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Expression of vasopressin receptors in hamster hypothalamus is sexually dimorphic and dependent upon photoperiod

(autoradiography/oxytocin/sex difference/Siberian hamster/ventral hypothalamus)

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ABSTRACT **The distribution of vasopressin receptors was studied in the brain of a photoperiodic animal, the Siberian hamster. Attention was focused on [^{3}H]vasopressin binding sites located in the hypothalamic ventromedial nucleus, medial tuberal nucleus, and ventral premammillary nucleus in males or females kept in long or short photoperiod conditions. Displacement experiments with structural analogs suggested that vasopressin receptors in the hamster hypothalamus are of the vasopressor (V_1) type. Quantitative data obtained with a gaseous detector of β-particles indicated that in the ventromedial nucleus and in the ventral premammillary nucleus of animals in long photoperiod, the number of β-particles emitted per unit area was significantly greater in males than in females. In the ventromedial hypothalamic nucleus, in both males and females, the number of β-particles emitted was significantly lower in short than in long photoperiod conditions. In the ventral premammillary nucleus, shortening of the photoperiod had a significant effect in reducing the amount of [^{3}H]vasopressin bound in females, but not in males. These data suggest that, in the hamster, the control of the expression of vasopressin receptors differs among various hypothalamic nuclei and may depend on the sex and/or on the level of circulating gonadal steroids.**

In photoperiodic species, the activity of the gonads is modulated by the length of the daily light phase and by its chronicity over weeks. Thus, in spring and summertime—during the long photoperiod (LP)—the Siberian hamster is sexually active, whereas in the short photoperiod (SP)—autumn and winter—its gonads atrophy, and the animals become sexually inactive. At the end of winter, the process is reversed and the gonads increase again in weight (1–3). Circulating gonadal steroids follow a parallel course: their level is lowered following shortening of the photoperiod, and this effect is reversed when the photoperiod increases again (4–8). These physiological modifications may be artificially induced in controlling the length of the photoperiod in animal husbandry (9). In the Siberian hamster, gonadal regression is directly under photoperiodic control: it is not dependent on the temperature or the amount of food available (1, 2, 10, 11).

In both male and female rats, oxytocin-binding sites in the hypothalamic ventromedial nucleus are down-regulated by castration, and they can be restored to precastration level by injections of sexual steroids (12–16). We investigated the distribution of vasopressin- and oxytocin-binding sites in the brain of male and female Siberian hamsters either in LP or SP conditions, using a newly developed gaseous detector of β-particles (17) and film autoradiography. We discovered that in Siberian hamsters, vasopressin receptors rather than oxytocin receptors are present in the ventromedial hypothalamic nucleus and that they are expressed differentially in the male and in the female. Furthermore, the density of the binding was found to be dependent on photoperiod.

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MATERIAL AND METHODS

Animals. Young adult Siberian hamsters (*Phodopus sungorus*) were maintained under either a LP (16/8-hr light/dark cycle) or a SP (8/16-hr light/dark cycle) for 10 weeks. Temperature was set at 20 ± 1°C and humidity at 60–70%. The animals received tap water and food pellets ad libitum.

Fourteen males (9 in LP, 5 in SP) and 11 females (7 in LP, 4 in SP) were used for the autoradiographic localization of vasopressin- and oxytocin-binding sites in the brain. Animals, 5 months old with a body weight of 30–50 g, were decapitated, and the testes were removed and weighed, or the uterus was inspected, to assess the activity of the gonads (2, 18). The brain was frozen in isopentane at −25°C, and 15-μm-thick coronal sections were cut in a cryostat, laid on gelatin/chromalum-coated slides, air-dried, and processed for light microscopic autoradiography.

Autoradiography. Autoradiography of vasopressin-binding sites was performed as described (19) with 1.5 nM [^{3}H]vasopressin as the ligand. Hydroxy[Thr^4,Gly^7]oxytocin, a selective oxytocin agonist (20), was added to the incubation medium to reduce binding of [^{3}H]vasopressin to oxytocin receptors. To assess the amount of nonspecific labeling, adjacent sections were treated in the same conditions, except that the incubation medium contained in addition 10 μM nonradioactive vasopressin. To determine whether the vasopressin-binding sites were of the V_1- or V_2-type, competition experiments were performed on sections containing the ventral hypothalamus. The incubation medium was then supplemented with 150 nM [Phe^2,Orn^8]vasotocin, a V_1 (vasopressor type) receptor agonist in which Orn = ornithine, or 1-deamino-[D-Arg^8]vasopressin (dDAVP), a peptide selective for V_2 (antidiuretic type) receptors (20).

Oxytocin-binding sites were detected by using as ligand 0.05 nM ^{125}I-labeled 8-ornithine/9-tyrosinamide-containing vasotocin (^{125}I-OTAVT) designated 1-deamino-[Cys(CH$_2$)$_5^1$, Tyr(Me)^2, Thr^4, Orn^8, Tyr^9-NH$_2$]vasotocin in which Cys-1 has been deaminated and substituted on the side chain with cyclopentamethylene to form [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*O*-methyltyrosine, 4-threonine, 8-ornithine, 9-tyrosinamide]vasotocin. This compound has also been called d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Tyr(NH$_2$)^9]orni-

Abbreviations: Orn, ornithine; OTAVT, 8-ornithine/9-tyrosinamide-containing vasotocin designated 1-deamino-[Cys(CH$_2$)$_5^1$, Tyr(Me)^2, Thr^4, Orn^8, Tyr^9-NH$_2$]vasotocin in which Cys-1 has been deaminated and substituted on the β-carbon with cyclopentamethylene to form [1-β-mercapto-β,β-cyclopentamethylenepropionic acid, 2-*O*-methyltyrosine, 4-threonine, 8-ornithine, 9-tyrosinamide]vasotocin.

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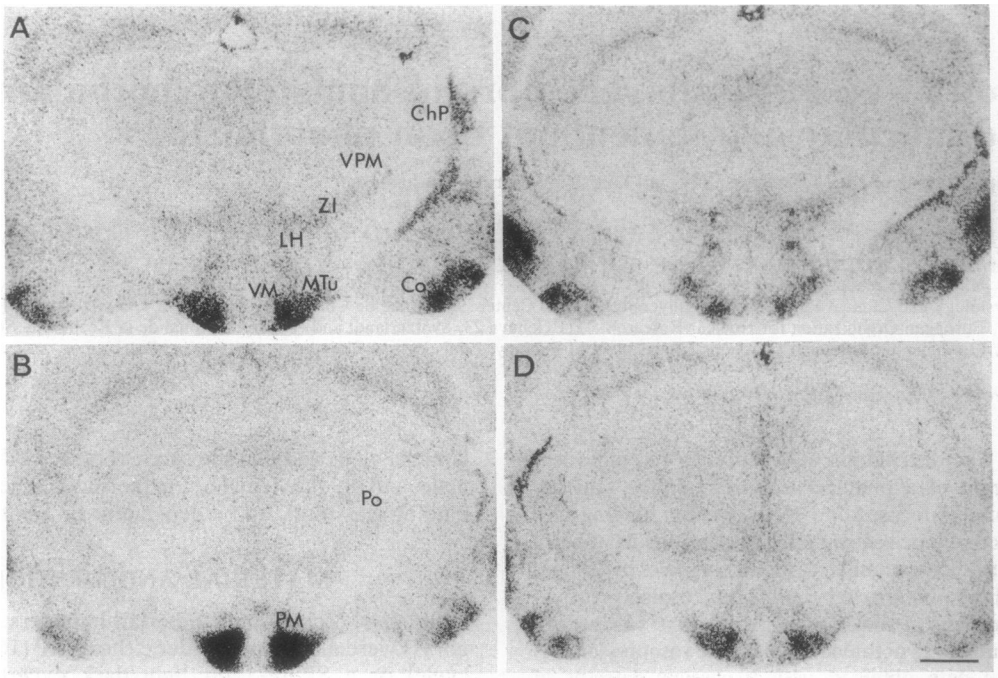

FIG. 1. Autoradiograms of [^{3}H]vasopressin binding in the ventral hypothalamus of a male (*A–B*) and a female (*C–D*) hamster raised in LP conditions (exposure time, 10 weeks). (*A* and *C*) Labeling in the caudal ventromedial hypothalamic (VM) and medial tuberal (MTu) nuclei. (*B* and *D*) Labeling at the level of the premammillary nucleus (PM). ChP, choroid plexus; Co, cortical amygdaloid nucleus; LH, lateral hypothalamic area; Po, posterior thalamic nuclear group; ZI, zona incerta; VPM, ventral posteromedial thalamic nucleus. (Bar = 1 mm.)

thine vasotocin (21, 22). The procedure used has been described (13). Nonspecific binding was evaluated by incubating adjacent sections with medium containing in addition 1 μM oxytocin.

The sections were placed in contact with a tritium-sensitive film (LKB Ultrofilm) in an x-ray cassette for 10 weeks at 4°C. Slides treated with ^{125}I-OTAVT were apposed to Amersham Hyperfilm-βmax film for 3–5 days. The films were developed in Kodak D19 for 5 min, and the sections were stained with cresyl violet. Brain structures were identified and named according to atlases of hamster and rat brains (23, 24).

Quantitative Analysis. Quantification of brain sections exposed to [^{3}H]vasopressin was carried out by using a gaseous detector of β-particles. The procedure described previously was simplified, as gold coating was no longer used (17). Data from slides placed in the gas-chamber detector were collected for 13 hr. Each β-particle generated a light spot of 1-mm diameter, which was read by a CCD (charged coupled device) camera. The number of counts per pixel was recorded with the coordinate of the center of gravity of the corresponding light spot. After binning of the counts, a map of sections was generated and stored as an Ascii file. These data were

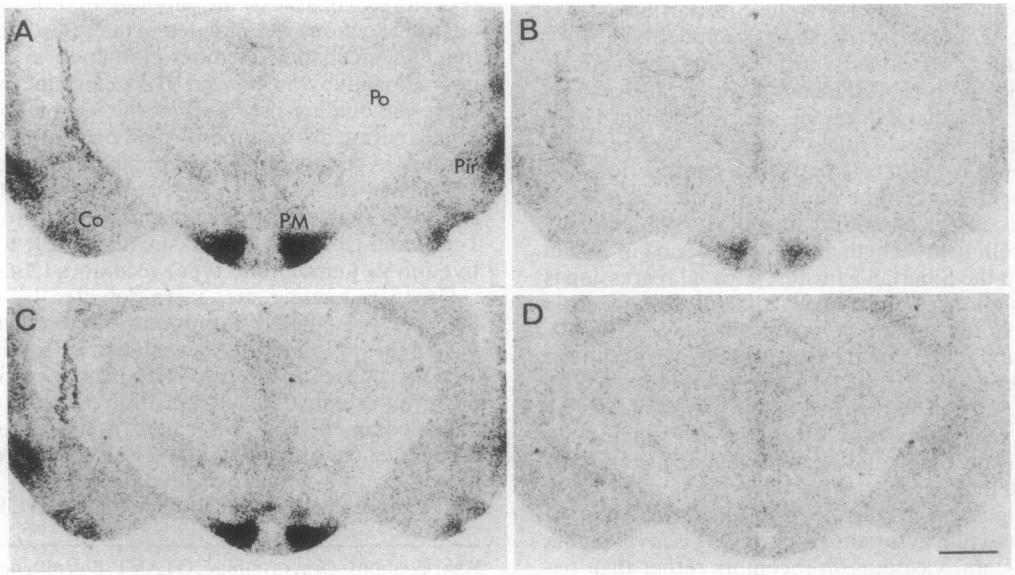

FIG. 2. Displacement of [^{3}H]vasopressin binding by synthetic structural analogs in a male hamster raised in LP conditions. (*A–D*) Autoradiograms from adjacent sections. [^{3}H]Vasopressin binding is shown in the ventral premammillary nucleus (PM), piriform cortex (Pir), and cortical amygdaloid nucleus (Co) (*A*). This binding was displaced by 150 nM of [Phe^2,Orn^8]VT (*B*). In contrast, 150 nM dDAVP was unable to displace the [^{3}H]vasopressin binding (*C*). All specific binding was displaced by 10 μM nonradioactive vasopressin (*D*). Po, posterior thalamic nuclear group. (Bar = 1 mm.)

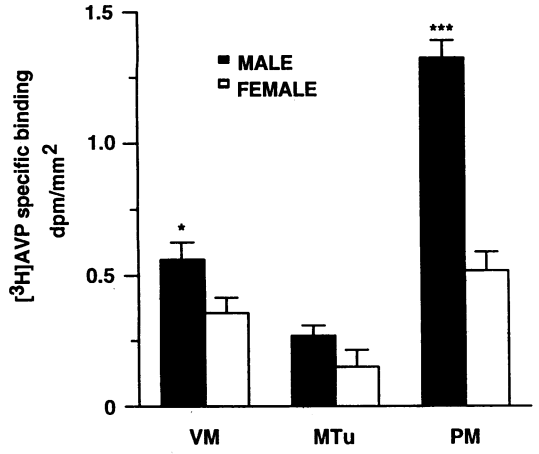

FIG. 3. Quantification of the 8-arginine [^{3}H]vasopressin ([^{3}H]AVP) binding in the hypothalamus of male and female hamster raised in the LP. The number of β-particles emitted per unit area were counted in 13 hr on brain sections containing the ventromedial (VM), medial tuberal (MTu), and premammillary (PM) nuclei and were expressed as dpm/mm^2. *, $P < 0.05$; ***, $P < 0.001$ when comparing males and females.

imported and analyzed in the Semper image analysis language (Synoptics, Cambridge, U.K.). The tritium activity in a given nucleus of the brain section was measured as counts/mm^2 per 13 hr and converted into dpm/mm^2. For each animal, measurements were carried out in the hypothalamus on both sides. Since for the same structure the values were found to be similar on both sides, we pooled them. The nonspecific binding of [^{3}H]vasopressin was measured in the same structures in displacement experiments. It amounted to 0.34 ± 0.028 dpm/mm^2 (mean ± SEM) and was similar in the various structures explored. This value was subtracted to yield the specific binding data shown in Figs. 3 and 6. The unpaired Student *t*-test was used for the statistics.

Chemicals. Tritiated 8-arginine vasopressin ([^{3}H]vasopressin; specific activity, 69 Ci/mmol; 1 Ci = 37 GBq) was purchased from DuPont/NEN. It was purified by HPLC and affinity chromatography on neurophysin bound to Sepharose-4B. The oxytocin antagonist OTAVT, the selective oxytocin agonist hydroxy[Thr^4,Gly^7]oxytocin, the vasopressin structural analogs [Phe^2,Orn^8]vasotocin and dDAVP were provided by M. M. Manning (Department of Biochemistry, Medical College of Ohio, Toledo). OTAVT was radioiodinated on Tyr-9 to a specific activity of ≈2000 Ci/mmol.

RESULTS

Localization of [^{3}H]Vasopressin Binding Sites in the Brain. In male hamsters maintained in the LP, specific [^{3}H]vasopressin binding sites were detected in the following regions of the brain: the olfactory bulb, the Calleja islands, the lateral septum, the bed nucleus of the stria terminalis, the fundus striati, the thalamic paraventricular nucleus, the cortical amygdaloid nucleus, the subiculum, the mesencephalic central grey, the tegmentum, the cerebral cortex (i.e., orbital, cingulate, piriform, and insular cortex), and the choroid plexus. In the hypothalamus, [^{3}H]vasopressin binding was detected in the lateral hypothalamic area, the zona incerta, and the ventral hypothalamus (Figs. 1*A*, 1*B*, and 2*A*). Along the anteroposterior axis, [^{3}H]vasopressin binding was present in the ventral hypothalamus from the middle of the ventromedial hypothalamic nucleus to the caudal part of the ventral premammillary nucleus, an area that encompassed the medial tuberal nucleus, the ventral premammillary nucleus, and the transitional zone between them. [^{3}H]Vasopressin binding was most intense in the caudal ventromedial hypothalamic nucleus (Fig. 1*A*) and in the ventral premammillary nucleus (Figs. 1*B* and 2*A*).

Characterization of Binding Sites in the Ventral Hypothalamus. The binding in the ventral hypothalamus was displaced partially by 150 nM [Phe^2,Orn^8]vasotocin (Fig. 2*B*) but was unaffected by 150 nM of dDAVP (Fig. 2*C*). Incubation with unlabeled 10 μM vasopressin displaced completely the [^{3}H]vasopressin bound (Fig. 2*D*).

In all animals, strong, specific ^{125}I-OTAVT binding was observed in the Calleja islands, the bed nucleus of the stria terminalis, and the central amygdaloid nucleus (not illustrated). In contrast, very light binding of ^{125}I-OTAVT was

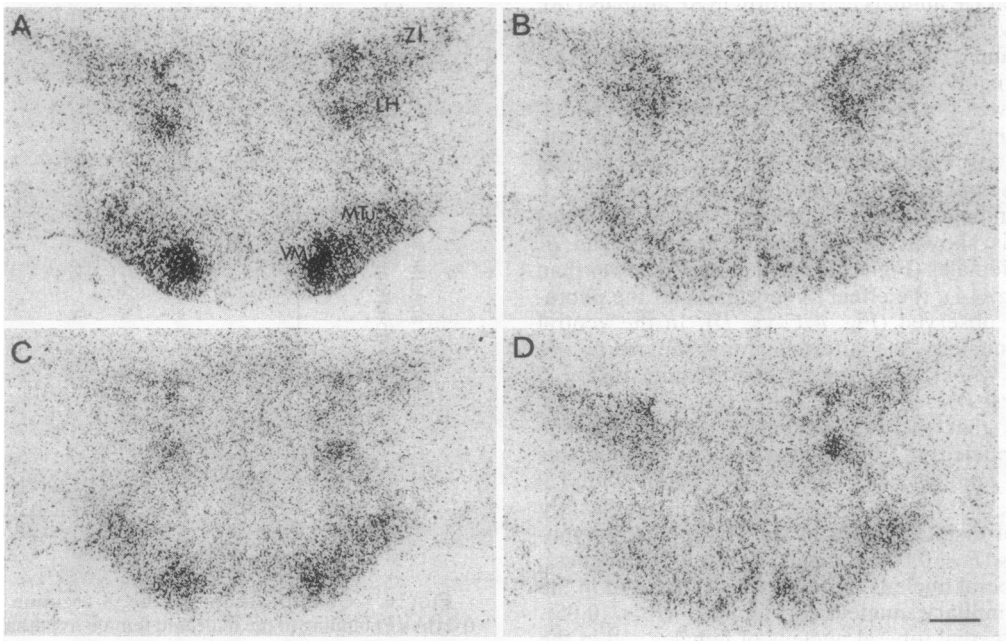

FIG. 4. Autoradiograms of [^{3}H]vasopressin binding in the ventromedial hypothalamic (VM) and medial tuberal (MTu) nuclei. The sexually dimorphic expression of [^{3}H]vasopressin binding sites is dependent of the photoperiod length in male (*A* and *B*) and female (*C* and *D*) hamsters. (*A* and *C*) LP. (*B* and *D*) SP. LH, lateral hypothalamic area; ZI, zona incerta. (Bar = 500 μm.)

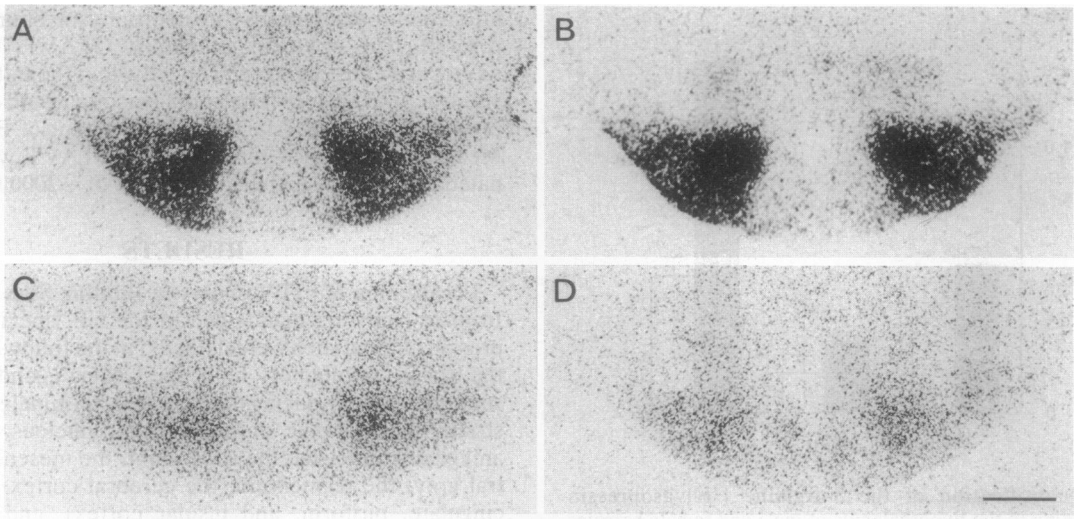

FIG. 5. Autoradiograms showing [^{3}H]vasopressin binding sites in the premammillary nucleus. (*A*) LP male. (*B*) SP male. (*C*) LP female. (*D*) SP female. (Bar = 400 μm.)

detected in the ventromedial hypothalamic nucleus in only a few animals.

Sexual Dimorphism. The counting of β-particles allowed a direct quantification of the density of [^{3}H]vasopressin binding in the ventral hypothalamus (Fig. 3). The number of disintegrations in the female ventromedial nucleus was significantly lower than in the males ($P < 0.05$ unpaired). In the medial tuberal nucleus, it was not statistically different between males and females. The activity measured in the female ventral premammillary nucleus was much lower than in the male nucleus, the difference being statistically highly significant ($P < 0.001$). Indeed, while the labeling was found to be similar in most brain regions in both males and females, a sexual difference was evident in the ventromedial, medial tuberal, and ventral premammillary hypothalamic nuclei, where the intensity of the autoradiographic labeling was lower in the female than in the male (compare in Fig. 1 *A* with *C* and *B* with *D*, in Fig. 4 *A* with *C*, and in Fig. 5 *A* with *C*).

Effects of the Length of the Photoperiod. The uterus was well developed in LP animals and filiform in SP animals (18). Pairs of testes weighed 748 ± 178 mg (mean ± SEM; $n = 9$) in LP animals and 37 ± 7 mg ($n = 5$) in SP animals. This difference was highly significant ($P < 0.001$).

A difference was observed in the ventromedial hypothalamic nucleus and the medial tuberal nucleus between animals in LP and SP conditions. In both sexes, the binding observed in these nuclei was lower in SP animals than in LP animals. The binding observed in these nuclei in LP males and SP males is shown respectively in Fig. 4 *A* and *B*. Although in LP females (Fig. 4*C*) the labeling was lower than in LP males (Fig. 4*A*), the effect of shortening of the photoperiod was still observed (Fig. 4 *C* vs. *D*). In the ventral premammillary nucleus, the autoradiograms showed no obvious difference when comparing LP and SP animals (Fig. 5 *A* vs. *B* and *C* vs. *D*).

Fig. 6 shows that the sex difference observed in the premammillary nucleus in LP animals was also evident in this structure in SP animals ($P < 0.001$). Compared with LP animals (Fig. 3), SP animals (Fig. 6) showed a significantly lower number of disintegrations in the ventromedial hypothalamic nucleus ($P < 0.001$ in males, $P < 0.05$ in females), in the medial tuberal nucleus of males ($P < 0.05$), and in the ventral premammillary nucleus of females ($P < 0.05$). Whereas in the medial tuberal nucleus of females and in the premammillary nucleus of males the trend was also a reduction of the number of disintegrations, the differences between LP and SP animals were not statistically significant.

DISCUSSION

Our study of [^{3}H]vasopressin binding sites in a photoperiodic animal, the Siberian hamster, led to two observations of particular interest. First, unlike what is seen in the rat and in the guinea pig, vasopressin receptors, rather than oxytocin receptors, are present in the ventromedial hypothalamic nucleus of the Siberian hamster. Second, the expression of vasopressin receptors in the ventral hypothalamus of the hamster is sexually dimorphic and under the control of the photoperiod.

[^{3}H]Vasopressin binding sites detected in the brain of the Siberian hamster are specific: the binding was suppressed in the presence of an excess of unlabeled vasopressin. Furthermore, the partial displacement of [^{3}H]vasopressin binding by a V_1 agonist, but not by a V_2 agonist, suggests that in the brain of the Siberian hamster, the vasopressin binding sites are of the V_1 subtype, as they are in other mammalian brains (19, 25).

The presence of specific vasopressin binding sites in the ventral hypothalamus of the Siberian hamster is somewhat surprising. Indeed, oxytocin receptors, but not vasopressin receptors, are present in the rat and guinea-pig ventromedial

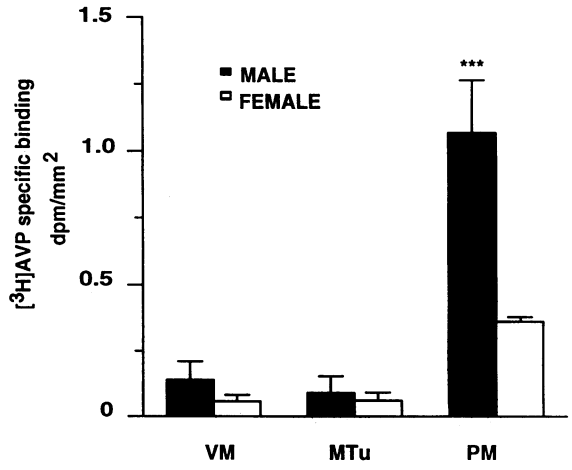

FIG. 6. Quantification of the 8-arginine [^{3}H]vasopressin ([^{3}H]AVP) binding in the male and female hypothalamus of hamsters raised in SP conditions. The number of β-particles emitted (dpm/mm^2) was counted on brain sections containing the ventromedial (VM), medial tuberal (MTu), and premammillary (PM) nuclei. ***, $P < 0.001$, males vs. females.

hypothalamic nucleus (12, 25, 26). In the rat, intracerebral injection of oxytocin has been shown to influence reproductive behaviors (27–30). Our findings suggest that vasopressin, rather than oxytocin, might affect reproductive behaviors in the Siberian hamster and play, in this species, the role exerted by oxytocin in the rat (31–37).

An influence of the photoperiod on the vasopressinergic innervation of the brain has been described (4) in the male European hamster, where the density of immunoreactive axones in the lateral septum is lower in SP than in LP conditions. A similar difference was observed in the male rat, where castration has been shown to reduce the amount of vasopressin immunoreactivity in the lateral septum (38). However the present study shows (*i*) a sexually dimorphic expression of neurohypophysial peptide receptors and (*ii*) a steroid dependency of the expression vasopressin receptors. The sexual dimorphism detected in the ventromedial hypothalamic nucleus was affected by the length of the photoperiod and thus probably by gonadal steroids (2, 5, 39). Indeed, in hamsters, the laterocaudal part of the ventromedial hypothalamic nucleus as well as the adjacent tuberal and ventral premammillary nuclei contain numerous estrogen-concentrating cells (40, 41). Thus, the expression of the vasopressin receptor gene may be regulated by sexual steroids via hormone-responsive elements (42). The sexually dimorphic expression of vasopressin receptors in the ventral hypothalamus may depend upon the conversion of androgens into 17β-estradiol, either around the time of birth (43, 44) or at puberty (13). Thus, the effect of neonatal castration should be examined in the future. In the Siberian hamster, the appearance of puberty may be delayed in raising the young in SP conditions (9). This affords the possibility of investigating the influence of the photoperiod on the development of the brain.

The number of disintegrations in the ventromedial hypothalamic nucleus and in the medial tuberal nucleus was much lower in SP animals than in LP animals. In the ventromedial hypothalamic nucleus, the sex difference in vasopressin binding seen in LP animals was lessened in SP animals, possibly because of the overall reduction of vasopressin binding in SP animals. In contrast, in the premammillary nucleus, a statistically significant sex difference was still observed in SP animals. This suggests that most of the vasopressin receptors in the premammillary nucleus, although expressed differentially in males and females, are not dependent on sexual steroids. Moreover, the control of the expression of vasopressin receptors apparently differs amongst the ventromedial and the premammillary nuclei.

The functional significance of the sexual dimorphism in vasopressin binding in the hypothalamus of the Siberian hamster remains unknown. Does the difference in [^{3}H]vasopressin binding between males and females reflect a difference in neuronal sensitivity to neurohypophysial peptides? Does it explain behavioral differences between males and females?

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