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Central vasopressin infusion prevents hibernation in the European hamster (*Cricetus cricetus*)

(temperature regulation/seasonal variation/gonadal hormones/lateral septum)

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ABSTRACT **The amount of immunocytochemically detectable vasopressin in the brain of the European hamster (*Cricetus cricetus*) shows a seasonal variation; i.e., dense vasopressin immunoreactivity is present in the lateral septum during summer but is absent in autumn and winter [Buijs, R. M., Pévet, P., Masson-Pévet, M., Pool, C. W., De Vries, G. J., Canguilhem, B. & Vivien-Roels, B. (1986) *Brain Res.* 371, 193–196]. In the winter period the European hamster hibernates. Since vasopressin in the lateral septum is known to be involved in the control of body temperature, we investigated whether infusion of vasopressin in the lateral septum during autumn–winter could influence hypothermic patterns normally seen in hibernating animals. Hamsters whose lateral septum was infused with vasopressin showed almost no periods of hypothermia, whereas hamsters treated with control infusions displayed a normal hibernation pattern. The results indicate that persistence of vasopressin release in the lateral septum of the European hamster during winter can prevent hibernation.**

Vasopressin-containing fibers in the brain have been shown to originate from cell bodies situated in several nuclei, such as the paraventricular hypothalamic nucleus, the suprachiasmatic nucleus, and the bed nucleus of the stria terminalis (BST) (1–5). The amount of vasopressin in fibers originating from the BST is dependent on the level of circulating testosterone: following castration there is a disappearance of immunostaining in vasopressin neurons in the BST as well as in vasopressin fibers in the lateral septum (LS) and the lateral habenula, while no changes in density of vasopressin labeling are seen in other brain regions (6, 7). To investigate the physiological significance of this vasopressin system we selected the European hamster (*Cricetus cricetus*), which under natural conditions displays seasonally changing levels of plasma testosterone. In this animal a seasonal variation in immunocytochemical vasopressin labeling in the brain is seen as well. From September to February, when circulating testosterone levels are low, almost no vasopressin can be detected in the projection areas of the BST (such as the LS), while in May, when plasma testosterone has reached high levels, dense vasopressin staining is obtained (8).

The changes in vasopressin labeling in the brain of the European hamster occur during a period in which there is an adaptation of several endocrine functions to enable the animal to cope with the changing environmental conditions (9). The most striking feature in the strategy of increasing the change for survival during winter is the expression of hibernation. This phenomenon is characterized by regularly occurring periods of hypothermia (10). The demonstration that vasopressin in the LS of several species is involved in the maintenance of a normal body temperature (11–14) led to the hypothesis that the observed disappearance of vasopressin immunoreactivity in the LS of the European hamster during winter could be implicated in the process of hibernation.

In a preliminary experiment in autumn, sexually inactive hamsters were implanted subcutaneously with a 20-mm Silastic capsule filled with testosterone to elevate plasma testosterone levels. It was investigated whether this treatment affected vasopressin immunoreactivity in the LS and the expression of hibernation. In the subsequent experiment, during autumn–winter, a continuous vasopressin release was established by prolonged infusion of this peptide in the LS. At the same time body temperature was registered to evaluate the effect of this vasopressin administration on the occurrence of hibernation.

MATERIALS AND METHODS

Sixty-six male European hamsters, caught during spring in the field near Strasbourg and kept under natural conditions of light and temperature, were used for this study. In the first experiment, at the beginning of their normal hibernation period, 14 animals were anesthetized with ether to receive a subcutaneous implant of a 20-mm Silastic capsule filled with testosterone (5 mg; Sigma) ($n = 7$) or an empty capsule ($n = 7$). Subsequently, the animals were transferred to a climate chamber with a temperature of 7 ± 1°C. The hamsters were submitted to a photoperiodic schedule of 10 hr of light and 14 hr of dark (light on from 0800 to 1800, provided by fluorescent strip lights yielding 400 lux at the bottom of the cage). The animals were tested for hibernation three times a week for up to 4 weeks, as determined by observation of the respiratory rate and reaction to sensory stimuli. Specifically, hibernating European hamsters breathe 5–10 times per minute and do not react to a touch on their skin. At the end of 4 weeks some of the testosterone-implanted animals were perfused and stained for vasopressin as described (8).

In the second experiment, 40 European hamsters were anesthetized by means of pentobarbital (Nembutal, 1 ml/kg of body weight, intraperitoneally) for bilateral implantation of an indwelling stainless steel cannula (o.d., 0.4 mm; i.d., 0.2 mm) in the LS (incisor bar 0; 2.2 mm anterior to bregma; 1.0 mm lateral to midline; 7.0 mm below skull surface). This cannula was connected via a polyethylene tube (Biotrol,

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Abbreviations: BST, bed nucleus of the stria terminalis; LS, lateral septum.

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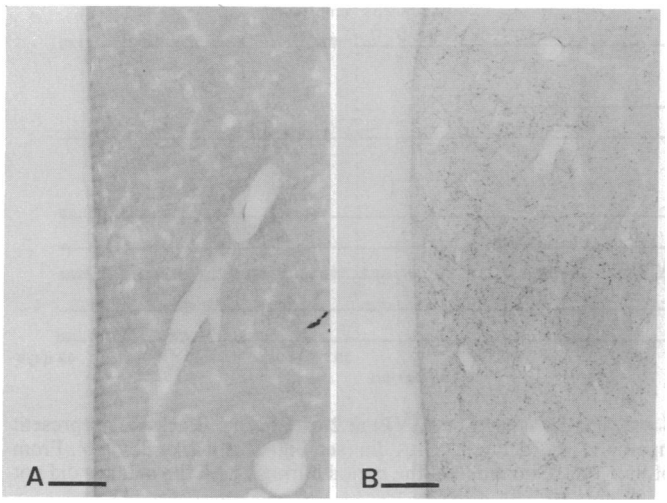

FIG. 1. Photomicrographs of transverse sections through the LS of the European hamster stained for vasopressin. (*A*) LS of a hamster perfused 4 weeks after subcutaneous implantation of an empty Silastic capsule. No vasopressin immunoreactivity is seen. (*B*) LS of a hamster perfused 4 weeks after subcutaneous implantation of a 20-mm testosterone-filled Silastic capsule. Dense vasopressin labeling can be observed. (Bars = 50 μm.)

Paris) to a subcutaneously placed Alzet osmotic minipump (type 2ML4, Alza) filled with Ringer solution containing [Arg^8]vasopressin (Sigma; 1 or 2 ng per 2.6 μl) or with Ringer solution alone. To serve as further controls a group of 5 animals underwent the same operation but did not receive any infusion in the septum, while in another group, consisting of 7 hamsters, only the osmotic minipump filled with Ringer solution containing vasopressin (2 ng/2.6 μl) was placed, without implantation of a septum cannula. In addition, in all the septum-implanted animals a temperature transmitter (model V, Mini-Mitter, Sunriver, OR) was placed intraperitoneally. After the operation the hamsters were transported to a separate climate room with a similar ambient temperature and photoperiodic schedule as described above. Each cage contained a wooden sleeping box and straw for nesting material. Food and water were available ad libitum.

All experiments were started at the beginning of November, the time at which European hamsters normally enter hibernation. For 1 month, 1 or 2 ng of vasopressin in 2.6 μl of Ringer solution per hour (or Ringer solution alone) was infused bilaterally into the septum. To extend the limited infusion time,[§] the pumps were replaced by new ones for another infusion period.

Of the 52 hamsters used in the second experiment, 10 died during the experiment. Together with 12 animals in which greatly enlarged ventricles or inflammatory foci in the septum were seen after termination of the experiment, they were excluded from the results.

RESULTS

Observation of the testosterone-implanted hamsters revealed that administration of this gonadal hormone results in complete prevention of hibernation. None of these animals showed hypothermia during the 4 weeks of observation. The plasma testosterone level obtained by subcutaneous implantation of 20-mm testosterone-filled Silastic capsules varied between 2.1 and 4.4 ng/ml, which is slightly below the values seen in sexually active animals (8). In contrast, the hamsters that had received a sham implant started hibernation 3 days after placement in the climate room. Until the end of the experiment, at each observation at least five of the seven control animals displayed deep hypothermia.

Immunocytochemical staining for vasopressin showed dense labeling in the LS of European hamsters that had a testosterone capsule implanted for 4 weeks, whereas no vasopressin-immunoreactive fibers could be detected in this brain region in control animals (Fig. 1).

The appearance of hypothermic bouts could be registered accurately by means of the temperature transmitters (Fig. 2). Bilateral infusion of the LS with vasopressin at 2 ng/hr resulted in nearly complete inhibition of hypothermia during the first infusion period (Fig. 3). In contrast, control animals infused with Ringer solution during the same period demonstrated frequent bouts of hypothermia [Fig. 4; 218.4 ± 39.7 hr (n = 5) vs. 16.5 ± 16.5 hr (n = 4) in hibernation for vasopressin-infused animals; mean ± SEM; $P < 0.01$, Student's t test]. No difference in time spent in hypothermia was seen between the group receiving Ringer solution and noninfused control animals [Fig. 4; 218.4 ± 39.7 hr (n = 5) vs. 263 ± 52.7 hr (n = 5)]. Because of dysfunctioning of the temperature transmitters during the second infusion period, only two of the vasopressin-infused animals were successfully recorded until the end of the

[§]The Alzet osmotic minipump type 2ML4 delivers at a constant pumping rate of 2.59 ± 0.1 μl/hr over a period of 28 days *in vitro* at 37 ± 0.5°C. After this period the pumping rate becomes unpredictable and falls rapidly to zero, leaving a residual volume of ≈315 μl in the pump (from specifications for Alzet osmotic minipumps; Alza).

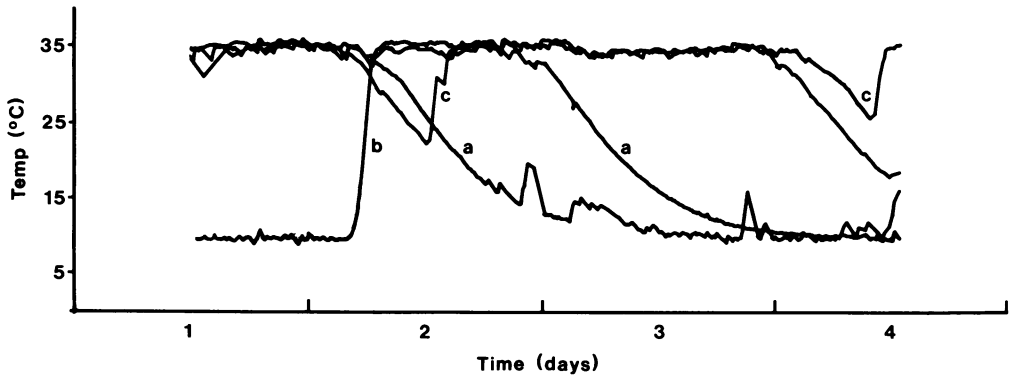

FIG. 2. Body temperature of four hamsters over a period of 3 days. The pulses generated by the intraperitoneally implanted transmitters were registered by antennas placed beneath each cage. With the use of a computer program developed in our laboratory, pulses over a period of 10 min were stored as one point. After 3–4 days the data were printed out as shown in this figure. A slow decrease in body temperature indicates entrance into a period of hypothermia (a), whereas the end of hypothermia is characterized by a rapid increase in temperature (b). On some occasions temperature dropped but soon returned to normal levels (c). This feature was interpreted as an attempt to enter hypothermia and was called a dip. In some cases the dip reached 15°C (not shown).

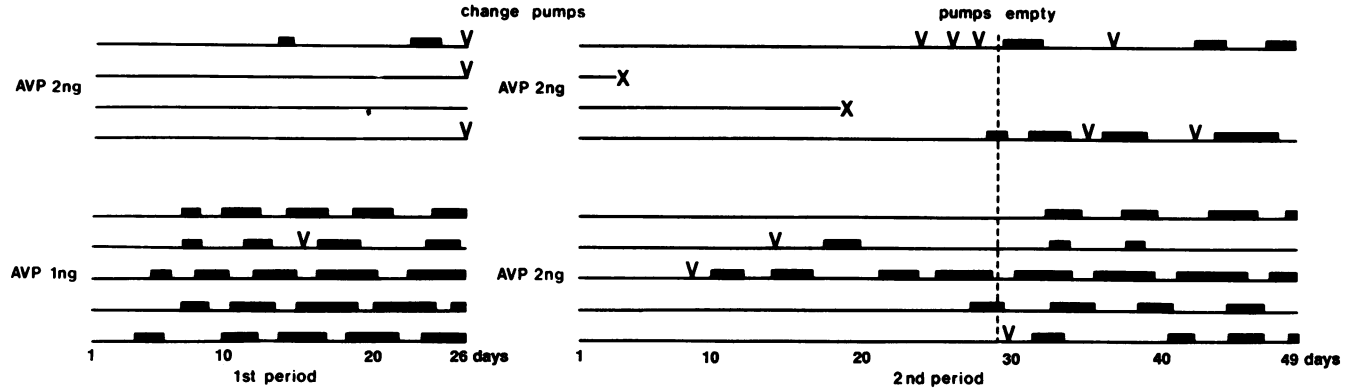

FIG. 3. Hibernation patterns of animals infused bilaterally in the LS with [Arg^8]vasopressin (AVP) at 2 or 1 ng/hr. Black bars represent periods of hypothermia, which were defined as times when the body temperature was below 15°C. A dip (see Fig. 2) is marked by a V. From left to right: the first and the second period of infusion and, to the right of the vertical dotted line, the period during which the animals did not receive vasopressin because the osmotic minipumps were empty.

experiment. They showed no hibernation until the osmotic minipumps were emptied. At that time both animals started to hibernate and resumed a normal pattern of hypothermia.

In an attempt to establish a dose-dependent decrease in frequency and/or duration of the bouts of hypothermia, in another group of animals vasopressin was infused into the septum at only 1 ng/hr. In contrast to the marked effect obtained with 2 ng/hr, vasopressin at 1 ng/hr appeared to have no influence on the pattern of hibernation (Fig. 3). All animals exhibited regularly occurring bouts of hypothermia, comparable to the animals receiving a Ringer infusion. However, administration of vasopressin at 2 ng/hr to the same animals after change of the pumps resulted in marked reduction of the time spent in hypothermia. Four out of five hamsters did not resume hibernation until the pumps were empty, while one hamster exhibited the same pattern of hypothermia as had been seen with administration of vasopressin at 1 ng/hr during the first period. The data from all animals infused with vasopressin at 2 ng/hr during the second period (Fig. 3) indicate that bilateral infusion of 2 ng of vasopressin per hr in the LS reduces significantly the time spent in hypothermia [53.7 ± 36 hr (n = 7) with vasopressin infusion as compared to 200.2 ± 50.8 hr (n = 5) in the case of Ringer infusion; $P < 0.05$, Student's t test]. Until the end of the experiment the control animals infused with Ringer solution continued to show a pattern of hibernation comparable to that of the noninfused controls.

Because of a lateral displacement of the cannula, in a number of animals an infusion was given either in the lateral ventricle or in the caudate putamen on one side, and in the medial septum on the other side. Hamsters infused with 2 ng of vasopressin per hr in these brain regions displayed a hibernation pattern comparable to that seen in Ringer-infused animals [Fig. 4; 195.5 ± 46 hr (n = 4) vs. 200.2 ± 50.8 hr (n = 5) in hypothermia]. Hamsters that received vasopressin into the peripheral circulation from subcutaneously placed osmotic minipumps showed no apparent disruption of hibernation either. Until 28 days after onset of the infusion, at each observation five out of a total of seven hamsters were found to be in hypothermia.

DISCUSSION

The present study shows that infusion of vasopressin in the LS of European hamsters during autumn–winter leads to a disruption of the process of hibernation. The lack of effect when this peptide is infused in other brain regions indicates that nonspecific effects of indwelling cannulas delivering vasopressin do not account for the observed effect of this peptide on hibernation. The results demonstrate that the

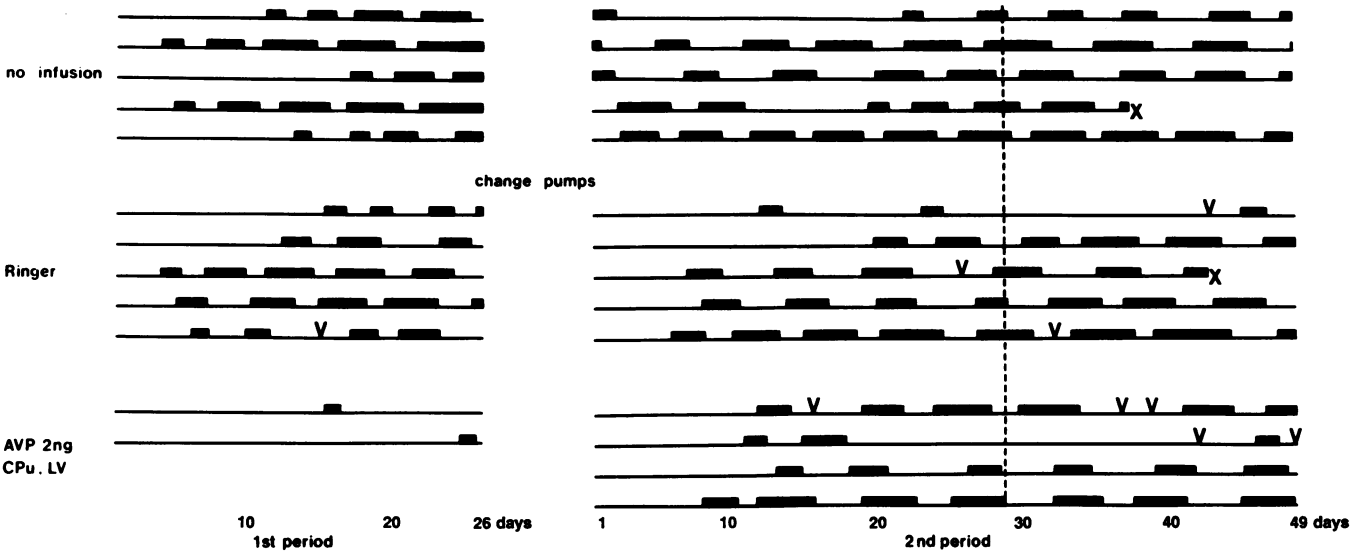

FIG. 4. Hibernation patterns of control animals that received no infusion, a bilateral infusion of Ringer solution, or a unilateral infusion of [Arg^8]vasopressin (AVP, 2 ng/hr) in the medial septum with a contralateral infusion in the caudate putamen (CPu) or lateral ventricle (LV). See legend to Fig. 3 for further explanation.

effect of vasopressin is exercised by a specific action on the LS.

The appearance of bouts of hypothermia directly after emptying of the pumps in most of the vasopressin-infused animals suggests that at that time vasopressin was still the only factor preventing hibernation. That the long duration of infusion did not affect the ability to hibernate can be concluded from the control animals infused with Ringer solution. Until the end of the experiment these hamsters showed a hibernation pattern similar to that seen in the noninfused hamsters.

The data show a dose-dependent effect of vasopressin, since a reduction in the amount of vasopressin infused in the septum resulted in a complete disappearance of its inhibitory effect on hibernation. A similar dosage effect was reported on hormonal vasopressin release for oxytocin infused in the A1 area (15). In both studies a maximal effect was obtained within a narrow micromolar concentration range.

The observation in several species, including hamsters, of a gonadal involution preceding the period of regular occurrence of hypothermia characteristic of hibernation suggested an important role of gonadal hormones in the hibernation process (16). The demonstration in the present study and by Goldman *et al.* (17) that subcutaneous implantation of testosterone-filled Silastic capsules prevents hibernation in European hamsters shows the necessity of low plasma testosterone levels for the expression of hibernation in this species. Moreover, the results indicate that the testosterone-dependent vasopressin neurotransmitter system is an important target for this hormone in its action of preparing the brain for entrance into hibernation. In view of the complex mechanism of hibernation and the widespread presence of binding sites for testosterone in the brain (18, 19) it is evident that other neurotransmitters may play an equally important role in this preparatory process. However, very localized changes in the vasopressin system are able to disturb the process of hibernation; to our knowledge, such an effect has not been reported for another neurotransmitter.

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