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Seasonal variation in levels of prostaglandins D_2, E_2 and $F_{2\alpha}$ in the brain of a mammalian hibernator, the Asian chipmunk

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Summary Seasonal changes in the in vivo levels of the prostaglandins (PGs) PGD_2, PGE_2, and $PGF_{2\alpha}$ were measured in the brain of the male Asian chipmunk, *Tamias asiaticus* (n = 111), which underwent hibernation during the period between November and March. The mean level of PGD_2 ranged from 36.0 to 85.2 pg/g tissue from June to October and remained essentially unchanged (80.5 pg/g tissue) in December. However, the mean PGD_2 level rose significantly to 128.6 pg/g tissue in February, and returned to 75.2 pg/g tissue in the following April, suggesting a correlation between PGD_2 and hibernation phenomenon. While PGE_2 level did not vary significantly throughout the year, $PGF_{2\alpha}$, which appeared to be the most abundant among the three prostanoids, showed a marked circannual rhythm with a trough of 51.6 pg/g tissue in July, rising to 391.6 pg/g tissue in February and reaching the peak value of 492.7 pg/g tissue in April, the reproduction period.

INTRODUCTION

Hibernation in mammals is always accompanied by a decrease in the body temperature and entered through a state during which slow-wave sleep predominates up to 80% or 90% of the recording time.[1–3] Based on behavioral and electrophysiological observations,[1,4–6] the hibernation–arousal phenomenon in mammals has been postulated to be a physiological extension of sleep–wake activities. Involvement in the hibernation physiology of such substances as norepinephrine, serotonin, and acetylcholine has also been reported;[7] and these substances have been implicated in the sleep–wake regulatory mechanisms as well as thermoregulation. Since the prostaglandins (PGs) PGD_2 and PGE_2 have been implicated in the physiological sleep–wake regulation in rats and monkeys,[8,9] and since both PGs also affect the brain body temperature,[10–12] it is a matter of interest whether PGD_2 and PGE_2 are involved in the hibernation phenomenon.

In the present study we measured the in vivo amounts of PGD_2, PGE_2, and $PGF_{2\alpha}$ in the brain of Asian chipmunks throughout the year, during which the experimental animals underwent hibernation between November and March. Despite the experimental limitations due to methodological difficulties in the hibernation study, as reviewed by Lyman,[13] in vivo levels of these three PGs in the brain were determined in this study, suggesting a correlation between PGD_2 and hibernation phenomenon. It also emerged that brain PGF_{2a} has a marked circannual rhythm with the maximum value in April, the reproduction season.

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MATERIALS AND METHODS

Male Asian chipmunks, *Tamias asiaticus* (n = 111), imported from China, were used in this study. The study period commenced in June 1991 and terminated in June 1992. From June to November 1991, the chipmunks were kept in cages (1 m × 1 m × 1 m) with access to open air and sunlight. In early November, the chipmunks were transferred to individual cages (diameter 0.30 m, height 0.25 m), which were supplied with pieces of cotton cloth for nest making and maintained inside a warehouse until the following mid-March. Thus, the entry of sunlight was prevented and the environmental temperature was kept between 0 and 12°C during the winter season. Food and water were supplied ad libitum throughout the experimental period.

The brains of the chipmunks were collected at 4- to 9-week intervals during the experimental year. The sampling of the brains was carried out as follows with efforts made to reduce any stress to the animals. The chipmunks were anesthetized by diethyl ether, and immediately decapitated between 10.00 and 12.00 h of the day to minimize possible circadian variation in the brain concentration of PGs. The decapitated heads were then frozen in liquid nitrogen and stored at −80°C until assayed.

Body weight of the animals at the time of sampling ranged between 80 and 140 g. During the hibernation season, which began in mid-November and continued for 4 months until the following March, only those chipmunks that had been hibernating for a continuous period of 5 days were taken from their respective cages and immediately decapitated in December and February. The hibernation state was determined by the curling posture continuing for several days without gross changes, and cessation of food and water consumption. The body temperature of the chipmunks which were in the hibernation state at the time of decapitation was 4–10°C.

The assays of PGD_2, PGE_2, and $PGF_{2\alpha}$ in the brain were conducted in July 1992, using the same reagents. The procedure described by Hiroshima et al[14] and Eguchi et al[15,16] was essentially followed. Briefly, the heads were kept in ethanol pre-cooled to −20°C for 7 min, and then the brains were rapidly removed and weighed (range, 1.3–2.3 g). Three brains were pooled to form a single sample for the simultaneous measurement of PGD_2, PGE_2, and $PGF_{2\alpha}$, because the in vivo amounts of these PGs in the brain were only in the order of pg/g wet tissue. Three brains combined were then homogenized in 20 ml of ethanol pre-cooled to −20°C. [^{3}H]PGD_2, [^{3}H]PGE_2, and [^{3}H]$PGF_{2\alpha}$ (each 10 000 dpm) were added to the homogenate as tracers for recovery estimation. After centrifugation at 1600 g for 10 min at 4°C, the supernatant fluid was evaporated and the residue was suspended in 20 ml of a 15% ethanol/1 N HCL (100:1, v/v) solution pre-cooled to 0°C. The three PGs were purified by SEP-PAK C_{18} cartridge (Waters Associates, Milford, MA) and HPLC (Cosmosil 5C_{18} column, 4.6 × 150 mm, Nacalai Tesque, Kyoto, Japan). The mobile phase for the HPLC consisted of a mixture of acetonitrile/water/acetic acid (40:60:0.01, v/v/v), and the flow rate was 1.0 ml/min. The retention times for $PGF_{2\alpha}$, PGE_2, and PGD_2 were 4.30–5.30, 5.30–6.40, and 6.40–7.50 min, respectively. PGs in the respective HPLC fractions were collected and extracted with ethyl acetate. Each extract was evaporated and the residues were suspended in either 300 µl (for PGD_2 or PGE_2) or 150 µl (for $PGF_{2\alpha}$) of respective assay buffer. The recovery of each tracer ranged between 22 and 38%. PGD_2 and PGE_2 were measured by radioimmunoassay (RIA) kits of Amersham and New England Nuclear, respectively. $PGF_{2\alpha}$ was measured by an enzyme immunoassay (EIA) kit from Cayman Chemical. Mean values of the results are presented with SEM in this study. Results were statistically analyzed by one-way ANOVA followed by Scheffe's F-test.

RESULTS

From mid-November, the chipmunks entered hibernation in an unsynchronized fashion by curling their bodies in the nest they had made in their respective cages. The number of chipmunks that were awake or easily awakened by sound or tactile stimulation was apparently larger in December than in February. This indicates that the chipmunks in general underwent stable hibernation in February. The body temperature of the chipmunks which were in the hibernation state at the time of decapitation ranged between 4 and 10°C. In late March, although unexposed to sunlight in the warehouse, chipmunks became active or were easily awakened by sound stimulation.

The mean brain PGD_2 level in the pre-hibernation months ranged between 36.0 and 85.2 pg/g wet tissue (Fig. 1). The level remained within this range (80.5 ± 7.2 pg/g wet tissue) in December, the early hibernation period, and became elevated markedly up to 128.6 ± 12.0 pg/g wet tissue in February, the month of stable and deep hibernation. In April 1992 of the post-hibernation period, the PGD_2 level returned to the pre-hibernation level, becoming 75.2 ± 11.8 pg/g wet tissue. The mean brain PGD_2 level was 71.3 ± 4.4 pg/g wet tissue (n = 29) during the non-hibernation months. Thus, a significant elevation in the brain PGD_2 level was observed specifically in February, during stable hibernation.

The PGE_2 level also showed apparent monthly fluctuations (Fig. 2) with the minimum level (37.0 ± 8.9 pg/g wet tissue) in December and the maximum one (93.2 ± 31.7 pg/g wet tissue) in February, during the hibernation period. However, since the detected levels were low

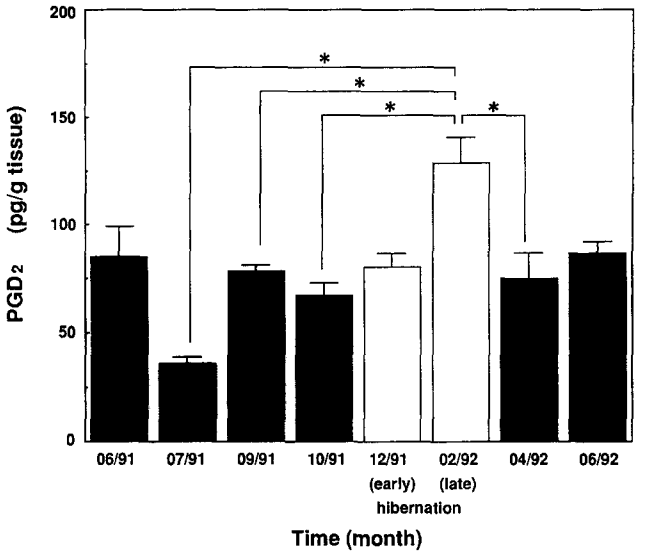

Fig. 1 Seasonal change in the in vivo content of PGD_2 in the brain of chipmunks. Data are shown as the mean ± SEM. Three brains were combined to form a single sample for the simultaneous measurement of the PGs; and the number of samples examined for the respective months of the study was 5 (June, July, September, October 1991, and June 1992) or 4 (December 1991, February and April 1992). Statistical significance between indicated months ($^*P < 0.01$) was obtained by Scheffe's F-test following one-way ANOVA.

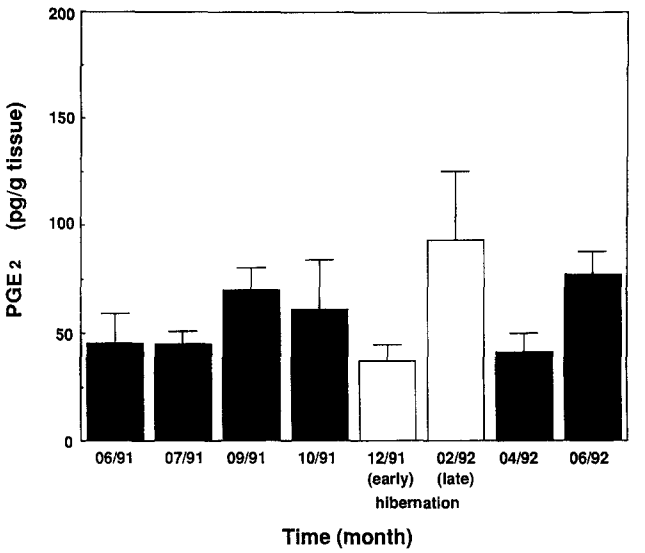

Fig. 2 Seasonal change in the in vivo content of PGE_2 in the brain of chipmunks. Data are shown as the mean ± SEM. Three brains were combined to form a single sample for the simultaneous measurement of the PGs; and the number of samples for the respective months was 5 (June, July, September, October 1991, and June 1992) or 4 (December 1991, February and April 1992).

throughout the year and showed relatively large variations, these fluctuations were not statistically significant. The mean level for the non-hibernation period was 57.3 ± 5.7 pg/g wet tissue ($n = 29$).

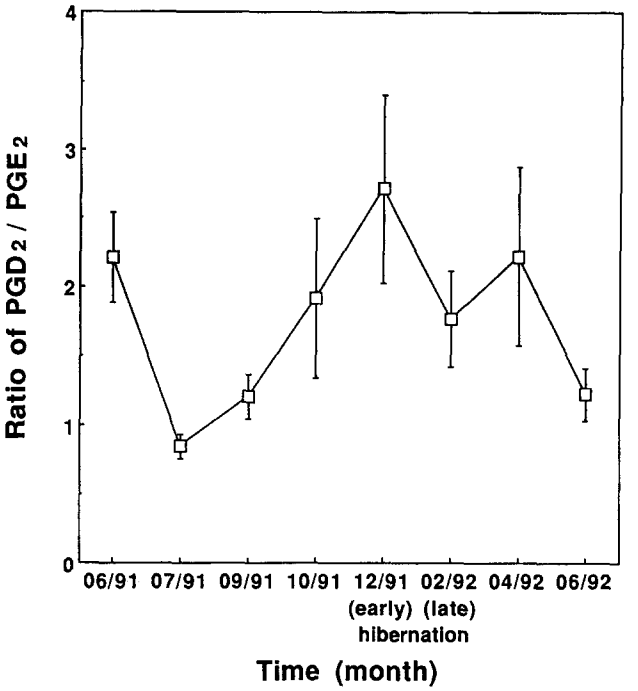

Fig. 3 Seasonal change in the PGD_2/PGE_2 ratio in the brain of chipmunks. Data are shown as the mean ± SEM. Three brains were combined to form a single sample for the simultaneous measurement of the PGs; and the number of samples for the respective months was 5 (June, July, September, October 1991, and June 1992) or 4 (December 1991, February and April 1992).

Since PGD_2 acts as an endogenous sleep-promoting factor[17–19] and PGE_2 as a factor for augmenting wakefulness[11,12,20,21] in the brain of mammals, the ratio of PGD_2/PGE_2 may have an important implication in the hibernation–arousal phenomenon of chipmunks, as has been postulated for the sleep–wake regulation in rats and monkeys.[8,9] The ratio of PGD_2/PGE_2 during the experimental period (Fig. 3) showed the maximum value of 2.7 in December, the early hibernation period, due to the relative decrease in the PGE_2 level (Fig. 2).

$PGF_{2\alpha}$ appeared to be the most abundant compound among the three prostanoids throughout the year (Fig. 4). The seasonal variation in the $PGF_{2\alpha}$ level showed its lowest value (51.6 ± 9.8 pg/g wet tissue) in July and its peak level of 492.7 ± 39.3 pg/g wet tissue in the following spring (April 1992). This elevation in $PGF_{2\alpha}$ level commenced in February (391.6 ± 26.1 pg/g wet tissue), the late hibernation period; whereas the level in December was 168.5 ± 9.5 pg/g wet tissue. The mean level for the non-hibernation and non-reproductive period was 162.2 ± 19.5 pg/g wet tissue ($n = 25$).

DISCUSSION

In the present study, three brains were pooled to form a single sample for the simultaneous measurement of

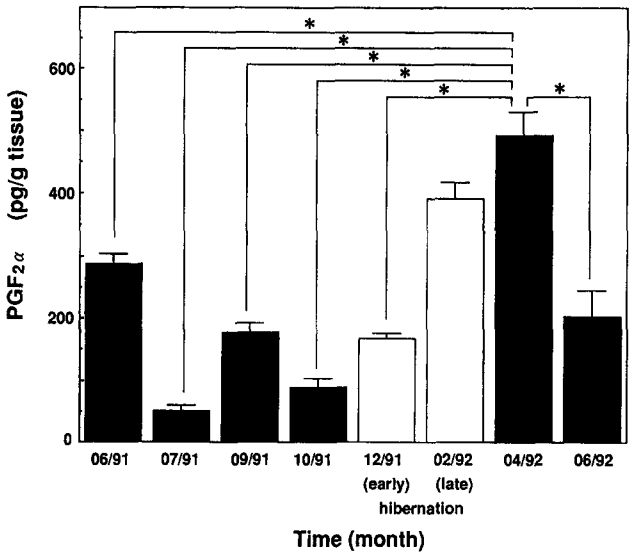

Fig. 4 Seasonal change in the in vivo content of $PGF_{2\alpha}$ in the brain of chipmunks. Data are shown as the mean ± SEM. Three brains were pooled to form a single sample for the simultaneous measurement of the PGs; and the number of samples for the respective months of the study was 5 (June, July, September, October 1991, and June 1992) or 4 (December 1991, February and April 1992). Statistical significance between indicated months (*$P < 0.01$) was obtained by Scheffe's F-test following one-way ANOVA.

PGD_2, PGE_2, and $PGF_{2\alpha}$ due to the low levels of these PGs. Therefore, the detection of subtle variations in the levels of PGs that may occur along with alteration in the behavioral, electrophysiological, and metabolic states of the chipmunks during hibernation[2,3,22] and non-hibernation seasons, could have been compromised. Thus, the reported data of this study represent gross seasonal changes rather than such subtle changes in the in vivo levels of brain PGs.

The in vivo level of PGD_2 in the brain of chipmunks during non-hibernation seasons was 71.3 ± 4.4 pg/g wet tissue (mean ± SEM, $n = 29$). The in vivo level of PGD_2 in the brain of male rats (Wistar) assessed by Hiroshima et al[14] by the same procedure as used by us was found to be 110 ± 30 pg/g wet tissue (mean ± SEM, $n = 6$). Thus, the non-hibernation level of PGD_2 in the brain of chipmunks appears to be within the same order of magnitude as the brain PGD_2 level in rats.

The brain PGD_2 content increased significantly during February, when the chipmunks underwent deep and stable hibernation. This elevated level cannot be a secondary phenomenon related to the experimental conditions such as prevention of light exposure and housing in small individual cages, because these conditions were the same for the chipmunks decapitated in December and February and the PGD_2 level in December was not elevated. It is also unlikely that this high amount of PGD_2 is a non-specific phenomenon secondary to a decreased metabolic rate in the hibernating brain, because no apparent increase was visible in the amount of PGD_2 in December, when the animals also underwent hibernation (Fig. 1). Furthermore, brain $PGF_{2\alpha}$ showed higher level in April in comparison with the levels in December and February. Therefore, the highest PGD_2 level detected in February probably reflects a functional change specifically occurring in the brain during the later period of hibernation season.

Feist and Galster[23] showed that the ratio of norepinephrine/serotonin in the hypothalamus was highest during the early arousal from hibernation in the Arctic ground squirrel (*Citellus undulatus*). Such a balance between endogenous substances may have an important implication in the hibernation–arousal phenomenon. In this study, the PGD_2/PGE_2 ratio in December was the highest throughout the year, although not significantly. A postulation that the earlier phase of hibernation correlates with the brain state resulting from a complex of high potency of sleep-promoting PGD_2[17–19,24] and inhibition of awake-augmenting PGE_2[11,12,20,21] remains for further investigation.

$PGF_{2\alpha}$ was the most abundant among the three prostanoids in the brain of chipmunks throughout the year. Förstermann et al[25] reported that, in spontaneously convulsing gerbils (*Meriones unguiculatus*), although PGD_2 was the major compound after the onset of convulsions, the basal amounts of PGD_2, PGE_2, and $PGF_{2\alpha}$ were 0.56, 0.75, and 1.55 ng/g tissue, respectively, indicating $PGF_{2\alpha}$ as the major PG. We also showed in the present study that the brain $PGF_{2\alpha}$ level has a marked circannual rhythm reaching its maximum in April, the reproductive season of the animal. Furthermore, this increment appeared to begin during the late hibernation phase. These data suggest that $PGF_{2\alpha}$ plays some specific roles in the central nervous system, correlating with reproductive physiology or other brain functions.

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