Repeated massage-like stimulation induces long-term effects on nociception: contribution of oxytocinergic mechanisms

Irène Lund,1 Long-Chuan Yu,2 Kerstin Uvnas-Moberg,1,3 Jing Wang,2 Cheng Yu,2 Mieko Kurosawa,4 Greta Agren,1 Annika Rosén,1 Magnus Lekman1 and Thomas Lundeberg1,5
1Department of Physiology and Pharmacology, Karolinska Institutet, SE-171 77 Stockholm, Sweden
2Department of Physiology, College of Life Science and National Laboratory of Biomembrane and Membrane Biotechnology, Peking University, Beijing 100871, China
3Department of Animal Physiology, Swedish University of Agricultural Sciences, SE-756 61 Uppsala, Sweden
4Department of Physiology, School of Health Science, International University of Health and Welfare, 2600–1 Kitakanemaru, Otawara, Tochigi 324–8501, Japan
5Department of Rehabilitation Medicine, Karolinska Hospital, SE-171 76 Stockholm, Sweden

Keywords: hot-plate, massage, oxytocin, PAG, Randall Selitto, RIA, sensory stimulation, stroking, withdrawal latencies

Abstract

Massage-like stroking induces acute antinociceptive effects that can be reversed by an oxytocin antagonist, indicating activation of oxytocin on endogenous pain controlling systems. We now demonstrate an increase in hindpaw withdrawal latencies (HWLs), in response to thermal and mechanical stimuli, which was present after six treatments of massage-like stroking every other day and which continued through the remaining seven treatments. Repeated massage-like stroking also resulted in increased oxytocin-like immunoreactivity (oxytocin-LI) levels in plasma and periaqueductal grey matter (PAG). Furthermore, increases in HWLs were also present after injections of oxytocin into the PAG (0.1, 0.5 and 1.0 nmol). Intra-PAG oxytocin injection of 1 nmol followed by 1 or 20 nmol of naloxone attenuated the increments in HWL. Also, there was a dose-dependent attenuation of the oxytocin-induced antinociceptive effects following intra-PAG injection of the µ-opioid antagonist β-funaltrexamine (β-FNA) and the κ-opioid antagonist nor-binaltorphimine (nor-BNI) but not the δ-opioid antagonist naltrindole. The long-term antinociceptive effects of massage-like stroking may be attributed, at least partly, to the oxytocinergic system and its interaction with the opioid system, especially the µ- and the κ-receptors in the PAG.

Introduction

Different modes of sensory stimulation have been shown to activate endogenous modulatory systems (Lundeberg et al., 1988; Andersson & Lundeberg, 1995; Hansson & Lundeberg, 1999). Manual massage, including sequences such as stroking and kneading, is probably one of the oldest and most appreciated modes of sensory stimulation used for the alleviation of pain, amelioration of disease symptoms and the promotion of well-being (Kamenetz, 1985; Haldeman, 1999). Despite a long and multicultural tradition there are a limited number of studies addressing the underlying mechanisms (Cawley, 1997; Furlan et al., 2001). Besides its use for the lessening of perceived pain, reduced levels of stress, anxiety and sleep disturbances have been demonstrated (Field, 1998), suggesting the initiation of broad spectrum of effects.

The diminished pain perception following massage probably results from increased activity in endogenous pain modulatory systems. The induced light-to-moderate mechanical stimulation of cutaneous and subcutaneous tissue results in increased activity in somatosensory neurons which may inhibit activity in pain-mediating neurons in the superficial layers of the spinal cord, in accordance with the gate control theory of pain (Melzack & Wall, 1965). When the stimulation intensity is increased there is a possibility of analgesia produced by more central mechanisms, such as activation of the descending pain inhibitory system with a proposed start in the periaqueductal grey matter (PAG) at the brainstem level (Basbaum & Fields, 1984) and with a continuum to the dorsal horn of the spinal cord furthering antinociception (Fields & Basbaum, 1994; Millan, 1999). The endogenous pain inhibition following peripheral stimulation is partly explained by opioid involvement (Harris, 1996) including activation of different types of opioids receptors, where the µ-, δ- and κ-receptors have been found in the PAG area (Mansour et al., 1995).

However, oxytocinergic mechanisms have also been shown to be activated and producing antinociception, especially in response to somatosensory stimulation that is non-noxious (Uvnas-Moberg et al., 1993). In a previous study we have demonstrated that single treatments of massage-like stimulation resulted in an increased withdrawal latency to heat stimulation that was reversed by the oxytocin antagonist 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin (Agren et al., 1995), indicating involvement of the oxytocinergic system. Thus, it has been demonstrated that oxytocin is involved in antinociception, and oxytocinergic terminals have been identified in the areas of pain modulation of the brain (Sofroniew, 1983). Also, a

Correspondence: Dr Irène Lund, as above.
E-mail: irene.lund@fyfa.ki.se

Received 14 February 2002, revised 5 May 2002, accepted 15 May 2002
possible interactive function between the oxytocinergic and opioid systems participates in the effects in response to sensory stimulation.

The aim of the present study was to investigate: (i) the antinociceptive effects of repeated massage-like stroking tested by the hind-paw withdrawal latency (HWL) to thermal and mechanical stimulation in a time-course study; (ii) the changes of oxytocin-like immunoreactivity (oxytocin-LI) concentrations in plasma and in PAG following repeated massage-like stroking; (iii) the antinociceptive effect of oxytocin directly injected into PAG and tested by HWLs; and (iv) the interaction between oxytocin and opioids on HWLs using intra-PAG injections of nonselective (naloxone) and selective (β-funaltrexamine, nor-binaltorphimine and naltrindole) opioid receptor antagonists mainly working at the μ-, κ- and δ-opioid receptors.

Materials and methods

Animal preparation

Male Sprague-Dawley rats (200–300 g, B & K Universal AB, Sollentuna, Sweden; Experimental Animal Center of Peking University, Beijing, P.R. China) were used in all experiments. The animals arrived 10 days before the start of experimentation, allowing them to adapt to their new environment. They were housed five or six per cage with free access to food and water. The light schedule was a 12 h light : 12 h dark cycle (lights on at 06.00 h) and the ambient temperature was 22 ± 2 °C. All experiments were performed in the daytime (10.00–15.00 h). The Ethical Committee for Animal Experiments in the respective universities approved of all experimental protocol. Initially the animals were assigned randomly to treatment, i.e. massage-like stroking and intra-PAG injections, and control groups.

The massaged rats were killed by decapitation and the intra-PAG injected rats by high doses of pentobarbital anaesthesia.

Massage-like stroking

Sensory stimulation comprised manual stroking for 5–10 min of the ventral (= 10 cm²) side with a low speed (≈ 20 cm/s) and frequency (0.67 Hz, i.e. stroking every 1.5 s or at 40 strokes/min), and with an estimated pressure of 100 mm Hg. The reproducibility of the massage-like stroking was tested and confirmed by similar application of pressure to a small pressure gauge (Kurosawa et al., 1995). During massage the rat was held across the scapula and neck region as described by Kanetake (1982).

The treatments were performed 3, 12 and 14 times on alternate days (n = 6, 6 and 10, respectively). Control animals (n = 6, 6 and 10) were held across the scapula and neck region when tested for the HWL, following the same testing schedule as the treated animals. The rats were naive to the massage-like stimulation before the experimentation started.

Collection of blood and tissue samples

Rats given treatments with massage-like stroking were killed one by one by decapitation in a separate room to avoid nonspecific stress responses (Brodin et al., 1994).

Trunk blood was collected from rats treated for 3 and 14 times with massage-like stroking, in tubes that were ice-chilled and contained 10 IU/mL of heparin (Lovens Låkemedel, Malmö, Sweden) and 500 IU/mL of Trasylol® (Bayer, Germany). Blood samples were centrifuged and the plasma was thereafter separated, frozen and stored at −20 °C until analysis.

In the rats treated repeatedly (12 times) with massage-like stroking, the whole rat brain was rapidly removed and placed in a mold where it could be sliced into 2.5-mm coronal sections with a thin (Ø = 0.07 mm) stainless steel wire. The tissue samples of PAG were quickly dissected out from slice numbers IV and V, weighed and immediately thereafter frozen on dry ice. The PAG tissue was stored at −70 °C until extraction and analysis.

Oxytocin-LI and radioimmunoassay: blood samples

Plasma (500 μL) was purified on Sep-Pak® C18 cartridges (Water Assoc. Inc., Milford, MA, USA) and dissolved in 500 μL of assay buffer (0.05 M phosphate buffer with 1.0% BSA, pH 7.5). Oxytocin-LI levels were determined by radioimmunoassay (RIA) using rabbit antiserum A19 (Euro-Diagnostica AB, Malmö, Sweden). Cross-reactivity with arginine–vasopressin was 0.01%, with lysine–vasopressin <0.01% and with a arginine–vasotocin 0.1%. A standard curve of oxytocin was prepared and a total of 50 μL of the antiserum was incubated with 100 μL of standard or purified sample at +4 °C for 24 h. Thereafter 50 μL of [125I]-Tyr2-oxytocin, specific activity 2200 Ci/mmol (DuPont NEN Research Products), was added and incubated at +4 °C for 48 h. Antiserum and [125I]-Tyr2-oxytocin were diluted in 0.05 M phosphate buffer with 0.1% BSA. The bound fraction was separated from the unbound by incubating the samples with a second rabbit antibody, Decanting Suspension 3 (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The samples were centrifuged for 10 min at +4 °C (1700 g) followed by the decanting of the supernatant and the measuring of radioactivity in the precipitate. The limit of the assay was 3.2 pmol/L and the intra-assay coefficient of variation was 13.9%.

Extraction, oxytocin-LI and RIA: PAG tissue samples

A combined neutral and acid extraction of dissected tissue was chosen. The samples of frozen PAG tissue were transferred to tubes containing 2 mL of boiling 0.05 M phosphate buffer, pH 7.4, for 10 min and then cooled before being homogenized on a vortex mixer with a steel rod in the tube. Thereafter the samples were centrifuged (+4 °C, 2800 g) for 10 min and the supernatant was taken off and poured into other tubes. The pellet was dissolved and mixed in 2 mL of 1.0 M acetic acid. The solution was again incubated at +100 °C for 10 min and the procedure with centrifugation, cooling and mixing was repeated. The supernatant from the extraction with acetic acid was pooled with the one from the neutral extraction and the samples were lyophilized overnight. The lyophilized sample was dissolved in 1 mL phosphate buffer and stored at −20 °C until RIA. Oxytocin-LI levels were determined by RIA, using rabbit antiserum solution RAS-8152 and tracer solution [125I]-Oxytocin, Y-8152 (Peninsula Laboratories, Inc., CA, USA). Cross-reactivity with [Arg 8]–vasopressin was 0%, [Lys8]–vasopressin <0.01%, LH–RH 0% and with CRF (human, rat) 0%. A standard curve of oxytocin was prepared. The antiserum solution was incubated with 100 μL of standard solution or extracted sample at +4 °C for 24 h. Thereafter 100 μL of the tracer solution was added and incubated at +4 °C for another 24 h. Separation of the bound from the unbound fraction was achieved by incubating the samples with 500 μL of a second rabbit antibody, Decanting Suspension 3 (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), for 30 min at room temperature. Incubation of the samples was interrupted by adding 1 mL of water to the tubes; the samples were then centrifuged for 17 min (+4 °C, 2800 g) and the supernatant decanted. The radioactivity in the precipitate was measured in a gamma counter for 6 min per sample. The limit of the assay was 7.8 pmol/L. All samples were assayed in duplicate.
Surgery for intra-PAG injection

Animals used for intra-PAG injections were anaesthetized with intraperitoneal sodium pentobarbital (45 mg/kg) and thereafter mounted on a stereotaxic frame. A sterilized stainless steel guide cannula with outer diameter of 0.8 mm was directed to the PAG and was fixed to the skull by dental acrylic. The stereotactic coordinates were AP –5.5, LM +0.5 and DV –6.0 mm measured from the skull surface according to Paxinos & Watson (1986). On the day of the experimentation a stainless steel needle 0.4 mm in diameter was directly inserted into the guide cannula, to extend 1 mm beyond its tip, and 1 µL of solution was thereafter infused into PAG over 1 min.

Chemicals and experimental design of intra-PAG injections

Solutions for intra-PAG administration were prepared with sterilized saline (0.9%) each with a volume of 1 µL and injected to rats: 0.01, 0.1, 0.5 and 1 nmol of oxytocin (Peninsula Laboratories), n = 8 per group or 1 µL of 0.9% saline as a control (n = 8).

Five minutes after injection of 1 nmol of oxytocin one of the following solutions and dosages in a volume of 1 µL each were injected in a volume of 1 µL each.

(i) Naloxone (naloxone hydrochloride; Sigma Chemical Company, St. Louis, MO, USA), 0.1, 1 or 20 nmol, n = 8 per group. [In another group, rats received intra-PAG administration of 0.9% saline, 5 min after 20 nmol of naloxone (n = 8)].

(ii) β-funaltrexamine (β-FNA; Tocris, Balwin, MO 63011, USA) 0.1, 0.5 or 1 nmol, n = 8 per group. [Another group of rats received intra-PAG injection of 1 µL of 0.9% saline 5 min after intra-PAG injection of 1 nmol of β-FNA (n = 8)].

(iii) Norbinaltorphimine (nor-BNI; Tocris) 0.5, 1 and 2 nmol, n = 8 per group. [In an additional group of rats intra-PAG injection of 1 µL of 0.9% saline was administered 5 min after intra-PAG administration of 2 nmol of nor-BNI (n = 8)].

(iv) Naltirindole (naltirindole hydrochloride; Sigma) 1 or 2 nmol, n = 8 per group [and 1 µL of 0.9% saline as a control in another group of rats (n = 8)].

Histology analysis

At the end of the intra-PAG injection experiments the rats were killed with a high dose of pentobarbital anaesthesia (80 mg/kg) and the rat heads were fixed in 10% formalin for one week with the guide cannula in situ before section. The location of the tip of the guide cannula was verified and only the measurements from nociceptive tests with the tips of the guide cannula properly placed within the PAG were used for statistical analysis.

Hindpaw withdrawal latency to thermal and mechanical stimulation

To decrease the stress caused by handling and measuring, all rats were acclimatized to the testing conditions on a daily basis for 3–5 days before starting the experiments. The hindpaw withdrawal latency, HWL, was measured in response to thermal and mechanical stimulation (Lundeberg et al., 1993; Agren et al., 1995; Yu et al., 1996a, 1996b; Agren et al., 1997) while the rats were held in a similar grip as during the sessions of stroking. All rats were tested with both thermal and mechanical stimulation.

The response to thermal stimulation was assessed in the hotplate test, by placing the entire plantar surface of the rat’s left or right hindpaw on an electrically heated copper plate, which was maintained at a constant temperature of 52 °C (51.8–52.2 °C). In the massage experiment a cutoff time of 50 s was imposed when the rats did not exhibit an aversive movement from the hotplate within that limit (Plone et al., 1996).

The Randall Sellito Test (UGO Basile, Type 21025, Comrio, Varese, Italy) was used to assess HWL in response to mechanical stimulation. A wedged-shaped pusher with a loading rate of 48 g/s was applied to the dorsal surface of the manually handled hindpaw and the mechanical stimulation required to initiate the struggle response was assessed.

The HWLs of the massaged rats were measured in 13 sessions. Measurements were carried out 20 min and immediately before treatment as well as post-treatment and 60 min after the end of treatment.

The injected rats’ HWLs were measured prior to treatment and repeatedly 5, 10, 20, 30 and 60 min after injection of oxytocin and saline. After injection of β-FNA, nor-BNI, naltirindole and saline, respectively, the rats were measured at 5, 10, 15, 20, 25, 30 and 60 min.

The HWLs are expressed in seconds, i.e. latency to withdrawal from start of stimulation. The average values of HWL obtained before massage-like stroking or intra-PAG injection was regarded as the 0 condition or basal HWL to thermal or mechanical stimulation. The HWLs recorded during subsequent experiments were expressed as a change from the 0 condition (Δ-value) or as a percentage change from the basal level for each rat (% change in HWL).

Statistical analysis

The results of the nociceptive tests are expressed as mean ± SEM. The difference between groups was determined by two-way analysis of variance (ANOVA) for repeated measures followed by Duncan’s multiple range post hoc test when appropriate.

In the massage experiment the effects within the different groups based on pretreatment values from every single treatment compared to the 0 condition were analysed by one-way ANOVA with repeated measures followed by Duncan’s multiple range test for post hoc comparison. To test the reactions over time the Pearson product–moment correlation was used.

One-way ANOVA was used in analysing the levels of oxytocin-LI in plasma, followed by Tukey’s honestly significant difference test for unequal sample sizes (Špidtovoll and Stoline test). The PAG tissue content of oxytocin-LI is expressed as median values and the range between max and min. This was further analysed with the Mann–Whitney U-test. P-values < 0.05 were regarded as statistically significant in all experiments.

Results

Repeated massage-like stroking: HWL

The effects of repeated treatments were evaluated against the measurements made 20 min before each one of the 13 treatments and showed differences between the groups, massage-like stroking and control, in the thermal and mechanical test: interaction (Time × Treatment) F<sub>12,216</sub> = 2.31, P < 0.01; and F<sub>12,216</sub> = 2.76, P = 0.01, respectively. The HWLs measured before the seventh treatment but also the tenth, 11th, 12th and 13th treatments were significantly increased in the massage-treated group as compared to the controls in the thermal test. In the mechanical test the measurements before the eleventh and 13th treatments were significantly changed as compared to controls, Fig. 1A and B.

Comparisons within the respective group (massage-like stimulation and control) between the baseline level and the first pretreatment
measurement of every treatment showed that there were significant increases of the HWLs in the massage-treated rats in response to thermal stimulation ($F_{12,108} = 3.05$, $P < 0.001$) and tested at the 7–13th treatments by the post hoc test. There was also an increase in response to the mechanical stimulation in the massaged rats ($F_{12,108} = 3.207$, $P < 0.001$) which was significantly raised at the seventh, 11th and 13th treatment. This reaction was not observed in the control group either in response to thermal ($F_{12,108} = 1.459$, ns) or mechanical stimulation ($F_{12,108} = 1.773$, ns); Table 1. These effects over time with a gradual increase in the HWLs in the massage-treated rats were also confirmed by the Pearson product–moment correlation test, thermal test ($r = 0.92$, $P < 0.001$) and the mechanical test ($r = 0.75$, $P < 0.01$), while no significant changes were found in the control group in response to neither of the tests (thermal test $r = -0.10$, $P = 0.74$ and mechanical test $r = 0.01$, $P = 0.97$) (Fig. 1C and D). There were no significant short-term changes in the HWL within 60 min following every treatment in any of the groups at any of the treatments (data not shown).

Repeated massage-like stroking: oxytocin-LI concentrations in plasma and PAG

The level of oxytocin-LI in plasma was changed after stroking treatment ($F_{3,28} = 25.5$, $P < 0.001$) and significantly higher after 14 repeated treatments than in those who received only three treatments $P < 0.001$, and than in the control animals ($P < 0.001$; Fig. 2A).

The content of oxytocin-LI in extracted tissue dissected from PAG was significantly raised in the group given massage-like stimulation on 12 occasions compared with the control group ($P < 0.05$; Fig. 2B).

Intra-PAG injections of oxytocin: HWLs

As shown in Fig. 3A and B, the HWLs in response to thermal and mechanical stimulation increased significantly after intra-PAG injection of oxytocin in comparison with the control group. Oxytocin 0.1 nmol: thermal test $F = 17.7$, $P < 0.001$; mechanical test $F = 57.6$, $P < 0.001$. Oxytocin 0.5 nmol: thermal test $F = 36.8$, $P < 0.001$; mechanical test $F = 68$, $P < 0.001$. Oxytocin 1 nmol:
Table 1. Changes in withdrawal latencies 20 min before each session in response to handling (control) or massage-like stroking of the abdominal area.

<table>
<thead>
<tr>
<th>Treatment number</th>
<th>Heat stimulation</th>
<th>Mechanical stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Massage group</td>
</tr>
<tr>
<td>1</td>
<td>10.7 ± 1.6</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>9.7 ± 1.4</td>
<td>7.9 ± 1.4</td>
</tr>
<tr>
<td>3</td>
<td>13.8 ± 2.1</td>
<td>10.8 ± 2.2</td>
</tr>
<tr>
<td>4</td>
<td>12.7 ± 2.3</td>
<td>12.0 ± 4.1</td>
</tr>
<tr>
<td>5</td>
<td>11.4 ± 2.0</td>
<td>14.0 ± 2.7</td>
</tr>
<tr>
<td>6</td>
<td>15.0 ± 2.8</td>
<td>12.2 ± 2.5</td>
</tr>
<tr>
<td>7</td>
<td>9.5 ± 1.6</td>
<td>15.4 ± 4.6*</td>
</tr>
<tr>
<td>8</td>
<td>12.3 ± 2.0</td>
<td>16.1 ± 4.1*</td>
</tr>
<tr>
<td>9</td>
<td>11.8 ± 2.6</td>
<td>16.2 ± 2.8*</td>
</tr>
<tr>
<td>10</td>
<td>7.4 ± 1.4</td>
<td>15.8 ± 3.2*</td>
</tr>
<tr>
<td>11</td>
<td>9.7 ± 2.3</td>
<td>19.1 ± 5.3**</td>
</tr>
<tr>
<td>12</td>
<td>12.2 ± 2.7</td>
<td>22.7 ± 5.4***</td>
</tr>
<tr>
<td>13</td>
<td>11.1 ± 2.8</td>
<td>17.3 ± 2.6*</td>
</tr>
</tbody>
</table>

Heat, responses to heat stimulation in the hot plate test; mechanical, responses to mechanical stimulation in the Randall Selitto test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. prestimulus values (treatment no. 1) by using one-way ANOVA with repeated measures and Duncan’s post hoc test; n = 10 per group.

Heat, responses to heat stimulation in the hot plate test; mechanical, responses to mechanical stimulation in the Randall Selitto test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. prestimulus values (treatment no. 1) by using one-way ANOVA with repeated measures and Duncan’s post hoc test; n = 10 per group.

Intra-PAG injections of naloxone (nonspecific opioid receptor antagonist) following oxytocin: HWLs

The HWLs to both thermal and mechanical stimulation increased after intra-PAG injection of 1 nmol of oxytocin. Compared to the control group, the oxytocin-induced increases in HWLs were attenuated by the administration, 5 min later, of naloxone in the following doses. Naloxone 1 nmol: thermal test F = 50.4, P < 0.001; mechanical test F = 55.4, P < 0.001. Naloxone 20 nmol: thermal test F = 132.6, P < 0.001; mechanical test F = 111.3, P < 0.001. However, 0.1 nmol of naloxone did not produce a significant attenuation: thermal test F = 0.02, P = 0.88; mechanical test F = 0.09, P = 0.76. The control group of rats who received intra-PAG administration of 0.9% saline, followed 5 min later by 20 nmol of naloxone, showed no marked changes in the HWLs to either type of stimulation, as shown in Fig. 4A and B.

Intra-PAG injections of opioid receptor antagonists following oxytocin: HWLs

The tests with three different types of opioid receptor antagonists were preceded 5 min earlier by 1 nmol of intra-PAG-administered oxytocin (which ensured an increase in HWL). The results presented are HWLs measured 20 min after injected opioid receptor antagonists.

B-FNA (μ-receptor antagonist)

In the thermal test the increased HWLs decreased significantly compared with the control group after intra-PAG injection of 1 nmol of β-FNA (F = 8.5, P < 0.01) but not after 0.5 nmol (F = 0.99, P = 0.33) or 0.1 nmol of β-FNA (F = 1.76, P = 0.21). In the mechanical tests the oxytocin-induced increase in HWLs decreased significantly compared to the control group after intra-PAG injection of 0.5 nmol (F = 9.96, P < 0.01) or 1 nmol (F = 14.73, P < 0.001), but not by 0.1 nmol (F = 3.42, P = 0.09). Another group of rats
which received intra-PAG injection of 1 µL of 0.9% saline 5 min after intra-PAG injection of 1 nmol of β-FNA showed no significant changes in HWLs to either stimulation (Fig. 5A and B).

**Nor-BNI (κ-receptor antagonist)**

The increased HWLs induced by oxytocin decreased significantly compared to the control group after intra-PAG injection of nor-BNI at a dose of 2 nmol (thermal test $F = 11.23$, $P < 0.01$; mechanical test $F = 16.87$, $P < 0.001$) but not at 1 nmol (thermal test: $F = 1.29$, $P = 0.28$; mechanical test $F = 2.49$, $P = 0.14$) or 0.5 nmol of nor-BNI (thermal test $F = 1.47$, $P = 0.25$; mechanical test $F = 2.04$, $P = 0.17$). In the group of rats that received intra-PAG injection of 1 µL of 0.9% saline 5 min before intra-PAG administration of 2 nmol of nor-BNI, no significant changes in HWLs to either stimulation were noticed (Fig. 5C and D).

**Naltrindole (δ-receptor antagonist)**

There were no significant changes of HWLs in response to either the thermal or mechanical stimulation in the group that received intra-PAG administration of 1 nmol of naltrindole after oxytocin-induced antinociception compared with the control group: thermal test $F = 0.53$, $P = 0.48$; mechanical test $F = 1.17$, $P = 0.12$ (Fig. 5E and F).

**Discussion**

The results of this study revealed no short-term effect in the HWLs in response to thermal or to mechanical stimulation after 10 min of massage-like stroking of the abdominal area. Instead, a cumulative effect appeared as a gradual rise in the HWLs in response to both thermal and mechanical stimulation compared to the baseline level...
and the controls after the treatment was repeated more than six times. The levels of oxytocin-LI in plasma and PAG were raised in the group treated repeatedly (i.e. 12 or 14 times) with the massage-like stimulation as compared to controls or only three treatments of massage-like stroking.

That PAG plays a crucial role in mediating the antinociceptive effects seen is supported by recent studies (Behbehani, 1995; Harris, 1996) and confirmed in this study showing significantly increases of the HWLs in response to thermal and mechanical stimulation following intra-PAG injections of oxytocin in doses of 0.1 nmol or more. These dose-dependent effects were partly reversed by intra-PAG injection of the nonselective opioid receptor antagonist naloxone. Another finding of this study is that the oxytocin-induced antinociceptive effect was attenuated by the μ-opioid antagonist β-funaltrexamine (β-FNA) and the κ-opioid antagonist nor-binaltorphimine (nor-BNI), but not by the δ-opioid antagonist naltrindole, indicating an oxytocin-opioid interactive function that works mainly through the μ- and κ-receptors of the opioid system.

A role for oxytocin in nociception is supported in studies demonstrating antinociceptive effects (Arletti et al., 1992; Uvnas-
The demonstrated increase in oxytocin-LI level in the plasma in this study is in parallel with results from previous studies using non-noxious sensory stimulation, e.g. touch, warmth, vibration and low-intensity electro-acupuncture (Stock & Uvnas-Moberg, 1988; Uvnas-Moberg et al., 1993). The increase in withdrawal latency seen in the tail-flick test was also due to oxytocin and reversed by the oxytocin antagonist 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin (Uvnas-Moberg, 1993), indicating influence from the oxytocinergic system. The heightened concentration of endogenous oxytocin possibly results in a greater synthesis of endogenous opioids because antinociception noted after repeated injections of oxytocin was temporarily reversed by the opioid antagonist naloxone (Petersson et al., 1996). Also, the content of various endogenous opioid peptides such as beta-endorphin, L-encephalin and dynorphin A₁₋₁₃ was raised in the cerebrospinal fluid after intrathecal treatment with oxytocin (Yang, 1994). It is also possible that the effects may be attributed to other neurotransmitters in the CNS including substance P, neuropeptide Y and neurokinin A (Bucinskaite et al., 1996) or to gamma-amino butyric acid (GABA) and purines at the level of the spinal cord (Melzack & Wall, 1965; De Konick & Henry, 1992).

It might be argued that the examiners’ holding of the rat by its neck and shoulders while applying the massage-like stimulation could be responsible for the analgesia because stress-induced analgesia has been demonstrated as an acute response in studies exploring nociception using immobilization as a stress challenge (Amir & Amit, 1978; Jorgensen et al., 1984; Fuchs & Melzack, 1996; De Kock & Meert, 1997). However, after the initial raising of latencies, Amir & Amit (1978) found them to diminish following repeated sessions of immobilization, which is in contrast to our findings. The results of the present study reflect effects, possibly centrally mediated, manifested as increases in the latencies to thermal and mechanical stimulation, plus an endogenous release of oxytocin both in plasma and the PAG in response to peripheral afferent massage-like stimulation, probably mediated through the Aβ-fibres, showing cumulative effects with repeated treatments. The increases could also be attributed to activation of touch-sensitive C-fibres (Vallbo et al., 1999) resulting in long-term effects at the synaptic level (Sandkühler, 2000).

The increase in hindpaw withdrawal latencies following massage-like stimulation and oxytocin injections, and also the endogenously increased release of oxytocin, are factors which could interact. Furthermore, attenuation of oxytocin-induced increase in HWLs by the intra-PAG injection of β-FNA and nor-BNI, but not naltrindole, indicates that μ- and κ-opioid receptors in PAG are involved in the oxytocin-induced antinociception in rats. Thus, further analyses of the mechanisms of the responses of withdrawal latencies to massage, including the contribution of the oxytocinergic system, are under investigation.

Acknowledgements

This study was supported by grants from The Foundation for Acupuncture and Alternative Biological Treatment Methods and Praktikertjäst AB. The valuable advice and skilful dissection of the PAG by Dr Viveka Hillegaart and the skilful technical assistance of Mrs Anja Finn are gratefully acknowledged.

Abbreviations

β-FNA, β-funtalretamine; HWL, hindpaw withdrawal latency; nor-BNI, norbinaltorphimine; oxytocin-LI, oxytocin-like immunoreactivity; PAG, periaqueductal grey matter; RIA, radioimmunoassay.

References


