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GLUCOCORTICOID RECEPTOR INVOLVEMENT IN PAIR BONDING IN FEMALE PRAIRIE VOLES: THE EFFECTS OF ACUTE BLOCKADE AND INTERACTIONS WITH CENTRAL DOPAMINE REWARD SYSTEMS

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Abstract—Induction of partner preferences in monogamous prairie voles (*Microtus ochrogaster*) was used to examine the possibility that blockade of glucocorticoid receptors may be rewarding in females of this species. We first examined the ability of either a mineralocorticoid receptor antagonist (spironolactone) or a glucocorticoid receptor antagonist (RU-486) to induce partner preferences in females. Peripheral administration of either of the antagonists was capable of inducing partner preferences, although the effective dose for RU-486 was an order of magnitude lower than that for spironolactone. We then examined a potential interaction of glucocorticoid receptor with central dopamine in pair bonding by treating females with i.c.v. dopamine receptor antagonists (haloperidol, SCH23390, or eticlopride) prior to peripheral administration of RU-486. All of the dopamine antagonists were capable of reversing the effects of glucocorticoid receptor blockade on pair bonding. These results establish the ability for acute blockade of glucocorticoid to induce pair bonds in female voles. Further, this effect appears to be mediated via an interaction with central dopamine systems. Together these findings support the possibility that, unlike other model systems, reductions in glucocorticoid receptor activity may enhance reward in female prairie voles. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: vole, social attachment, corticosterone, stress, *Microtus*, monogamy.

Pair bonding in prairie voles (*Microtus ochrogaster*) has been used extensively as a model for investigating the neural basis of social attachment. Both in the field and in the laboratory, this species displays characteristics associated with a monogamous life strategy (Getz et al., 1981), the most prominent of which is the formation of pair bonds between males and females. In the laboratory such pair bonds are manifested by a robust preference to associate with the familiar partner versus with a conspecific stranger (Getz et al., 1981). Importantly, such partner-preferences can be readily quantified in an experimental setting and provide a benchmark by which the effects of experimental manipulations can be assessed.

Recently, increasing attention has been paid to the role of reward processing in pair bond formation. The involvement of the prefrontal cortex (Young et al., 2001), nucleus accumbens (Aragona et al., 2003a; Gingrich et al., 2000; Liu and Wang, 2003; Wang et al., 1999), and ventral pallidum (Lim et al., 2004; Pitkow et al., 2001) in pair bonding supports the suggestion that activation of central "reward circuitry" plays a critical role in pair bond formation (Insel, 2003). Consistent with this notion, mating, which facilitates pair bond formation in this species, also is known to activate brain regions associated with reward processing (Mermelstein and Becker, 1995; Pfaus et al., 1990). Thus, pair bond formation by prairie voles may present an excellent model in which to examine the effects of potentially rewarding stimuli.

A number of studies have identified neurochemical systems that are important for pair bond formation and/or expression. Although there are sex-specific sensitivity differences, neurochemicals associated with pair bonding such as dopamine, vasopressin, and oxytocin appear to act similarly in both sexes in the regulation of social attachment (Cho et al., 1999; Aragona et al., 2003b; Gingrich et al., 2000). An exception to this pattern is found in the effects of the adrenal stress hormone corticosterone for which the effects on pair bonding in monogamous voles are sexually dimorphic (DeVries et al., 1996). Under normal circumstances, the formation of a pair bond by monogamous voles requires many hours of exposure to the partner. However, when corticosterone levels are chronically reduced via adrenalectomy, female prairie voles may form pair bonds within 1 h of exposure to a male (DeVries et al., 1995). In contrast, acute increases in corticosterone enhance, while adrenalectomy inhibits, the formation of pair bonds in male prairie voles (DeVries et al., 1996).

The information above suggests that, in male voles, increased glucocorticoid receptor activation enhances the reward value of the female. Such a response would be in accord with an extensive literature showing that the effects of stress or elevated corticosterone augment reward (Marinelli and Piazza, 2002). In contrast, adrenalectomy enhances pair bonding in females, suggesting that *reductions* in glucocorticoid receptor activation may be rewarding in female prairie voles, a suggestion that is contrary to the widely accepted view that corticosterone enhances reward. Most of the work examining the effects of stress on reward processing has been done in males and there are few studies directly comparing the effects of stress on reward processing between males and females. In one such study, Haney et al. (1995) showed that social stress affected self-administration of cocaine similarly in both

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Abbreviations: ANOVA, analysis of variance; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; NAcc, nucleus accumbens; VTA, ventral tegmental area.

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sexes. In general, although several studies reported sex differences in the magnitude of responses to stress, we could find no reports in which stress produced opposite effects on reward processing in males and females in other species. Thus, the first goal of the present study was to test the possibility that reduction in glucocorticoid receptor activation is rewarding in female voles by examining the effects of Type I, mineralocorticoid receptor (MR) and Type II, glucocorticoid receptor (GR) blockade on pair bond formation. Since pair bond formation likely involves a significant reward component, facilitation of pair bonding by GR blockade would provide evidence that GR blockade is rewarding in female prairie voles.

Any effects of GR blockade on reward processing likely involve interactions with central dopamine systems. Dopamine exerts its effects via activation of two families (D_1 and D_2) of receptors (Missale et al., 1998) and a number of studies have examined roles for each in reward processing. For example, the reward associated with self-administered cocaine is reduced by administration of a D_1 type antagonist into the ventral tegmental area (VTA) in rat (Ranaldi and Wise, 2001). Similarly, blockade of D_2 type dopamine receptors in the VTA reduces self-administration of morphine (David et al., 2002). Finally, Ikemoto et al. (1997) showed that concurrent activation of both types of dopamine receptors in nucleus accumbens plays a role in reward processing, possibly via a synergistic interaction. These studies show that blockade of either type of dopamine receptor potentially can reduce the reward value of certain stimuli. Thus, in the second part of this study, we test the ability of a variety of dopamine receptor antagonists to counteract the effects of GR blockade, thus providing further evidence that GR blockade may enhance reward in female prairie voles.

EXPERIMENTAL PROCEDURES

All procedures followed accepted animal care and use guidelines and were approved by the Institutional Animal Care and Use Committee at Florida State University. Care was taken to minimize the number of animals used and to minimize discomfort. All drugs were purchased from Sigma-Aldritch, St. Louis, MO, USA.

Subjects were female offspring of the F4 generation of a laboratory colony of prairie voles (*Microtus ochrogaster*) originating from Illinois. After weaning at about 21 days of age, pups were kept in same-sex sibling pairs until used in experiments. All animals were housed in plastic shoebox style cages (29×19×13 cm) under a 14/10-h light/dark cycle with *ad libitum* food and water. All subjects were about 70 days of age at the time of the experiments.

Neither of the GR antagonists we employed (RU-486 and spironolactone) is water-soluble. Previously we have used sesame oil as a vehicle for non-soluble compounds, however, in those cases, animals received oil at least 1 day prior to experimental manipulations (Curtis et al., 2001). In the present experiment however, animals receiving acute injections of the oil vehicle displayed a partner preference (see results, experiment 1). Thus we instead used propylene glycol as a vehicle for drugs injected i.p. Propylene glycol has previously been used in voles with no apparent effect on behavior (Lonstein, 2002). Dosages were calibrated such that each animal received 100 μl of propylene glycol/40 g body weight.

Experiment 1

Females were randomly assigned to experimental groups that received i.p. injections of either vehicle or vehicle containing the GR antagonist RU-486 (0.4, 4.0 or 40 mg/kg) or the MR antagonist spironolactone (4.0 or 40 mg/kg). An additional group received a mixture containing 4.0 mg/kg of RU-486 and 5.0 mg/kg of progesterone to control for the possibility that effects of RU-486 were via reduction of progesterone activity. Immediately after drug administration each female was paired with a male for 6 h of non-sexual cohabitation. Throughout the cohabitation period, the animals' interactions were videotaped (Panasonic time-lapse video recorder (12:1 compression) and low-light camera) for detailed behavioral analysis. Videotapes were subsequently examined to verify the absence of mating.

At the end of the 6 h cohabitation period, each female was tested for a partner preference. The apparatus for the partner preference test consisted of a central cage (20×25×45 cm) joined by hollow tubes (7.5×16 cm) to two identical parallel cages. One of these latter cages contained the familiar male partner, and the other contained an unfamiliar, conspecific male. The males were tethered to restrict their movements to their respective cages and thus had no direct contact with each other. The female was released into the central cage and had free access to all cages. All cages contained food and water. A customized computer program (R. Henderson, Florida State University) using a series of light beams across the connecting tubes was used to monitor movement of the female among the cages. The computer program recorded the amount of time the female spent in each cage and the number of transits between cages. Throughout the test, the animals again were videotaped for behavioral analysis. Variables included the time spent by the female in each male's cage, number of transits between cages (measures of activity to ensure that treatments did not affect locomotor behavior), and the frequency and amount of time the female spent in direct contact with each male. The amount of time females in each group spent in the neutral cage was assessed as a measure of time spent in isolation. Each test lasted for 3 h. For each group, comparisons of time spent in direct contact with the partner vs. that with the stranger (partner preference) were made using a paired *t*-test. Between groups treatment effects on other behavioral measures were evaluated using one-way analysis of variance (ANOVA).

Spironolactone can alter body fluid regulation (Rahmouni et al., 1999), and thus may alter the activity of oxytocin and vasopressin systems, both of which, in turn, can impact pair bonding (Cho et al., 1999). Therefore, any effects of spironolactone on pair bonding could be indirect. Thus, in a separate experiment, 13 female voles were given access to graduated drinking tubes containing 0.15 M NaCl for 1 week to acclimate them to the presence of a sodium source and then were given a less palatable 0.25 M NaCl solution for 24 h. Six hour baseline intakes of 0.25 M NaCl then were recorded. The following day, females were injected with 40 mg/kg of spironolactone (n=7) or with vehicle (n=6) and 6 h saline consumption again was recorded. Saline intakes were compared using two-way repeated measures ANOVA.

RESULTS

As mentioned, all groups that received i.p. injections of sesame oil displayed partner preferences regardless of whether RU-486 was present or not (Table 1). Replacing sesame oil with propylene glycol as an injection vehicle eliminated this confound (Fig. 1).

Both spironolactone and RU-486 were capable of affecting partner preferences in female prairie voles (Fig. 1). As expected vehicle treatment did not produce partner