Antinociceptive role of oxytocin in the nucleus raphe magnus of rats, an involvement of μ-opioid receptor

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Received 27 January 2003; received in revised form 12 May 2003; accepted 13 May 2003

Abstract

Recent studies showed that oxytocin plays an important role in nociceptive modulation in the central nervous system. The present study was undertaken to investigate the role of oxytocin in antinociception in the nucleus raphe magnus (NRM) of rats and the possible interaction between oxytocin and the opioid systems. Intra-NRM injection of oxytocin induced dose-dependent increases in hindpaw withdrawal latencies (HWLs) to noxious thermal and mechanical stimulation in rats. The antinociceptive effect of oxytocin was significantly attenuated by subsequent intra-NRM injection of the oxytocin antagonist 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin. Intra-NRM injection of naloxone dose-dependently antagonized the increased HWLs induced by preceding intra-NRM injection of oxytocin, indicating an involvement of opioid receptors in oxytocin-induced antinociception in the NRM of rats. Furthermore, the antinociceptive effect of oxytocin was dose-dependently attenuated by subsequent intra-NRM injection of the μ-opioid antagonist h-FNA, but not by the κ-opioid antagonist nor-binaltorphimine (nor-BNI) or the δ-opioid antagonist naltrindole. The results demonstrated that oxytocin plays an antinociceptive role in the NRM of rats through activating the oxytocin receptor. Moreover, μ-opioid receptors, not κ and δ receptors, are involved in the oxytocin-induced antinociception in the NRM of rats.

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Keywords: Oxytocin; Oxytocin receptor; μ-opioid receptor; Nucleus raphe magnus (NRM); Antinociception

1. Introduction

Descending pain inhibitory pathways from midbrain to dorsal horn of the spinal cord play a crucial role in the transmission of nociceptive information from the periphery to central nervous system [1,2]. One major relay station is the nucleus raphe magnus (NRM) [1,3]. It is well known that the NRM plays an important role in pain modulation [1,3–5]. There is a mono-synaptic connection between the periaqueductal grey (PAG) and the NRM [6]. The main descending pain inhibitory pathway is from the periaqueductal grey to the dorsal longitudinal tract to the dorsal horn of the spinal cord [1–3].

Oxytocin was demonstrated to be involved in the modulation of pain at different levels in the central nervous system [7–13]. Intrapertoneal or intracisternal injection of oxytocin produced antinociceptive effects in rats or in mice [14]. Intracerebroventricular injection of oxytocin induced antinociception [9]. Administration of oxytocin has also been shown to increase colonic pain thresholds in patients with irritable bowel syndrome [15] and to induce analgesia in patients with low back pain [16].

Recent studies in our laboratory showed that intra-PAG injection of oxytocin induced dose-dependent antinociceptive effects in rats [11,13]. Furthermore, opioid receptors were involved in the oxytocin-induced antinociception in the PAG of rats [11]. The aim of the present study was to investigate the role of oxytocin in antinociception and the possible interaction between oxytocin and opioids in the NRM of rats.

2. Materials and methods

2.1. Animals

All experiments were performed on freely moving male Wistar rats (200–300 g; Experimental Animal Center of Peking University, Beijing, China). The rats were housed...
in cages with free access to food and water, and maintained in a room temperature of $24 \pm 2 \, ^\circ C$ with a 12-h light–dark cycle. All experiments were conducted according to the guideline of the animal ethical committee of Karolinska Institutet and every effort was made to minimize both the animal suffering and the number of animals used.

2.2. Nociceptive tests

All rats were accustomed to the test condition for 5 days before the experiment was conducted. The latencies to hindpaw withdrawal during the thermal and mechanical stimulation were measured and expressed in seconds to be referred to as the hindpaw withdrawal latency (HWL). The response to noxious thermal stimulation was assessed by the hot plate test \[17,18\]. The entire ventral surface of the rat’s hindpaw was placed manually on the hot plate, which was maintained at a temperature at $52 \, ^\circ C$ ($51.8–52.4 \, ^\circ C$). The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to noxious mechanical stimulation \[17,18\]. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of the manually handled hindpaw and the latency required to initiate the withdrawal response was assessed and expressed in seconds. In both of the tests, 15 s was the cut-off time to prevent possible tissue damage. The average values obtained before intra-NRM injection were regarded as the basal HWL. The HWLs recorded during subsequent experiments were expressed as percentage changes of the basal level for each rat (% changes of the HWL). Each rat was tested with both types of stimulation.

2.3. Intra-NRM injection

The animals were anaesthetized by intraperitoneal pentobarbital (40 mg/kg) and were mounted on a stereotaxic instrument. A stainless steel guide cannula of 0.8 mm outer-diameter was directed into the NRM (AP, $-10.0$; LR, 0.4; V, 8.5 mm from the surface of the skull. AP, anterior (+) or posterior (−) to Bregma; L or R, lateral to midline; V, ventral to the surface of skull) or into the decussation of superior cerebellar peduncles (DSCP; AP-7.0, L 0.6, V 12.0 mm from the surface of the skull) according to Paxinos and Watson \[19\] and was fixed to the skull by dental acrylic. On the day of experiment, a stainless-steel needle with 0.4 mm diameter was directly inserted into the guide cannula, with 1.5 mm beyond the tip of the latter. One microliter of solution was thereafter infused into NRM over 1 min.

At the end of the experiments, the rat was killed by a high dose of pentobarbital (80 mg/kg) and the rat heads were fixed in 10% formalin for 1 week with the injecting tube in situ before section. The location of the tip of the injecting tube was verified and all the tips of the injecting tube were in NRM area of rats in the present study.

2.4. Chemicals

Solutions for intra-NRM injection were prepared with sterilized saline (0.9%), each with a volume of 1 µl: (1) 0.1, 1 or 3 nmol of oxytocin (Peninsula Laboratories, USA); (2) 3 nmol of 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin (Ferring, Malmo, Sweden); (3) 0.3, 1.5 or 3 nmol of naloxone hydrochloride; Sigma, St. Louis, MO); (4) 0.3, 1 or 3 nmol of β-funaltrexamine (β-FNA hydrochloride; Tocris, Balwin, MO 63011, USA); (5) 3 nmol of naltrindole (naltrindole hydrochloride; Tocris); (6) 3 nmol of nor-binaltorphimine (nor-BNI dihydrochloride; Tocris).

2.5. Statistical analysis

Data from nociceptive tests were presented as mean ± S.E.M. The two-way analysis of variance (ANOVA) was used to evaluate the difference in HWLs between two groups ($F_{left/left}$ is the $F$ value of the two groups: the left HWL of the first group compared with the left HWL of the second group). *$P<0.05$, **$P<0.01$ and ***$P<0.001$ were considered as significant differences.

3. Results

3.1. Effects of intra-NRM injection of oxytocin on HWLs to noxious thermal and mechanical stimulation in rats

Rats received intra-NRM injection of 0.1 ($n = 8$), 1 ($n = 8$) or 3 nmol of oxytocin ($n = 8$), or 1 µl of 0.9% saline as a control ($n = 8$). As shown in Fig. 1, the HWLs to thermal and mechanical stimulation increased significantly after intra-NRM injection of 3 (thermal test: $F_{left/left} = 113.86, P < 0.001$; $F_{right/right} = 51.68, P < 0.001$; Randall Selitto test: $F_{left/left} = 32.61, P < 0.001$; $F_{right/right} = 51.25, P < 0.001$) or 1 nmol of oxytocin (thermal test: $F_{left/left} = 39.29, P < 0.001$; $F_{right/right} = 42.26, P < 0.001$; Randall Selitto test: $F_{left/left} = 65.15, P < 0.001$; $F_{right/right} = 40.40, P < 0.001$), but not 0.1 nmol of oxytocin (thermal test: $F_{left/left} = 2.63, P = 0.13$; $F_{right/right} = 1.30, P = 0.27$; Randall Selitto test: $F_{left/left} = 5.35, P = 0.05$; $F_{right/right} = 9.78, P < 0.01$), compared with the control group. The antinoceptive effect of oxytocin reached the peak at 10 min after intra-NRM injection, and then recovered to the basal line at 60 min.

One group of rats ($n = 8$) received of 3 nmol of oxytocin into the DSCP. Compared with the group receiving intra-NRM injection of 0.9% saline, there were no significant changes in HWLs during 60 min after the injection of oxytocin (thermal test: $F_{left/left} = 0.71, P = 0.41$; $F_{right/right} = 1.20, P = 0.29$; Randall Selitto test: $F_{left/left} = 0.003, P = 0.96$; $F_{right/right} = 0.32, P = 0.58$). Compared with the group of rats received intra-NRM injection of 3 nmol of oxytocin, there were significant changes in HWLs during 60 min after the intra-DSCP injection of oxytocin (thermal
3.2. Blockade effects of intra-NRM injection of the oxytocin antagonist 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin on the oxytocin-induced increases in HWLs

Rats received intra-NRM injection of 3 nmol of oxytocin, followed 5 min later by 3 nmol of 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin \((n = 7)\), or 1 \(\mu\)l of 0.9\% saline as a control \((n = 7)\). The results are shown in Fig. 2. Compared with the control group, the increased HWLs to both thermal and mechanical stimulation were blocked significantly after administration of 3 nmol of 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin (thermal test: \(F_{left/left} = 86.09, P < 0.001; F_{right/right} = 12.96, P < 0.01\); Randall Selitto test: \(F_{left/left} = 29.59, P < 0.001; F_{right/right} = 15.61, P < 0.01\)). Another group of rats \((n = 8)\) received intra-NRM injection of 1 \(\mu\)l of 0.9\% saline, followed 5 min late by intra-NRM injection of 3 nmol of 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin. There were no marked changes in HWLs during 60 min after the injection.

3.3. Blockade effects of intra-NRM injection of naloxone on the oxytocin-induced increases in HWLs

Rats received intra-NRM injection of 3 nmol of oxytocin, followed 5 min later by 0.3 \(n = 8\), 1.5 \(n = 8\) or 3 nmol of naloxone \((n = 7)\), or 1 \(\mu\)l of 0.9\% saline as a control \((n = 7)\). The results are shown in Fig. 3. Compared with the control group, the increased HWLs to both thermal and mechanical stimulation were blocked significantly after administration of 3 \(n = 8\), 1 \(n = 8\) or 3 nmol of naloxone (thermal test: \(F_{left/left} = 82.16, P < 0.001; F_{right/right} = 18.71, P < 0.001; F_{right/right} = 23.26, P < 0.001\) or 1.5 nmol of naloxone (thermal test: \(F_{left/left} = 61.90, P < 0.001; F_{right/right} = 16.13, P < 0.01\); Randall Selitto test: \(F_{left/left} = 27.50, P < 0.001; F_{right/right} = 22.82, P < 0.001\)), but not 0.3 nmol of naloxone (thermal test: \(F_{left/left} = 0.62, P = 0.44; F_{right/right} = 0.69, P = 0.42\); Randall Selitto test: \(F_{left/left} = 0.39, P = 0.54; F_{right/right} = 0.08, P = 0.78\)). Another group of rats \((n = 8)\) received intra-NRM injection of 1 \(\mu\)l of 0.9\% saline, followed 5 min late by intra-NRM injection of 3 nmol of naloxone. There were no marked changes in HWLs during 60 min after the injection.

3.4. Influence of \(\mu\)-opioid receptor on the oxytocin-induced increases in HWLs

Rats received intra-NRM administration of 3 nmol of oxytocin, followed 5 min later by 0.3 \(n = 6\), 1 \(n = 8\) or 3 nmol of \(\beta\)-FNA \((n = 7)\), or 1 \(\mu\)l of 0.9\% saline as a control \((n = 6)\). The results are shown in Fig. 4. Compared with the control group, the increased HWLs to both thermal and mechanical stimulation were blocked significantly after the administration of 3 nmol of \(\beta\)-FNA (thermal test: \(F_{left/left} = 33.07, P < 0.001; F_{right/right} = 21.92, P < 0.001\); Randall Selitto test: \(F_{left/left} = 39.27, P < 0.001; F_{right/right} = 41.52, P < 0.001\)), but not after the administration of 1 (thermal...
test: $F_{\text{left/left}} = 9.79, P < 0.05; F_{\text{right/right}} = 2.96, P = 0.11$; Randall Selitto test: $F_{\text{left/left}} = 4.51, P = 0.05; F_{\text{right/right}} = 1.52, P = 0.24$) or 0.3 nmol of β-FNA (thermal test: $F_{\text{left/left}} = 1.15, P = 0.31; F_{\text{right/right}} = 0.01, P = 0.97$; Randall Selitto test: $F_{\text{left/left}} = 0.48, P = 0.50; F_{\text{right/right}} = 3.25, P = 0.15$). Another group of rats ($n = 6$) received intra-NRM injection of 1 µl of 0.9% saline, followed 5 min later by intra-NRM injection of 3 nmol of β-FNA. There were no marked changes in HWLs during 60 min after the injection.

Fig. 2. Blockade effects of intra-NRM administration of oxytocin antagonist (1-deamino-2-α-Tyr-(Oet)-4-Thr-8-Orn-oxytocin) on the oxytocin-induced increases in HWLs to thermal (A and B) and mechanical stimulation (C and D) in rats. Left HWL: A and C; right HWL: B and D. Time = 0 min: intra-NRM administration of 3 nmol of oxytocin; time = 5 min: intra-NRM administration of 3 nmol of oxytocin antagonist (1-deamino-2-α-Tyr-(Oet)-4-Thr-8-Orn- oxytocin) or 1 µl of 0.9% saline as the control group. Data are presented as mean ± S.E.M., % change of HWL (vertical axis). Horizontal axis indicates minutes (min) after the injection. The statistical difference between groups was evaluated by two-way ANOVA. OT, oxytocin; NRM, nucleus raphe magnus; HWL, hindpaw withdrawal latency. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with control group.

Fig. 3. Inhibitory effects of intra-NRM administration of naloxone on the oxytocin-induced increases in HWLs to thermal (A and B) and mechanical stimulation (C and D) in rats. Left HWL: A and C; right HWL: B and D. Time = 0 min: intra-NRM administration of 3 nmol of oxytocin; time = 5 min: intra-NRM administration of 0.3, 1.5 or 3 nmol of naloxone, or 1 µl of 0.9% saline as the control group. Data are presented as mean ± S.E.M., % change of HWL (vertical axis). Horizontal axis indicates minutes (min) after the injection. The statistical difference between groups was evaluated by two-way ANOVA. OT, oxytocin; NRM, nucleus raphe magnus; HWL, hindpaw withdrawal latency. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with control group.
3.5. Influence of δ-opioid receptor on the oxytocin-induced increases in HWLs

Rats received intra-NRM administration of 3 nmol of oxytocin, followed 5 min later by 3 nmol of naltrindole (\(n=7\)), or 1 \(\mu\)l of 0.9% saline as a control (\(n=7\)). There were no significant differences in HWLs after the injection of naltrindole compared with the saline group (thermal test: \(F_{\text{left/left}} = 2.92, P=0.11; F_{\text{right/right}} = 0.001, P=0.97\); Randall Selitto test: \(F_{\text{left/left}} = 2.11, P=0.17; F_{\text{right/right}} = 3.07, P=0.10\), as shown in Fig. 5.
3.6. Influence of κ-opioid receptor on the oxytocin-induced increases in HWLs

Rats received intra-NRM administration of 3 nmol of oxytocin, followed 5 min later by 3 nmol of nor-BNI (n = 8), or 1 μl of 0.9% saline as a control (n = 7). There were no significant changes in HWLs after the injection of nor-BNI compared with the saline group (thermalsal test: \( F_{\text{left/offset}} = 2.80, P = 0.12; F_{\text{right/offset}} = 0.31, P = 0.59 \); Randall Selitto test: \( F_{\text{left/len}} = 1.06, P = 0.32; F_{\text{right/len}} = 2.24, P = 0.16 \), as shown in Fig. 5.

4. Discussion

The present study demonstrated that the nociceptive response latencies increased significantly after intra-NRM administration of 1 or 3 nmol of oxytocin in rats, but not 0.1 nmol. Administration of 3 nmol of oxytocin out of NRM (into the DSCP) showed no marked changes in HWLs during 60 min after the injection, indicating oxytocin plays an important role in antinociception in the NRM. Similar results have been obtained when 1 nmol of oxytocin was injected into periaqueductal grey [11]. It is known that 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin, a selective antagonist of oxytocin receptor [20]. In the present, the oxytocin-induced antinociception were blocked by subsequent administration of 3 nmol of the selective oxytocin antagonist 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin. The results indicate that oxytocin-induced antinociception was mediated by the oxytocin receptor located in the NRM. Furthermore, in the present study, the results demonstrated that the oxytocin-induced antinociception was dose-dependently attenuated by following intra-NRM administration of 3 the opioid antagonists naloxone, indicating that there is an interaction between endogenous opioids and oxytocin in the modulation of nociception in the NRM of rats. In the present study the antinociceptive effect of oxytocin was attenuated by subsequent intra-NRM injection of the selective μ-opioid receptor antagonists β-FNA, but not by nor-BNI and naltrindole, indicating that the μ-opioid receptors, not κ- or δ-opioid receptors, are involved in the oxytocin-induced antinociception in the NRM of rats.

Our results are supported by previous studies which showed that oxytocin is involved in antinociception at the supraspinal level [9,11,13]. It has been reported that intracerebroventricular and intra-PAG injection of oxytocin produced a significant antinociceptive effect [9,11]. The antinociceptive effect of oxytocin was blocked by intracerebroventricular and intra-PAG injection of selective oxytocin antagonist, suggesting that oxytocin induces antinociception by activating oxytocin receptors in the brain [9,11]. Studies showed that in the central nervous system, the oxytocin gene is primarily expressed in magnocellular neurons in the hypothalamic paraventricular nucleus (PNV) and supraopticus nucleus (SON) [21]. Action potentials in these neurosecretory cells trigger the release of oxytocin from their axon terminals in the neurohypophysis [22]. Also, studies showed that oxytocin fibers and endings have been described in the NRM of rats [21], suggesting the presence of endogenous oxytocin in the NRM. Histological studies showed that the NRM region of the rat brain showed constant expression of oxytocin receptor mRNA throughout development and in the adult, suggesting the presence of oxytocin receptor in the NRM [23,24]. The present study demonstrated that intra-NRM administration of oxytocin produced dose-dependent antinociception, suggesting that oxytocin is involved in the endogenous antinociception system. Furthermore, our study showed that the oxytocin-induced antinociception was blocked by subsequent intra-NRM injection of the oxytocin antagonist 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin, suggesting that the oxytocin receptors are involved in the antinociceptive effect induced by oxytocin in the NRM. In addition, intra-NRM administration of 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin alone did not produce any effects, suggesting that there is no tonic release of oxytocin in the NRM under normal conditions.

It is well known that opioid peptides play a key role in antinociception in the central nervous system, including the PAG and the NRM [1,3,25]. A widely used chemical in opioid research is the non-selective opioid receptor antagonist naloxone [3]. Also, the analgesic effect of intra-NRM injection of morphine was reversed by systemic administration of naloxone, indicating an involvement of opioid peptides in the NRM [26]. In the present study the results demonstrated that the antinociceptive effect of oxytocin was attenuated by intra-NRM administration of naloxone, suggesting that there is an involvement of opioid receptors in oxytocin-induced modulation of nociception in the NRM of rats. It is known that there are three types of opioid receptors in the central nervous system, the μ-, δ- and κ-opioid receptors [27–29]. It has been reported that predominantly μ and κ receptor mRNA expression was observed in the NRM area [30]. Our study demonstrated the antinociceptive effect of oxytocin was attenuated by subsequent intra-NRM injection of the selective opioid receptor antagonists β-FNA, but not by nor-BNI and naltrindole, indicating that μ-opioid receptors, not κ- and δ-opioid receptors, is involved in the oxytocin-induced antinociception in the NRM.

The NRM is one of the major brainstem sources of axons that project to the spinal cord [3,31]. It is known that supraspinal opioid administration activates descending antinociceptive controls [3]. There are three classes of neuron in the NRM: the on-cells, the off-cells and the neutral cells [3,32]. The on-cells are excited and the off-cells are inhibited by noxious stimuli [3,32]. Studies showed that the off-cells were excited and the on-cells were inhibited by intra-NRM administration of opioid agonists, suggesting that the off-cells inhibit and the on-cells facilitate nociceptive transmission [32,33]. There is evidence that endogenous opioids acting on the μ-opioid receptor mediated local
circuit in the NRM provide a necessary link for the PAG-NRM-spinal cord pain-modulating pathway [3]. It is possible that oxytocin administered to the NRM excites opioid-ergic interneurons, then opioid actions activate the off-cells and inhibit the on-cells, thus activate descending antinoceptive pathways.

Acknowledgements

This study was supported by funds from the National Natural Science Foundation of China (NSFC) and the Karolinska Institute Foundation.

References