig. 3. Relation between the survival time of mice under normobaric hypoxia and body temperature. Points for each variable are the means for the ad libitum fed (○) and feeding-restricted (●) mice shown in Fig. 2 and reference 12. The regression line ($y = -1.043x + 44.20$) was calculated by the method of least squares, and a correlation coefficient ($r = 0.700$, $P < 0.01$) was obtained.

Fig. 4. Circadian rhythm of blood glucose in mice. ○: Ad libitum fed mice ($N = 10$), ●: feeding-restricted mice ($N = 7$). Significant differences were as follows: [ad libitum fed mice] $P < 0.01$: 21 hr vs. 6, 12, 15, 18 and 24 hr, 9 hr vs. 6, 12, 15 and 18 hr, 3 hr vs. 12, 15 and 18 hr, 18 hr vs. 6, 12 and 24 hr; $P < 0.05$: 24 hr vs. 9 and 15 hr, 3 hr vs. 6 hr, 15 hr vs. 18 hr, [feeding-restricted mice] $P < 0.01$: 15 hr vs. 6 and 9 hr, 9 hr vs. 3, 12 and 24 hr, 21 hr vs. 6 and 9 hr, 18 hr vs. 6 and 9 hr; $P < 0.05$: 6 hr vs. 3, 12 and 24 hr (Duncan's multiple range test).

Fig. 5. Relation between the survival time of mice under normobaric hypoxia and blood glucose. Points for each variable are the means for the ad libitum fed (○) and feeding-restricted (●) mice shown in Figs. 2 and 4. The regression line ($y = -0.038x + 10.71$) was calculated by the method of least squares, and a correlation coefficient ($r = -0.608$, $P < 0.05$) was obtained.

ture were in agreement with previous reports (13, 14). Furthermore, as shown in Fig. 3, there was a negative correlation between the survival time in mice exposed to normobaric hypoxic gas and body temperature. A similar correlation was also obtained between the survival time in mice exposed to hypobaric hypoxia and body temperature ($r = -0.870$, $P < 0.01$, $N = 8$).

Figure 4 shows the circadian rhythm of blood glucose levels. In ad libitum fed mice, blood glucose was increased during the dark period, being particularly high immediately after the dark period, and was then gradually decreased during light period. When food presentation was restricted to the light phase (9:00–15:00), the circadian pattern of blood glucose was shifted, depending on the time of food intake. These results agreed with the previous reports (15, 16).

Figure 5 shows the negative correlation between the survival time in mice exposed to normobaric hypoxic gas and blood glucose. Additionally, a similar negative correlation was also observed between the survival time under hypobaric hypoxia and blood glucose ($r = -0.718$, $P < 0.05$, $N = 8$).

DISCUSSION

The present findings demonstrated that there was a circadian rhythm in the survival time in mice exposed to either hypobaric hypoxia or normobaric hypoxia, which

was shifted by the restriction of food presentation. The circadian rhythms of the survival time exhibited a reverse relationship to those of body temperature under both ad libitum and restricted feeding conditions, and there was a negative correlation between the survival time and body temperature. It is known that hypothermia protects animals from hypoxia, whereas a negative relation between the survival time and body temperature has previously been demonstrated in mice whose body temperature was artificially modified by either some drug or ambient temperature (8, 9), present results indicated that the survival time is intimately associated with circadian rhythm of body temperature. From the findings, in the screening test of drugs during the light (rest) period, caution must be paid to minimize an artificial increase of body temperature by either excessive handling or other stimuli in mice.

While the injection of glucose was reported to increase the resistance to hypoxia (10, 11), the survival time in mice exposed to normobaric hypoxic gas was negatively correlated to the blood glucose levels in the present study. The discrepancy may be explained by the following concepts: first, the blood glucose attained by food intake may be insufficient to increase the resistance to hypoxia, and secondly, the status of brain energy metabolism rather than blood glucose level may be the critical factor in the resistance to hypoxia under the present conditions. In this regard, Kwarecki et al. (7) reported that in experiments using 24-hr starved rats, the increased resistance to hypoxia during the light period may be related to a heightened ability to mobilize glucose from the liver glycogen store, and that this is also in agreement with the changes of blood glucose levels. However, since in the ad libitum fed mice and the feeding-restricted mice, the change in blood glucose was mainly dependent on the time of food intake, the circadian variation of blood glucose in this study differed from that under starvation, as in the above report.

In the previous paper, we reported that there was a circadian rhythm in the duration of decapitation-induced gasping in mice and the duration of the gasping response may depend on the status of energy reserves via changes in the cerebral metabolic rate (12). The circadian patterns of the resistance to hypoxia were in agreement with those of the duration of the gasping response in both mice with ad libitum feeding and mice with restricted feeding, and regression analysis revealed a positive correlation between the resistance to hypoxia and decapitation-induced gasping ($r=0.727$, $P<0.01$, $N=16$). This may suggest that possible common mechanisms lie in the responses by either hypoxia or decapitation.

It was known that hypothermia decreased oxygen consumption in the brain, and the protective effect of hypothermia to hypoxia was due to the depression of cerebral metabolic rate (17). The decreased metabolism

may be thought to allow for a longer survival of mice exposed to hypoxia. Oxygen consumption was known to follow a circadian rhythm, being low during the rest period and high during activity period in rats (18). Under our experimental conditions, the locomotor activity in mice was found to closely parallel the body temperature (12). The above evidence suggests that the rate of loss of brain energy reserves during hypoxia (10) is altered depending on the changes of energy requirements, namely oxygen consumption during the rest-activity cycle, resulting in the changes of survival time. Hereafter, further studies are required to confirm this point.

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