BRES 25929

Short Communications

Induction of arousal in hibernating European hamsters (*Cricetus cricetus* L.) by vasopressin infusion in the lateral septum

M.L.H.J. Hermes^{a,b,*}, A. Kalsbeek^{a,b}, R. Kirsch^a, R.M. Buijs^b, P. Pévet^a

^a CNRS, URA 1332, Neurobiologie des fonctions rythmiques et saisonnières, Université Louis Pasteur, 12 rue de l'Université, 67000 Strasbourg, France

^b Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

(Accepted 7 September 1993)

Key words: Immunocytochemistry; Seasonal variation; Testosterone; Hypothermic period

Vasopressin immunostaining in the lateral septum of the European hamster (*Cricetus cricetus* L.) disappears in autumn, at the time of the first appearance of hypothermic periods characteristic to hibernation. Previous results have shown that chronic administration of vasopressin in the lateral septum during winter prevents the expression of hypothermic periods, suggesting a role for this peptide in hibernation. It is now observed that acute infusion of vasopressin, and in 50% of the cases, of a specific vasopressin V₁ receptor agonist, during a hypothermic period results in an immediate termination of hypothermia. Infusion of oxytocin or a vasopressin V₂ receptor agonist were without effect. The results indicate that the seasonal variation in central vasopressin activity, possibly through an interaction with V₁ receptors, may play an important role in the expression of hibernation in the European hamster.

Following the detection of a sex difference in the amount of vasopressin (VP) immunostaining in the central nervous system of the rat⁷, several experimental findings have corroborated the suggestion that the biosynthesis of VP in the rat brain is regulated by plasma testosterone. Briefly, castration of adult male rats resulted in complete disappearance of VP immunoreactivity and VP mRNA expression in neurons in the bed nucleus of the stria terminalis and the medial amygdaloid nucleus, and of VP staining in efferent fibers to the lateral septum (LS). Re-establishment of physiological plasma levels of testosterone restored the normal level of immunoreactivity and hybridization signal^{6,15,16,23}. Functionally, the steroidal regulation of the central VP innervation indicates that this peptidergic system is involved in brain processes that are influenced by gonadal activity, such as sexual behavior (for a review see ref. 5).

Contrary to the laboratory rat, several rodent species living in the wild display endogenous, annual, fluctua-

tions in plasma testosterone levels which induce seasonal changes in the density of central VP immunostaining^{2,10}. This observation implies that central VP might be involved in certain gonadal dependent *seasonal* functions that are not readily expressed in laboratory rats, such as hibernation^{4,9,18}. A possible role of central VP in hibernation was recently indicated by the observation that continuous administration of VP into the LS of European hamsters in winter (when VP immunoreactivity is absent in this brain region) prevents the appearance of hypothermic periods that are characteristic to hibernation¹¹. The results suggest that the disappearance of central VP may be a prerequisite for the expression of hypothermic periods.

Conversely, a coincident decrease in duration of hypothermic periods and gradual increase of central VP immunostaining at the end of winter (Hermes, personal observation) rises the possibility that this peptide may be involved in the termination of hibernation in early spring as well. To extend the evidence for an

* Corresponding author. Present address: Neuroscience Unit, Loeb Research Institute, Ottawa Civic Hospital, 1053 Carling Avenue, Ottawa, Ontario, Canada K1Y 4E9. Fax: (1) (613) 798-0724.

implication of central VP in hibernation, we investigated the influence of infusion of VP into the LS of European hamsters during a hypothermic period.

In early spring, male European hamsters were trapped near Strasbourg (France) and housed individually in cages in the open air. Food and water were available *ad libitum*. At several times during the hibernation season, ranging from early December to early April, a total of 20 hamsters was anesthetized with intraperitoneal sodium pentobarbitone (Nembutal, 1 ml/kg body weight) for bilateral stereotaxic implantation of an in-dwelling stainless steel cannula (o.d. 0.3 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). Each cannula was connected to the lower end of a Y-shaped silicon tubing construction (Dow Corning, Midland, MI, USA) to allow replacement of the solution in the tubing without infusion into the brain. The (four) tubes were led subcutaneously to an interscapular exit and sealed by stainless steel stylets. In later experiments, the tubes were directed out of the

cage across a stainless-steel, articulated protection to facilitate the infusion procedure. Temperature transmitters (model V, Mini-Mitter, Sunriver, OR, USA) were placed intraperitoneally for remote continuous recording of the body temperature, as described previously¹¹. Briefly, signals emitted by the transmitters were received by antennas placed underneath each cage and transferred to individual AM receivers in a nextdoor room. The sum of impulses over a period of 10 min was stored in a computer and, with the use of transmitter specific standards, converted into body temperatures.

Two days after the operation, the hamsters were placed in a climate chamber with a temperature of $7 \pm 1^\circ\text{C}$ and a 10/14 h light-dark cycle (lights on from 08.00 h to 18.00 h). After expression of at least one hypothermic period, infusion experiments were performed during the day using hypothermic animals that had started a hypothermic period two nights earlier. Fifteen min before the start of the experiment the tubing was flushed with a fresh solution and connected

Fig. 1. Photomicrograph showing cannula tract lesions (arrows) in the lateral septum (LS) where infusion of vasopressin and the vasopressin V_1 receptor agonist $[\text{Phe}^2, \text{Orn}^8]\text{VT}$ induced an immediate termination of hypothermia. Bar = 320 μm .

to a Harvard micro-injection pump for a 10 min infusion into each LS of 1.0 μ l of either Ringer solution or Ringer containing 4.6×10^{-6} M of VP or oxytocin (OT; both Sigma chemicals). To determine the receptor type through which a possible effect of VP was mediated, additional infusions were done with compounds that specifically reacted with VP V_1 and V_2 receptors, [Phe², Orn⁸]VT and dDAVP (gifts from Dr. M. Manning), respectively. Since the biological activity of the V_1 agonist at the V_1 receptor is one-third of that of VP at equimolar concentrations, doses of this agonist three times that of VP were administered^{13,14}.

The European hamsters presented in the results displayed a normal hibernation pattern characterized by an occurrence of hypothermic periods with a minimum duration of 3 days alternated with relatively short euthermic intervals (not exceeding 3 days). Incidentally, hypothermic periods of short duration (1–2 days) and prolonged euthermic periods (5 days or more) were observed. A possible correlation between the prolonged euthermic intervals and preceding infusions could not be established, since in several cases experimentation with neighbouring animals might have prevented entrance into a hypothermic period.

Infusion of micromolar concentrations of VP induced arousal from hypothermia in 13 out of 14 hamsters with cannula placements in the anterior two-thirds of the LS (bregma 0.70 mm to 0.20 mm in ref. 17; Fig. 1; Table I). Typically, 20–30 min after termination of the infusion basal frequency of respiration started to increase gradually, rising from an initial 3–9 to 40–50 excursions per minute after 2 h, while at that time also a constant shivering of the animal became obvious. Frequently, no postural movements were seen until 2.5 h after termination of the infusion. The time to complete the temperature increase from 8 ± 1 °C to 36 ± 1 °C was 4.5 ± 0.5 h, similar to that of spontaneous arousals (Fig. 2). Administration of equimolar concentrations of OT (tested in 10 animals) or of Ringer solution (tested in 4 animals) in the LS did not result in arousal from hypothermia (Table I). Infusion of the VP

Fig. 2. Time course of the rise in the body temperature of a hypothermic European hamster following infusion of 4.6×10^{-6} M vasopressin (VP) into the lateral septum.

V_1 receptor agonist [Phe², Orn⁸]VT induced arousal with a similar time course in 4 out of 8 cases, whereas the VP V_2 receptor agonist dDAVP was without effect in 6 hamsters (Table I). In European hamsters (6) which had the cannulas placed in the caudal one-third of the LS (bregma -0.26 mm to -0.40 mm in ref. 17) all compounds were without any effect on the expression of hypothermia (data not shown).

The results show that septal infusion of VP is able, possibly through an interaction with V_1 receptors, to induce a premature and immediate termination of hypothermic periods in hibernating European hamsters. The observation that only the VP V_1 receptor agonist, and not the VP V_2 receptor agonist, was able (albeit partially) to mimic the effect of VP agrees with autoradiographic receptor and electrophysiological data indicating a presence of VP V_1 , and not V_2 , receptors in the LS of the rat^{19,21,24}. Moreover, the present results extend the evidence for functional VP V_1 receptors in the LS by showing that activation of these receptors, next to influencing cellular activity²¹, is also able to induce changes at a systemic neuronal level. It should be mentioned that compensatory changes in VP receptors following disappearance of the ligand (VP) in autumn probably do not play a major role in the present and previous results¹¹, since, in contrast to certain diencephalic nuclei in the Siberian hamster⁸, no apparent influence of gonadal hormones on the density or sensitivity of VP receptors in the LS was observed in rats^{20,22}.

The central events through which VP in the LS induces arousal from hypothermia are unknown. The pronounced decrease in body temperature during hypothermia is the result of a change in multiple (central and peripheral) processes, both occurring before and during the decrease of the body temperature. The disappearance of VP from the LS preceding the actual

TABLE I

Collective data of the response (arousal vs. no arousal) of hypothermic European hamsters following infusion of different substances into the lateral septum

Treatment	No. of animals	Arousal	No arousal
Vasopressin	14	13	1
Oxytocin	10	0	10
Ringer	4	0	4
V_1 agonist	8	4	4
V_2 agonist	6	0	6

appearance of hypothermic periods in autumn suggests that this peptide is implicated in preparatory mechanisms and thus permits a decrease of the body temperature, and is not involved in the regulation of the body temperature per se. Similar conclusions were derived from experiments in rats that showed that central administration of VP did not affect the body temperature by itself but was able to modulate the temperature response to various fever-inducing agents^{1,3,12}.

In conclusion then, the results indicate that the gradual reappearance of VP immunoreactivity in the LS of the European hamster (Hermes, personal observation) induced by the progressive increase in plasma testosterone levels² may be involved in the decrease in duration, and ultimately, permanent termination of hypothermic periods at the end of the hibernation season. Together with previous data showing that VP infusion also prevents entrance into hypothermia, it is suggested that the seasonal change in central VP activity plays an important role in the seasonal expression of hibernation.

The authors thank Prof. J.J. Dreifuss for his kind suggestion for this work, Prof. D.F. Swaab for his helpful comments on the manuscript, Mr. D. Bonn for animal care, and Mr. R. Brenklé and Ms. M. Rijkers for technical assistance. This study was supported by grants from Action Thématique Programée – Centre National de la Recherche Scientifique – 'Europe' and the European Community (Twinning Contract ST2 00 271).

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