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Vasopressin mediates enhanced offspring protection in multiparous rats☆

Benjamin C. Nephew*, Elizabeth M. Byrnes, Robert S. Bridges

Department of Biomedical Sciences, Tufts University, Cummings School of Veterinary Medicine, 200 Westboro Rd, North Grafton, MA 01536, USA

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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 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.

1. 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., in press-a), but the mechanism that mediates this experience-dependent phenomenon has not been identified. The study of this robust increase in endogenous maternal aggression in multiparous mothers 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., in press-a). 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,b; 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

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* Corresponding author.
E-mail address: bcnephew@aol.com (B.C. Nephew).

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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., in press-a). 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.

2. Methods

2.1. Animals

Female Sprague–Dawley (Crl:CD[SD]BR) rats (175–200 g) were obtained from Charles River Laboratories (Wilmington, MA) and maintained in temperature (21–25 °C) and light (14:10 LD cycle; lights on at 0500 h) controlled rooms. Food and water were available *ad libidum* throughout the studies. Pregnancies were confirmed by the presence of sperm in a vaginal lavage. Two weeks after raising an initial litter of 8–10 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). 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.

2.2. Experimental Procedures

2.2.1. ICV cannulations

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 = −0.8 mm, ML = −1.5 mm). 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.

2.2.2. Infusions

Ten minutes prior to testing on d5, implanted females were administered either (icv) saline vehicle (2 μl) or one of three AVP (Sigma) doses (0.5, 2.5, 12.5 ng in 2 μl 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 μl of saline). All treatments were infused over 60 s. 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 h 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 min, 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.

2.2.3. Behavioral testing

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 s bins, and also generates frequency and duration summaries for all behavioral measures over the 15 min 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.

2.3. 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 $p < 0.05$.

3. 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 (Figs. 1A and 2A). Attack duration during the 15 min

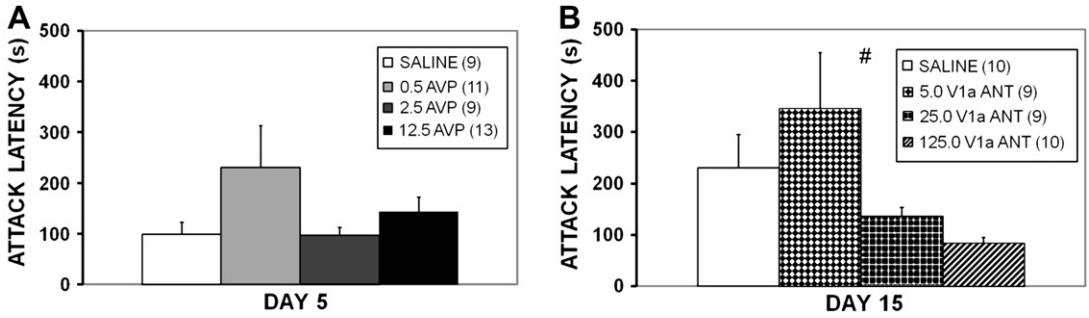

Fig. 1. (A–B) Mean (+SEM) seconds(s) for attack latency during 15 min maternal aggression trials in multiparous rats treated with (A) icv saline, 0.5, 2.5, or 12.5 ng AVP on day 5 of lactation, and (B) icv saline, 5.0, 25.0, or 125.0 ng V1a receptor antagonist on d15 of lactation. Sample sizes for each treatment are listed in parentheses. # indicates a significant overall effect of treatment ($p < 0.05$).