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NOTE *Physiology*

Extension of Time until Cardiac Arrest after Injection of a Lethal Dose of Pentobarbital in the Hibernating Syrian Hamster

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ABSTRACT. The aim of the present study was to examine whether entry of peripherally injected drugs into the central nervous system is reduced during hibernation. When a lethal dose of pentobarbital was injected intraperitoneally, the time until cardiac arrest was significantly longer in hibernating hamsters than in active controls. The time difference was not a consequence of low body temperature or diminished circulation, because mimicking these parameters in artificial hypothermia did not prolong the time. In contrast, there was no difference in the time until cardiac arrest after intracerebroventricular injection of the anesthetic. These results indicate that entry of peripherally injected anesthetics into the central nervous system may be suppressed during hibernation.
KEY WORDS: anesthesia, blood brain barrier, heart, hibernation, hypothermia.

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Mammalian hibernation is an adaptive phenomenon for survival of the severe environments during winter. During hibernation, the metabolic rate is drastically suppressed, and the body temperature drops to a few degrees above ambient temperature. Many physiological systems, including brain and renal metabolism, respiration and cardiac function, are arrested or substantially reduced during hibernation [3, 5, 6, 11]. Immune responses are also suppressed in this unique physiological phenomenon [1, 7, 13]. It has been demonstrated that treatment of hibernating squirrels with bacterial lipopolysaccharide (LPS) does not induce fever, although an intracerebroventricular injection of prostaglandin (PG) E_2 does [10]. Since the PG produced at circumventricular sites is the final mediator of LPS-induced fever [12], it is possible that cytokines and other humoral mediators, which are produced peripherally in response to LPS, cannot access sites where they trigger production of PGE_2 in hibernating animals. However, few studies have examined the accessibility of peripheral mediators to the central nervous system (CNS) during hibernation. If accessibility to the CNS is limited in hibernating animals, the effects of peripherally injected drugs, the action of which is mediated via the CNS, would be diminished during hibernation. Therefore, the aim of the present study was to determine whether the time until cardiac arrest after peripheral injection of a lethal dose of pentobarbital is extended in the hibernating hamster.

Male Syrian hamsters were used in the present study. The procedures for breeding and induction of hibernation have been previously described [9]. The experimental procedures were approved by the Animal Care and Use Committee of Gifu University. Both hibernating hamsters and active controls were intraperitoneally administered a lethal dose of sodium pentobarbital (200 mg/kg) and were kept in a room maintained at 27°C. The time until cardiac arrest was then individually measured. In a series of experiments in which artificial hypothermia was induced before injection of a lethal dose of pentobarbital, hamsters were injected with a non-lethal dose of pentobarbital (80 mg/kg, ip) and then transferred to a cold room and kept at 2°C. This procedure allowed induction of hypothermia that is comparable with natural hibernation [9]. After establishing hypothermia, animals were transferred to a room kept at 27°C and then additionally given the anesthetic by intraperitoneal injection (120 mg/kg) or intracerebroventricular injection into the 4th ventricle at a constant rate of 250 μg/head/min. The same dosage regimen was also applied to hibernating animals. In all experiments, cardiac arrest was judged by ECG tracing, and the point at which the QRS complex disappeared was defined as cardiac arrest. Statistical differences between the groups were determined by the Kaplan-Meier method and a subsequent logrank test. $P<0.05$ was considered significant.

Figure 1 shows Kaplan-Meier curves for the survival percentages of the hibernating and active control hamsters injected with a lethal dose of pentobarbital (200 mg/kg). Although pentobarbital caused cardiac arrest in both hibernating and active hamsters, the time until cardiac arrest was significantly longer in hibernating hamsters than in active ones. The average times until cardiac arrest in the hibernating and active hamsters were 161 ± 15 min and 30 ± 2 min, respectively. These results suggest that the sensitivity of hibernating animals to pentobarbital is less than that of their active counterparts. However, considering that the heart rates of hibernating hamsters are much lower than those of active animals [9], it is likely that the difference depends on reduced circulation rather than limited entry of the anesthetic into the CNS.

To address this issue, we employed artificial hypother-

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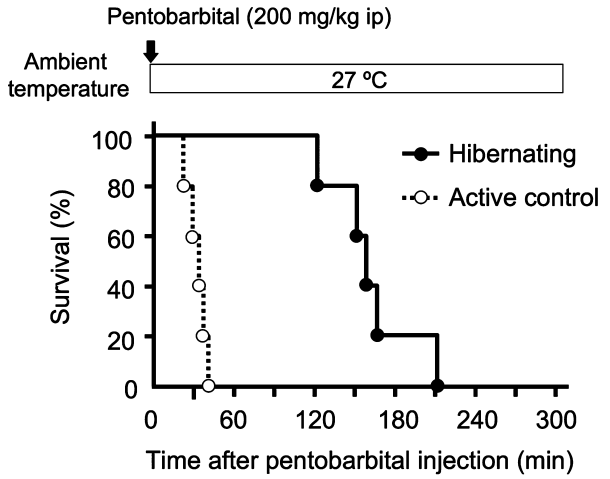

Fig. 1. Kaplan-Meier curves for the survival percentages of the hibernating and active control hamsters intraperitoneally injected with a lethal dose of pentobarbital. After injection of a lethal dose of pentobarbital (200 mg/kg, ip), the time until cardiac arrest was measured. There was a significant difference between the times until cardiac arrest of the hibernating (●) and active control (○) hamsters.

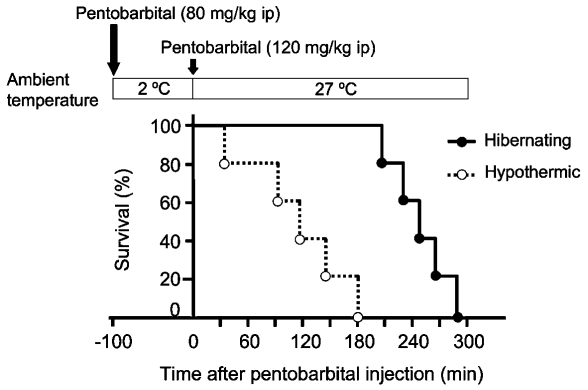

Fig. 2. Kaplan-Meier curves for the survival percentages of the hibernating and artificially induced hypothermic hamsters intraperitoneally injected with a lethal dose of pentobarbital. Control hamsters were anesthetized with pentobarbital (80 mg/kg, ip) and cooled in a refrigerator, allowing induction of hypothermia. Hibernating hamsters were also injected with the same dose of pentobarbital and kept for 100 min. Additional pentobarbital (120 mg/kg, ip) was then injected, and the time until cardiac arrest was measured. There was a significant difference between the times until cardiac arrest of the hibernating (●) and hypothermic (○) hamsters.

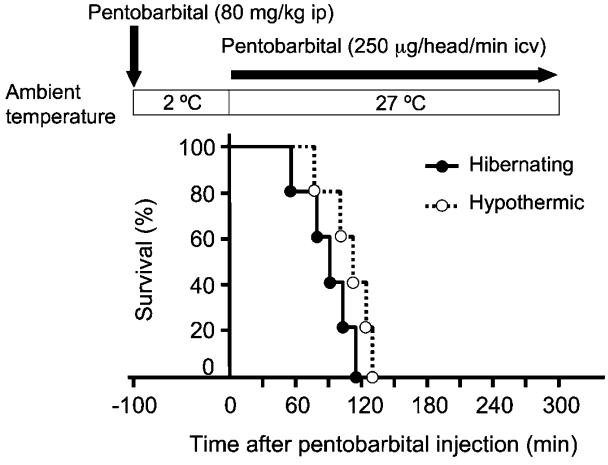

Fig. 3. Kaplan-Meier curves for the survival percentages of the hibernating and artificially induced hypothermic hamsters intracerebroventricular injected with pentobarbital. Both the hibernating and artificially induced hypothermic hamsters were intracerebroventricularly injected with pentobarbital (250 μg/head/min). There was no significant difference between the times until cardiac arrest of the hibernating (●) and hypothermic (○) hamsters.

mia. Hamsters were anesthetized with pentobarbital (80 mg/kg, ip) and cooled in a refrigerator. This procedure reduced their body temperatures and heart rates to levels comparable to those of hibernating animals within 100 min [9]. No hamster showed cardiac arrest after induction of hypothermia. For proper comparison, hibernating hamsters were injected with pentobarbital at the same dose (80 mg/kg, ip) and kept for 100 min. Additional pentobarbital (120 mg/kg, ip) was then injected to induce cardiac arrest in both the hibernating and artificially hypothermic hamsters. Just before the additional pentobarbital injection, the body temperatures, heart rates and respiratory rates of the hibernating and artificially hypothermic hamsters were 7.5 ± 0.6 and 8.4 ± 0.4°C, 14.6 ± 46 and 21.3 ± 7.1 beats/min and 2.1 ± 1.3 and 2.5 ± 1.8 times/min, respectively. Although these parameters were not significantly different, the time until cardiac arrest of the hibernating hamsters was still significantly longer than that of the artificially hypothermic hamsters (Fig. 2). The mean times until cardiac arrest in the hibernating and artificially hypothermic hamsters were 252 ± 13 min and 113 ± 24 min, respectively. These results indicate that the extension of time until cardiac arrest after injection of pentobarbital in hibernating hamsters is not due to low body temperature or reduced circulation.

The low sensitivity of hibernating hamsters to pentobarbital might be due to suppression of access of the anesthetic to the CNS. Alternatively, it is possible that the sensitivity of the $GABA_A$ receptor, through which pentobarbital exerts its anesthetic action, is reduced during hibernation [8]. To validate these possibilities, we examined the effect of central administration of the anesthetic to hibernating and artificially hypothermic hamsters. In the artificially hypothermic animals, the time until cardiac arrest after initiation of the central infusion of pentobarbital (250 μg/head/min) was not significantly different from that after intraperitoneal injection. As shown in Fig. 3, there was no significant difference in the Kaplan-Meier curves for survival percentage. The average times until cardiac arrest after initiation of the pentobarbital injection into the 4th ventricle in the hibernating and artificially hypothermic hamsters were 85 ± 12 min and 110 ± 9 min, respectively. It is therefore

most probable that reduction of receptor sensitivity is not, if any, a major cause of the delayed cardiac arrest in hibernating hamsters.

The extension of time until cardiac arrest after injection of a lethal dose of pentobarbital in hibernating hamsters can be explained by assuming that metabolism of the exogenously injected drugs is accelerated during hibernation. However, this seems unlikely because metabolic processes in hibernating animals are greatly suppressed [2, 4]. Alternatively, it is possible that transfer of the anesthetic to the CNS is reduced in hamsters during hibernation. This view would conflict with the finding in an earlier study that a hypothermic condition increases permeability of the blood brain barrier in rats [14]. This apparent conflict can be explained by the assumption of operation of a hibernation-specific mechanism that limits access of peripherally injected drugs to the CNS. It should be noted, however, that our results do not necessarily provide generalized evidence for suppression of accessibility to the CNS. Since limitation of access of peripheral factors to the CNS would be beneficial in protecting the brain from unfavorable factors accumulating in the blood during hibernation, additional studies should address this possibility.

In summary, the results of the present study demonstrate that the time until cardiac arrest after peripheral injection of a lethal dose of pentobarbital is extended in the hibernating hamster. Considering that this extension was not observed when the anesthetic was injected into the CNS, it is likely that entry of pentobarbital into the CNS is reduced. Further study is needed to clarify the nature of this mechanism.

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