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Vasopressin Mediates Enhanced Offspring Protection in Multiparous Rats ¹

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Abstract

Maternal aggression is highly expressed during lactation and serves to protect the developing young from intruders that may injure the offspring. One neurochemical modulator of maternal aggression appears to be arginine vasopressin (AVP). Earlier research supports a role for AVP in maternal aggression in rats as treatment with an AVP antagonist in lactating, primiparous rats stimulates the mother's aggression towards intruders the second half of lactation, but that AVP itself was without major effects during early lactation. Recent behavioral findings indicate that during a second lactation (multiparous) mothers display higher levels of maternal aggression than do first time mothers (primiparous). The present study was designed to assess the involvement of AVP as mothers acquire reproductive experience. Therefore, the involvement of AVP in maternal aggression in multiparous mothers was measured after intracerebroventricular (ICV) treatment with both AVP and a V1a receptor antagonist. Behavior was assessed during early lactation when aggression levels are very high in multiparous mothers as well as during late lactation when aggression levels are lower. The results demonstrated that ICV infusions of AVP significantly reduced maternal aggression in multiparous females on day 5 of lactation, whereas V1a antagonist infusions increased aggression on day 15 of lactation. These findings suggest that the role of AVP in maternal aggression may be amplified as reproductive/lactational experiences increase, and support the involvement of the central AVP system as a key modulator of maternal protection of the young.

Introduction

Maternal aggression is a distinct form of aggression found in lactating rats (Erskine et al., 1978) and is critical for the protection of altricial young (Fleming, 1979; Maestripieri and Alleva, 1990; Numan and Insel, 2003; vom Saal et al., 1995; Wolff, 1985, 1993; Wolff and Peterson, 1998). There is evidence that the level of maternal aggression changes as a function of the number of births. In mice, maternal aggression increases across the first three litters (Svare and Gandelman, 1976). Recent findings in our laboratory demonstrate that multiparous rats (second lactation) are also more aggressive than primiparous females during early lactation (Nephew et al., 2009a), but the mechanism that mediates this experience-dependent

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phenomenon has not been identified. The study of this robust increase in endogenous maternal aggression may provide insight into the control of aggression in maternal mammals.

One neurohormone involved in the expression of maternal aggression is arginine vasopressin (AVP). Investigations of AVP in primiparous lactating rats indicate that this peptide inhibits the display of maternal aggression (Nephew and Bridges, 2008b). Furthermore, AVP mRNA expression is decreased in the paraventricular nucleus (PVN) of multiparous rats during early lactation compared to primiparous animals, a time when multiparous mothers are significantly more aggressive. During late lactation, both aggression levels and PVN mRNA expression are similar across parity (Nephew et al., 2009a). Although AVP's involvement in maternal aggression has only recently been explored (Bosch and Neumann, 2008; Nephew and Bridges, 2008b), other behavioral studies have established the significance of AVP in the modulation of a range of aggressive displays in rodent species (Compaan et al., 1993; Delville et al., 1996a; Delville et al., 1996b; Elkabir et al., 1990; Ferris et al., 1997; Ferris and Potegal, 1988; Stribley and Carter, 1999; Winslow et al., 1993).

Given the role of AVP in primiparous maternal aggression and the higher levels of maternal aggression found in multiparous mothers, it was postulated that neural AVP may be involved in multiparous aggression as well. The prior evidence for a role for AVP in maternal aggression is based primarily upon the finding that central administration of an antagonist to the AVP V1a receptor to primiparous rats stimulates maternal aggression. Only transient or negligible inhibitory actions of AVP itself were detected (Nephew and Bridges, 2008b). The inability to demonstrate a substantial inhibitory action of AVP during early lactation in primiparous lactating rats may be due to the fact that aggression in primiparous rats during early lactation is significantly lower compared to age-matched, multiparous rats (Nephew et al., 2009a). Thus, a “floor effect” may be present in the primiparous mothers. Therefore, in the current study, we selected multiparous rats to test with AVP during early lactation to further examine the actions of AVP as a possible inhibitor of maternal aggression. We also examined the effects of central administration of V1a receptor antagonist on multiparous maternal aggression during later lactation to evaluate the involvement of the AVP neural system in aggression throughout lactation in these experienced females. It was hypothesized that AVP would decrease aggression in multiparous lactating rats during early lactation when aggression levels are high, and a V1a receptor antagonist would increase aggression during late lactation when aggression levels are reduced. The results confirm this hypothesis and indicate that AVP plays a key role in the regulation of offspring protection throughout lactation in multiparous dams.

METHODS

Animals

Female Sprague-Dawley (CrI:CD[SD]BR) rats (175–200g) were obtained from Charles River Laboratories (Wilmington, MA) and maintained in temperature (21–25°C) and light (14:10 LD cycle; lights on at 0500h) controlled rooms. Food and water were available *ad libitum* throughout the studies. Pregnancies were confirmed by the presence of sperm in a vaginal lavage. Two weeks after raising an initial litter of 8 to 10 pups to weaning, dams were remated to generate a set of multiparous subjects. There was no formal testing of maternal behavior for the first litters, but full maternal behavior (retrieval, grouping, crouching over pups) was present in all experimental subjects during the first lactation. Sample sizes were 9–13 per treatment group, and all multiparous females whose litters were culled the day after parturition to 8 pups received one randomly selected treatment on day 5 of lactation (d5), and one randomly selected treatment on day 15 of lactation (d15).

ICV Cannulation Surgery

Females were anesthetized with isoflurane on day 20 of their second pregnancy and implanted with unilateral guide cannulae directed into the right lateral ventricle (coordinates relative to bregma: AP = -0.8mm, ML = -1.5mm). Guide cannulae were 3 mm long, and the injectors were 3.5 mm. Females were allowed to recover in individual home cages, and were kept with their litters throughout the experiment. To habituate the animals to the infusion procedure, all females were handled once a day for 4 days prior to the treatment infusions.

Treatment infusions

Ten minutes prior to testing on d5, implanted females were administered either (icv) saline vehicle (2 μ l) or one of three AVP (Sigma) doses (0.5, 2.5, 12.5 ng in 2 μ l of saline). Two hours prior to behavioral testing on d15, the same females received either the saline vehicle or one of three AVP V1a receptor antagonist (Sigma) doses (5, 25, or 125 ng of d(CH₂)₅Tyr(Me) AVP in 2 μ l of saline. All treatments were infused over 60 seconds. These doses were based on earlier studies of the behavioral effects of AVP (Engelmann et al., 1996; Goodson and Bass, 2001), personal communication with M. Manning, as well as previous studies in primiparous females (Nephew and Bridges, 2008b). All treatments were randomized on both days such that each subject received one of the 4 treatments for d5 and d15. Rats from at least 3 of the d5 treatment groups were included in each d15 treatment group, resulting in 2–4 animals from each d5 treatment in d15 treatment groups. The two hour delay in behavioral testing after the V1a receptor antagonist treatment was designed to avoid potential agonistic activity of the antagonist (Ferris et al., 1985). Since AVP V1a receptor antagonist has been shown to delay aggression for 18 hours in prairie voles (Winslow et al., 1993), we were confident that we would not miss behavioral effects due to the timing of the treatment infusions. Furthermore, initial behavioral pilot studies indicated that handled animals return to typical undisturbed behavioral patterns within 10 minutes, so effects of the infusion procedure on behavior were minimal. Cannulae placements were confirmed at the end of the study by icv injection of India ink. Only animals that had successful and secure icv cannula placements throughout the study were included in the statistical analyses.

Animals in this study were maintained in accordance with the guidelines of the Committee of the Care and Use of Laboratory Animals Resources, National Research Council. The research protocol was approved by Tufts University's Institutional and Animal Care Use Committee.

Behavioral Variables

Maternal aggression testing was conducted between 1330 and 1630 h. Dams in their home cages were moved to an empty behavioral observation room one day prior to testing. A digital video camera (Panasonic PV-GS180) allowed for behavioral observation without human interference. Fifteen minute behavioral trials began when a slightly smaller intruder male (50–70 days old) was placed into the female's clear plastic home cage. Upon conclusion of the aggression trials, the digital videotapes were scored by an observer that was blind to the treatments using ODlog video analysis software (Macropod Inc.). The ODlog software records continuous data in 5 seconds bins, and also generates frequency and duration summaries for all behavioral measures over the 15 minute observation period.

Behaviors observed included maternal aggression, grooming, pup-directed maternal behavior, and activity. Maternal aggression included the scoring of both frontal and lateral attacks. Attacks consisted of bites, kicking with forelimbs or hind limbs, and pinning the intruder to the floor of the cage. Latency to initiate, number, and duration of attacks were recorded. A single attack started upon contact between the male and female, and concluded when they separated. Grooming consisted of cleaning and/or manipulation of the dams own fur with mouth or paws. Pup-directed maternal behavior included the retrieval and gathering of pups,

nest building, pup licking, and crouching over the pups. Activity included any change in position not involved in aggression, grooming, or maternal behavior.

Statistics

Lactation day 5 and 15 data were analyzed separately by one-way ANOVA for treatment followed by Tukey's post-hoc tests for pair wise multiple comparisons, if significant treatment and/or day effects were identified (SigmaStat 2.03). If the data were not normally distributed, a Kruskal-Wallis one-way ANOVA on ranks was used, followed by Dunn's pair wise comparisons. All results are presented as means + SEM, and the level of statistical significance was $p < 0.05$.

RESULTS

There were no effects of d5 treatment on d15 behavior (all p 's > 0.2). AVP treatment did not affect attack latencies or number of attacks (fig. 1A and fig 2A). Attack duration during the 15 minute aggression test was decreased on d5 following icv AVP ($F_{[3,41]} = 8.90$, $p < 0.01$, fig. 3A), as all three doses caused a significant attenuation of aggression ($p < 0.01$ for all 3 doses). V1a receptor antagonist treatment significantly shortened d15 attack latencies ($F_{[3,37]} = 3.33$, $p < 0.05$, fig. 1B). This effect was most evident following the 25 and 125 ng infusions, although post hoc comparisons to the saline control were not significant. The V1a receptor antagonist treatment also increased the number of attacks on d15 of lactation ($F_{[3,37]} = 3.84$, $p < 0.05$, fig. 2B), and this effect tended to be stronger at the higher doses as well (p 's = 0.09 for saline vs. 25 and 125 ng). Overall attack duration on d15 increased as a result of V1a receptor antagonist treatment ($F_{[3,37]} = 3.47$, $p < 0.05$, fig. 3B) with the 125 ng treatment having the greatest stimulatory effect ($p < 0.05$). As shown in Table 1, there were no effects of the AVP or V1a receptor antagonist treatments on grooming, maternal behavior, or the number of activity bouts during the 15 minute aggression trials. Activity duration levels were elevated following only the 0.5 ng dose of AVP on day 5 ($F_{[3,41]} = 3.33$, $p < 0.05$) when compared to saline controls.

DISCUSSION

In the present study, central AVP treatment decreased maternal aggression during early lactation and V1a receptor antagonist treatment increased aggression during late lactation. These findings indicate that central AVP modulates maternal aggression during both early and late gestation in multiparous rats. The data support the previously proposed hypothesis that decreased central AVP activity is associated with elevated maternal aggression. Intracerebroventricular infusion of a V1a receptor antagonist increases maternal aggression during both early and late lactation in primiparous rats (Nephew and Bridges, 2008b), and AVP mRNA expression is down-regulated on d5 of lactation in the PVN of multiparous females compared to the less aggressive primiparous animals (Nephew et al., 2009a).

The present data, in conjunction with the recent AVP mRNA data, suggest that parity-enhanced maternal aggression may be regulated by endogenous alterations in central AVP activity. A comparison of the aggression levels of the present multiparous females with those of primiparous mothers indicates that during early lactation the multiparous mothers are more sensitive than primiparous mothers to the actions of icv AVP in suppressing maternal aggression, i.e. attack duration. This effect is likely due to the higher baseline levels of maternal aggression present in day 5 lactating multiparous dams (Byrnes et al., 2008), and it is likely that the suppressive effect of AVP may be more easily detected given the higher baselines in these animals. The general lack of treatment effects on grooming duration, maternal behavior, and activity (where only the 0.5 ng AVP dose increased activity) suggests that the modulation of central AVP activity was specifically affecting the display of maternal aggression.

Although much of the research on the neuroendocrine role of AVP in aggression has focused on stimulatory actions in males, there are several studies which suggest a potential inhibitory role for central AVP. AVP levels in the lateral septum are negatively correlated with intermale aggression in male wild type rats (Everts et al., 1997) as well as male mice (Compaan et al., 1993). In male prairie voles, highly aggressive individuals have decreased AVP activity in the bed nucleus of the stria terminalis and medial amygdala, as well as decreased AVP receptor levels in the lateral septum (LS) when compared to less aggressive meadow voles (Insel et al., 1994; Wang and De Vries, 1995; Young et al., 1997). There are also previous reports suggesting that AVP inhibits maternal behavior.

Lesions of the paraventricular nucleus (PVN) in maternal females have been shown to both increase (Giovenardi et al., 1998) and decrease (Consiglio and Lucion, 1994) maternal aggression. However, the lesions in the Consiglio et al. study involved both magnocellular and parvocellular neurons, which may explain the different effects in the two studies. A more recent investigation of the effects of peripherally administered novel V1a receptor antagonist (AZN 576) adds further support to the hypothesis that AVP has inhibitory actions in maternal females. In Long Evans rats, the intraperitoneal injection of AZN 576 increased both the number and total duration of attacks towards a novel male intruder without affecting other behaviors, such as grooming and locomotor activity (Nephew et al., 2009b). Although these peripheral injections and the current icv injections cannot specifically identify where these AVP manipulations are acting, functional MRI studies of primiparous females presented with a male intruder (Nephew et al., 2009c) and primiparous females presented with a male intruder while being administered icv V1a receptor antagonist (Caffrey et al., 2009) have identified several relevant nuclei to target for future study, such as the somatosensory cortex, cortical amygdala and ventromedial hypothalamus. Furthermore, it is argued that the effectiveness of nonspecific treatments supports future translational work on AVP and aggression.

One alternate hypothesis to explain the role of AVP in maternal aggression is that it is acting through its effects on anxiety. AVP increases anxiety in rats (Beiderbeck et al., 2007; Landgraf et al., 1995; Landgraf et al., 2007), and it is possible that AVP is suppressed during early lactation to allow for increased maternal aggression. Although anxiety was not measured in the current study, multiparous females are less anxious than primiparous females (Walf and Frye, 2008). Single nucleotide polymorphisms in the AVP promoter regions of high anxiety behavior (HAB) and low anxiety behavior (LAB) rats control the over-expression of AVP gene in HAB rats, and under expression in LAB rats (Landgraf et al., 2007). Although HAB females show increased maternal aggression, it is the LAB males, which have decreased AVP activity, which show greater intermale aggression (Beiderbeck et al., 2007; Veenema et al., 2007). In addition, short aggression latency male mice are more aggressive and have a decreased AVP fiber network in the LS when compared to the long aggression latency males (Compaan et al., 1993). In other mouse studies, chronic social defeat increased anxiety-associated behavior and was correlated with changes in PVN AVP mRNA levels (Erhardt et al., 2008), and chronic restraint stress increases anxiety and decreases maternal aggression (Maestripietri et al., 1991). Although further study targeting maternal rats is needed, the current literature on AVP and anxiety suggests that stress-induced changes in maternal aggression and anxiety may be mediated through central AVP activity, possibly in the PVN.

Overall, these findings support recent studies which conclude that AVP mediates the display of maternal aggression, and may be a factor in parity dependent changes in offspring protection. Although the current study focused on maternal aggression, additional evidence that AVP is involved in other aspects of parental care is supported by findings that AVP regulates the expression (Bosch and Neumann, 2008) and retention (Nephew and Bridges, 2008a) of pup-directed maternal behaviors. Further investigations of the roles of AVP in maternal aggression and maternal care should provide valuable insights into the regulation of these complex

behaviors, and may provide a framework for understanding disorders which involve depression and/or anxiety.

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Figure 1A–B.

Mean (+SEM) seconds(s) for attack latency during 15 minute maternal aggression trials in multiparous rats treated with **(A)** icv saline, 0.5, 2.5, or 12.5 ng AVP on day 5 of lactation, and **(B)** icv saline, 5.0, 25.0, or 125.0 ng V1a receptor antagonist on d15 of lactation. Sample sizes for each treatment are listed in parentheses. # indicates a significant overall effect of treatment ($p < 0.05$).

Figure 2A–B.

Mean (+SEM) number of attacks during 15 minute maternal aggression trials in multiparous rats treated with **(A)** icv saline, 0.5, 2.5, or 12.5 ng AVP on day 5 of lactation, and **(B)** icv saline, 5.0, 25.0, or 125.0 ng V1a receptor antagonist on d15 of lactation. Sample sizes for each treatment are listed in parentheses. # indicates a significant overall effect of treatment ($p < 0.05$).

Figure 3A–B.

Mean (+SEM) seconds(s) for cumulative time spent attacking during 15 minute maternal aggression trials in multiparous rats treated with (A) icv saline or 0.5, 2.5, 12.5 ng AVP on day 5 of lactation, and (B) icv saline or 5.0, 25.0, 125.0 ng V1a receptor antagonist on d15 of lactation. Sample sizes for each treatment are listed in parentheses. * indicates a significant difference when compared to same day saline controls ($p < 0.05$).

Mean ± SEM values for grooming, maternal behavior (MB), and locomotor activity during 15 minute maternal aggression trials following icv injection of saline 0.5, 2.5, 12.5ng AVP on day 5 of lactation, or saline, 5.0 25.0, or 125.0ng V1a antagonist on lactation day 15. # refers to the number of bouts of each behavior. Duration units are seconds. Bold p-values indicate a significant effect of treatment, with significantly different values also in bold.

Table 1

Behavioral Variable	DAY 5				P	DAY 15				P
	SALINE	0.5 AVP	2.5 P AVP	12.5 AVP		SALINE	5.0 ANT	25.0 ANT	125.0 ANT	
Groom #	10.9±2.3	7.4±1.3	10.3±1.8	8.0±1.3	0.38	5.5±1.1	4.7±1.0	4.3±1.0	5.3±1.0	0.86
Groom Duration (s)	90.4±24.8	62.2±15.7	87.2±18.2	92.1±27.8	0.77	36.3±8.9	43.8±14.0	28.9±6.3	30.2±7.8	0.69
MB #	8.7±1.8	10.4±2.5	10.1±2.9	11.5±1.6	0.83	8.2±2.5	7.7±2.0	11.2±2.9	7.8±1.6	0.67
MB Duration (s)	173.2±59.3	33.9±40.4	198.7±62.3	174.0±49.3	0.86	73.2±22.8	130.3±40.3	211.3±68.7	110.0±44.5	0.21
Activity #	27.4±4.1	31.1±3.5	27.1±3.7	22.1±2.1	0.23	38.4±5.6	30.3±2.4	27.9±3.9	35.2±4.4	0.33
Activity Duration (s)	81.5±11.5	162.9±34.3	1104.9±11.4	102.1±8.7	0.04	149.1±25.5	144.6±33.2	115.5±10.7	110.8±12.4	0.51