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Apomorphine Stimulation of Male Copulatory Behavior Is Prevented by the Oxytocin Antagonist $d(CH_2)_5Tyr(Me)$-Orn^8-Vasotocin in Rats

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ARGIOLAS, A., M. COLLU, P. D'AQUILA, G. L. GESSA, M. R. MELIS AND G. SERRA. *Apomorphine stimulation of male copulatory behavior is prevented by the oxytocin antagonist $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin in rats.* PHARMACOL BIOCHEM BEHAV **33**(1) 81–83, 1989.—The effect of the intracerebroventricular (ICV) injection of the oxytocin antagonist $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin on the stimulation of copulatory behavior induced by the dopamine (DA) agonist apomorphine was studied in male rats. Apomorphine (80 μg/kg SC) given 5 min before mating tests decreased intromission frequency and ejaculation latency in experienced male rats. Such effects were abolished and reversed by pretreatment with 50 and 1000 ng of the oxytocin antagonist given ICV 5 min before apomorphine. The peptide per se markedly increased intromission and ejaculation latency and abolished ejaculation in control rats. The results suggest that brain oxytocin is implicated in the expression of sexual behavior, and apomorphine might improve male copulatory behavior by releasing oxytocin in brain.

Apomorphine Oxytocin antagonists Copulatory behavior

THE existence of a neuronal dopamine (DA)-oxytocin link in the central nervous system involved in the regulation of sexual behavior is suggested by the ability of both DA agonists and oxytocin to induce repeated episodes of penile erection in male rats (1, 3, 9, 10) and to improve copulatory behavior of vigorous sexually experienced male rats in the presence of a female in estrus (5, 6, 11). In agreement with this hypothesis we have recently found that penile erection induced either by the DA agonist apomorphine or by oxytocin is prevented by the intracerebroventricular (ICV) injection of the potent oxytocin antagonist $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin (2). To provide further evidence for the the existence of such DA-oxytocin link, we have studied the effect of $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin on the facilitatory effect of apomorphine on copulatory behavior in vigorous sexually experienced male rats.

METHOD

The methods are the same as those previously reported (4,11). Male Wistar rats (Charles River, Como, Italy) 3 months old and weighing 275–300 g at the beginning of the experiments were used. The animals were individually caged at 24°C, 60% humidity under reversed 12-hr light/dark cycle (lights from 11:00 p.m. to 11:00 a.m.). A population of rats was selected which reached at least 2 ejaculations in the last 4 of 8 mating tests performed at weekly intervals, with an ovariectomized Wistar female rat brought into estrus by subcutaneous injections of oestradiol benzoate (200 μg/rat in oil) and progesterone (0.5 mg/rat, in oil), 48 and 6 hr before the mating test, respectively [see (11)]. Mating tests were carried out during the dark phase of the cycle, from 4.00 to 6.00 p.m. in a room lit by a dim red light. A female was introduced into the male's home cage, and the following measures of copulatory behavior were recorded on an event recorder (Esterline Angus) for 30 min. Intromission latency: the time which elapsed from the introduction of the female into the male's cage until the first intromission. Ejaculation latency: the time from the first intromission until the first ejaculation. Mount frequency and intromission frequency: the number of mounts or intromissions in a series. Ejaculation frequency: total number of ejaculations during 30-min observation. Postejaculatory interval: the time from the first ejaculation to the next intromission. Ejaculation refers to the behavioral response and not seminal emission. Each test included an equal number of saline- and drug-treated rats. Each rat in a given group received randomly each of the treatments reported in the table.

ICV injections were performed through stainless steel guide cannulas aimed at one lateral ventricle stereotaxically implanted under chloral hydrate anaesthesia 5 days before the experiments (coordinates: 1 mm anterior to bregma, 1 mm lateral to midline, and 2 mm ventral to dura) (12) as previously described (4). One week after surgery, rats were retested for sexual behavior and only those that were found to be as vigorous as before were used. The oxytocin antagonist $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin (7) (a generous gift by Dr. M. Manning, Toledo University, OH), dissolved in

TABLE 1

EFFECT OF $d(CH_2)_5Tyr(Me)$-Orn^8-VASOTOCIN (oxy ant) ON THE COPULATORY BEHAVIOR OF SALINE- AND APOMORPHINE-TREATED MALE RATS WITH RECEPTIVE FEMALES

	Saline			Apomorphine		
Copulatory Parameters	0	50 ng ICV oxy ant	1000	0	50 ng ICV oxy ant	1000
Introm. Lat.	60 ± 7	214 ± 99*	232 ± 77*	50 ± 10	110 ± 29†	308 ± 85*†
Ejacul. Lat.	678 ± 175	>1800	>1800	394 ± 96*	740 ± 110†	>1800
Mount Freq.	6.6 ± 0.6	—	—[c]	3.5 ± 1.0*	6.0 ± 0.9†	—[c]
Introm. Freq.	13.4 ± 0.5	—[c]	—[c]	5.2 ± 2.6*	10.4 ± 3.0†	—[c]
Ejacul. Freq.	2.7 ± 0.3[a]	0	0	2.9 ± 0.3[a]	2.3 ± 0.2[a]	0
Postejac. Int.	455 ± 25	—	—	534 ± 60	580 ± 98	—

A group of 30 sexually vigorous male rats was used. Each rat randomly received each of the treatments at 7-day interval. Apomorphine (80 μg/kg SC) or saline (60 μl for each 100 g of body weight SC) was injected 5 min before the mating test. ICV treatments were: saline 5 μl (oxy ant = 0); oxy ant 50 and 1000 ng dissolved in 5 μl of saline, 10 min before the mating test. Values are means ± S.E.M. for the same rats under different treatment conditions. Intromission frequency, ejaculation latency and postejaculatory interval are expressed in seconds. *$p<0.001$ with respect to saline not receiving oxy ant; †$p<0.001$ with respect to apomorphine not receiving oxy ant (one-way analysis of variance followed by the comparison of the means by Student's *t*-test).

[a]All rats ejaculated at least twice; [c]cannot be calculated.

saline or saline alone (5 μl/rat) was injected ICV 10 min before the mating test. Five min later, the rats received in the back of the neck a subcutaneous injection of 60 μl of saline with or without freshly dissolved apomorphine-HCl (Sigma) (1 mg/7.5 ml) for each 100 g of body weight. The statistical significance of the data was analyzed by one-way analysis of variance followed by comparison of the means by Student's *t*-test.

RESULTS

Sexually experienced male rats attained ejaculation with receptive females after 8–14 penile intromissions and within 8–14 min after the first intromission, and ejaculated at least twice in a 30-min mating test. As shown in Table 1, the ICV injection of 5 μl of saline 5 min before the mating test failed to induce any significant change in the above parameters. In agreement with previous studies (11), 80 μg/kg SC of apomorphine given 5 min before the mating test markedly decreased intromission frequency and ejaculation latency ($F=52.6$ and 78.8, respectively, $p<0.001$). These effects of apomorphine were abolished by pretreatment with 50 ng ($F=35.6$ and 34.8, respectively, $p<0.001$) and 1000 ng of ICV oxytocin antagonist given 5 min before apomorphine, respectively. In particular, the dose of 1000 ng abolished ejaculation in all apomorphine-treated rats. As already reported (4), the oxytocin antagonist increased significantly the intromission latency ($F=54.6$, $p<0.001$) and abolished ejaculation in saline-treated rats.

DISCUSSION

The present results show that the ICV administration of the potent oxytocin antagonist $d(CH_2)_5Tyr(Me)$-Orn^8-vasotocin not only impairs in a dose-dependent manner sexual performance of male experienced rats in the presence of a receptive female (4), but also prevents the facilitatory effect of apomorphine on copulatory behavior. Although the peptide per se inhibits copulatory behavior, it is unlikely that the reversal of apomorphine effect is due to a nonspecific depressant effect of the peptide, since it inhibits copulatory behavior without inducing any other gross behavioral change (4). The finding is in agreement with the hypothesis that a central DA-oxytocin link is involved in the expression of sexual behavior. Accordingly, both apomorphine and oxytocin have been found able to induce penile erection and to improve sexual performance in male rats (see Introduction). Since the blockade of oxytocin receptors prevents both the improvement of sexual performance (present results) and penile erection induced by apomorphine and oxytocin (2), it is likely that these effects of DA agonists are mediated by an activation of the central oxytocinergic transmission. Conversely, DA receptor blockers prevent the stimulatory effect of apomorphine on sexual performance (11) and penile erection (13), but not that of oxytocin (1). The finding well correlates with the ability of low doses of apomorphine (that induce penile erection and facilitate copulatory behavior) to increase the circulating concentration of oxytocin (Melis *et al.*, submitted) and that plasma oxytocin levels are increased after ejaculation in rabbits (14) and during sexual response in humans (8,15).

Other than provide further support for a neurotransmitter role of oxytocin [see (1) and enclosed references], the ability of oxytocin antagonists to abolish the decrease in intromission frequency and ejaculation latency induced by DA agonists might be of clinical significance. Accordingly, since the sexual response to DA agonists is considered to be an animal model of premature ejaculation (11), synthetic centrally acting oxytocin antagonists able to cross the blood-brain barrier might be a valid tool for the control of this sexual disturbance.

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