Oxytocin is a nonapeptide produced in the paraventricular and supraoptic nuclei of the hypothalamus. Oxytocin neurons project to the posterior pituitary gland from where the peptide is released into the circulation. The hypothalamic neurons also project to the olfactory bulb and to the superficial laminae of the spinal cord, areas known to be involved in the regulation of autonomic processes as well as nociception.1

Both nociceptive and anti-nociceptive effects of central (10 ng–10 μg i.t. or 150 ng–1 μg i.c.v.) administration of oxytocin have been reported in rats and mice.2–4 The conflicting results are probably a result of different dose, route of administration or the model used. Varying results on nociception have also been reported following systemic administration of oxytocin (0.05–1.5 mg/kg), although administration i.p. or s.c. in high doses has generally been reported to induce anti-nociceptive effects in both rats and mice.2,6–8 In humans significant relief from intractable central neurogenic pain9 and from low back pain10 has been reported following the administration of oxytocin i.c.v. and i.t., respectively.

We recently demonstrated an olfactorily induced tail skin temperature drop in saline-injected rats exposed to an oxytocin-injected cagemate, an effect abolished by olfactory impairment. Treatment with oxytocin may induce both nociceptive and anti-nociceptive effects. The contrasting effects likely depend on the model and dosage used. Here we report an increased hindpaw withdrawal latency in response to nociceptive heat following the subcutaneous administration of oxytocin (1 mg/kg). An increased withdrawal response latency was also found in the untreated cagemates of an oxytocin-treated rat. The anti-nociceptive effect was abolished in oxytocin-antagonist-injected cagemates. Our results suggests that an olfactorily induced oxytocinergic mechanism is activated in the cagemates of an oxytocin-injected rat promoting anti-nociception.

Key words: Heat withdrawal thresholds; Olfactory stimulation; Oxytocin; Oxytocin antagonist

Olfactory cues from an oxytocin-injected male rat can induce anti-nociception in its cagemates

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Material and Methods

Animals and housing conditions: Thirty-two male Sprague–Dawley rats (350–390 g; B&K Universal AB, Sollentuna) were housed in a room maintained at 21°C, with a 12:12 h light:dark cycle, lights on at 07.00 h. The rats were kept in permanent groups of four males throughout the study, and they were provided with food and water ad lib.
Olfactory and drug treatments: Specific olfactory stimulation of the experimental rats was accomplished by injecting one ‘odour-donor’ rat in their cage with oxytocin (1 mg/kg, s.c.). Thus, in the cages of the experimental rats, three rats received either a saline injection, or an oxytocin-antagonist injection (1 mg/kg; 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin, Ferring AB, Malmö, Sweden), and the fourth rat received an oxytocin injection. In control groups all rats in a cage received saline, and in one cage all rats received oxytocin injections. The drugs were dissolved in saline, and all rats were injected s.c. using the same volume (1 ml/kg).

Hindpaw withdrawal latency assessments: The rats were accustomed to handling for 5 days before being tested. They were then moved to a novel room where they were allowed to settle down for 3 h. When measuring the withdrawal latencies the ventral surface of one hind paw was placed on a hot-plate maintained at 51.8–52.2°C. A timer indicated the latency (in seconds) for the rat to withdraw the paw from the hot-plate. The withdrawal latency to heat was assessed repeatedly in the rats over 2 h on two consecutive days, before they were tested under drug influence. The rats were then assigned to either of the four treatment conditions (Table 1). When tested under drug influence, each rat’s pre-injection withdrawal latency was assessed, followed by eight post-injection assessments every 15 min for 2 h after the injections.

Results

Effects of saline and oxytocin injections on repeated hindpaw withdrawal responses: The variation in the successive withdrawal latencies in response to heat was significant in the saline-injected control rats ($\chi^2 = 16.2, n = 8, df = 8, p = 0.04$), in that they showed a minor, but significant decrease over 95 min (Fig. 1) of the 2 h test. In the oxytocin-injected rats the latencies varied significantly (Friedman two-way ANOVA; $\chi^2 = 24.7, n = 9, df = 8, p < 0.002$), but with significantly increased withdrawal latencies within 15 min of the injection. The latencies remained significantly increased for 75 min.

Effects of sensory cues from an oxytocin-injected cage-mate on repeated hindpaw withdrawal responses in saline and oxytocin antagonist-injected rats: The saline-injected cage-mates showed a significant variation in withdrawal latencies in response to heat over the 2 h test ($\chi^2 = 17.7, n = 9, df = 8, p = 0.02$). The latencies were significantly increased 30 min after the saline injection and remained increased for about 75 min after the injections (Fig. 1).

The withdrawal latencies of the oxytocin-antagonist-injected cage-mates of an oxytocin-injected rat did not vary significantly over time.

Between group comparisons of the development of the anti-nociceptive responses: Although both the oxytocin- and their saline-injected cage-mates showed an increase in withdrawal latencies over time, the latencies of the oxytocin-injected rats reached a maximum within 30 min of the injections, while those of their saline-injected cage-mates reached the maximum 60–75 min after the injections (Fig. 1). At 15 min the latencies of the oxytocin-injected rats were signifi-
cantly longer than their saline-injected cagemates \((z = 2.21, n_1 = n_2 = 9, p < 0.03)\). At 75 min the withdrawal latencies of the oxytocin-injected rats and their saline-injected cagemates were not significantly different.

The opposite was true in the saline-injected cagemates vs saline-injected control rats; by 75 min the latencies of experimental saline-injected rats were significantly longer than those of the saline-injected control rats \((z = 3.32, n_1 = 8, n_2 = 9, p < 0.001)\), but this was not seen at 15 min.

**Discussion**

The results of the present study demonstrate increased hindpaw withdrawal latencies in saline-injected adult male rats when these are cagemates of an oxytocin-injected rat, contrary to saline-injected rats without an oxytocin-injected cagemate. The effect was abolished in cagemate rats given a specific oxytocin antagonist.

One obvious problem is how this anti-nociceptive effect spread from one rat to another. Although not demonstrated in this study, olfactory stimulation is the most likely pathway. In a parallel study we have demonstrated an olfactorily induced tail skin temperature reduction in saline-injected rats exposed to an oxytocin-injected cagemate, an effect abolished by olfactory impairment.14 However other sensory modalities, for example ultrasonic stimulation,15,16 cannot be ruled out.

Rats can discriminate between odour released from stressed and unstressed conspecifics.17–19 When exposed to the odour of an electric-shock stressed rat, they become analgesic.20 This stress-induced analgesia is reversed by the opioid antagonist naltrexone.20 Since the anti-nociceptive effect observed in the cagemates was abolished following oxytocin antagonist administration in the present study, an endogenous oxytocin release and an oxytocinergic modulatory effect is implicated.

High doses of oxytocin may induce a stress response for ~45 min,19 which may explain the peak in withdrawal latencies at 30 min in the present study. However, recent findings suggest that an oxytocin-inhibited response to the nociceptive stimuli can be related to a state of inhibited stress or anxiety rather than stress. This idea is supported by previous studies showing that the administration of oxytocin in lower doses (0.1 mg/kg) may shift the activity of a male rat from the periphery to the centre of an open field, which is viewed as a sign of anxiolysis.21 Estrogen-primed female mice given oxytocin also demonstrate anxiolysis in terms of an increased percentage of time spent in the open arms of an elevated plus-maze.22 Evidence is accumulating to show that lactation is associated with reduced physiological and behavioural responses to stress, as well as a central release of oxytocin.23 Oxytocin decreases olfactory processing in the olfactory bulb in female rats,12 and thereby induces a gating process to aversive stimuli from newborn pups.13 The effect was proposed to be due to an endogenous release of oxytocin. Finally, maternal olfactory cues have been proposed to induce an endogenous oxytocin release affecting rat pups’ behaviour, since the behavioural effect was abolished by intracranial injections of an oxytocin antagonist (d(CH2)[tyr(Me2,Thr4,Tyr(NH9)2]-ornithine vaso-
tocin).11 Thus, the injection of oxytocin in this study is proposed to induce olfactory signals in the adult male rat similar to those in lactating females. The saline-injected adult male cagemates in turn are proposed to respond by an endogenous oxytocin release and stress reduction like that observed with rat pups.11 Anti-nociception may then occur when stress responses are inhibited as well as under stressed conditions. Anti-nociception under stress is known to be mediated by opioids.25 This study demonstrates the involvement of an oxytocinergic mechanism in anti-nociception.

In conclusion, the gradually developing anti-nociceptive effect in saline-injected cagemates of an oxytocin-injected rat in this study is consistent with a mechanism dependent on an olfactorily induced oxytocin release, since the effect was abolished by oxytocin antagonist treatment.

**Conclusion**

Systemic administration of oxytocin resulted in an increased hindpaw withdrawal response to heat in male rats. Their untreated (saline) cagemates showed a similar increase, but not following treatment with an oxytocin antagonist. This implies that an oxytocinergic mechanism was activated olfactorily. Olfactorily induced stress and opioid reversed analgesia has previously been reported in rats.20 Since low-dose systemic oxytocin administration and central oxytocin administration in the olfactory bulb have previously been shown to induce stress inhibition,11,13,21,24 we suggest that anti-nociception may be related to both stress and anti-stress states. While anti-nociception under stress is due to opioids, the latter involves endogenous oxytocin release.

**References**

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