Hormones and Behavior 60 (2011) 128–138

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Effects of reproductive status on behavioral and endocrine responses to acute stress in a biparental rodent, the California mouse (*Peromyscus californicus*)

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ARTICLE INFO

Article history:
Received 14 December 2010
Revised 29 March 2011
Accepted 14 April 2011
Available online 30 April 2011

Keywords:
Corticosterone
Lactation
Maternal care
Paternal care
Biparental care
Stress
HPA reactivity
Monogamy

ABSTRACT

In several mammalian species, lactating females show blunted neural, hormonal, and behavioral responses to stressors. It is not known whether new fathers also show stress hyporesponsiveness in species in which males provide infant care. To test this possibility, we determined the effects of male and female reproductive status on stress responsiveness in the biparental, monogamous California mouse (*Peromyscus californicus*). Breeding (N = 8 females, 8 males), nonbreeding (N = 10 females, 10 males) and virgin mice (N = 12 females, 9 males) were exposed to a 5-min predator-urine stressor at two time points, corresponding to the early postpartum (5–7 days postpartum) and mid/late postpartum (19–21 days postpartum) phases, and blood samples were collected immediately afterwards. Baseline blood samples were obtained 2 days prior to each stress test. Baseline plasma corticosterone (CORT) concentrations did not differ among male or female groups. CORT responses to the stressor did not differ among female reproductive groups, and all three groups showed distinct behavioral responses to predator urine. Virgin males tended to increase their CORT response from the first to the second stress test, while breeding and nonbreeding males did not. Moreover, virgin and nonbreeding males showed significant behavioral changes in response to predator urine, whereas breeding males did not. These results suggest that adrenocortical responses to a repeated stressor in male California mice may be modulated by cohabitation with a female, whereas behavioral responses to stress may be blunted by parental status.

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Introduction

Lactational hyporesponsiveness, or blunted responsiveness to stress in lactating females, has been described in a number of mammalian species, including sheep (*Ovis aries*), flying foxes (*Pteropus hypomelanus*), Columbian ground squirrels (*Spermophilus columbianus*), Norway rats (*Rattus norvegicus*), and humans (Deschamps et al., 2003; Hubbs et al., 2000; Lightman, 1992; Reeder et al., 2004; Shanks et al., 1997; Tilbrook et al., 2006; Tu et al., 2005; Windle et al., 1997a). This phenomenon has been characterized most thoroughly in rats. Compared to virgin females, lactating female rats have been found to exhibit reduced adrenocorticotropic hormone (ACTH) and corticosterone (CORT) responses to a variety of stressors, including immobilization, endotoxins, acoustic startle, predator urine, and open-field tests (Deschamps et al., 2003; Lightman, 1992; Shanks et al., 1997; Toufexis et al., 1999a; Windle et al., 1997a). Lactating rats also exhibit reduced anxiety-like and stress-related behavior in open-field, elevated plus maze, noise stress, and acoustic startle response tests, compared to virgin females (Fleming and Luebke, 1981; Lonstein, 2005; Toufexis et al., 1999b; Windle et al., 1997a).

At the central level, lactating female rats show reduced stress-induced activation of afferent projections to the paraventricular nucleus of the hypothalamus (PVN), which activates the hypothalamic-pituitary-adrenal (HPA) axis through the release of the ACTH secretagogues corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) (da Costa et al., 2001; Toufexis and Walker, 1996; Toufexis et al., 1998). In addition, baseline CRH mRNA levels are lower, and AVP mRNA levels higher, in the PVN of lactating compared to non-lactating female rats (Fischer et al., 1995; Walker et al., 2001), while lactating rats express lower CRH mRNA levels in the PVN in response to hypertonic saline and restraint stress, compared to virgin females (da Costa et al., 2001; Lightman and Young, 1989). Lactating rats have also been found to exhibit reduced pituitary sensitivity to CRH compared to virgin females, thus partly explaining their lower CORT responses to stress (Toufexis et al., 1999a).

The functional significance of neuroendocrine hyporesponsiveness to stress during lactation is unknown. One possibility is that it could protect infants from high glucocorticoid concentrations in the mother's

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0018-506X/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.yhbeh.2011.04.002

milk (Lightman et al., 2001; Slattery and Neumann, 2008). CORT treatment of rat dams during lactation has long-term effects on HPA activity and anxiety-related behavior in their offspring (Brummelte et al., 2006; Casolini et al., 1997; Catalani et al., 1993, 2000). Another possibility is that reduced peripartum stress responsiveness functions to preserve energy essential for lactation (Numan and Insel, 2003), or to buffer parental behavior from disruption by stressors, as hormones of the HPA axis are thought to suppress parental behavior (Brunton et al., 2008; Carter et al., 2001; Lightman et al., 2001; Rivier and Rivest, 1991; Saltzman and Abbott, 2009; Wingfield and Sapolsky, 2003).

In approximately 6% of mammalian species, fathers, in addition to mothers, engage in parental care (Kleiman and Malcolm, 1981). The neuroendocrine basis of paternal behavior in rodents is unclear, and the role of hormones in the regulation of paternal behavior is currently under debate (Schradin, 2007; Wynne-Edwards and Timonin, 2007). Moreover, little is known of the neuroendocrine and affective consequences for fathers of engaging in paternal behavior. If, as described above, lactational hyporesponsiveness evolved to buffer parental behavior from stress, a reasonable hypothesis is that fathers of dependent offspring, in addition to lactating females, exhibit reduced neuroendocrine and behavioral responses to stress in biparental species.

We tested this hypothesis in a biparental rodent, the California mouse (*Peromyscus californicus*). California mice are socially and genetically monogamous, and males engage in extensive parental behavior upon the birth of their pups (Gubernick and Alberts, 1987; Ribble, 1991). The presence of the father accelerates pup development and increases pup survival, especially under energetically challenging conditions (Cantoni and Brown, 1997; Dudley, 1974; Gubernick et al., 1993; Gubernick and Teferi, 2000; Wright and Brown, 2002). Importantly, both circulating prolactin concentrations and AVP-ir within the brain have been shown to correlate with paternal behaviors in California mouse fathers (Bester-Meredith and Marler, 2003; Gubernick and Nelson, 1989). Given that both of these peptides are involved in the modulation of stress responsiveness, it is plausible that they may play a dual role in facilitating paternal behavior and ameliorating stress-reactivity in new fathers (Appenrodt et al., 1998; Bales et al., 2004; Everts and Koolhaas, 1999; Landgraf, 2006; Parker and Lee, 2001; Torner et al., 2002).

We characterized behavioral and CORT responses to predator-odor stress in breeding male and female California mice as compared to (1) virgin males and virgin females housed in same-sex pairs, and (2) nonbreeding (vasectomized) males and their female pairmates housed in heterosexual pairs. We chose to use predator urine as a stressor because it is ecologically relevant and because predator odors are known to be potent stressors in a variety of mammalian species (Figueiredo et al., 2003; Kavaliers et al., 2001; Ward et al., 1996; Zhang et al., 2003). Specifically, we used urine from bobcats and coyotes, two predators that are sympatric with California mice through at least part of the latter's range (including the region from which our colony was derived, the Santa Monica Mountains in southern California), and that prey heavily on rodents (Fedriani et al., 2000). We tested the breeding animals in the presence of their pups to avoid separation-induced stress (Lonstein, 2005); however, conflicting data describe the effect of the infants' presence during exposure to a stressor as either eliminating (rats: Deschamps et al., 2003) or further accentuating (sheep: Tilbrook et al., 2006) neuroendocrine hyporesponsiveness in mothers.

Previous studies of stress hyporesponsiveness in other species focused on females that were either pregnant or lactating, but not both (Lightman and Young, 1989; Shanks et al., 1999; Walker et al., 1992, 1995). In California mice and many other rodents, however, females commonly undergo a postpartum estrus and conceive shortly after giving birth, so that pregnancy and lactation coincide (Gubernick, 1988). Therefore, we characterized stress responsiveness in female California mice that were concurrently pregnant and lactating, in order to study the animals under more naturalistic reproductive conditions. In rats, the stage of lactation affects females' HPA responses to stress; early lactating females (≤1 week postpartum) display higher CORT and ACTH responses to stress than mid-lactating females (approximately 2 weeks postpartum; Deschamps et al., 2003; Walker et al., 1995). We therefore tested breeding pairs during both the females' early (5–7 days) and mid/late (19–21 days) postpartum periods, which coincided with early- and mid-pregnancy.

Materials and methods

Animals

We used male and female California mice purchased as adults from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA). Mice were maintained as described previously (de Jong et al., 2009, 2010). Briefly, animals were housed in transparent, 44×24×20 cm polycarbonate cages, with aspen shavings as bedding, cotton wool as nesting material, and food (Purina Rodent Chow 5001) and water available ad libitum. Lights were on from 0500 h to 1900 h (14:10), and room temperature and humidity were maintained at approximately 18–26 °C and 60–70%, respectively. Animals were inspected daily and weighed twice per week to monitor health and pregnancies, and cages and water bottles were changed once per week. Mice had been weaned at 25–35 days of age, initially housed in same-sex groups of 2–3 animals, and then housed in same-sex pairs upon their arrival in our lab until the start of the study. Animals had been ear-punched at weaning, and were marked with non-toxic hair color (Bigen, Hoyu Co. Ltd., Nagoya, Japan) at the start of the study for rapid identification. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the University of California, Riverside (UCR) IACUC. UCR is fully accredited by AAALAC.

Design

Male and female mice were randomly assigned to three reproductive groups (same-sex siblings were assigned to different conditions): breeding pairs, nonbreeding pairs, and virgins. Breeding pairs (N = 8) comprised a female (breeding females) and sham-vasectomized male (breeding males). Nonbreeding pairs (N = 10) comprised a female (nonbreeding females) and vasectomized male (nonbreeding males). Virgin males (sham-vasectomized; N = 9) and virgin females (N = 12) were maintained in unrelated same-sex pairs from the time of arrival in our laboratory and throughout the experiment.

Each pair of mice underwent two stress tests (see below) separated by 14 days, with each test immediately followed by collection of a blood sample; a baseline blood sample was collected two days before each stress test. Breeding males and breeding females underwent their first baseline blood sample 2–5 days after the birth of their first litter of pups. Breeding females gave birth to their first litter 42.3 ± 6.3 days after pair formation (mean ± SE), and all but two gave birth to their second litter 14–23 (16.2 ± 3.0) days after the second stress test. The schedule of baseline blood samples and stress tests for nonbreeding males, nonbreeding females, virgin males and virgin females was established by matching each nonbreeding or virgin pair to specific breeding pairs. Cohorts of animals containing mice from each reproductive group were tested concurrently. Time from pair formation (first day of pair-housing) to the first baseline blood sample did not differ between breeding (48.8 ± 6.2 days) and nonbreeding (44.1 ± 2.5 days) pairs (P = 0.463).

Mean age of males (381.4 ± 16.1 days) at the time of the first stress test did not differ significantly among the three reproductive groups (P = 0.259), and there were no significant differences in the mean length of time between surgery and the first stress test (61.4 ± 7.4 days) across male reproductive groups (P = 0.147). Mean age of females, however, differed among breeding (385.1 ± 20.4 days), nonbreeding (312.9 ± 12.0 days), and virgin (341.4 ± 12.9 days) females (F[2,29] =