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Copulation-induced antinociception in female rats is blocked by atosiban, an oxytocin receptor antagonist

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ABSTRACT

Aims: We hypothesized that copulation-induced temporary anti-nociception in female rats is mediated by the activation of central and/or peripheral oxytocin receptors. To test this hypothesis, we assessed the effects of intraperitoneal (ip), intrathecal (it), and intra-cerebroventricular (icv) administration of an oxytocin receptor antagonist (atosiban), on copulation-induced temporary anti-nociception in estrous rats.

Main methods: The treatment groups were ovariectomized rats pre-treated subcutaneously (sc) with 10 μg of estradiol benzoate (EB) followed 24 h later by an sc injection of 5 μg EB, and 4 h later, by an sc injection of 2 mg progesterone (P4). Rats were then administered saline vehicle (ip, it, or icv: control groups) or atosiban (500 μg/kg ip; 500 ng it; or 500 ng icv: experimental groups). Thirty minutes after drug or saline administration, their sexual behavior was tested by pairing with a sexually-experienced male rat. Brief pulse trains of 50 Hz, 300 ms duration, supra-threshold tail electrical shocks (STS) were delivered before and during copulatory activity i.e., while the female was receiving mounts, intromissions, or ejaculations, and we recorded whether vocalization occurred in response to each STS.

Key findings: Replicating our previous findings, the vocalization response to STS in control rats was significantly attenuated during intromissions and ejaculations, compared to their baseline (pre-mating) response, indicative of anti-nociception. By contrast, rats pre-treated with atosiban (each route of administration) failed to show an attenuation of the vocalization response to shock.

Significance: These findings provide evidence that the temporary anti-nociceptive effect of copulation in female rats is mediated by copulation-induced release of endogenous oxytocin in brain, spinal cord and periphery.

1. Introduction

Oxytocin has diverse biological effects on multiple reproductive behavior patterns. Oxytocin also inhibits nociception (Lundeberg et al., 1994). In rats, nociception is inhibited by concurrent sexual behavior: female rats vocalized in response to tail shocks less during the male's intromissions and ejaculation than during non-copulatory intervals (Gómora et al., 1994). This antinociceptive effect is induced by vaginocervical stimulation (via mating or artificially) and was blocked by transection of the pelvic and hypogastric nerves, but not the pudendal nerve (Gómora et al., 1994; Cunningham et al., 1991). Vaginocervical stimulation releases oxytocin within the spinal cord in rat (Sansone et al., 2002). Such release is probably accompanied by release of oxytocin in brain (Eliava et al., 2016).

Since the antinociceptive effect of vaginocervical stimulation described above could be mediated by endogenous oxytocin release within the brain, spinal cord, and/or into the peripheral circulation, we hypothesized that the antinociceptive effect of vaginocervical stimulation is due to endogenous central and/or peripheral oxytocin release. In accordance with this hypothesis, we predicted that intromission-induced antinociception in female rats would be attenuated by atosiban, an oxytocin receptor competitive antagonist that does not cross the blood-brain barrier (BBB) (Meisenberg and Simmons, 1983). The BBB was bypassed by administering the atosiban i.c.v. or i.t. By administering the atosiban also i.p., we assessed whether peripheral oxytocin receptors might be involved in the antinoception.

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2. Material and methods

2.1. Animals

Adult female Sprague-Dawley rats of 200–250 g were housed 4 per cage (45 × 30 × 20 cm) under a 14:10 h light: dark cycle, 22 ± 2 °C, and with Purina rat chow and water ad libitum. Our institutional ethics committee approved all animal procedures. 50 adult rats were anesthetized with ketamine 80 mg/kg (Bristol Laboratories, IP) and xylazine (Haver Lockhart, 4 mg/kg, IP). An incision was made in the ventrum, the ovaries were removed, and the abdominal cavity was closed with sutures. Penicillin (100 I.U., intramuscularly) was administered, and the animal was returned to the home cage.

2.2. Implantation of intrathecal catheter

One week after ovariectomy, sixteen rats were anesthetized as described above, then a 7.5 cm catheter (Clay Adams PE-10 tubing; Fisher Chemical) was inserted permanently in the subarachnoid intrathecal space through an incision made in the atlanto-occipital region according to the technique of Yaksh and Rudy (Yaksh and Rudy, 1976). The catheter extended to the lumbar level of the spinal cord. After surgery, animals were given an injection of penicillin (100 I.U.) and housed individually.

2.3. Implantation of intracerebroventricular cannula

One week after of the ovariectomy, eighteen female rats were anesthetized as described above and placed in a Kopf stereotaxic instrument (Tujunga, CA, USA) for implantation of a stainless Steel cannula (22 ga, 17 mm length) into the right lateral ventricle according to coordinates from the atlas of Paxinos and Watson (Paxinos and Watson, 1998) (antero-posterior +0.80 mm, mediolateral 1.5 mm, dorsoventral −3.5 mm with respect to bregma). A stainless steel screw was fixed to the skull, and cannula and screw were attached to the bone with dental cement. A dummy cannula (30 ga) provided with a cap was introduced into the guide cannula to prevent clogging and contamination. Immediately after surgery, animals were given an injection of penicillin (100 I.U.) and housed individually.

2.4. Drug administration

In Experiment 1, atosiban (Sigma Aldrich, 0.5 mg/ml in saline) was administered ip at a dose of 500 μg/kg body weight.

Atosiban (1 mg/kg bw) was reported to inhibit oxytocin-induced analgesia (Abbasnezhad et al., 2016). We observed that when we administered this dose of atosiban to female rats pretreated with EB and P, their sexual behavior was inhibited. For this reason, we reduced the atosiban dose that we used to 500 μg/kg bw. At this dose, we observed that female sexual behavior was expressed normally, but the copulation-induced analgesia was significantly reduced (unpublished findings). In Experiment 2, atosiban was administered intrathecally (it, 500 ng in 5 μl saline) according to the method of Hylden and Wilcox (Hylden and Wilcox, 1980). The drug was administrated over a period of 120 s using a 25 μl microsyringe mounted on a microinjector. The 7.5 cm length PE10 catheter contained 7 μl of saline, plus 5 μl of atosiban or saline, plus 7 μl of saline as a flush. In Experiment 3, atosiban was administered intracerebroventricularly (icv; 500 ng in 1 μl) using a 10 μl microsyringe, over a period of approximately 50–60 s, according to the method previously described (Gómora et al., 1994).

2.5. Experimental procedure

2.5.1. Induction of sexual behavior

Two weeks after ovariectomy, animals received a sc injection of 10 μg estradiol benzoate (EB; Sigma Aldrich), followed 24 h later with another sc injection of 5 μg EB. Then, 24 h after the final EB injection, animals received a sc injection of 2 mg progesterone (P; Sigma Aldrich). This hormonal treatment reliably induces estrous behavior (Beyer et al., 1988). Four hours after the P injection, sexual receptivity was tested by placing the female inside a circular (50 cm diameter) Plexiglas arena together with a vigorous male. Females received ten mounts by the male and only those showing a lordosis quotient [LQ = (number of lordosis/number of mounts) × 100] of at least 80 were used in this experiment; this criterion eliminated approximately 10% of the animals. All observations were performed by an observer blind to the treatments, during the dark phase of the diurnal cycle under dim red light.

2.5.2. Determination of the vocalization threshold to tail shock

Five minutes after the sexual behavior test, the vocalization response to the tail shock in the same arena was measured. A pair of electrodes were attached to the base of the tail and connected to a DC stimulator (Coulbourn model E 13–51) through a switch as described previously (González-Mariscal et al., 1992). The parameters of the stimulus were: pulses of 50 Hz, with a train duration of 300 ms, the current intensity was increased in steps of 100 μA until the rat vocalized (audible to the human ear) and then the intensity was decreased in steps of 100 μA until the vocalization in response to the tail shock did not occur. This process was repeated three times. The threshold of vocalization was calculated as the average of the inflection points. Then, ten shocks of 20% intensity above the threshold (supra-threshold shocks: STS) were administered to the tail of the female rat at intervals of 10 s, and the number of times that vocalization occurred to each of the 10 shocks was recorded as the baseline response.

2.5.3. Copulation-induced antinociception and effect of atosiban

Saline or atosiban was administered immediately after establishing the baseline vocalization response. Thirty minutes later, a sexually experienced male rat was introduced into the arena. A single 20% STS was given to females at the onset of each mounting train. Behavior patterns were defined as follows: a mount (M) consisted of the male rat climbing onto the female's rump and grasping the flanks with the forelegs, followed by approx. 15 to 20 rapid thrusts to the perineal region; intromission (I) was a mount motor pattern with insertion of the penis into the vagina; ejaculation (E) was an intromission motor pattern with a duration of approx. 640 ms (Beyer et al., 1981). The percent of STS applications that elicited vocalization following M, I, or E across the entire copulatory series was calculated for each animal, and the percent of females vocalizing after a single ejaculation ($n = 8$) was also determined. Thus, the timing of the shocks and the total number of shocks that each female received was determined by the male's behavior, rather than by a predetermined schedule, since the experimenter delivered each shock upon initiation of the mounting train. With this stimulation schedule, a single shock train was administered 100–150 msec after initiation of each copulatory event. A subgroup of sexually unreceptive rats (ovx and treated only with saline, 1 ml/kg body weight; $n = 8$) was also tested, in order to determine if mounting trains without intromissions affected the vocalization response in unreceptive females.

2.6. Statistical analysis

In both control and atosiban groups, the effect of M, I or E on antinociception was analyzed, by the ANOVA one way test (Siegel and Castellan, 1988), followed by a *t*-test comparing the baseline vocalization response to the response when the females received M, I or E from the male.

3. Results

In the ip saline-treated rats, the vocalization response was significantly reduced in association with intromissions ($t = 5.18$; df = 14,

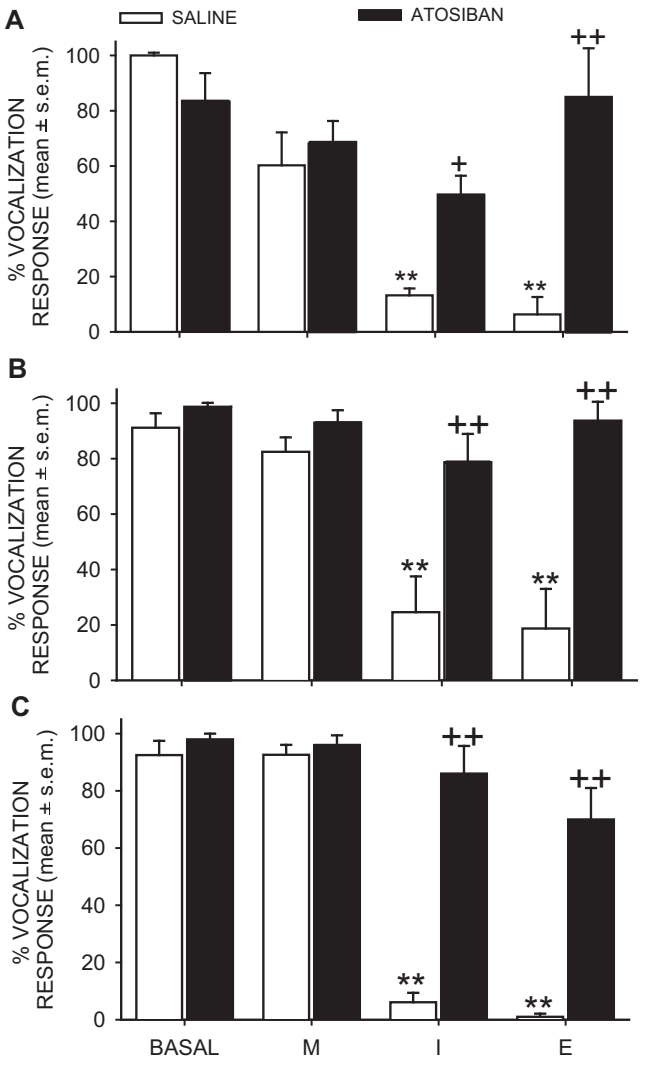

Fig. 1. Intraperitoneal, intrathecal or intracerebroventricular administration of atosiban: Effect on the vocalization response to suprathreshold shocks (20% above the vocalization threshold) during copulation in female rats. * = $p < 0.05$, ** = $p < 0.01$ comparison of basal vocalization response vs Intromissions or Ejaculations. + = $p < 0.05$, + + = $p < 0.01$ comparison between atosiban group vs control group.

$p < 0.001$) and ejaculation ($t = 5.17$; $p < 0.001$) compared to the baseline vocalization response. By contrast, in females that received ip atosiban, the vocalization response to STS was not significantly reduced during mounts, intromissions or ejaculations, compared to their baseline vocalization response. However, compared to saline treatment, the antinociceptive effect was significantly reduced in the atosiban treated females during both intromissions ($t = 3.5$; df = 14, $p < 0.01$) and ejaculations ($t = 5.1$; df = 14, $p < 0.01$) (Fig. 1A). A separate group of females that did not receive EB or P (and therefore were not sexually receptive and did not display lordosis) showed no changes in vocalization response in association with the male's mounts (intromissions or ejaculations did not occur). Similar results were obtained with atosiban or saline delivered it or icv. In animals that received it saline, the vocalizations in response to STS were significantly lower when the rats received intromissions ($t = 5.18$; df = 14 $p < 0.01$), or ejaculations ($t = 5.17$; df = 14, $p < 0.01$), compared to their baseline response. By contrast, atosiban-treated rats did not display a significant reduction in vocalizations in response to intromissions or ejaculations (Fig. 1B). Thus, the antinociceptive effect induced by mounts ($t = -3.2$; df = 14, $p < 0.05$) intromissions ($t = -3.5$; df = 14, $p < 0.01$) and ejaculations ($t = -5.1$; df = 14, $p < 0.01$) was significantly reduced in atosiban-treated females, compared to saline controls (Fig. 1B). Animals that received icv saline displayed significantly reduced vocalizations in response to intromissions ($t = 14.7$; df = 14, $p < 0.01$) and ejaculations ($t = 18.8$; df = 14, $p < 0.01$), compared to their baseline response. However, in females that received icv atosiban, the vocalizations in response to intromissions and ejaculations did not differ significantly from the number of their baseline vocalization responses. Thus, the antinociceptive effect induced by intromissions ($t = -6.9$; df = 16, $p < 0.01$) and ejaculations ($t = -5.6$; df = 16, $p < 0.01$) was significantly reduced in atosiban-treated females, compared to saline controls (Fig. 1C). Cohen's d effect size analysis revealed that the effect size was greater during intromissions or ejaculations than during mounts in all groups, and greater in the icv group than the other groups during mounts as well as intromissions and ejaculations (Table 1).

Table 1
Effect size of the atosiban intraperitoneal, intrathecal or intracereroventicular injection in EB plus progesterone treated females.

Atosiban treatment	Mounts	Intromissions	Ejaculations
Ip	−0.19	−0.76	−0.71
It	−0.17	−0.75	−0.74
Icv	−0.21	−0.93	−0.85

Values are comparisons between saline vs atosiban.

4. Discussion

During copulation, the female rat receives several different types of sensory stimulation, including flank and perineal stimulation during the male's mounts and vaginocervical stimulation in response to penile intromission. This stimulation most likely activates the pelvic (Peters et al., 1987) and vagus nerves (Komisaruk et al., 1996), which in turn stimulate the release of oxytocin from magnocellular and parvocellular neurons of the paraventricular nucleus of the hypothalamus (Swanson and Kuypers, 1980). Since both vaginocervical stimulation and exogenous oxytocin administration have antinociceptive effects, we hypothesized that the antinociceptive effects of vaginocervical stimulation are due to endogenous central and/or peripheral oxytocin release. We tested this hypothesis by assessing the effects of systemic, intrathecal or intracerebroventricular administration of atosiban. Replicating our previous findings, the number of vocalizations in response to STS was markedly reduced during intromissions and ejaculations. This antinociceptive effect was prevented (i.e., significantly reduced) by atosiban administered intracerebroventricularly or intrathecally, as well as systemically. These results provide evidence that both central and peripheral oxytocin receptor sites can mediate the antinociceptive action of vaginocervical stimulation.

Animal models that examined effects of exogenous oxytocin administration show that intracerebral oxytocin receptors can mediate antinociception. Oxytocin infused into the ventrolateral orbital cortex produced anti-allodynia, an effect that was inhibited by atosiban (Taati and Tamaddonfard, 2018). Furthermore, oxytocin infusion into the caudate nucleus or periaqueductal gray increased pain thresholds; in the periaqueductal gray this effect was mediated in part by endogenous opioid release (Yang et al., 2011). Infusion of oxytocin into the central nucleus of the amygdala or nucleus accumbens in rats reduced sensitivity to noxious thermal and mechanical stimulation of the paw, effects that were blocked by an oxytocin antagonist, and, in the case of the nucleus accumbens, mediated by activation of opioid receptors (Han and Yu, 2009).

There is evidence that spinal cord or peripheral oxytocin receptors also mediate antinociception. Parvocellular oxytocinergic neurons of the paraventricular nucleus descend into the spinal cord where they release oxytocin (Rojas-Piloni et al., 2010). Electrical stimulation of the

PVN as well as topical application of oxytocin onto the dorsal horn region inhibited A-delta and C-fiber responses; these effects were blocked by an oxytocin antagonist (Sansone et al., 2002; Rojas-Piloni et al., 2010). Intravenous administration of oxytocin reduced action potentials of C-type nociceptive fibers, and stress-induced analgesia was reduced by intravenous administration of an oxytocin antagonist (Juif and Poisbeau, 2013). Oxytocin receptors in the spinal cord are involved in the inhibition of the firing of A-delta and C fibers, while immunohistological findings indicated that oxytocin receptors are located in specific nociceptive nerve terminals (González-Hernández et al., 2017).

In the present study, we provide evidence that intromissions and ejaculations induced antinociception in female rats through the endogenous release of oxytocin, an effect that could be mediated by oxytocin receptors within the brain, spinal cord, and the periphery. Recently, an independent study demonstrated that, in male rats, copulatory behavior was likewise associated with an antinociceptive response to tail shock, and this effect was inhibited by the intracerebroventricular administration of an oxytocin receptor antagonist (Futagami et al., 2016). Together, these studies provide evidence of a significant role for endogenous oxytocin release in modulating pain sensitivity in response to copulatory stimulation.

Conflict of interest statement

All authors affirm that there are no conflicts of interest associated with carrying out this study.

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