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BRIEF COMMUNICATION

Oxytocin Inhibits Male Sexual Behavior in Prairie Voles[1]

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MAHALATI, K., K. OKANOYA, D. M. WITT AND C. S. CARTER. *Oxytocin inhibits male sexual behavior in prairie voles.* PHARMACOL BIOCHEM BEHAV **39**(1) 219–222, 1991.—Intracerebroventricular (ICV) injection of oxytocin (300 ng) produced an immediate cessation in sexual behavior in sexually active male prairie voles (*Microtus ochrogaster*). Other social behaviors including social contact, aggression, and autogrooming were not significantly affected by oxytocin, but males that received oxytocin ICV, versus injections that missed the ventricles, showed more sleep postures. Sexual behavior remained inhibited for at least 24 hours and was not activated in tests with a novel receptive female. Sexual and social behavior were not signficantly altered in animals in which the oxytocin injection missed the ventricles or in saline-treated males. These findings are consistent with the hypothesis that oxytocin plays a role in sexual satiety.

Prairie voles Oxytocin Male sexual behavior

OXYTOCIN is best known as a posterior pituitary hormone, with major effects in parturition and lactation. However, there is recent interest in the behavioral activity of this polypeptide. Oxytocin has been implicated in male sexual behavior (1, 2, 15), and there are indications that it can both faciliate (4, 12, 16, 23) and inhibit male sexual performance (12,23). Genital stimulation releases oxytocin (7, 17, 21–24). Oxytocin receptors have been identified in brain areas that regulate sexual behavior (13,28). In both male and female rats, intracerebroventricular (ICV) injection of oxytocin has been associated with increases in lordosis behavior (3,5). In contrast, in estrous female prairie voles, oxytocin injections were followed by a dose-dependent elimination of lordosis behavior, and there is at present no evidence that oxytocin enhances sexual behavior in this species (27). The purpose of the present study was to examine the effect of oxytocin (OT) on sexual and social behaviors in male prairie voles (*Microtus ochrogaster*).

Prairie voles are small rodents that exhibit a complex social organization including many features of monogamy (8,9). We have previously observed in this species that sexual interactions, characterized by mounting, can continue intermittently over a day or more (26). The hormonal condition of the female plays a major role in regulating the duration of sexual interactions. However, relatively little is known regarding the physiological factors that regulate male sexual activity and satiety in this or any other species (20).

In the present study, male behavior was observed before and after ICV administration of oxytocin or saline. The dose of oxytocin chosen (300 ng) was one which has been shown to produce a partial inhibition of female sexual behavior (27). In some cases injections missed the ventricles and behavioral comparisons also were made as a function of the site of injection. Each male was tested before and after injection with an estrous female, and some animals were monitored (using time-lapse videotaping) for 24 hours. After 24 hours each male also was tested with his original female versus a fresh estrous female in a 10-minute choice test. This test assessed the sexual satiety of each male as a function of prior oxytocin exposure, and also examined the sexual preferences of the male for a familiar versus an unfamiliar partner.

METHOD

Animals

Prairie voles were reared from stock originally captured near Urbana, IL. Animals were housed in $25 \times 25 \times 45$ cm polycarbonate cages with pine chip bedding. Purina rabbit chow and

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water were provided ad lib. A 14:10-h light:dark cycle was maintained with lights on at 0500 hours.

Upon weaning at 21 days of age, animals were housed in unisexual sibling groups of two to six animals until 60–90 days of age when they were randomly assigned to an experimental group. Males were prescreened for sexual activity in three 10-minute tests with behaviorally estrous females. Only males that showed mounts with intromissions during at least two of these tests were used here.

Oxytocin Treatment

Oxytocin (Peninsula) was suspended in 2:1 saline/India ink solution (300 ng/0.1 microliters/injection). Control injections consisted of an equal volume of the saline-India ink vehicle. Injections were administered directly into the the right lateral cerebral ventricle (ICV) using the method of Popick [(18), as modified for voles (27)].

Behavioral Tests

Behavioral tests were conducted in a 10-gallon glass aquarium with food and water available at all times. In tests of sexual behavior, ovariectomized stimulus females were brought into estrus by prior treatment with at least two daily injections of estradiol benzoate (EB, 1 μg in sesame oil, given subcutaneously). Females were screened for lordosis prior to use. Males were pretested for 1 hour for sexual behavior with an estrous female before treatment, allowed a one-week rest period, and tested, after injection with saline or oxytocin, for 1 hour. In addition to a 1-hour posttreatment test, which was directly observed and recorded, the 24-hour period following ICV injection was recorded on time-lapse videotape for a randomly sampled subset of animals from each group. Behaviors in all tests were recorded by an experimentally blinded observer, on a computerized event recorder. Among the behaviors recorded were frequencies and/or durations of mounting (without vaginal insertion), intromission (mounts with vaginal penetration), anogenital sniffing, side by side contact, autogrooming, and aggression, defined by biting, chasing, or wrestling behavior, apparent sleeping (immobile with head down) and sitting alone. Methods used in videotape recording and behavioral definitions are identical to those described in Witt et al. (26,27).

Pretests

Treatment males were placed in a 10-gallon aquarium for 10 minutes after which the stimulus female was placed in the aquarium and the behaviors described above were observed and recorded for 1 hour. After one hour of testing animals were individually housed. Only males that showed sexual behavior in this test were used for the next stage of the experiment.

Posttreatment Tests

Animals were anesthetized with metofane, injected ICV with saline or oxytocin, and tested approximately 15 minutes following injection. Behavioral interactions with an estrous female were observed directly for 1 hour and filmed for 24 hours using time-lapse videotape. At the end of 24 hours together the male was given an additional 10-minute choice test. In the latter test his original (familiar) female and a new estrous (unfamiliar) female were tethered at opposite ends of a 10-gallon glass aquarium. Each female could move over approximately one-third of the aquarium, while the male was free to move throughout. All behaviors and the position of the male within the aquarium were recorded.

To confirm the injections following testing, animals were deeply anesthetized and then decapitated. The brain was removed from the skull and placed in formalin solution for several days, followed by hand sectioning and microscopic examination. If dye was detected only in the ventricles, that animal was classified as an "ICV" subject. Animals in which dye was detected in cortex or meninges were classified as a "miss."

Analysis

In the saline group only two animals were classified as "misses," prohibiting the analysis of data for this subgroup, although their behavior was similar to that of animals receiving ICV saline. A Kruskal-Wallis one-way analysis of variance was used to determine group differences among the "saline," "oxytocin ICV," and "oxytocin miss" groups. Within group comparisons (differences before and after injection in one group) were made using the Mann-Whitney U-test. Tests were two-tailed and an alpha of 0.05 was required for significance throughout.

RESULTS

ICV administration of oxytocin eliminated male sexual behavior in the posttest (Table 1). However, when saline was injected or when oxytocin injections missed the ventricles, most males continued to show normal levels of copulatory behavior. During the twenty-four-hour videotaping males receiving ICV oxytocin did not resume sexual behavior and they did not mate when presented with a fresh female in the preference test. Data for other behavioral measures on tests before injection and immediately following injection are shown in Table 1. Oxytocin injection within the ventricles also was associated with reductions in anogenital sniffing toward the female and these males spent more time sitting still in a posture that was interpreted as sleep.

The analysis of 24-hour time-lapse videotapes for three saline males indicated that these males continued to mount intermittently throughout almost the entire test with an average of 72 mounts across the 24 hours. In contrast, in three oxytocin-ICV males, one showed only one mount during the 24 hours of testing and the other males did not mount at all. Other behaviors including anogenital investigation, time sitting, side by side contact, and aggression, were similar in oxytocin- and saline-treated animals.

In the posttreatment choice test (24 hours after treatment) males did not exhibit a significant preference for either female. Even in the presence of a fresh estrous female, oxytocin-ICV males continued to fail to show sexual behavior, although other behavioral patterns did not differ significantly among groups on this test.

DISCUSSION

ICV administration of 300 ng of oxytocin in sexually active male prairie voles immediately inhibited sexual behavior and sexual activity did not return during the 24 hours of these observations. In contrast, oxytocin injections that missed the ventricles and ICV injection of the vehicle solution did not significantly affect sexual performance. Males receiving ICV oxytocin showed decreased levels of anogenital sniffing toward the estrous test female and increased the time they spent in sleep-like postures during the first hour after injection. In males observed on time-lapse videotape for 24 hours following injection, behaviors other than sexual behaviors were apparently normal and in males

TABLE 1

SEXUAL AND SOCIAL BEHAVIORS IN MALE PRAIRIE VOLES BEFORE AND AFTER TREATMENT WITH OXYTOCIN (OT ICV), OR OXYTOCIN WHICH MISSED VENTRICLES (OT-Miss) OR SALINE (ICV)

Behavior	OT-ICV Pretest N=7	OT-ICV Posttest N=7	OT-Miss Pretest N=6	OT-Miss Posttest N=6	Saline Pretest N=7	Saline Posttest N=7
Mount (f)	27 ± 10	0*	39 ± 6	34 ± 11	45 ± 15	40 ± 17
Intro. (f)	52 ± 22	0*	73 ± 23	106 ± 50	39 ± 13	34 ± 12
Ejac. (f)	2 ± 0	0*	2 ± 0	3 ± 0	3 ± 0	2 ± 0
Aggres. (f)	5 ± 2	5 ± 2	3 ± 3	12 ± 7	4 ± 2	2 ± 1
S × S (d)	762 ± 400	916 ± 497	339 ± 189	862 ± 525	1199 ± 448	915 ± 241
Sit. (d)	36 ± 17	940 ± 409*	200 ± 135	462 ± 257	319 ± 229	662 ± 604
Sleep. (d)	42 ± 42	288 ± 159*	121 ± 119	51 ± 51	191 ± 137	58 ± 41
Autog. (f)	49 ± 9	54 ± 10	45 ± 7	61 ± 19	43 ± 7	74 ± 11

Values are mean ± sem. Abbreviations: f = frequency and d = duration (in s) per 60-minute test. Mount = mounting, intro. = intromissions, ejac. = ejaculations, aggres. = aggression, S × S = side by side contact, sitting = sitting alone, sleep. = apparent sleep, autog. = autogrooming. *$p<0.05$, pretest versus posttest, Mann-Whitney U-test.

tested 24 hours following injection.

In rats the serum-half life of oxytocin is only a few minutes (19). Comparable measures for voles and as a function of ICV injection are not available; however, it is possible that the behavioral effects of oxytocin continued beyond the time when injected oxytocin was present. Long-term effects have been reported for other behavioral measures (14). Such effects could represent long-lasting receptor changes or other biochemical consequences of exposure to oxytocin.

Behavioral changes were not observed in males receiving oxytocin injections that did not hit the ventricles. However, these results do not exclude possible peripheral effects of oxytocin. The densely packed nature of brain tissue may prevent movement into the ventricles or into peripheral circulation (11). A recent study in this laboratory (25) revealed that peripheral (IP) injections of 500 ng oxytocin immediately eliminated male sexual behavior in 8 of 12 males tested, while 50 ng of IP oxytocin did not affect male sexual behavior.

Male sexual behavior in prairie voles is apparently more sensitive to the inhibitory effects of oxytocin than is female sexual behavior. ICV injection of 300 ng of oxytocin in female prairie voles inhibited lordosis behavior in about half of the females tested, while 1000 ng produced a total inhibition of female sexual behavior. In prairie voles large doses of IP oxytocin (1000 to 10,000 ng) did not inhibit female sexual behavior (27).

Several investigators have suggested that oxytocin release might participate in sexual satiety (10, 12, 23). The present finding offers tentative support for this hypothesis. However, it also is possible that malaise or soporific effects of oxytocin injections contributed indirectly to the observed inhibition of sexual behavior.

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