Oxytocin decreases carrageenan induced inflammation in rats

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Abstract

The effects of oxytocin on carrageenan-induced inflammation in rat hindpaw was examined. Oxytocin at 100 (P < 0.05) and 1000 µg/kg s.c. (P < 0.05), but not at 1 and 10 µg/kg s.c., reduced the edema of the paw when measured up to 10 h after the injection. An additional experiment showed that the effect was comparable to the effect of the glucocorticoid dexamethasone. No effect was found by oxytocin i.c.v.

In addition, rats with carrageenan-induced inflammation given oxytocin (1000 µg/kg s.c.) responded differently to nociceptive mechanical stimulation (P < 0.05) and had a reduced amount of myeloperoxidase (marker for neutrophil recruitment) in the paw (P < 0.01).

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1. Introduction

Oxytocin is a nonapeptide produced in the paraventricular (PVN) and the supraoptical (SON) nuclei in the hypothalamus. Oxytocin is mainly associated with uterine contraction during parturition and the milk-ejection reflex during lactation, but it is also implicated in for example cardiovascular and natriuretic regulation [9,15,17], various behaviors such as maternal, social and sexual behavior [3] as well as modulation of the release of adrenocorticoid hormones [18]. In addition, oxytocin has been suggested to be involved in the modulation of immune and inflammatory processes, since oxytocin and oxytocin receptors are located in the thymus [6,7], and the oxytocin receptor gene contains response elements for acute phase reactants and interleukins [10]. In vitro, oxytocin may decrease the release of some interleukins [23]. We have recently found that oxytocin increases the survival of ischaemic skin flaps in rats. An effect which might be related to an oxytocin induced activation of growth factors or anti-inflammatory mechanisms [14]. Against this background, we wanted to investigate if oxytocin could affect acute inflammation. For this purpose a model of carrageenan induced inflammation was used. Oxytocin was administered immediately before the injection of carrageenan and the volume of the edema in the rat hindpaw was measured regularly for 6–10 h. Since glucocorticoids have a strong anti-inflammatory effect we also gave dexamethasone to one group of rats for comparison with the oxytocin treated groups. In addition, the effects of oxytocin on carrageenan-induced hyperalgesia and neutrophil accumulation in the hindpaw were measured.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (260–300 g for s.c. injected and 340–380 g for i.c.v. injected) were used (B&K Universal AB, Sollentuna, Sweden). The animals arrived at least one week before experiments and were housed three-four per cage (except animals provided with i.c.v. cannulas that were housed individually) with free access to food (R36, Ewos, Södertälje, Sweden) and water. The light schedule was a 12/12 h light/dark cycle, and ambient temperature was 20 ± 2°C.

2.2. Drugs

Oxytocin and the oxytocin antagonist (1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin) (Ferring, Malmö, Sweden) were dissolved in physiological saline and injected in...
a volume of 1 ml/kg s.c. in the dorsal neck. Dexamethasone (Decadron®, Merck, Sharp & Dome, USA) was administered intramuscularly (i.m.). Oxytocin given i.c.v. was dissolved in a volume of 5 μl physiological saline and slowly injected over a period of 1 min through the i.c.v. guide cannula via a 25 G stainless-steel injection needle connected to a 10 μl Hamilton syringe via a polyethylene tube. Controls received saline in the same amounts.

2.3. Surgery for i.c.v. injections

The animals were anaesthetized with sodiumpentobarbital (50 mg/kg) (Apoteksbolaget, Sweden) injected intraperitoneally (i.p.). The scull was uncovered, a hole was drilled in the right parietal bone and a guide cannula (21 G) was fixed stereotactically to the scull by means of acrylic dental cement. The coordinates were 1.00 mm posterior and 1.30 mm lateral to the bregma. The guides reached but did not penetrate the dura mater. The injection needles (25 G) reached 3.80 mm below the dura mater, with the tip of the needle in the right lateral ventricle. The animals were allowed one week of recovery after the operation. At the end of the experiment, the placement of the guide cannula was checked by injection of 2 μl of toluidine blue.

2.4. Nociceptive thresholds

The response to mechanical stimulation was determined using the Randall Selitto Test (Ugo Basile, type 7200, Italy). The mechanical stimulus was applied to the dorsal surface of the hindpaw by a wedged-shape pusher at a loading rate of 48 g/s and the pressure required to initiate the struggle response was measured. All rats were trained on three consecutive days before testing.

2.5. Myeloperoxidase assay

To determine the recruitment of neutrophils in response to the carrageenan induced inflammation in the rat hindpaw, the paws were weighed and homogenized in 10 ml 0.5% hexadecltrimethyl-ammonium bromide (Sigma Chemical Co, USA), and freeze-thawed, whereafter the myeloperoxidase activity of the supernatant was assessed. The enzyme activity was determined spectrophotometrically as the change in absorbance at 650 nm (25°C) occurring in the redox reaction of H2O2-tetramethylbenzidine (Sigma Chemical Co, USA) catalyzed by myeloperoxidase. Values are expressed as myeloperoxidase units/g tissue.

2.6. Experimental design

To produce acute inflammation, carrageenan, 2 mg in 0.1 ml saline, was injected s.c. into the plantar region of the rat right hindpaw.

1. The rats were treated s.c. with oxytocin (1.0, 10, 100 or 1000 μg/kg (n = 8 in each group) or saline (n = 8+8) immediately before the carrageenan injection. The edema of the right hindpaw was measured (volume in ml) using a plethysmometer (Ugo Basile, type 7150, Florence, Italy) before treatment, half an hour after, one hour after, and then every hour up to 6 or 10 h after treatment. Nociceptive thresholds were measured in the rats given oxytocin 1000 μg/kg s.c. and their controls.

2. The rats were treated s.c. with oxytocin (1000 μg/kg) and the oxytocin antagonist (1000 μg/kg) (the antagonist was given 30 min before oxytocin) (n = 8), the oxytocin antagonist alone (1000 μg/kg) (n = 8) or saline (n = 8) immediately before the carrageenan injection. Measurements as in experiment 1.

3. The rats were treated i.c.v. with oxytocin (1.0 μg/kg) (n = 6) or saline (n = 7) immediately before the carrageenan injection. Measurements as in experiment 1.

4. The rats were treated i.m. with dexamethasone (10 mg/kg) (n = 6) or saline (n = 6) immediately before the carrageenan injection. The edema of the right hindpaw was measured using the plethysmometer before treatment, and at 2, 4 and 6 h after treatment.

5. The rats were treated s.c. with oxytocin (1000 μg/kg) (n = 6) or saline (n = 6) immediately before the carrageenan injection. The accumulation of myeloperoxidase in the rat right hindpaw was measured 6 h after the treatment with oxytocin or saline, and carrageenan.

2.7. Statistical analysis

The results are presented as means ± SD. Statistical analysis was performed by means of a 1-way ANOVA, followed by Fisher’s test for post-hoc comparisons. In the analysis of myeloperoxidase accumulation, a Student’s t test was used. P-values of 0.05 or less were regarded as statistically significant.

3. Results

3.1. The effects of oxytocin s.c. on carrageenan induced edema

Oxytocin (1000 μg/kg s.c.) decreased the carrageenan induced edema significantly at 1–3 h after the injection compared to saline injected controls (1 h: 21 ± 16% vs. 43 ± 11%; P < 0.05, 2 h: 30 ± 17% vs. 61 ± 22%; P < 0.05 and 3 h: 41 ± 16% vs. 79 ± 21%; P < 0.05) (values calculated as percentage increase from pretreatment values) (ANOVA; F(1,84) = 5.72, P = 0.031) (Fig. 1).

A 10-fold lower dose of oxytocin (100 μg/kg s.c.) re-
duced the edema significantly 4–10 h after the injection (ANOVA; F(1,140) = 5.09, P = 0.041) (Fig. 2).

No effect was observed in response to oxytocin 1.0 and 10 μg/kg s.c. (data not shown).

The oxytocin antagonist (1000 μg/kg s.c.) did not abol-

ish the oxytocin (1000 μg/kg s.c.) induced effect on carra-
geenan induced edema. In this experiment, a significant reduction of the hindpaw volume compared to controls was seen 1–10 h after the injection of carrageenan (ANOVA; F(1,140) = 20.82, P = 0.0004) (Fig. 3). The significant difference was gone when measured at 24 h (data not shown). The oxytocin antagonist (1000 μg/kg s.c.) administered alone did not induce any effect (data not shown).

3.2. The effects of oxytocin i.c.v. on carrageenan induced edema

Oxytocin administered i.c.v (1.0 μg/kg) did not decrease the paw edema (ANOVA; F(1,110) = 2.73, P = 0.13) (data not shown).

3.3. The effects of dexamethasone i.m. on carrageenan induced edema

Dexamethasone (10 mg/kg i.m.) decreased the carrageenan induced edema significantly when measured at 2, 4 and 6 h after the injection (2 h: 35 ± 9.3% vs. 48 ± 10%; P < 0.05, 4 h: 54 ± 14% vs. 78 ± 12%; P < 0.05 and 6 h: 71 ± 15% vs. 105 ± 11%; P < 0.01) (Fig. 4).

3.4. The effects of oxytocin s.c. on carrageenan induced hyperalgesia

Carrageenan decreased nociceptive thresholds significantly (ANOVA; F(1,84) = 22.6, P = 0.0001). Oxytocin
treated rats (1000 µg/kg s.c.) responded significantly different to the mechanical stimulation (ANOVA; F(1,84) = 56.68, P = 0.022) and showed an increase in withdrawal latency of the paw at 1 h and 6 h after the injection, compared to controls (P < 0.05) (Fig. 5).

3.5. The effects of oxytocin s.c. on carrageenan induced myeloperoxidase accumulation

Oxytocin 1000 µg/kg s.c. reduced the neutrophil content, as measured as myeloperoxidase activity, in the carrageenan induced inflammation significantly. The myeloperoxidase concentration (i.e. the neutrophil recruitment) in the hind-paw was 3.5 ± 0.72 units/g tissue in the oxytocin-treated rats compared to 5.4 ± 1.1 units/g in the saline-treated controls (P < 0.01) (Fig. 6).

4. Discussion

The present study showed that oxytocin administered s.c. reduced experimentally induced inflammation, as measured as the volume of the carrageenan induced edema and the myeloperoxidase activity in the rat paw.

The model of inflammation used, the carrageenan induced edema, is a commonly used model for studies of inflammation and for testing novel anti-inflammatory drugs [28]. Since neutrophil recruitment is one important mediator of inflammation, and the enzyme myeloperoxidase, which is abundant in neutrophil leukocytes [26], has been found to be a reliable marker for the detection of neutrophil accumulation in inflamed skin in vivo [22], we measured the activity of myeloperoxidase in the rat paw injected with carrageenan.

Carrageenan is also known to induce hyperalgesia [21], and earlier studies have found that oxytocin has an antinociceptive effect which is most prominent 30–60 min after the injection [12]. In this study we found that oxytocin-treated rats had significantly higher nociceptive thresholds both at 1 and 6 h after the injection of oxytocin. Thus oxytocin seems to have diminished the carrageenan induced hyperalgesia.

Oxytocin has been shown to affect several mediators involved in the formation of inflammation. As mentioned in the introduction, the oxytocin receptor gene contains response elements to acute phase proteins and interleukins [10]. Oxytocin may also decrease the release of interleukin-6 (IL-6) [23], and influence the coagulation and the fibrinolytic system [1]. In addition, oxytocin releases prostacyclin, which inhibits platelet aggregation [27]. A possible mediator behind the decrease in myeloperoxidase activity in response to oxytocin could be nitric oxide. Nitric oxide can be released by oxytocin and inhibits adhesion and aggregation of neutrophil leukocytes [24].
we previously have shown that oxytocin at 1 g/kg i.c.v. on the experimentally induced inflammation was that pated in the effect. Only, although corticosterone could of course have partici-

tory effect on inflammation. Additionally, also oxytocin levels acutely in rats [19], and therefore it could be argued that the anti-inflammatory effect of oxytocin was caused by a rise in corticosterone. However, the effect of oxytocin in this study was equally potent as the effect of dexamethasone in high doses, may increase corticosterone levels acutely in rats [8], and therefore it could be argued that the activity of myeloperoxidase was significantly lower when measured 6 h after the injection of oxytocin 1 mg/kg s.c. alone and since oxytocin at a 10-fold lower dose induced a similar long-lasting effect as oxytocin and the oxytocin antagonist administered together.

In conclusion, this study showed that oxytocin may have anti-inflammatory effects in vivo, an effect of oxytocin that to our knowledge not previously has been shown. Such an effect of oxytocin would be physiologically suitable to protect females against inflammation during parturition and breastfeeding, which are periods when oxytocin is released in high amounts.

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The study was approved by the Stockholm Ethical Committee for Experiments in Animals.

References


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Fig. 6. The accumulation of myeloperoxidase in the right hindpaw of rats 6 h after s.c. treatment with NaCl (n = 6) or oxytocin (1.0 mg/kg) (n = 6) right before induction of acute inflammation by means of a s.c. carrageenan injection into the hindpaw. The results are shown as means ± SD. Statistical evaluation was performed by a Student’s t-test. ** P < 0.01.

Growth factors may also have been involved in the anti-inflammatory effect of oxytocin. Insulin like growth factor-I (IGF-I) has been found to modulate inflammatory responses, by stimulating phagocyte migration and phagocyte production of cytokines [20], and oxytocin has been found to influence plasma levels of both IGF-1 and growth hormone (GH) [2,14]. Since glucocorticoids are potent anti-inflammatory agents we examined the effect of dexamethasone on the carrassgean induced inflammation as a complement to the oxytocin experiments. As expected, dexamethasone reduced the carrassgean induced edema significantly. Oxytocin, when administered in high doses, may increase corticosterone levels acutely in rats [8], and therefore it could be argued that the anti-inflammatory effect of oxytocin was caused by a rise in corticosterone. However, the effect of oxytocin in this study was equally potent as the effect of dexamethasone, and also the lower dose of oxytocin (100 μg/kg s.c.), which does not induce a similar increase in corticosterone levels as 1000 μg/kg of oxytocin [16], had a strong inhibitory effect on inflammation. Additionally, also oxytocin administered i.c.v. may increase corticosterone levels acutely in rats [19]. Thus, the oxytocin administered i.c.v. in the present study should have decreased inflammation if the effect was mediated through an increase in corticosterone levels. Therefore, it is not likely that oxytocin caused its anti-inflammatory effect through a rise in corticosterone only, although corticosterone could of course have participated in the effect.

Another reason for examining the effect of oxytocin i.c.v. on the experimentally induced inflammation was that we previously have shown that oxytocin at 1 μg/kg i.c.v.


