The antinociceptive effect of non-noxious sensory stimulation is mediated partly through oxytocinergic mechanisms

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The objective of the present study was to investigate whether oxytocinergic mechanisms may contribute to the antinociceptive effect of non-noxious sensory stimulation. To test this hypothesis, oxytocin levels in plasma and cerebrospinal fluid (CSF) were measured in control rats as well as in rats exposed for 30 min to electro-acupuncture (2 Hz), thermal stimulation (40 °C) or vibration (100 Hz). All modes of stimulation induced significant elevations of oxytocin levels in plasma and/or in CSF, 30 or 90 min after the end of stimulation. Secondly, the antinociceptive effects of these treatments were investigated in the tail-flick test with and without prior administration of the oxytocin antagonist l-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin (1 mg kg⁻¹ i.p.). All three modes of stimulation caused a significant delay of the tail-flick latency to the same degree as that caused by injection of oxytocin 1 mg kg⁻¹ i.p. (electro-acupuncture \( P < 0.01 \), thermal stimulation and vibration \( P < 0.05 \)). In all cases, the delay was reversed by administration of the oxytocin antagonist (1 mg kg⁻¹ i.p.). These findings suggest that analgesic effects induced by non-noxious sensory stimulation may, in part, be mediated through activation of oxytocinergic mechanisms.

Key words: analgesic effects, non-noxious somatosensory stimulation, oxytocin, oxytocin antagonist.

The existence of pain modulatory systems was first proposed clearly in 1965 by Melzack & Wall in the Gate control theory of pain. Descending systems also contribute to pain modulation, since pain suppression can be obtained following electrical stimulation of discrete brain sites. It is established that endogenous opioids may play an important role in these pain-alleviating systems (Basbaum & Fields 1978). Opioid-mediated analgesia can be physiologically activated in animal models e.g. by prolonged muscle activity, by noxious stimulation or by a variety of stressful stimuli (Lewis et al. 1982).

However, parallel pain-modulating pathways without opioid links also exist. In a previous study it has been shown that oxytocin administered to rats elevates the nociceptive threshold, evidenced by a prolonged latency in the tail-flick test and that the oxytocin antagonist l-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin (1 mg kg⁻¹ i.p.) which is specific for the uterine oxytocin receptor not only reverses the effect induced by oxytocin but by itself reduces the tail-flick latency, indicating that endogenous oxytocin may modulate nociception (Uvnäs-Moberg et al. 1992c). The fact that oxytocinergic neurons

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from the paraventricular nucleus (PVN) project to areas within the brain involved in pain regulation such as the raphe nuclei, the periaqueductal grey and the spinal cord (Sawchenko & Swanson 1982) supports a pain-modulating role for oxytocin.

Activation of sensory nerves caused by e.g. vaginal stimulation, vibration (100 Hz), warm temperature (40 °C) and electro-acupuncture (2 Hz) is known to induce pain alleviation (Crowley et al. 1977, Lundeberg & Ottoson 1982, Lundeberg 1984, Lundeberg et al. 1988). Given the facts that oxytocin, which elevates pain threshold, is released by vaginal stimulation, low intensity electrical stimulation of the sciatic nerve and brushing in anaesthetized rats (Stock & Uvnäs-Moberg 1988), the pain relief induced by non-noxious somatosensory stimulation may well involve oxytocinergic mechanisms.

To test this hypothesis, oxytocin levels in plasma and CSF were measured following three kinds of non-noxious sensory stimulation, i.e. warm temperature (40 °C), vibration (100 Hz) or electro-acupuncture (2 Hz), and the antinociceptive effect of these stimuli was tested before and after administration of an oxytocin antagonist specific for the uterine receptor I-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin.

METHODS

Experiments were performed on male Sprague-Dawley rats (270–320 g, ALAB, Laboratorytjänst AB, Sollentuna, Sweden). The animals were maintained under controlled conditions of light–dark cycle (12:12 h, lights on 06.00 h), temperature 20 ± 2 °C and relative humidity (55–60%). The animals were anaesthetized with chloral hydrate (500 mg kg⁻¹) (blood samples) or anaesthetized with 1.0–1.1% halothane (Tokeda, Japan) (tail-flick experiments).

Sensory stimulations

All stimulations were performed for 30 min. The electro-acupuncture points chosen were UB11 (the trapezius muscle close to the shoulder joint) and UB54 (the gluteus maximus muscle close to the hip joint) bilaterally. Vibratory stimulation (100 Hz) was carried out with the electromechanical vibrator (Somedic AB, Stockholm, Sweden) with a probe area of 9 cm² which was applied with constant pressure (3 kPa) to the thorax and abdomen of the rat. Thermal stimulation was applied to the skin of the thorax and abdomen of the rat using a feedback controlled contact thermode. The thermode had a contact surface of 9 cm², was fixed to a glass chamber with a temperature of 40 °C and applied with a constant pressure of 3 kPa. For a more detailed description of these methods, see Uvnäs-Moberg et al. (1992b).

Oxytocin determinations. Blood (5 ml) was collected by decapitation. Samples were collected in tubes containing heparin (10 IU ml⁻¹) and Trasylol (500 IU ml⁻¹) and were centrifuged. The plasma was removed and frozen at −20 °C. CSF samples were collected as previously described (Uvnäs-Moberg et al. 1992c).

Blood samples were collected at 0 or 60 min after the stimulations. Samples were also collected 330 min after the end of the electro-acupuncture. Control samples were collected from anaesthetized animals at time point 0 min. Six animals were included in each group. CSF samples were collected 60 min after the end of stimulation. Five animals were included in each group.

The concentration of oxytocin in plasma or CSF was measured with specific radio-immunoassay (RIA) using the antibody KA19 (Milab, Malmö, Sweden). Samples were extracted on SEP-PAK C₁₅ cartridges (Millipore Corp., Bedford, MA, USA) prior to assay. The limit of oxytocin detection was 2 fmol ml⁻¹ and the intra- and inter-assay coefficients of variation were 11.2 and 13%, respectively (Stock & Uvnäs-Moberg 1985).

Nociceptive testing. The first group of animals was given an i.p. injection of saline, oxytocin (1.0 mg kg⁻¹) (Ferring AB, Malmö, Sweden), the oxytocin antagonist 1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin (1.0 mg kg⁻¹) (Ferring AB) or oxytocin together with the oxytocin antagonist and was then anaesthetized with halothane for 30 min without receiving any sensory stimulation. The second group of animals was given an i.p. injection of saline or the oxytocin antagonist (1 mg kg⁻¹) and was then anaesthetized with halothane for 30 min during which period they received sensory stimulation (electro-acupuncture, vibration or thermal stimulation). Twelve animals were included in each group in this type of experiment.

The tail–flick test was performed 15 min after the end of anaesthesia. During the test, the rats were restrained in a cylinder attached to a thermostatically controlled hot water bath. The noxious stimuli were applied by immersing the rats' tail in the hot water (50 °C). The time taken for the first brief flick of the tail and for the later strong flexion of the tail was recorded. The prolongation of the response time was used as a measure of the antinociceptive effect of the treatment applied.

Statistical evaluations. The hormone levels in the figures are presented as mean values ± SD. Comparisons between groups were evaluated with the Kruskal-Wallis one-way analysis of variance
The antinociceptive effect of sensory stimulation is oxytocin mediated

(ANOVA) and subsequent two-group comparisons were made with the Mann Whitney U-test. Two-tailed tests were used and P-values < 0.05 were considered significant. * P < 0.05, ** P < 0.01.

RESULTS

Oxytocin levels

Electro-acupuncture caused a rise of oxytocin levels in plasma from 30 to 64 pm (P < 0.05) immediately after stimulation. Levels were reduced 60 min later and completely reversed to basal after 6 h. Vibration caused a rise from 28 to 44 pm at 0 min (P < 0.05), thermal stimulation (40 °C) failed to raise plasma levels of oxytocin (Figs 1a, b, c).

CSF levels of oxytocin showed a different response pattern. From a control value of 22 ± 6 pm, electro-acupuncture caused an increase to 31 ± 4 pm (P < 0.05) and vibration and thermal stimulation (40 °C) raised oxytocin levels to 33 ± 10 pm (P < 0.05), 46 ± 20 pm (P < 0.01).

Tail-flick latency (Fig. 2)

In saline-treated rats the first and second flicks occurred after 4.1 and 4.5 s, respectively. Administration of the oxytocin antagonist alone caused a slight but insignificant shortening of the latency. Oxytocin (1 mg kg⁻¹) caused a prolongation of the latency of both the first and the second flick (P < 0.01) which was reversed in experiments in which the antagonist was also given.

All three types of sensory stimulation significantly delayed the occurrence of the first and second flick (electro-acupuncture P < 0.01 and thermal stimulation and vibration P < 0.05). The responses were completely abolished by previous administration of the oxytocin antagonist.

DISCUSSION

The major findings in the present study were that three types of non-noxious, sensory stimulation caused a release of oxytocin and that the analgesic effect caused by these stimuli was reversed by an oxytocin antagonist, thus suggesting that oxytocinergic mechanisms may be involved in pain relief caused by certain types of somatosensory stimulations.

Several kinds of non-noxious sensory stimulation such as electro-acupuncture (2 Hz), vibration (100 Hz) and thermal stimulation (40 °C) are known to induce pain alleviation (Lundeberg & Ottoson 1982, Lundeberg 1984, Lundeberg et al. 1988). The mechanisms involved are not well...
known but endogenous opioid systems have been implicated in the analgesic effect of electro-acupuncture (Thorén et al. 1990).

Oxytocin neurons from the paraventricular nucleus (PVN) project to many areas within the brain including regions known to be involved in the control of pain, e.g. the Raphe nuclei, the brain stem and the dorsal horn of the spinal cord (Sawchanko & Swanson 1982). Recently it has been shown that oxytocin given i.p. to male rats increases the latency in the tail-flick test. These effects were antagonized by an oxytocin antagonist specific for the uterine receptor. The antagonist by itself shortened the latency in the tail-flick test, suggesting that endogenous oxytocinergic mechanisms may be involved in the modulation of nociception (Uvnås-Moberg et al. 1992c). In another study it has been shown that opioid mechanisms do not mediate the antinociceptive effects of oxytocin, since naloxone did not block them. Furthermore, oxytocin was shown to act at a central site since intrathecal administration of oxytocin to mice delayed the latency in the hot plate test (Lundeberg, personal communication). The reason why effects of oxytocin or the antagonist can be obtained following i.p. administration is that about 1 permille of these drugs passes the blood brain barrier, but higher doses must be given (1 mg kg⁻¹ versus 1 μg kg⁻¹ following i.t. administration) (Jones & Robinson 1982).

Since oxytocin exerts antinociceptive effects and can be released by suckling, vaginocervical stimulation (Crowley et al. 1977, Gintzler et al. 1983) or in response to low intensity electrical stimulation of the sciatic nerve (Stock and Uvnås-Moberg 1988), the authors wanted to investigate whether the antinociceptive effects of some types of non-noxious sensory stimulations could be mediated by oxytocin. Three different kinds of afferent stimulations known to activate specific populations of sensory nerve fibres were applied in this study. Vibratory stimulation at 100 Hz activates different types of low threshold mechanoreceptors in the stimulated area and the activity is transmitted through A-δ fibres (Merzenich & Harrington 1969). A-δ and C-fibres are activated in response to a thermode of 40 °C (Kenshalo 1970) and electro-acupuncture at 2 Hz activates several types of mechanoreceptors within the muscle, among them the so-called ergoreceptors. The activity set up in these receptors is transmitted in A-δ fibres (Kiiffki et al. 1981).

These data show that electro-acupuncture and vibration increased plasma levels of oxytocin and that all three treatments were followed by a release of oxytocin into the CSF, indicating that oxytocin neurons terminating in the posterior pituitary and in the brain were activated by these stimulations. In addition, the prolongation of the tail-flick latency caused by all three modes of sensory stimulation was antagonized by administration of the oxytocin antagonist. Together

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**Fig. 2.** Effect of various treatments on tail-flick latency (a, first flick; b, second flick). A, NaCl; B, oxytocin antagonist (oxy ant) (1 mg kg⁻¹ i.p.); C, oxytocin (oxy) (1 mg kg⁻¹ i.p.); D, oxy + oxy ant; E, electro-acupuncture (EA) (2 Hz) for 30 min; F, EA + oxy ant, G, warm temperature (WT) (40 °C) for 30 min; H, WT + oxy ant; J, vibration (V) (100 Hz) for 30 min; K, V + oxy ant. (n = 12 in each group). *P < 0.05, **P < 0.01.
these data support the hypothesis that an oxytocinergic mechanism may play a role in analgesia caused by the different modes of sensory stimulation used in this study. The tail-flick pattern in the present study differs from that observed in previous studies in that the second flick occurred closer to the first one (4.5 s vs. 6.9 s). The change in the tail-flick pattern is likely to be a consequence of the exposure to halothane anaesthesia, which is a prerequisite for sensory treatments. Since controls were included in the study, it is believed that the results obtained between groups are still valid.

How the somatosensory stimuli used in this study activate the oxytocin system in the PVN is not yet known, but investigations are in progress to trace the afferent pathways involved. As mentioned in the Introduction, several parallel pain relieving systems may exist. Opioid mechanisms have been shown to be involved in pain relief caused by noxious stimulation and by a variety of stressful stimuli (Lewis et al. 1982). Oxytocinergic mechanisms may instead be involved in some less stressful physiological situations, e.g. during labour and following suckling and in response to non-noxious somatosensory stimulations. As stated above, opioid mechanisms do not appear to be involved in the effects of oxytocin (Lundeborg, personal communication). The suggestion that noxious and non-noxious types of somatosensory stimulations may act via different mechanisms is supported by the fact that these two types of somatosensory stimulations induce different endocrine responses. Pinching gives rise to a secretion of catecholamines and corticosteroids in anaesthetized rats, whereas brushing is followed by a decrease in the levels of these hormones (Sato 1987, Tsuchiya et al. 1991). In addition, vagally controlled gastrointestinal hormones are released following suckling (Lindén et al. 1987), by low intensity afferent stimulation of the sciatic nerve (Uvnäs-Moberg et al. 1986) as well as by the types of non-noxious stimulations used in this study (Uvnäs-Moberg et al. 1992b).

In conclusion, the main observations in the present study are that oxytocin levels are raised in plasma and/or CSF in response to different modes of non-noxious sensory stimulation and that the antinociceptive effect caused by these stimuli is reversed by an oxytocin antagonist. These data raise the possibility that oxytocinergic transmission is involved in the analgesic effect caused by non-noxious sensory stimulation. Further studies are needed to explore this idea.

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REFERENCES


