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Circannual Control of Hibernation by HP Complex in the Brain

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SUMMARY

Seasonal hibernation in mammals is under a unique adaptation system that protects organisms from various harmful events, such as lowering of body temperature (Tb), during hibernation. However, the precise factors controlling hibernation remain unknown. We have previously demonstrated a decrease in hibernation-specific protein (HP) complex in the blood of chipmunks during hibernation. Here, HP is identified as a candidate hormone for hibernation. In chipmunks kept in constant cold and darkness, HP is regulated by an individual free-running circannual rhythm that correlates with hibernation. The level of HP complex in the brain increases coincident with the onset of hibernation. Such HP regulation proceeds independently of Tb changes in constant warmth, and Tb decreases only when brain HP is increased in the cold. Blocking brain HP activity using an antibody decreases the duration of hibernation. We suggest that HP, a target of endogenously generated circannual rhythm, carries hormonal signals essential for hibernation to the brain.

INTRODUCTION

One of the most curious biological phenomena in mammals is their ability to hibernate circannually, which allows them to survive unusually low body temperatures (Tb) at or near 0°C. As lowering Tb brings about dysfunction of various cells and organs, such as heart and brain, hibernators are required to develop a capacity for maintaining regulated functions at potentially lethal low Tb during hibernation. It has already been shown that during hibernation, organisms are protected not only from hypothermia (Hochachka, 1986) but also from ischemia (Frerichs and Hallenbeck, 1998), muscle disuse (Harlow et al., 2001), bacterial infection (Sharapov, 1983), and tumorigenesis (Kemper and Ruben, 1982), suggesting that hibernation is a unique physiological adaptation for preventing lethal damage and diseases caused by various harmful events. Such an adaptation can be assumed to be accomplished prior to the onset of and sustained during hibernation because central and peripheral organs would rapidly lose functions at low Tb if the adaptation were not accomplished in advance. In fact, in the heart of chipmunks, a rodent hibernator, the capacity of intracellular stores to take up cytosolic calcium ions, which is critical for avoiding intracellular calcium overload evoked at low Tb (Hochachka, 1986), is greatly enhanced prior to the onset of and during hibernation (Kondo and Shibata, 1984; Kondo, 1986a, 1987, 1988). This enhancement is also caused throughout the hibernation season even in animals prevented from experiencing a lowered Tb by keeping them constantly warm (Kondo, 1987; Kondo and Kondo, 1992b), indicating that cellular adjustment in the heart occurs on a seasonal basis without a lowering of Tb. Furthermore, several physiological and molecular changes, such as decreased locomotor activity and food intake, depressed endocrine functions (Wang, 1982), and the regulated expression of a few proteins (Kondo and Kondo, 1992a; Srere et al., 1992), have been seasonally observed before the beginning of hibernation. These studies suggest the existence of a systemic adaptation mechanism for hibernation independent of Tb changes, which led us to consider the possibility of a seasonally controlled molecular network through which the functions of principal organs would be modulated for maintaining the organism in a healthy state during the hibernation season. For decades, many studies have been carried out to explore factors responsible for hibernation that are seasonally regulated, especially hormones which play a central role in

physiological adaptation to various internal and external changes (Wang, 1982, 1988). However, no factor absolutely critical for hibernation has been discovered.

In a previous study, we found a protein complex (140 kDa MW) specific for hibernation (hibernation-specific protein, HP) in the blood of chipmunks, as a complex that was decreased in the blood during hibernation (Kondo and Kondo, 1992a). This complex is composed of four proteins, three of which are structurally homologous proteins with a collagen-like domain in the N-terminal regions (HP20, 25, and 27) and form a complex called HP20c by triple-helix formation of this domain. In blood, HP20c is further associated with the fourth protein, HP55, which is homologous to α 1-antitrypsin, a member of the serpin superfamily. HP20c associated with HP55 (HP complex, HPc) is known to be produced specifically in the liver and secreted into the blood (Takamatsu et al., 1993, 1997).

Herein, we demonstrate that HP complex, whose concentration in the blood and brain is regulated by an endogenously generated circannual rhythm, is a candidate hormone that may be essential for hibernation.

RESULTS

Free-Running Hibernation Rhythm in Chipmunks

Although an exact characterization of hibernation rhythm is of prime importance for studying the mechanisms of circannual control of hibernation, there is little evidence for its rhythmicity under constant laboratory conditions throughout life. Therefore, in 27 male chipmunks kept under constant cold (5°C) and dark conditions throughout their lives, the precise rhythmicity was examined by monitoring surface Tb changes using a method we originally developed for determining the accurate onset, termination, and duration of hibernation (Figure 1). The characterization and evaluation of hibernation were based on this method throughout the study. Twenty of twenty-seven animals exhibited a clear hibernation rhythm with individually constant cycles and durations (Figure 2A), whereas the other seven animals (26% of animals tested) never hibernated until they died naturally within a few years. The individual hibernation rhythm was sustained throughout their life spans, the maximum of which was 11 years, a duration surprisingly longer than that of rats (about 4-fold longer). The mean period of the rhythm was 313 days, and the maximum and minimum periods were 391 and 157 days, respectively (Figure 2B). The period varied widely between animals but was less than a year in most (17/20 animals). Such a period shorter than a year has been observed in golden-mantled ground squirrels kept in the cold with a 12 hr LD photoperiod (Pengelley and Asmundson, 1974). Thus, in chipmunks, hibernation is strictly controlled by an individual circannual rhythm throughout life under constant cold conditions. Interestingly, the timing and duration of hibernation is little affected by aging under laboratory conditions.

Circannual Regulation of HP Complex (HPc) Correlates with Hibernation

As the results of a previous study showed that HPc levels in the blood were markedly decreased during hibernation (Kondo and Kondo, 1992a), the relationship between HPc changes in the blood and circannual hibernation rhythms was examined throughout the entire life of the animal. HPc levels in blood collected monthly from the above 27 animals kept in the cold and dark were analyzed. In 20 animals exhibiting hibernation rhythm, the HPc concentration in the blood (479 ± 38 nM; n = 13) started to decrease prior to the onset of and remained low during hibernation (76 ± 19 nM; n = 13), following which hibernation was terminated with an increase in HPc (Figure 2C). Such an association between HPc and hibernation was sustained throughout their lives. However, the seven animals that never underwent hibernation did not exhibit an HP rhythm even though HPc was present at normal levels in the blood (512 ± 56 nM; n = 7; Figures 2D and 2E). Thus, the regulation of HPc in the blood appears to correlate with circannually controlled hibernation throughout life.

Circannual Regulation of HPc Is Independent of Tb Changes

In order to clarify whether a decrease in HPc that correlates with hibernation is due to low Tb during hibernation or not, HP rhythm in the blood was examined in animals in which a decreased Tb was prevented by keeping them under conditions of constant warmth (23°C) and a 12 hr LD cycle. Among 29 animals in which a shallow torpor was not detected (see Experimental Procedures), 22 generated circannual HP rhythms (Figure 3A) while the remaining seven had no HP rhythm, like the animals unable to hibernate (see Figure 2D). The mean period of HP rhythm between peaks detected by Western blotting of blood collected monthly, which corresponds to that of hibernation rhythm, was 10 months (Figure 3B). The maximum and minimum periods were 13 and 6 months, respectively. These rhythm characteristics were similar to those in animals with a low Tb during hibernation season, suggesting that HPc is regulated by endogenous circannual rhythms responsible for hibernation and not by changes in Tb and/or environment and that the timing of this rhythm may be insensitive to Tb changes.

To examine whether the physiological state during HPc reduction allows organisms to lower Tb as hibernating animals do, animals with or without decreased HPc levels were exposed to cold conditions (5°C). Whenever a circannual decrease in HPc in the blood was attained, Tb was lowered within a few days (1.43 ± 0.25 days; n = 10) after the cold exposure (Figure 3C). However, the cold conditions never lowered Tb without decreasing HPc. In fact, a few animals that did not generate HP rhythm throughout their life underwent neither low Tb nor a decrease in HPc even by prolonged exposure to cold and died within a few years (data not shown). These results indicate that only during regulated reduction of HPc did the physiological state allow the organisms to survive low Tb. The timing