Circadian Periodicity in the Duration of Decapitation-Induced Gasping in Mice

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ABSTRACT—The circadian variation of decapitation-induced gasping was investigated by measuring the gasping duration of isolated mouse head after decapitation under both normal and restricted feeding conditions. In the normally fed mice, there was a circadian periodicity in the gasping duration: it was longer during the light period than during the dark period. The circadian periodicity was completely reversed by the restriction of food. The circadian periodicities of the gasping duration were conversely parallel to those of body temperature in both normal and feeding restricted mice, and regression analysis revealed a negative correlation between the gasping duration and body temperature. Furthermore, pentobarbital and ethanol, agents that caused hypothermia, markedly prolonged the gasping duration. These findings suggest that there is a circadian periodicity in the brain reactivity after complete ischemia, which may be associated with the changes of body temperature.

Decapitation instantaneously induced a condition of irreversible complete brain ischemia, in which the behavior of the isolated head was characteristic: a fairly rapid, but shallow gasping lasting about 20 sec until the onset of terminal gasping. Although the mechanism of decapitation-induced gasping still remains unclear, a number of cerebral protecting drugs have been reported to prolong the duration of the gasping in mice (1-4), implying that the gasping duration is one of the indices of brain reactivity after complete brain ischemia. However, there is yet no available information about how this index is related to brain reactivity in experimental animals, although several studies indicated that there is a relation between the time of day and the onset of human ischemic stroke (5-9). This led us to examine the circadian variation in the duration of decapitation-induced gasping in mice to determine if there is a time-related reactivity of the brain to decapitation-induced ischemia.

MATERIALS AND METHODS

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

The mice were decapitated, and the gasping duration of the isolated head was determined.

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

Locomotor activity was determined by making a 30-min observation every 3 hour as described by Irwin (10). The degree of locomotor activity was assessed by the following scoring system: nil (sleeping) −, rarely +, occasionally ++, frequently +++.

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

RESULTS

The gasping duration of isolated mouse head after decapitation was determined every 3 hr over a 24-hr period. As shown in Fig. 1, a circadian variation was found in the gasping duration of normally fed mice. The gasping duration was longer during the light period than during the dark period. A peak of gasping duration was found at 12–18 hr, and the minimum was at 21–3 hr. When the access of mice to food was restricted to 9–15 hr, the gasping duration was inversely shorter during the light period than during the dark period. Gasping duration peaked at 24–6 hr, and the minimum was at 9–15 hr. In the food restricted mice, the striking finding was about a 12-hr shift in the peak of gasping duration.

Under the same conditions, body temperature exhibited a typical circadian periodicity (Fig. 2). The rhythmicity of body temperature also showed a similar shift in food restricted mice. These circadian periodicities of body temperature were in agreement with previous reports (11, 12). Furthermore, as shown in Fig. 3, there was a negative correlation between the gasping duration and body temperature.

To assess the role of body temperature in the gasping tests, the gasping duration in mice administered pentobarbital or ethanol, both agents known to cause hypothermia (13, 14), was investigated at 12:00 (Table 1). Pentobarb-
bital (60 mg/kg) prolonged the gasping duration and caused the hypothermia under our conditions. Gasping duration in ethanol-treated mice was 2–3 fold larger than that in control mice and the concomitant decrease of body temperature was greater. These results may also support the relationship between the gasping duration and body temperature.

Under normal feeding conditions, there was a marked increase in locomotor activity during the dark period and little activity during the light period (Table 2). Under the restricted feeding condition, there was a marked increase in locomotor activity during the light period, unlike under the normal feeding condition, and the activity decreased significantly during the dark period as compared with that of normally fed mice. These results were in agreement with the previous report (11).

**DISCUSSION**

Present findings demonstrated that there was a circadian periodicity in the gasping duration which was shifted by the restriction

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**Table 1. Effects of pentobarbital and ethanol on the gasping duration and body temperature in mice**

| Drug               | Gasping duration (sec) | Body temperature (°C) |
|--------------------|------------------------|-----------------------|
| Control (Saline)   | 19.2 ± 0.42 (10)       | 37.4 ± 0.09 (8)       |
| Pentobarbital 40 mg/kg | 26.1 ± 0.95 (10)       | 36.6 ± 0.22 (8)       |
| Pentobarbital 60   | 31.8 ± 1.46* (10)      | 35.7 ± 0.21* (8)      |
| Ethanol 3.75 g/kg  | 39.1 ± 1.18* (10)      | 35.1 ± 0.22* (8)      |
| Ethanol 7.50       | 70.4 ± 2.08* (10)      | 34.6 ± 0.21* (8)      |

Drug was injected intraperitoneally 30 min before each determination. Significantly different from the control (*P < 0.01).

**Table 2. Circadian periodicity of the locomotor activity in mice**

| Feeding conditions | 9   | 12  | 15  | 18  | 21  | 24  | 3  | 6  |
|--------------------|-----|-----|-----|-----|-----|-----|----|----|
| Normal feeding     | +   | -   | -   | +   | +++ | +++ | +++| +++|
| Restricted feeding | +++ | ++  | ++  | +   | +++ | ++  | ++ | -  |

Nil −, rarely +, occasionally ++, frequently +++.
of food presentation. Since a number of cerebral protecting drugs, which were effective in hypoxia and/or other types of cerebral ischemic models, were reported to be effective in this gasping test, the gasping duration may be one of the indices of the reactivity of brain tissue toward complete ischemia. Since the present results indicate that there may be a circadian periodicity in the reactivity of the brain against complete ischemia, experiments on brain ischemia for the evaluation of cerebral protecting drugs, especially the gasping test, must be carried out within a relatively short time to avoid variation of the results.

The circadian periodicities of the gasping duration exhibited a reverse relationship to those of body temperature under both normal and restricted feeding conditions, and there was a negative correlation between the gasping duration and body temperature. Pentobarbital and ethanol which caused hypothermia prolonged the gasping duration. Thus, these findings strongly suggest that body temperature may be a critical factor that affects the duration of decapitation-induced gasping. With regards to locomotor activity, it was closely parallel to the changes of body temperature. The duration of gasping was markedly decreased at 21 hr in normally fed mice and at 9 hr in food restricted mice, and this time coincided with the early time of the feeding period, namely the activity period, suggesting a negative relationship between gasping and the locomotor activity. The gasping duration was reported to depend on brain energy reserves from experiments using glycolytic inhibitors and glucose (15, 16). Furthermore, hypothermia was demonstrated to decrease cerebral oxygen consumption (17). Thus, the gasping duration may depend on the status of energy reserves via changes in the cerebral metabolic rate.

In the study reported by Marler et al. (8), there was a circadian variation of the onset of ischemic stroke, and the frequency of onset of stroke was higher at 10–12 hr than during other 2-hr intervals. There were several similar reports supporting that the onset of stroke was usually late in the morning (5–7), although there are a few conflicting reports (9). When the nocturnal mice data are compared with the human data, the decrease of the duration of decapitation-induced gasping seems to coincide with the onset of human stroke. Although the gasping of an isolated head could not be interpreted as having anything to do with ischemic vulnerability, the present results suggest that the status of brain energy reserves may play a role as one of the time-related factors governing the occurrence of ischemic brain injury. Experiments using physiologically and reproducible stroke models are necessary to investigate the time-related reactivity of brain against ischemia.

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