Journal of Neuroendocrinology, 2000, Vol. 12, 111–120

Hypothalamic Vasopressin Gene Expression Increases in Both Males and Females Postpartum in a Biparental Rodent

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Key words: paraventricular nucleus, supraoptic nucleus, ventromedial hypothalamic nucleus, monogamy.

Abstract

In previous studies, the closely related neuropeptide hormones oxytocin and vasopressin have been implicated in the central mediation of parental behaviour. Several studies in rats and sheep have demonstrated a role for oxytocin in the initiation of maternal behaviour. Recently, a few studies in a biparental species, the prairie vole (*Microxytocinus ochrogaster*) have suggested that vasopressin is important for paternal care. The present study investigated this latter possibility by measuring changes in vasopressin and oxytocin hypothalamic gene expression 1 day and 6 days following parturition in prairie voles which show paternal care and in montane voles (*M. montanus*) which lack paternal care. In prairie voles, vasopressin gene expression increased in both males and females postpartum, relative to sexually naive controls. In the non-paternal montane vole, no change in vasopressin gene expression was observed in either sex. In contrast to this species difference in vasopressin gene expression, hypothalamic oxytocin gene expression increased in both prairie and montane vole females, but not in males of either species. To augment measures of gene expression, we assessed vasopressin (V1a) and oxytocin receptor binding in both species. Although forebrain vasopressin V1a receptor binding was not altered following parturition in either species, oxytocin receptor binding increased in the ventromedial nucleus of the hypothalamus in females, but not males, in both prairie and montane voles. In summary, vasopressin gene expression increases in both males and females postpartum in a biparental species and oxytocin gene expression and receptor binding increase selectively in females. These results are consistent with earlier reports of a role for vasopressin in paternal care and for oxytocin in maternal behaviour.

The neuropeptides oxytocin and vasopressin are primarily synthesized in the hypothalamus, especially the paraventricular nucleus and supraoptic nucleus, and transported to the posterior pituitary where both are released into the circulation. Oxytocin is important in reproductively related events such as uterine contraction at parturition (1) and milk ejection during lactation (2), whereas vasopressin regulates plasma osmolality and vascular tone (3, 4). Oxytocin and vasopressin neurones also project into areas within the central nervous system (CNS), where they act on specific membrane-bound receptors to influence cognition and behaviour.

Among the many central actions of these hormones, effects on parental care have received intense study. Several studies in rats and sheep have demonstrated that oxytocin facilitates, and oxytocin antagonists or lesions of oxytocin neurones inhibit, the onset of maternal behaviour (5–7). At parturition, oxytocin is released in central pathways (8) and oxytocin receptors increase in limbic regions of the forebrain (9). Although recent studies with an oxytocin knockout mouse have cast some doubt on the necessity of this hormone for maternal behaviour (10, 11), it now seems likely that oxytocin's effects are species-specific. That is, oxytocin appears to be essential for species, like rats and sheep, that become maternal at parturition.

The importance of vasopressin in reproduction is less recognized. Although vasopressin was found to increase maternal behaviour in an earlier study describing oxytocin's effects on maternal behaviour in rats (6) and the hypothalamic vasopressin mRNA increases postpartum (12, 13), there has been little systematic study of vasopressin and parental

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care. As vasopressin has been associated with male territorial behaviours (14, 15) and many of vasopressin's behavioural effects appear to be androgen-dependent (16), one might consider that vasopressin would be important for paternal behaviour. Unfortunately, paternal behaviour does not occur naturally in most laboratory species.

The arvicoline rodents, also known as voles, provide a powerful comparative approach for studies of both male and female parental care. Voles show remarkable species diversity in social organization, ranging from biparental species such as the prairie vole (*Microxytocinus ochrogaster*) to species without paternal care such as the montane vole (*M. montanus*) (17–19). Comparative studies of these different vole species have demonstrated a number of contrasts in oxytocin and vasopressin neurotransmission. Prairie and montane voles differ in the distribution of oxytocin and vasopressin receptors (20–22) and in their response to centrally administered oxytocin and vasopressin (23). In male prairie voles receiving central injections, vasopressin increases, and a vasopressin antagonist reduces, paternal behaviour as well as social attachment to a female partner (24, 25). Mating, which appears to be associated with the release of vasopressin in prairie voles, also increases paternal behaviour (26–28). However, it is not yet clear that vasopressin is involved with the natural expression of paternal behaviour in the postpartum period.

To investigate physiological changes in vasopressin and oxytocin following the birth of the litter, we measured hypothalamic vasopressin and oxytocin mRNA in both male and female prairie voles 1 day and 6 days postpartum. To determine if changes in gene expression in prairie voles were specific for a biparental species, we performed parallel experiments in male and female montane voles. Previous studies have demonstrated the importance of receptor differences for behavioural effects of these neuropeptide hormones. Therefore, we also measured oxytocin and vasopressin (V1a) receptor binding in males and females of both species.

Materials and methods

Subjects

The prairie voles (*M. ochrogaster*) and montane voles (*M. montanus*) used in the present study were the F3 generation of a laboratory breeding colony that started with field-captured animals. At 21 days of age, subjects from each species were weaned from parents and housed as same-sex sibling pairs in plastic cages ($44 \times 24 \times 20$ cm) which contained cedar chip bedding. Water and food were provided *ad lib*. The cages were maintained on a 14 : 10 h light : dark photoperiod with lights on at 07.00. The temperature in the colony room was about 20 °C.

At 60–70 days of age, subjects from each species were randomly assigned into three experimental groups. In the first group, males and females were paired and females gave birth around 21–23 days after pairing. Six days later (postpartum day 6), brains were harvested from both females and males (n = 5/sex for prairie voles and n = 4/sex for montane voles). In the second group, males and females were also paired and brains were harvested within 12 h of the birth of their litter (postpartum day 1; n = 5/sex for prairie voles and n = 6 or 5 for female and male montane voles). In the third group, male and female subjects were continuously housed as same-sex sibling pairs (sexually naive) before harvesting brains around 85–90 days of age (parallel to group 1 and 2) (n = 5/sex for prairie voles and n = 8 or 4 for female or male montane voles). All brains were removed quickly and frozen on dry ice. Brains were then cut into 20 μm sections on a cryostat, thaw-mounted on Superfrost/Plus slides (Fisher), and stored at −80 °C. Oxytocin and vasopressin *in-situ* hybridization and receptor binding were performed separately for each species.

Oxytocin and vasopressin in-situ hybridization

For each species, one set of slide-mounted brain sections through the hypothalamus at 100 μm intervals was processed for oxytocin mRNA *in-situ* hybridization and alternate sections were processed for vasopressin mRNA *in-situ* hybridization. Slides were air-dried, fixed in 4% paraformaldehyde for 5 min, and rinsed in 0.1 M phosphate buffered saline (pH 7.4) for 2 min, both at 4 °C. After one rinse in 1.5% triethanolamine (TEA), slides were treated in 0.25% acetic anhydride in TEA (pH 8.0) for 10 min, and rinsed twice with 0.03 M Na citrate in 0.3 M NaCl ($2 \times$ SSC), pH 7.0. Slides were subsequently defatted by incubating them 2 min each in 70%, 95%, and 100% ethanol, 5 min in chloroform, 2 min each in 100% and 95% ethanol, after which they were air-dried. For each slide 100 μl hybridization solution was applied, covered with parafilm, and placed in a humidified incubator 14 h at 37 °C.

The hybridization solution contained 50% formamide, $4 \times$ SSC, $1 \times$ Denhardt's solution, 2.5 mg/ml yeast tRNA, 10% dextran sulphate (MW = 50 000), 0.3 M NaCl, 10 mM Tris, 10 mM dithioxytocinhreitol (DTT), and an oligodeoxyribonucleoxytocinide probe for either oxytocin or vasopressin mRNA hybridization. The oxytocin probe consisted of 42 bases and was complementary to the rat mRNA encoding amino acids 82–96 of the oxytocin-associated neurophysin peptide (29). The vasopressin probe which consisted of 48 bases and was complementary to the vasopressin mRNA sequence encoding amino acids 129–144 of the vasopressin precursor peptide was used previously in a vole study (28). Both probes were labelled at the 3′-end with 35S-dATP (New England Nuclear-Dupont, Boston, MA, USA) using terminal deoxynucleoxytocinidyl transferase to a specific activity of 1×106/pmol, purified and mixed with the hybridization buffer at a concentration around 4 pmol/ml. After incubation, the parafilm was removed. Slides were washed in SSC 4×15 min at 55 °C and 60 min at room temperature, and then dehydrated in a series of graded concentrations of ethanol. Slides were put on the BioMax MR film (Eastman Kodak Company, Rochester, NY, USA) for 6 h to generate autoradiograms. Thereafter, slides were dipped in Kodak NTB-2 track emulsion (1 : 1 with 0.6 M ammonium acetate, pH 3.5) under safelight, and stored desiccated in light-tight boxes at 4 °C. Four weeks later, slides were developed, lightly counterstained with 0.2% cresyl violet in 0.1 M acetate buffer, and coverslipped. Controls for specificity included treating the sections with a sense probe for each peptide, which did not produce any labelling.

Oxytocin and vasopressin receptor binding

Oxytocin and vasopressin receptor binding was performed according to the procedure reported previously (20, 22). For each species, two sets of slide-mounted sections at 100 μm intervals were thawed for 30 min at room temperature, and preincubated in 50 mM Tris-HCl buffer (pH 7.4) for 10 min $\times 2$. Sections were then placed in incubation buffer consisting of 50 mM Tris-HCl (pH 7.4) with 10 mM $MgCl_2$, 0.1% BSA, 0.05% bacitracin, and 50 pM tracer for 60 min in room temperature. The tracer was ^{125}I-oxytocin ligand (New England Nuclear-Dupont, Boston, MA, USA) for oxytocin receptor binding, or ^{125}I-linear-vasopressin ligand (Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH2; New England Nuclear-Dupont, Boston, MA, USA) for vasopressin receptor binding. Both ligands were previously characterized and employed in the studies of voles and rats (20, 22, 30, 31). Following incubation, sections were lightly fixed in 50 mM Tris-HCl with 10 mM $MgCl_2$ and 0.1% paraformaldehyde for 2 min in room temperature, washed in 50 mM Tris-HCl with 10 mM $MgCl_2$ for 4×5 min at 4 °C, and then washed in the same solution with stirring for 30 min in room temperature. Sections were dipped in water and then immediately dried under a stream of cool air. In control sections, 1 μM of the selective V1a, d(CH2)5[Tyr(Me)]vasopressin or oxytocin ligand was added into the incubation buffer to define non-specific binding. After drying, the slides were exposed to a BioMax MR film (Kodak) along with ^{125}I plastic standards (I-125) microscales; Amersham, Piscataway, NJ, USA) for 2 days to generate autoradiograms.

Data quantification and analysis

For each peptide *in-situ* hybridization or receptor binding, slides from all subjects in each species were processed simultaneously. All autoradiograms were coded to obscure the identity of the tissue. Sections on the autoradiograms were visually inspected and then three sections that contained the highest density of the mRNA labelling or receptor binding for each brain area were measured bilaterally for each subject to provide individual means. For *in-situ* hybridization, oxytocin or vasopressin mRNA expression in the paraventricular nucleus and supraoptic nucleus was measured as optical density with a computerized image program (NIH IMAGE 1.60) standardized using a

Kodak density step wedge. Both oxytocin and vasopressin receptor binding was quantified in the lateral septum, BNST, ventromedial nucleus of the hypothalamus, and amygdaloid nucleus. The autoradiograms were analysed with a computerized image program, permitting the conversion of optical density to d.p.m/tissue equivalents from the standard curve derived from coexposed standards. Non-specific binding was subtracted from total binding to yield values for specific binding. For each species, data for oxytocin or vasopressin mRNA labelling or receptor binding were analysed by using a two-way ANOVA with sex and group as between subject variables. Significant group or sex by group interaction ($P<0.05$) were further evaluated by a Newman Keul's *post-hoc* test (SNK).

Results

Hypothalamic vasopressin mRNA

As expected, a dense cluster of vasopressin mRNA hybridization was found in the paraventricular nucleus and supraoptic nucleus with antisense; little label was evident following sense strand hybridization. The hypothalamic vasopressin mRNA labelling differed among treatment groups. The density of the vasopressin mRNA labelling was significantly higher in prairie voles on postpartum day 1 or day 6 relative to sexually naive controls in the paraventricular nucleus ($F=10.25$, d.f. = 2/24, $P<0.001$) and in the supraoptic nucleus of the hypothalamus ($F=14.49$, d.f. = 2/24, $P<0.001$; Figs 1, 2A, B). No differences were found between the day 1 and day 6 groups. In addition, these increases were equal in males and females as neither sex nor sex-by-group differences were detected in hypothalamic vasopressin mRNA in prairie voles.

Although vasopressin mRNA hybridization was evident in the same regions of the montane vole, no group differences were detected. That is, there were no changes in hypothalamic vasopressin mRNA following parturition in either male or female montane voles (Fig. 2C, D). No sex difference was detected in the level of vasopressin mRNA in montane voles.

Hypothalamic oxytocin mRNA

Oxytocin mRNA, like vasopressin mRNA, was clearly detected in the paraventricular nucleus and supraoptic nucleus of the prairie vole brain. The density of oxytocin mRNA labelling in the paraventricular nucleus changed across treatment groups ($F=5.49$, d.f. = 2/24, $P<0.05$) and this effect differed between males and females ($F=7.71$, d.f. = 2/24, $P<0.01$). A *post-hoc* test indicated that females on postpartum day 1 or day 6 had a higher density of oxytocin mRNA labelling in the paraventricular nucleus than sexually naive females or males under any conditions (Fig. 3A). A sex-by-group interaction was also found in the supraoptic nucleus ($F=13.44$, d.f. = 2/24, $P<0.01$), in which females on postpartum day 1 had a level of oxytocin mRNA labelling higher than sexually naive females but lower than females on postpartum day 6 (Fig. 3B). No group differences were detected in the hypothalamic oxytocin mRNA levels in either of the brain regions in male prairie voles.

Although montane voles differ from prairie voles in several measures of social behaviour, the species showed similar changes in hypothalamic oxytocin mRNA. In montane voles, a significant sex-by-group interaction on oxytocin mRNA was detected in the paraventricular nucleus ($F=9.89$, d.f. = 2/27, $P<0.001$) and the supraoptic nucleus ($F=7.62$, d.f. = 2/27, $P<0.01$). In each brain region, sexually naive females had a lower density of oxytocin mRNA labelling than females on parental day 1 or day 6, both of which, in turn, did not differ from each other (Fig. 3C, D). No group differences were found in hypothalamic oxytocin mRNA levels in males.

Oxytocin and vasopressin receptor binding

To determine if parturition or parental experience was associated with changes in receptor expression, we measured binding to both oxytocin and vasopressin (V1a) receptors. The distribution pattern of ^{125}I-oxytocin and ^{125}I-linear-vasopressin binding in the prairie vole brain resembled those observed previously (20, 22). No sex or group differences were detected in the vasopressin receptor binding in any of the examined brain regions (Table 1). For oxytocin receptor binding, no sex or group differences were detected in the lateral septum, the bed nucleus of the stria terminalis (BNST), or the amygdaloid nucleus. In the ventromedial nucleus of the hypothalamus, however, females had a higher density of oxytocin receptor binding than males ($F=7.68$, d.f. = 1/24, $P<0.05$). This sex difference was due to the fact that females on postpartum day 1 had significantly more oxytocin receptor binding than sexually naive females or males under any conditions ($F=6.13$, d.f. = 2/24, $P<0.01$; Figs 4, 5).

As expected, ^{125}I-oxytocin and ^{125}I-linear-vasopressin bound to different regions of the montane vole brain relative to the pattern observed in prairie voles (20, 22). However, as in the prairie voles, montane vole females on postpartum day 1 had a higher density of oxytocin receptor binding in the ventromedial nucleus of the hypothalamus compared to the sexually naive females or males in any conditions ($F=7.66$, d.f. = 2/27, $P<0.01$; Figs 4, 6). No group differences were detected either in oxytocin receptor binding in any other brain regions or in vasopressin receptor binding in all measured brain areas (Fig. 6 and Table 2). However, a sex difference was found in which female montane voles showed a higher density of oxytocin receptor binding in the lateral septum ($F=5.48$, d.f. = 1/28, $P<0.05$) or the BNST ($F=9.26$, d.f. = 1/28, $P<0.01$) than male montane voles.

Discussion

The present study examined the effect of parturition and parental experience on the expression of hypothalamic vasopressin and oxytocin mRNA and receptor binding in prairie and montane voles—microtine species that differ in parental behaviour. Hypothalamic vasopressin mRNA increased in biparental prairie voles but not in montane voles, which fail to show paternal behaviour. In contrast to these changes in vasopressin gene expression, oxytocin mRNA and receptor binding increased postpartum in females from both species, suggesting that a common mechanism exists for the regulation of the hypothalamic oxytocin activity postpartum.

The distribution pattern of oxytocin and vasopressin mRNA and receptors

In the present study, the pattern of *in-situ* hybridization for vasopressin and oxytocin resembled the patterns of

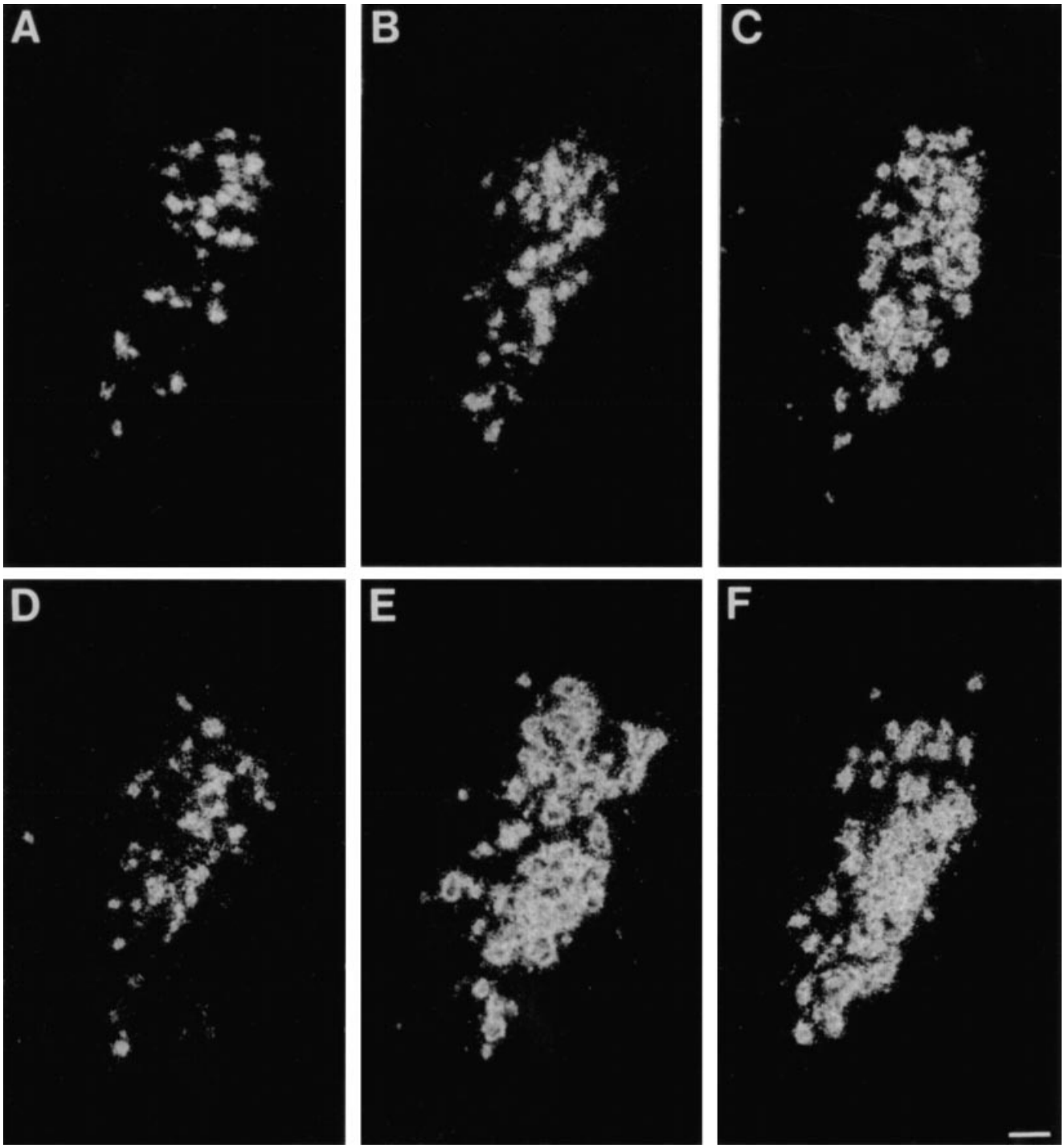

FIG. 1. Photomicrographs displaying vasopressin mRNA labelling in the paraventricular nucleus in male (top) and female prairie voles (bottom) that were sexually naive (A, D), on postpartum day 1 (B, E), or day 6 (C, F). Both males and females on postpartum day 1 or day 6 had a significant increase in vasopressin mRNA labelling in the paraventricular nucleus relative to sexually naive prairie voles. Scale bar = 0.5 mm.

immunoreactivity for both hormones previously reported in voles (26, 28, 32) and similar to patterns reported for common laboratory rodents. It is noticeable that neither sex nor species differences were detected in oxytocin or vasopressin mRNA labelling in naive animals.

The distribution pattern of oxytocin and vasopressin receptors resembled the pattern found in our early studies (20–22). Although a direct species comparison was not performed due to the fact that the binding assay was performed separately for each species, the two species showed the expected differences in regional oxytocin and vasopressin receptor binding. For example, the montane vole showed denser vasopressin receptor binding in the lateral septum than the prairie vole. We reported previously that species differences in regional

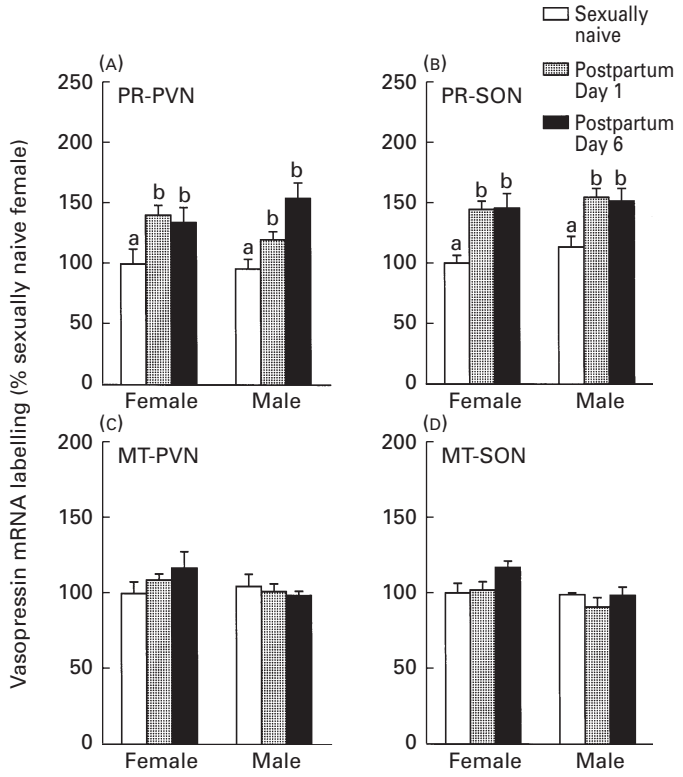

FIG. 2. Vasopressin mRNA labelling in the paraventricular nucleus and supraoptic nucleus of the hypoxytocinhalamus in prairie voles (PR) and montane voles (MT). Data are presented as the percentage of changes relative to sexually naive females for each brain area per species. Both female and male prairie voles had a significant increase in vasopressin mRNA labelling in the paraventricular nucleus (A) and supraoptic nucleus (B) on postpartum day 1 or day 6 compared to their sexually naive counterparts. No significant changes in the hypothalamic vasopressin mRNA were found in either sex of montane voles (C, D). Each bar represents the mean (SEM from 4–6 animals. Bars labelled with different letters differ from each other significantly ($P<0.05$).

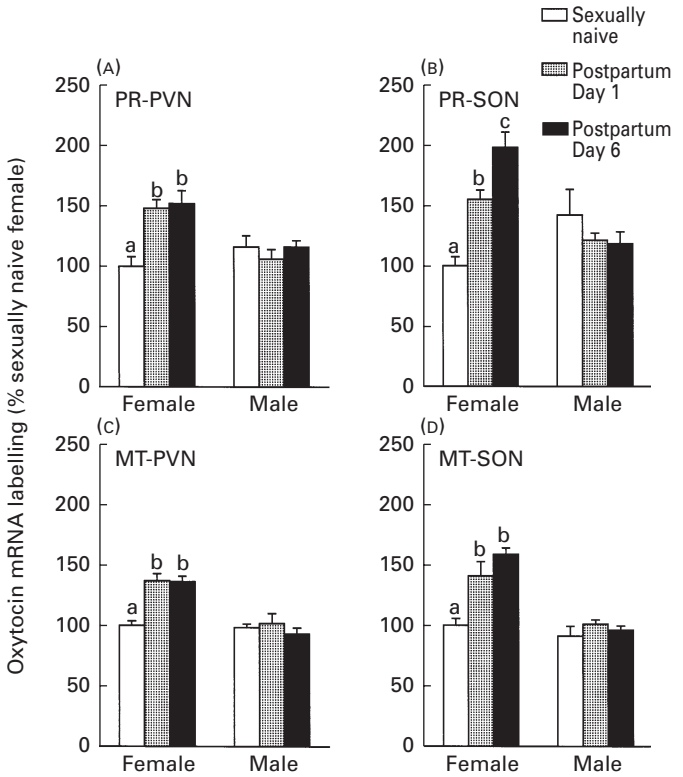

FIG. 3. Oxytocin mRNA labelling in the paraventricular nucleus and supraoptic nucleus of the hypothalamus in prairie voles (PR) and montane voles (MT). Data were presented as the percentage of changes over sexually naive females for each brain area per species. Females in both species had a significant higher level of vasopressin mRNA labelling in the paraventricular nucleus and supraoptic nucleus on postpartum day 1 or day 6 *vs* their sexually naive counterparts. No changes in the hypothalamic oxytocin mRNA were found in the male of either species. Each bar represents the mean $\pm$SEM from four to six animals. Bars labelled with different letters differ from each other significantly ($P<0.05$).

oxytocin and vasopressin receptor binding in voles were associated with differences in promoter (not coding) sequences of these genes, leading to differences in regional receptor gene expression (23, 33). In the current study, we noticed a species-specific gender difference in the regional oxytocin receptor binding: female montane voles had a higher density of oxytocin receptors in the lateral septum and the BNST than male montane voles. Such differences seemed to be due to a slight decrease in the regional vasopressin receptor binding in males following parturition of their mates and does not appear to be evident in sexually naive montane voles.

A species-specific increase in the hypothalamic vasopressin mRNA

A significant increase in vasopressin mRNA in the paraventricular nucleus and supraoptic nucleus following parturition and during lactation was found in both male and female prairie voles but not montane voles. Several studies have examined the postpartum increase in hypothalamic vasopressin (12, 13, 34) and its hormonal regulation in female rats (35). Increased hypothalamic vasopressin postpartum was not the result of suckling (36). Although an increased percentage of hypothalamic oxytocin neurones synthesize vasopressin during lactation (37), the role of vasopressin in the reproduction is still not clear. A commonly accepted hypothesis is that released vasopressin may help the animals to compensate for loss of water during lactation (37). In female rats, maternal anogenital licking results in the ingestion of pup's urine (38, 39), which may be an osmotic challenge. Clearly, the osmolality in female's urine increases during lactation (37); and treatment with hypertonic saline increases hypothalamic vasopressin (40, 41). Together these data suggest that increased hypothalamic vasopressin mRNA may compensate for increased water loss and elevated osmolality during lactation in females.

If the osmotic challenge of lactation and anogenital licking accounts for increased hypothalamic vasopressin mRNA in female prairie voles, why did we fail to find an increase in vasopressin mRNA in female montane voles that also lactate and display maternal licking? There are currently no data to provide a simple answer to this question. Behavioural studies indicate that prairie vole mothers spend twice the amount of time than montane vole mothers in displaying maternal

TABLE 1. ^{125}I-Linear-Vasopressin Binding (d.p.m./mg Tissue Equivalent × 100) in Selected Brain Areas in Prairie Voles.

	Female			Male		
	Sexually naive	Parental day 1	Parental day 6	Sexually naive	Parental day 1	Parental day 6
LS	56.3±16.4	47.2±21.9	24.0±3.6	53.5±11.5	17.2±3.5	28.1±14.9
BNST	88.6±19.2	70.0±10.8	78.5±13.5	75.9±11.0	53.2±3.5	80.6±5.8
VMH	19.9±2.8	23.1±5.5	24.1±6.1	21.8±4.3	31.1±14.3	17.7±2.4
AMYG	93.3±11.8	75.4±12.7	82.5±13.2	77.5±9.0	82.0±3.9	80.2±12.2

LS, lateral septum; BNST, bed nucleus of the stria terminalis; VMH, ventromedial hypothalamus; AMYG, amygdaloid nucleus.

behaviour (42, 43). The two voles may also differ in some subtle aspects of maternal behaviour and physiology so that they may face different osmotic challenges during lactation or have different regulating mechanisms. It is also possible that increased vasopressin synthesis is involved in the regulation of behavioural changes occurring around parturition in one species but not the other, irrespective of osmotic changes. Further studies are needed to clarify this issue.

It is interesting that male prairie voles also showed a 'postpartum' increase in hypothalamic vasopressin mRNA identical to the pattern of their female mates. Males do not lactate and thus this particular challenge cannot be inducing their vasopressin gene. Detailed behavioural analysis has shown that male prairie voles also display extensive paternal behaviour including anogentially licking their young (44). This paternal licking results in the ingestion of pup's urine, which has been found in the prairie vole (45) and in another monogamous rodent, California mice (*Peromyscus californicus*) (46). Thus, the increased hypothalamic vasopressin in male prairie voles may be due to their paternal experience with offspring. This notion is supported by finding that male prairie voles with pups removed neonatally had a lower level of the hypothalamic vasopressin mRNA on postpartum day 6 than males with pups (45). In addition, male montane voles that do not display paternal behaviour (43) showed no increase in their hypothalamic vasopressin mRNA although they were exposed to the offspring.

Following mating or after becoming parents, male prairie voles display a significant increase in selective aggression towards conspecific male or female intruders, but such increased aggression was not found in male montane voles (18, 24). Vasopressin treatment induces aggression in sexual naive male prairie but not montane voles (23). In addition, treatment of an vasopressin V1a receptor antagonist blocks mating-induced aggression in male prairie voles (24). Could it be possible that increased hypothalamic vasopressin mRNA postpartum in male prairie voles is also associated with their increased aggression? In an earlier study, injections of an vasopressin V1a antagonist into the brain did not block aggression in male prairie voles from the established breeders, suggesting that vasopressin is not necessary, at least for the maintenance of postpartum aggression in males (24). Sexually experienced (breeder) female prairie voles also display aggressive behaviour towards strangers of both sexes (47, 48). The role of vasopressin, if any, in the regulation of postpartum aggression in female prairie voles needs to be further studied.

Vasopressin neurones in the supraoptic nucleus project almost exclusively to the pituitary whereas the neurones in the paraventricular nucleus also project into various brain areas (49, 50). Therefore, increased hypothalamic vasopressin in both brain regions indicates an increase in peripheral as well as in central vasopressin release.

A postpartum increase in the oxytocin mRNA and receptor binding

Our data indicated that on the day of (postpartum day 1) or 6 days after litter birth (postpartum day 6), female voles showed a significant increase in oxytocin mRNA labelling in the paraventricular nucleus and supraoptic nucleus in comparison to their sexually naive counterparts. In addition, females on postpartum day 1 or day 6 did not differ from each other on the levels of their hypothalamic oxytocin mRNA. These data suggested that physiological events associated with pregnancy, parturition and lactation were probably responsible for an increased and sustained hypothalamic oxytocin mRNA in voles. A similar postpartum increase in the hypothalamic oxytocin activity has been reported in other rodents. In female rats, for example, a significant postpartum increase in oxytocin mRNA, immunoreactivity, or release was found in the paraventricular nucleus and supraoptic nucleus (12, 13, 34, 40). Such increased oxytocin activity was sustained by suckling during lactation (51) and was associated with decreased oxytocin storage in the pituitary and increased peripheral oxytocin release (52).

In rats, an increase in the hypothalamic oxytocin mRNA reached a peak even before parturition, suggesting that it is not the parturition *per se* but the hormonal changes associated with pregnancy that enhance oxytocin activity in the hypothalamus (12, 52, 53). The notion that steroid hormones regulate oxytocin expression is supported by the presence of an oestrogen response element (54) and a glucocorticoid response element (which can bind glucocorticoid and/or progesterone) (55) in the oxytocin gene. In addition, the sequential changes of oestrogen and progesterone that occur during pregnancy and parturition have been shown to increase hypothalamic oxytocin mRNA in female rats (52, 56). Although the ovarian and uterine weights and protein levels showed predictable changes during reproductive cycles in prairie voles (57), hormonal profiles during pregnancy and lactation are still unknown in voles. Nevertheless, similarities in the dynamic pattern of the postpartum hypothalamic oxytocin mRNA between rats and voles and between voles with different life strategy and behaviour suggest that a

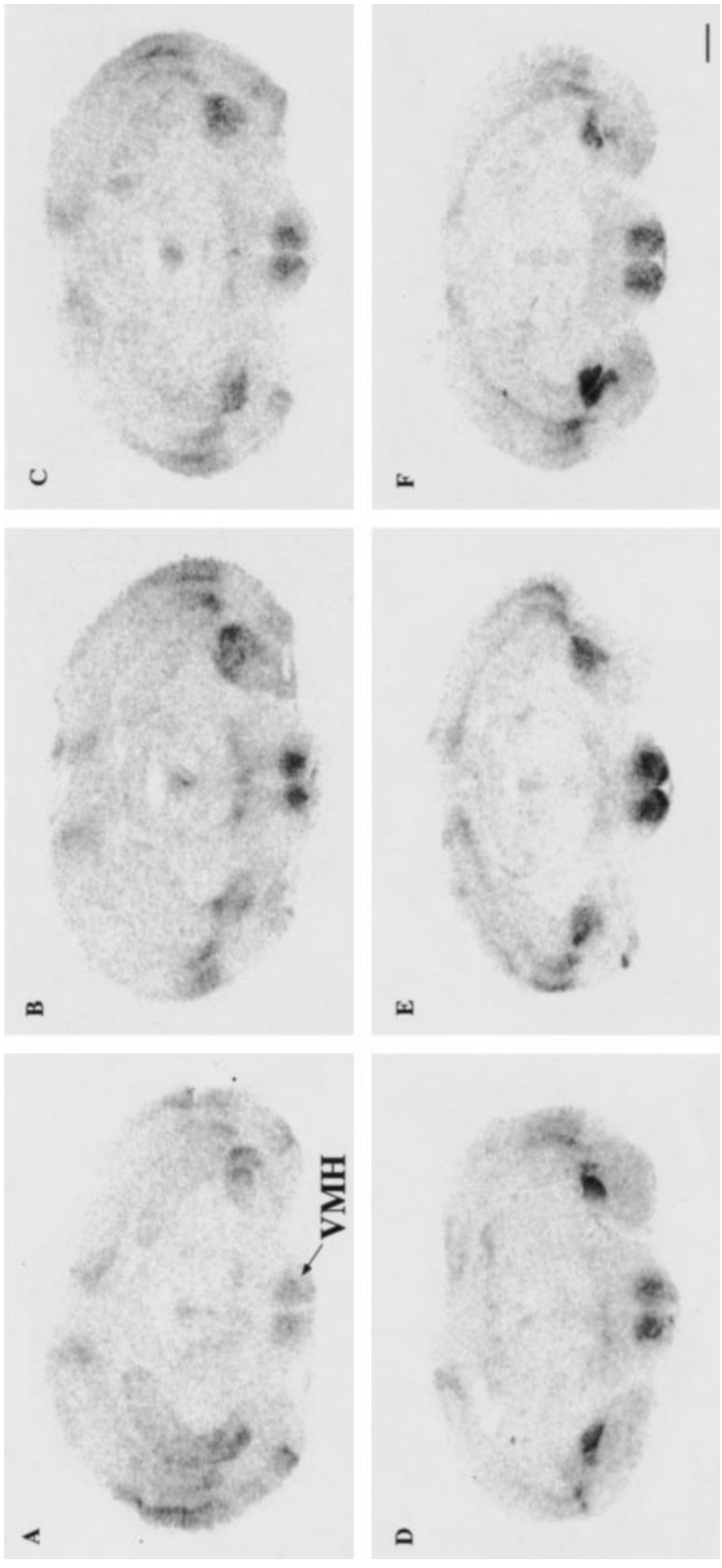

FIG. 4. ^{125}I-oxytocin binding in the ventromedial nucleus of the hypothalamus of female prairie voles (top) and montane voles (bottom) that were sexually naive (A, D), on the day of litter birth (B, E), or 6 days postpartum (C, F). Females of both species on the day of litter birth had denser ^{125}I-oxytocin binding in the ventromedial nucleus of the hypothalamus than sexually naive females. VMH = ventromedial hypothalamus. Scale bar = 1 mm.

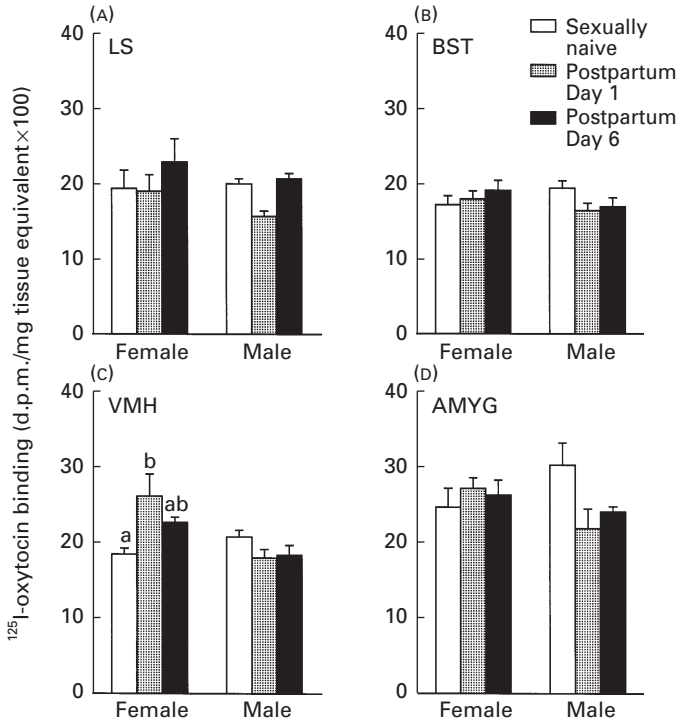

FIG. 5. ^{125}I-oxytocin binding in the lateral septum (LS; A), the bed nucleus of the stria terminalis (BNST; B), ventromedial nucleus of the hypoxytocinhalamus (VMH; C), and amygdaloid nucleus (AMYG; D) of male and female prairie voles. Females on postpartum day 1 had a higher density of ^{125}I-oxytocin binding in the ventromedial nucleus of the hypothalamus than the sexually naive females. Each bar represents the mean ± SEM from four to six animals.

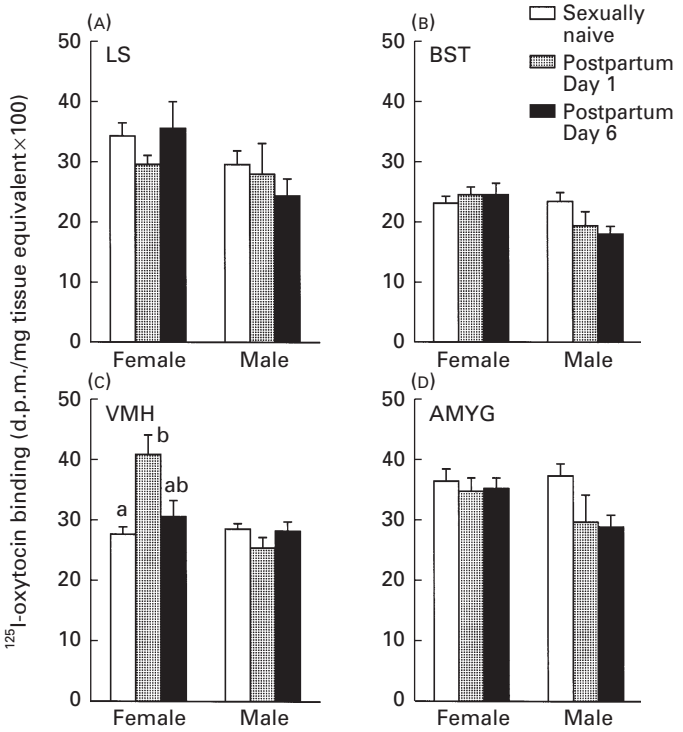

FIG. 6. ^{125}I-oxytocin binding in the lateral septum (LS; A), the bed nucleus of the stria terminalis (BNST; B), ventromedial nucleus of the hypoxytocinhalamus (VMH; C), and amygdaloid nucleus (AMYG; D) of male and female montane voles. Females on postpartum day 1 had a higher density of ^{125}I-oxytocin binding in the ventromedial nucleus of the hypothalamus than the sexually naive ones. Each bar represents the mean ± SEM from four to six animals.

common mechanism exists for the regulation of the hypothalamic oxytocin gene expression postpartum.

Although the hypothalamic oxytocin mRNA increased in female voles following litter birth, an increase was not found in males of either species. Immediately following litter birth, male voles mate with the female that shows postpartum estrus (57). Although oxytocin has been found to have controversial effects on male sexual behaviour in rats (58, 59), it inhibits mating in male prairie voles (60). Thus, the lack of an increase in the hypothalamic oxytocin may be a physiological adaptation that allows male voles to mate postpartum.

In the present study, a significant increase in oxytocin receptor binding was found in the ventromedial nucleus of the hypothalamus in female voles on the day of litter birth, but this increase did not persist 6 days later: a pattern similar to the pattern reported in rats (61, 62). In rats and mice, steroid hormones play an important role in the regulation of oxytocin receptors in the ventromedial nucleus of the hypothalamus in a species-specific manner (63–66). In prairie voles, however, neither a prolonged male exposure which increased endogenous oestradiol levels nor ovariectomy with subsequent oestradiol treatment altered oxytocin receptor binding in the ventromedial nucleus of the hypothalamus (67). One would assume that endogenous factors other than hormonal changes at parturition were responsible for enhanced ventromedial nucleus of the hypothalamus oxytocin receptor binding in voles.

Increased oxytocin receptors in the ventromedial nucleus of the hypothalamus just after parturition coincides with the onset of postpartum oestrus in both rats and voles. In rats, oxytocin activation in the ventromedial nucleus of the hypothalamus is correlated with elevated oestrogen and progesterone and has been related to lordosis (68). In voles, however, the functional significance might be different as progesterone is not required for oestrus induction (69) and behavioural oestrus was not correlated with increased oxytocin receptor binding in the ventromedial nucleus of the hypothalamus (67). Surprisingly, although prairie and montane voles differ quantitatively in oxytocin receptors in the (20, 33), the two voles showed virtually identical patterns of a postpartum increase in the ventromedial nucleus of the hypothalamus oxytocin receptor binding, providing further evidence to support the notion that a common mechanism exists for the postpartum regulation of oxytocin activity.

In summary, the present study used a comparative approach to address the role of vasopressin in paternal behaviour. Following parturition, vasopressin gene expression was increased in both male and female prairie voles, a biparental species. In montane voles, which do not exhibit paternal behaviour, vasopressin mRNA did not increase in either sex after parturition. In contrast to this species-specific pattern of vasopressin synthesis, oxytocin mRNA increased in the paraventricular nucleus and supraoptic nucleus and oxytocin receptor binding increased in the ventromedial

TABLE 2. ^{125}I-Linear-Vasopressin Binding (d.p.m./mg Tissue Equivalent × 100) in Selected Brain Areas in Montane Voles.

	Female			Male		
	Sexually naive	Parental day 1	Parental day 6	Sexually naive	Parental day 1	Parental day 6
LS	33.1±2.3	29.2±2.3	34.7±4.7	36.0±5.8	31.2±6.9	33.8±3.8
BNST	20.3±1.1	22.4±0.9	22.3±1.7	19.7±2.0	19.6±1.7	19.9±2.1
VMH	26.5±1.4	28.2±0.6	24.9±2.1	25.9±1.9	26.7±2.3	25.9±2.2
AMYG	25.9±1.2	26.5±0.2	23.1±1.6	23.7±0.8	26.4±2.3	26.8±2.0

LS, lateral septum; BNST, bed nucleus of the stria terminalis; VMH, ventromedial hypothalamus; AMYG, amygdaloid nucleus.

nucleus of the hypothalamus in females of both species. These increases in oxytocin mRNA and receptors may be associated with events during parturition and lactation that are common to both species. Along with earlier studies that have demonstrated vasopressin effects on paternal behaviour in prairie voles, these new data suggest that vasopressin but not oxytocin may be important for male parental care.

Acknowledgements

We wish to thank Dr Fred Stephan for a critical reading of the manuscript. This research was supported by NIMH grant 54554 and 56539.

Accepted 24 July 1999

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