The interaction between the oxytocin and pain modulation in headache patients

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ABSTRACT

Oxytocin (OXT), a nonapeptide hormone of posterior pituitary, reaches the central nervous system from systemic blood circulation with a difficulty because of the blood–brain barrier (BBB). The interest has been expressed in the use of the nasal route for delivery of OXT to the brain directly, exploiting the olfactory pathway. Our previous study has demonstrated that OXT in the central nervous system rather than the blood circulation plays an important role in pain modulation. The communication tried to investigate the interaction between the OXT and pain modulation in Chinese patients with headache to understand the OXT effect on human pain modulation. The results showed that (1) intranasal OXT could relieve the human headache in a dose-dependent manner; (2) OXT concentration in both plasma and cerebrospinal fluid (CSF) increased significantly in headache patients in relation with the pain level; and (3) there was a positive relationship between plasma and CSF OXT concentration in headache patients. The data suggested that intranasal OXT, which was delivered to the central nervous system through olfactory region, could treat human headache and OXT might be a potential drug of headache relief by intranasal administration.

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1. Introduction

Oxytocin (OXT), a nonapeptide hormone of posterior pituitary, is mainly synthesized and secreted in the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON). This hormone, combined with an apparent carrier protein (neurophysin), is transported along the hypothalamo-hypophysial pathway to the neurohypophysis, where it is stored for subsequent release (Antunes and Zimmerman, 1978). The remarkable functions of OXT include uterine contraction during parturition, milk-ejection reflex during lactation, cardiovascular regulation, sex activity, learning and memory (McEwen, 2004).

Many studies demonstrated that OXT in central nervous system was related with the pain modulation (Yang et al., 2007a,b). Intraventricular injection (iv) of OXT has an analgesic effect in a patient

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with intractable cancer pain (Brown and Perlowski, 1998). OXT in the spinal cord relates to antinociception in the dog (Madrazo et al., 1987) and OXT takes part in the chronic and acute low back pain in human (Yang, 1994). Our previous study has discovered that the pain threshold is elevated by OXT following iv or intrathecal injection (it), and reduced by anti-OXT serum (iv or it), but the pain threshold is not altered by intravenous injection (iv) of OXT or anti-OXT serum (Yang et al., 2007b). Pain stimulation induces the SON release of OXT, which can be transferred to the locus coeruleus (LC), nucleus raphe magnus (NRM), caudate nucleus (CdN) and spinal cord (Yang et al., 2007a). Although some reports showed that OXT (intraperitoneal injection, ip) increased the pain threshold, which was prevented by OXT antagonist (icv) (Uvnas-Moberg et al., 1998), this OXT pharmacological analgesic effect in the peripheral system needed a very high dose OXT (10–200 mg OXT/rat) and a very long period (60 days) to treat the animal.

OXT reaches the central nervous system from systemic blood circulation with a difficulty because of the blood-brain barrier (BBB) (Antunes and Zimmerman, 1978). For developing OXT-related drugs in the field of pain relief, it is very important to find one way that rapidly delivers OXT from systemically administration to central nervous system. The interest has been expressed in the use of the nasal route for delivery of peptides to the brain directly, exploiting the olfactory pathway (Dhuria et al., 2010; Ilum, 2004; Pietrowsky et al., 1996; Veronesi et al., 2011). However, few studies reported that intranasal OXT influenced on headache in Chinese patients. The communication tried to investigate the effect of intranasal OXT on the human headache, so as to understand the interaction between the OXT and pain modulation in Chinese patients with headache.

2. Materials and methods

2.1. Materials

OXT was obtained from Peninsula Laboratories, San Carlos, CA, USA. 125Iodine was from Amersham Pharmacia, Buckinghamshire, UK. The other chemicals were from Sigma Co., St Louis, MO, USA. Rabbit anti-human OXT serum was made by Department of Neurobiology, Second Military Medical University, Shanghai, China (Song et al., 1987). The specificity of the antisera was 99.99% cross-reactivity with OXT and less than 0.01% cross-reactivity with arginine vasopressin, lypressin-vasopressin, vasotocin, vasoactive intestinal peptide, neuropeptide, leucine-enkephalin, methionine-enkephalin, beta-endorphin and dynorphin A1-17. The dilution of the antisera was more than 1:40,000 for radioimmunoassay.

2.2. Participants

2.2.1. Headache patients

One hundred and twelve outpatients including 49 male and 63 female, 20–62 years old, average 44.5 ± 8.2 years old, whose suffered with headaches, were asked to participate in the study between May 2010 and November 2011. The patients were only diagnosed as tension-type headache and migraine. The patients were classified as 1–4 pain level depending on the International Headache Society's International Classification of Headache Disorders (ICHD). The patients, which headache history was 4–12 months (average 5.4 ± 2.1 months), did not receive any treatments before the experiment.

2.2.2. Health volunteers

One hundred and three health volunteers including 42 male and 61 female, 19–64 years old, average 45.6 ± 8.1 years old were asked to participate in the study between May 2010 and November 2011. They have not been suffering from any headaches.

2.2.3. Inclusion criteria

Inclusion criteria in those were as follows: (a) agreement to sign the informed consent form; (b) eligibility was checked before the experiments (exclusion criteria: pregnancy, menstrual period, tumor, cardiovascular, gastrointestinal, respiratory, brain, endocrine, psychiatric or other diseases, smoking, intake of drugs); (c) participants were asked not to drink any alcohol, caffeine containing beverages and analgesic medication during the experiment; (d) participants were asked not to eat anything before collecting the blood and cerebrospinal fluid during the day of sample collection; (e) all experimental sessions were carried out between 08:00 am and 09:00 am; and (f) over 18 years old.

All experiments were approved by the relative hospital Ethics Committees and carried out according to the Declaration of Helsinki.

2.3. Procedure

The experiments were only carried out during the patients filling ill of headache. Participants were instructed to abstain from smoking, caffeine and analgesic medication. Subsequently, participants completed a set of questionnaires and were checked with the physical examination. The experimental sessions were conducted in a double-blind and placebo controlled within-subject cross-over design. OXT or the placebo was administered intranasal. Following a standardized protocol, the participants self-administered three puff of OXT per nostril, which were controlled in the volume with a different OXT concentration (100, 200, 400 ng OXT) or placebo (containing all ingredients except for the peptide) under the supervision of the study coordinator. The total time of an experimental session was 3 h. The health volunteers were done as the patients except the headache. All participants received monetary compensation after completion of the study.

2.4. Sample collection

2.4.1. Blood sample

Blood was taken by vein-puncture between 08:00 am and 09:00 am. The blood was collected using the EDTA-Na2-treated vacutainer and immediately placed on ice.

2.4.2. Cerebrospinal fluid (CSF) sample

CSF was taken by lumbar puncture between 08:00 am and 09:00 am. The CSF was collected using the silicone oil-treated tube and immediately placed on ice.

2.4.3. Sample treatment

After the centrifugation at 10,000g for 20 min at 4 °C, the supernatants were withdrawn and stored at −80 °C until OXT determination.

2.5. OXT assay

OXT concentration was measured by radioimmunoassay with specific rabbit antisera against human OXT. OXT was labeled 125Iodine using the chloramines-T method and iodinated peptide was purified by Sephadex G-50. The assay sensitivity of OXT was 0.8 pg/tube and the normal range for plasma OXT was 1–64 pg/mL. The intra- and inter-assay coefficients of variation were less than 3.8% and 6.5%, respectively.
2.6. Statistical analysis

Data were expressed as mean ± standard error of the mean (S.E.M.) and performed with the SPSS 17.0 statistical package, with two-way analysis of variance (ANOVA) followed by the Bonferroni test and multivariation analysis of difference followed by the $\chi^2$ test. Significance was accepted at $p < 0.05$.

3. Results

3.1. Effect of intranasal OXT on human headache

Intranasal OXT relieved the human headache in a dose-dependent manner. The effect of intranasal OXT 400 ng in 28 cases of headache patients was complete remission 20 cases (71.4%), partial remission 8 cases (28.6%) and invalid remission 0 case (0.0%); the effect of intranasal AVP 200 ng in 28 cases of headache patients was complete remission 14 cases (50.0%), partial remission 12 cases (42.9%) and invalid remission 2 cases (7.1%); the effect of intranasal OXT 100 ng of headache patients in 28 cases was complete remission 9 cases (32.1%), partial remission 10 cases (35.8%) and invalid remission 9 cases (32.1%); and the effect of intranasal placebo in 28 cases of headache patients was complete remission 2 cases (7.1%), partial remission 7 cases (25.0%) and invalid remission 19 cases (67.9%); in which $\chi^2$ tests for the comparison between two groups showed all $p < 0.01$ (Table 1).

3.2. Change of plasma OXT concentration in headache patients

Comparing with the healthy volunteers, plasma OXT concentration was increased significantly in headache patients ($18.95 \pm 4.83$ pg/ml vs. $9.43 \pm 2.32$ pg/ml, $p < 0.01$) (Fig. 1). It showed a positive relationship between headache level and plasma OXT concentration in headache patients ($Y = 5.616X + 3.506$, $R = 0.861$, $p < 0.001$) (Fig. 2).

3.3. Change of CSF OXT concentration in headache patients

Comparing with the healthy volunteers, CSF OXT concentration was increased significantly in headache patients ($33.21 \pm 6.37$ pg/ml vs. $16.38 \pm 4.53$ pg/ml, $p < 0.01$) (Fig. 3). It showed a positive relationship between headache level and CSF OXT concentration in headache patients ($Y = 9.898X + 7.614$, $R = 0.950$, $p < 0.001$) (Fig. 4).

3.4. Relationship between plasma and CSF OXT concentration in headache patients

In headache patients, there was a positive relationship between plasma and CSF OXT concentration ($Y = 1.647X + 2.015$, $R = 0.926$, $p < 0.001$) (Fig. 5).

4. Discussion

OXT in the central nervous system rather than the blood circulation plays an important role in rat pain modulation (Yang et al.,

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Effect (Case)</th>
<th>Partial remission</th>
<th>Invalid remission</th>
<th>$\chi^2$ tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intranasal OXT 400 ng</td>
<td>28 (20)</td>
<td>8</td>
<td>0</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Intranasal OXT 200 ng</td>
<td>28 (14)</td>
<td>12</td>
<td>2</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Intranasal OXT 100 ng</td>
<td>28 (9)</td>
<td>10</td>
<td>9</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>Placebo</td>
<td>28 (2)</td>
<td>7</td>
<td>19</td>
<td>$p &lt; 0.01$</td>
</tr>
</tbody>
</table>

Fig. 1. Change of plasma oxytocin (OXT) concentration in the headache patients. The results are shown as mean ± SEM. **$p < 0.01$** is used for the comparison of plasma OXT concentration from healthy volunteer group ($N = 103$) and headache patient group ($N = 112$).

Fig. 2. The relationship between the headache level and plasma oxytocin (OXT) concentration in headache patients.

Fig. 3. Change of cerebrospinal fluid (CSF) oxytocin (OXT) concentration in the headache patients. The results are shown as mean ± SEM. **$p < 0.01$** is used for the comparison of plasma OXT concentration from healthy volunteer group ($N = 15$) and headache patient group ($N = 17$).
intranasal OXT in humans. Evidence from 38 randomised controlled trials, nine studies in vulnerable groups, showed no side-effects specific to OXT versus placebo, and no adverse reactions. The most frequently reported sensation was a feeling of calmness, but again this did not differ between OXT and placebo. The short-term use of intranasal OXT (1) produces minimal side-effects (2) produces no detectable differences in side-effects reported by either placebo or OXT participants, (3) produces no detectable subjective changes in recipients and, (4) preliminary is equally safe to use with vulnerable populations as with healthy adults. A systematic search for adverse reactions found only three reported incidents, two due to misuse of the nasal spray OXT and one linked with long term application (MacDonald et al., 2011).

SON is a nucleus of magnocellular neurosecretory cells in the hypothalamus of the mammalian brain. The nucleus is situated at the base of the brain, adjacent to the optic chiasm. SON neurons, which produce OXT and arginine vasopressin (AVP), are gathered in a long (about 1 mm) and narrow (about 100–200 μm) arrangement. There are no paracerebral subdivisions within SON, and this nucleus consists almost entirely of magnocellular neurons (McEwen, 2004). Electrical stimulation of the SON or microinjection of a small dose of γ-glutamate sodium into the SON, which only excites neurons, elevates the nociceptive threshold in a dose-dependent manner, while cauterization of the SON decreases the nociceptive threshold (Yang et al., 2008a,b). The data suggested that SON played an important role in pain modulation. Our previous work has pointed that SON acts through OXT rather than AVP to influence the pain process (Yang et al., 2011a,b,c,d). Microinjection of γ-glutamate sodium into the SON increases OXT concentrations in the SON perfusion liquid; pain stimulation induces OXT, but not AVP release in the SON; and pretreatment with anti-OXT serum, but not anti-AVP serum inhibits the antinociceptive role of SON stimulation (Yang et al., 2011c). The present results showed that OXT concentration in both the plasma and the CSF increased significantly in headache patients in relation with the pain level; and there was a positive relationship between the plasma and the CSF OXT concentration. The data suggested that blood OXT might be from the brain during the headache.

Pain stimulation induces SON release of OXT, which is transferred to the locus coeruleus (LC), periaqueductal gray (PAG), nucleus raphe magnus (NRM), caudate nucleus (CdN) and spinal cord, but no change in the hypothalamic paraventricular nucleus (PVN) (Yang et al., 2007a,b). Intra-PAG, CdN or NRM injection of OXT increases the pain threshold, whereas the local administration of the high specific OXT receptor antagonist, desGly-NH2, d(CH2)5[D-Tyr2, Thr-sup-4]OVT decreases the pain threshold in a dose-dependent manner; pain stimulation can elevate OXT concentration in the PAG, CdN or NRM perfusion liquid (Yang et al., 2011a,b,c,d; Pan et al., 2012). The data suggest that OXT in the PAG, CdN or NRM are involved in the antinociceptive process through the OXT receptor. Our previous studies have discovered that OXT could enhance the PAG synthesis and secretion of the endogenous opiate peptides including leu-enkephalin, met-enkephalin, β-endorphin and dynorphin A1–13 (Yang et al., 2011b). OXT (Rh) induces analgesia in low back pain involving the endogenous opiate peptide system also (Yang, 1994). It may be the neurobiological mechanism of central OXT regulating pain process. Bypassing the BBB to the brain through olfactory region, intranasal OXT may influence the endogenous opiate peptide to regulate pain process in human brain. However, it was very difficult to prove which brain structures and bioactive substances intranasal OXT related with in human headache relief in the present study. It needs to be studied in near future.

In conclusion, our present study made it clear that (1) intranasal OXT could relieve the human headache in a dose-dependent manner; (2) OXT concentration in both plasma and CSF increased

![Graph 1](image1.png)

Fig. 4. The relationship between the headache level and oxytocin (OXT) concentration in cerebrospinal fluid (CSF) in headache patients.

![Graph 2](image2.png)

Fig. 5. The relationship between plasma oxytocin (OXT) concentration and cerebrospinal fluid (CSF) OXT concentration in the headache patients.

2007a,b). However, few studies have reported that OXT regulates the pain process in Chinese human, because a major barrier to entry of OXT into the central nervous system is low bioavailability and presence of the brain-blood barrier (BBB). Intranasal delivery of OXT provides a potentially promising alternative to other routes administration. Since a direct pathway exists between the olfactory neuroepithelium and the brain, Petrovsky et al. (1996) reported that effect of OXT was facilitated after intranasal as compared to intravenous administration in human brain. The present study showed that intranasal OXT relieved the human headache in a dose-dependent manner. The data suggested that intranasal OXT effected on pain process in human.

Following the discovery that intranasal administration of neuropeptides can reach the central nervous system, a growing number of studies applied intranasal OXT paradigms to demonstrate the possible effects of OXT on social and emotional processes. The three-step paradigm typically included: OXT administration, a 45-min waiting period, and approximately 1-h period of active drug effects when experimental manipulations are applied. Yet, this schedule has not been put to systematic validation. Utilizing a double-blind placebo-control within-subject design, ten individuals were administered OXT or placebo and salivary OXT was measured ten times, at baseline and nine times over four consecutive hours. OXT administration induced substantial increases in salivary OXT across the entire period. OXT rose dramatically 15 min after administration (from 6.9 pg/ml at baseline to 1265.4 pg/ml), reached plateau at 45–120 min (range = 131.6 and 105.3 pg/ml), and did not return to baseline by 4 h (Weisman et al., 2012). Salivary levels of oxytocin remain elevated for more than two hours after intranasal oxytocin administration (Huffman et al., 2012).

In the present study, the data showed that more than 100 ng OXT for intranasal administration could treat the headache.

MacDonald et al. (2011) gave a systematic review of safety and side effects data in research investigating the central effects of
significantly in headache patients in relation with the headache level; and (3) there was a positive relationship between plasma and CSF OXT concentration in headache patients. The data suggested that brain OXT, which was delivered through olfactory region, could treat human headache and OXT might be a potential drug of pain relief by intranasal administration.

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