抑えた．また，この培養液が示した抑制効果は，培養液にフォリスタチン抗体を添加しておくと中和され，アクチビン作用が復元した．以上の結果から，PACAPは濾胞星状細胞の性腺刺激ホルモン産生細胞に対するパラクリン作用を調節できることが示唆された．PACAPは下垂体の内分泌細胞に対する直接的な作用に加え，下垂体細胞間の相互作用の制御因子としての役割を有していると推察された．

一年以上の間，室温22度，14時間明：10時間暗の照明条件下で飼育されたシマリスを短日照明(10時間明：14時間暗)と低温条件に暴露することにより季節外冬眠を誘起した．我々は一年のどの時期でもこの季節外冬眠を誘起できた．この季節外冬眠は季節間において，冬眠—覚醒インターバルや，それぞれの冬眠や覚醒時間には有意な差を認めなかったが，冬眠に入るまでの期間の長さにおいて夏のみ約60日を要し，他の季節の平均30日より長かった．さらに，覚醒インターバルでの覚醒時刻は冬では明期に起こるのに対し，春では明期と暗期でほぼ等しい割合で起こった．これらの結果はシマリスの冬眠がサーカディアンリズム(概日リズム)とサーカニュアルリズム(概年リズム)の両者にリンクしていることを示唆している．夏の季節外冬眠において，セロトニン枯渇剤であるパラクロロフェニルアラニン(PCPA)の冬眠中での慢性投与は冬眠を阻止し，非冬眠動物への投与は逆に冬眠を誘発した．一方，オピオイドのアンタゴニストであるナロキソンの投与は覚醒時間の延長を起こした．これらの結果は，セロトニンによる冬眠誘起や維持機構がサーカニュアル(概年リズム)システムと独立したものであることを示唆している．

公衆衛生学：

生後4カ月の男児に下痢が認められ糞便から *Salmonella* Virchowが分離された．有効薬剤を投与したにもかかわらず1カ月以上に渡り同菌が分離された．乳児宅で飼育されていた室内犬3頭のうち，2頭から *S.* Virchowが分離された．乳児を入院させ治療したところサルモネラは陰性に転じた．乳児および犬から分離されたサルモネラの薬剤感受性および制限酵素 *Xba* Iを用いたPFGEパターンが完全に一致したことから，本事例は犬が感染源と考えられた．

外　科　学：

2歳の犬が食欲減少，腹囲膨満を主訴に来院した．開腹術により卵円形で長径31 cmの左側卵巣部腫瘤と直径11 cm・類円形の左側腹膜後腔部腫瘤を外科的に切除した．両腫瘤は，病理組織学的に管状構造をなす気管支および腸粘膜，毛包，皮脂腺，アポクリン汗腺，神経組織と，その間隙に介在する軟骨，骨，脂肪組織などからなり，奇形腫と診断された．本例は犬の腹膜後腔原発奇形腫の最初の報告例と思われる．

臨床繁殖学：

急性乳房炎を発症した泌乳期のホルスタイン種乳牛の乳房内にオゾンガスを注入し，その治療効果を評価した．オゾンガスは専用のオゾン発生装置を用いて発生させ，罹患分房の乳頭口から分房内に注入した．供試牛19頭のうち，15頭に対してオゾンガスの

NOTE *Physiology*

Induction of Unseasonable Hibernation and Involvement of Serotonin in Entrance into and Maintenance of Its Hibernation of Chipmunks *T. asiaticus*

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ABSTRACT. Chipmunks that had been housed at 22°C under a light-dark cycle of 14L:10D for at least one year were exposed to a short photoperiod (10L:14D) and low temperature to induce unseasonable hibernation. We were able to induce hibernation at any time of year and there was no significant difference in the duration of the hibernation bout, the duration of interbout euthermia and duration of bouts of torpor throughout the year; however entrance into hibernation took about 60 days in summer but only about 30 days in any other seasons. In addition, interbout euthermia predominantly occurred during the light phase in winter, whereas in spring interbout euthermia occurred equally in the light and dark phases. These results suggest that both the circadian and circannual systems are linked to hibernation in chipmunks. Subcutaneous infusion of a serotonin antagonist, para-chlorophenylalanine (PCPA), facilitated entrance into and interrupted hibernation in aroused and hibernating chipmunks in summer, respectively. On the other hand, opioid antagonist, naloxone, did not affect hibernation, but extended the period of interbout euthermia. These results suggest that the role of serotonin in entrance into and maintenance of hibernation in chipmunks is independent of the circannual system, and that opioid system may not be involved in hibernation in chipmunks.

KEY WORDS: circadian rhythm, circannual rhythm, hibernation.

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In natural environments, hibernation of chipmunks is a seasonally adapted physiological state that is triggered by changes such as short days, low temperature and decreased food availability, which usually occur in autumn and winter. Even when chipmunks are housed under artificial conditions of constant low temperature, constant dim light and readily available food they enter hibernation only during the autumn to winter season [16]. On the other hand, hibernation can be induced at any time of year in ground squirrel by moving them from 22°C to a low temperature room [23]. These facts suggest that although the endogenous circannual system is strongly linked to a mechanism for entrance into hibernation, the trigger for hibernation may be complex [13, 29]. However, there is no report examined whether the short photoperiod and low temperature could induce hibernation at all seasons in chipmunks as well as ground squirrel. If we could do it, unseasonable hibernation of chipmunks may be useful to analyze the mechanism of hibernation. First of all, therefore, we tried to induce the unseasonable hibernation in chipmunks by changing the photoperiod and temperature.

Biochemical analysis has revealed that serotonin (5-HT) and/or opioid system may be involved in the neurochemical process of hibernation in ground squirrel [2, 4–6, 12, 21, 27]. For examples, 5-HT levels of several brain areas increase before entrance into hibernation [20, 22] and reducing the brain level of 5-HT, either by lesion of the median raphe, or by injection of para-chlorophenylalanine (PCPA) or 5, 7-dihydroxytryptamine inhibits hibernation [6, 9]. Opioid levels, opioid immunoreactivity and receptors for several opioids increase in specific brain areas during hibernation [19]. Furthermore, administering an opioid receptor antagonist reduces the duration of hibernation or inhibits hibernation [1, 17, 18, 28]. In the second study, therefore, we investigated whether 5-HT or opioids are directly involved in hibernation by studying unseasonable hibernation in chipmunks, using continuous administration of PCPA and naloxone by osmotic mini pump.

We studied 80 male Asian chipmunks (*T. asiaticus*) that had been raised and bred in a pet shop (Miyazaki, Japan). At about 40 days of age the chipmunks were transferred to a climate-control room at Miyazaki University, in March 1996. They were housed under a 14-hr light: 10-hr dark cycle at 22 ± 2°C for at least 14 months. Tap water and pelleted chipmunk chow (Kuroda Animal Co., Japan) were provided *ad libitum*. Hibernation was induced from the next end of March by transferring the chipmunks to a cold room. The temperature was held at 15 ± 2°C for 10 days and then reduced to 6 ± 1°C. The light-dark cycle was changed to 10L:14D (lights on 05:00 hr) on the day that the temperature was changed to 6°C. Individual locomotor activity was measured by using a rat locomotor activity recording system (Muromachi Co., Tokyo) [19]. Data were collected at intervals of 30 min. Body temperature (infrared reflected heat) was examined every day. Complete hibernation was confirmed when the body temperature was less than 6°C and the respiratory rate was less than 10 breaths/min. As locomotor activity record by infrared sensors was very sensitive and corresponded to change in body temperature, euthermic or hibernating times was able to judge by activity record.

For the second experiment, an incision and tunnel was made in the skin on the back of each chipmunk under ether anesthesia just before they were moved from 22°C to 15°C. A mini-osmotic pump (model 2002, Alzet) was implanted subcutaneously via the tunnel with light ether anesthesia in hibernating or euthermic chipmunks in cold room. PCPA (30 mg in 200 μl of saline), naloxone (60 mg in 200 μl of saline) or saline (200 μl) was delivered at 0.5 ± 0.1 μl/hr for 14 days.

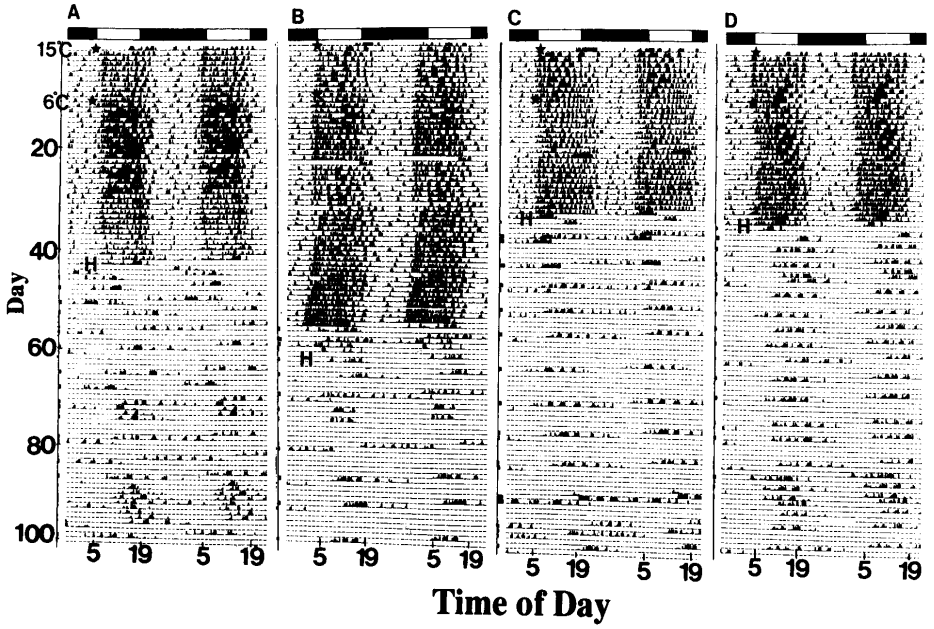

Fig. 1. Seasonal effect on locomotor activity of chipmunks exposed to a temperature decrease. The figure represents the double plotted (48 hr) locomotor activity record. Chipmunks that had been housed at 22°C were moved to a cold room (asterisks) in spring (A), summer (B), autumn (C), and winter (D). The white and black bar represents the daily light-dark cycle. H indicates the initiation of hibernation. The small black mark on the vertical line on the left side of each column indicates the day on which the body surface temperature increased over 6°C.

Therefore, the drugs were released at constant rates for 2 weeks: 75 μg /hr for PCPA and 37.5 μg/hr for naloxone. These doses do not significantly affect locomotor activity or body weight and food intake in intact chipmunks, except slight decrease of food. The each sample size for each treatment was shown in results. Two weeks after implantation the pumps were removed. The data were analyzed by Student's t test after angular transformation to compare the effects of PCPA and naloxone with that of saline.

Low temperature induced hibernation in all seasons (Figs. 1 and 2) without any significant difference in euthermic intervals (average; 30.65 hr in spring, 31.02 hr, in summer, 31.63 hr, in autumn, 31.65 hr in winter: $p>0.05$) and bout duration (average; 3.65 days in spring, 3.54 day in summer 4.01 days in autumn, 4.03 days in winter, $p>0.05$), and so on between four seasons. Although there was no difference in the number of days until entrance into hibernation between spring, autumn and winter, in summer entrance took twice as long as in the other seasons ($p<0.01$; Fig. 3A). In addition, timing of interbout euthermia coincided with the light period 95% of the time during winter and only 50% of the time in spring; the frequencies for summer (67%) and autumn (77%) were intermediate (Fig. 3B). For examples, as shown in Fig. 1D (winter), interbout euthermia begins almost constantly at middle of light phase.

When PCPA was administered to six hibernating chipmunks, hibernation was interrupted in all six animals (Figs. 4A and 4B) within 4–9 days, whereas saline had no effect (Fig. 4E; $p<0.05$, PCPA vs saline). Interrupted animals by PCPA never re-entered into hibernation for at least one month

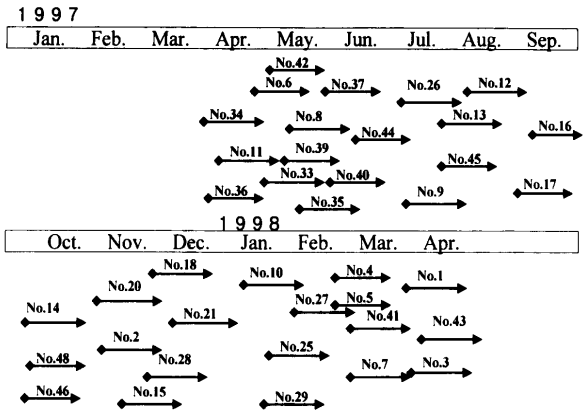

Fig. 2. Summary of initiation times of hibernation in various seasons. Each diamond symbol attached the arrow shows the initiation of hibernation. The number shows individual number of each chipmunks.

after removal of pump. Administration of PCPA to non-hibernating chipmunks housed at 6°C facilitated entrance into hibernation in five of seven animals, whereas saline had no effect (Figs. 4C, 4D and 4F; $p<0.05$, PCPA vs saline). Those five animals entered into hibernation within 10 days of pump implantation. The other two animals entered incomplete hibernation. The non-hibernating animals that received saline entered into hibernation 3 weeks after pump implantation.

Naloxone did not affect hibernation, but did extend the length of interbout euthermia. During naloxone administration the periods of interbout euthermia were 1.5 times longer

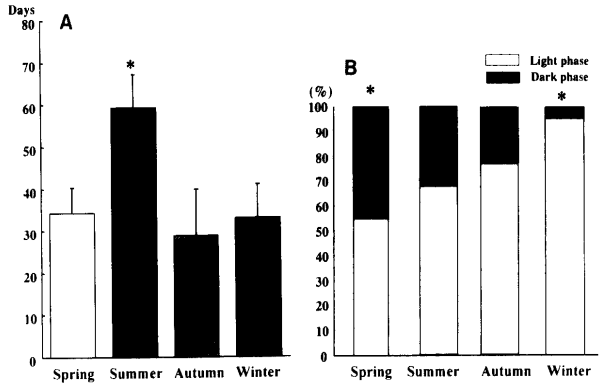

Fig. 3. Seasonal and photoperiod effects on entrance into hibernation and interbout euthermia. A: Comparison of the number of days until hibernation in each season. An asterisk indicates a significant difference ($p<0.01$ vs the other seasons). B: Frequencies of interbout euthermia in the light and dark periods during hibernation. A significant difference ($p<0.05$) was observed between spring and winter.

than those seen before or after naloxone treatment (Fig. 5B). After the osmotic pump was removed on day 14 of implantation, interbout hibernation became longer (Fig. 5A).

The present study demonstrates that hibernation of chipmunks can be induced by exposure to low temperature in all seasons, as well as ground squirrel [23]. However, the time until entrance into unseasonable hibernation was longer in summer than in any other season. This finding indicates that the endogenous circannual system is tightly coupled with entrance into hibernation in chipmunks, even when animals were kept under 14L:10D and at 23°C for over one year. On the other hand, it has been reported that chipmunks housed at constant low temperature enter hibernation only during winter [16]. This observation seems contradictory to our observation of hibernation in summer. However, the present study indicates that a temperature drop can override the circannual system for entrance into hibernation. In winter, spring and autumn, about 30 days, but not immediately, of low temperature were required to enter into hibernation, suggesting that a delay is required for adaptation to low temperature or gradual preparation for hibernation. In addition, the present study showed that both the circannual system and the circadian system are related to hibernation. Timing of interbout euthermia by judging from locomotor activity differed between seasons (As mentioned before, the locomotor activity record has very sensitive and corresponds to change in body temperature). Surprisingly, 95% of interbout euthermia occurred during the light periods in winter. These results indicate that the circadian system functions during hibernation and is entrained to the light-dark cycle. On the other hand, timing of interbout euthermia was divided equally between the light and dark periods in spring, so entrainment to the light-dark cycle was very weak during unseasonable hibernation. Many studies have indicated that the suprachiasmatic nucleus (SCN), a circadian pacemaker, plays an important role in the timing of arousal from hibernation in ground squirrel [3, 7, 8, 11, 14, 15,

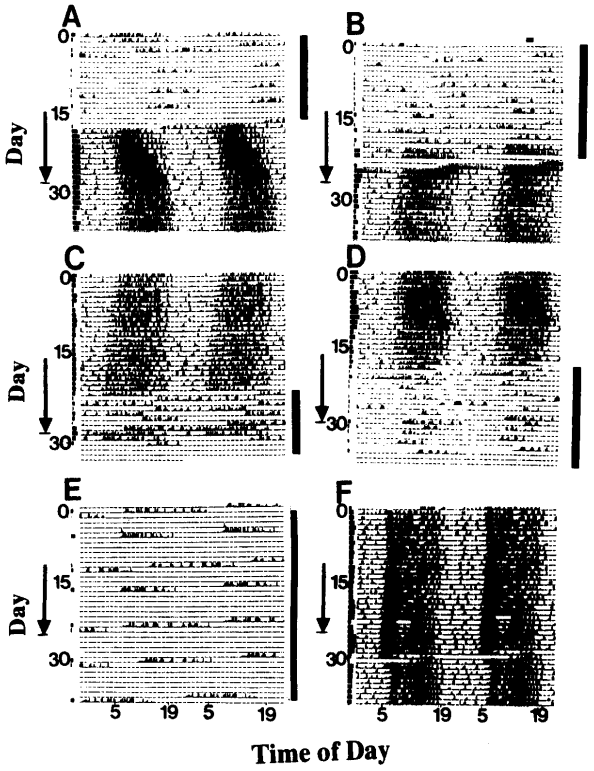

Fig. 4. Representative locomotor activity records of chipmunks treated with PCPA or saline. Chipmunks were implanted mini-osmotic pumps. An arrow represents the period of infusion. The vertical black bar indicates the period of hibernation. Infusion of PCPA interrupted hibernation (A and B) or induced hibernation (C and D). Saline had no effect (E and F). The day 0 indicates the initiation on cold exposure.

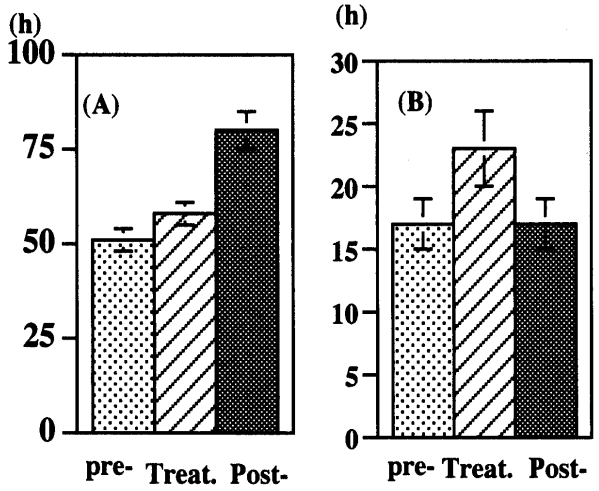

Fig. 5. Effect of naloxone on the length of interbout hibernation and euthermia. Naloxone was infused with a mini-osmotic pump. The lengths of interbout hibernation (left panel: A) and euthermia were measured (right panel: B) for 3 weeks before pump implantation (pre-), during the 2-week infusion (treat.) and for 3 weeks after removal of the pump (post-). Asterisks indicate significant differences ($p<0.05$ vs the other group).

24]. It may be necessary to examine the effect of lesion of SCN on the unseasonable hibernation in chipmunks.

Subcutaneous infusion of PCPA in non-hibernating chipmunks housed at 6°C facilitated entrance into hibernation in five of seven animals in summer. Those five animals entered hibernation within 10 days of the beginning of drug infusion. Saline-treated controls did not enter into hibernation within 3 weeks after the infusion began. These results support the finding of Spafford and Pengelley [25] that brain 5-HT decreases during entrance into hibernation compared with the euthermia 5-HT level in ground squirrel. Both findings suggest that reduced 5-HT is an important trigger for entrance into hibernation in chipmunks. However, it has been reported that 5-HT levels increase in several brain areas before entrance into hibernation [20, 22]. Therefore, we tried to increase the 5-HT content of the brain by adding tryptophan to the drinking water, but we could not induce hibernation in tryptophan-treated chipmunks (unpublished data). A possible explanation for this apparent discrepancy may be that brain 5-HT levels increase just before entrance into hibernation and then rapidly decrease. When PCPA was administered to six hibernating chipmunks, hibernation was interrupted in all of the animals, whereas saline had no effect. This result agrees with previous observations reported by ground squirrel that a decrease of 5-HT level by lesion of the median raphe or injection of PCPA or 5,7-dihydroxytryptamine, inhibits hibernation [6, 9]. Therefore, 5-HT may play an important role in the maintenance of hibernation in chipmunks as well as ground squirrel.

Opioid levels, opioid immunoreactivity and receptors for several opioids increase in specific brain areas during hibernation [18]. Administration of an opioid receptor antagonist (naloxone or naltrexone) is reported to reduce the incidence and duration of hibernation [1, 17, 18, 28]. In the present study, however, treatment with naloxone did not interrupt hibernation in chipmunks, but did lengthen interbout euthermia. The reason why effect of naloxone on the hibernation is different between chipmunks and ground squirrel is unknown in this study. In chipmunks, opioid system may not be involved in hibernation, or the dose of naloxone given in this study may be too low to interrupt hibernation.

In conclusion, the biochemical and physiological features of unseasonable hibernation are similar to those of natural hibernation, except for seasonal differences in the timing of interbout euthermia. In addition, the role of 5-HT in entrance into and maintenance of hibernation in chipmunks appears to be independent of the circannual system. That is, decrease of 5-HT by agents can induce the hibernation in all season.

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