ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6): 488 – 492
http://www.chinaphar.com

Effect of centrally administered oxytocin on gastric and duodenal ulcers in rats

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KEY WORDS oxytocin; atosiban; ulcer; indomethacin; histamine; cysteamine

ABSTRACT

AIM: To investigate the effect of centrally administered oxytocin and its receptor antagonist, atosiban, on gastric acid secretion and on experimentally induced gastric and duodenal ulcers. **METHODS**: The acute gastric ulcer models, such as pylorus ligation, indomethacin-induced and ethanol-induced gastric ulcers were used. Chronic gastric ulcers were induced by acetic acid and duodenal ulcers by cysteamine HCl. **RESULTS**: In pylorus ligated rats, oxytocin (10 μg/kg, icv) showed significant antisecretory and antiulcer activity ($P < 0.01$). However, it aggravated the ethanol-induced gastric ulcers and did not show any effect on indomethacin-induced gastric ulcers. Oxytocin increased gastric ulcer healing in acetic acid-induced chronic gastric ulcers. The effect of oxytocin was reversed by atosiban (10 μg/kg, icv), a selective oxytocin receptor antagonist. Atosiban when given alone increased gastric acid secretion and ulcer index in pylorus-ligated rats and also aggravated acetic acid-induced chronic gastric ulcers. It seems the antiulcer activity of oxytocin was due to its anti-secretory effect. **CONCLUSION**: Centrally administered oxytocin possesses gastric anti-secretory and anti-ulcer activity and oxytocin antagonist, atosiban, is pro-ulcerogenic in rats.

INTRODUCTION

Central neurons that synthesize oxytocin are located in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus. Magnocellular neurons in both nuclei project to the posterior pituitary gland, whereas parvocellular oxytocinergic neurons project centrally from the PVN to terminal fields within the brain[1]. It was reported that electrical stimulation of PVN could reduce gastric acid secretion in rats[2]. In addition, nanomolar quantities of oxytocin injected into PVN produced a reduction in gastric acid output[3]. However, oxytocin when injected into the dorsal medullary nucleus of the vagus (DMX) increased gastric acid secretion[4]. Recently, Petersson *et al*[5] reported that repeated administration of oxytocin by subcutaneous route reduced plasma concentration of vagally controlled hormones such as gastrin, cholecystokinin, and insulin.

Oxytocin is a known anti-stress agent[6] and centrally administered oxytocin has been reported to reduce stress-induced gastric lesions in rats[7]. This study was carried out to investigate the effect of centrally administered oxytocin and its receptor antagonist, atosiban, on gastric acid secretion of various experimentally induced gastric and duodenal ulcers.

MATERIALS AND METHODS

Animals Wistar rats (130 – 150 g) of either sexes obtained from Central Animal House of Jawaharlal Institute of Post-graduate Medical Education and Research were used. During fasting, animals were placed in cages having wire grid bottoms to prevent coprophagia while they had free access to water. The animals were randomly divided in fifteen different treatment groups consisting of six animals in each group.

Drugs Oxytocin (Novartis India Ltd, Goa, India) and atosiban (1-deamino-2-*D*-Tyr-(Oet)-4-Thr-8-Orn-oxytocin) (Ferring, Malmo, Sweden) were intracerebroventricularly (icv) administered at a dose of 10 μg/kg body weight[8]. The drugs were dissolved in saline and injected in a volume of 25 μL/kg. Control group received vehicle in the same amount. Indomethacin (Sigma, USA), ethanol (Bengal Chemicals, India), and cysteamine hydrochloride (Hi-media, India) were

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Received 2000-12-12 Accepted 2001-03-22

used.

Surgery for icv injections Following anaesthesia with ketamine HCl (100 mg/kg, ip), the skull was uncovered and a guide cannula (20 G) was sterotactically fixed to the skull by acrylic dental cement. The coordinates were 1.0 mm posterior and 1.3 mm lateral to the bregma. The injection needle (27 G) reached 3.8 mm below the surface of the skull, with the needle tip in the lateral ventricle. The animals were allowed one week of recovery after the operation. At the end of the experiment, the placement of the cannula was checked by injecting 2 μL of Indian ink.

Acute gastric ulcers

Pylorus ligated (Shay) rats[9] Rats were fasted for 48 h and pylorus ligation was performed under light ether anaesthesia. Oxytocin (1 μg/kg or 10 μg/kg, icv) and/or atosiban (10 μg/kg, icv) were administered at 0, 6, and 12 h after pylorus ligation. Atosiban was given 10 min before each dose of oxytocin. Nineteen hours after pylorus ligation animals were sacrificed, stomach was isolated from the body and the contents were collected, measured, and centrifuged (100 × *g* for 5 min). The contents were subjected to free and total acidity estimation. The stomach was then cut open and the surface area was examined for ulceration. The ulcer index was calculated using the equation:

Ulcer index = 10 × Total ulcerated area/Total mucosal area.

Indomethacin-induced gastric ulcers[10] Rats were fasted for 36 h. Indomethacin suspended in 1 % carboxymethylcellulose (CMC) was given orally at a dose of 20 mg/kg body weight. Four hours later, animals were sacrificed and their stomachs were examined for lesions and ulcer index was determined as described above. Oxytocin (10 μg/kg, icv) was given 30 min before indomethacin.

Ethanol-induced gastric ulcers[11] Rats fasted for 36 h were given 80 % ethanol at a dose of 1 mL per rat orally. Oxytocin (10 μg/kg, icv) was pretreated 1 h before ethanol. Animals were sacrificed 1 h later and ulcer index was determined.

Chronic gastric ulcers

Acetic acid-induced gastric ulcers[12] Rats were starved for 24 h prior to the experiment. Under light ether anaesthesia, a midline epigastric incision was made and stomach was exposed. Using a cylindrical mould (6.0 mm internal diameter), 0.05 mL of glacial acetic acid was applied topically and was allowed to remain there for 60 s. The acetic acid was then removed by rinsing the mould with 0.9 % saline to prevent possible damage to the surrounding tissues close to the point of application. The abdomen was closed and from the second day after the operation, oxytocin (10 μg/kg, icv) and/or atosiban (10 μg/kg, icv) were given twice daily to the respective treatment group for 9 d while the control group recieved only saline. Atosiban was given 10 min before oxytocin. Rats were sacrificed on the 10th day, stomachs were removed and cut open and the ulcer index were determined.

Histopathological examination[13] The stomachs were subsequently fixed in 10 % formalin and processed for histological examination. Sections (5 μm thick) were taken and stained with haematoxylin and eosin (HE), periodic acid schiff's (PAS) or Masson's trichrome stain. Three indices were selected to reflect the rate and quality of ulcer healing.

Regenerated glandular epithelium width Epithelium was judged to be regenerated on the basis of cystic dilation, positive PAS stain reaction and number of collagen fibres. Regenerated glandular epithelium width was defined as the average distance from the origin of regenerated glands to ulcer edge on two sides of the ulcer.

Capillary density within the granulation tissues of the ulcers Capillary density was determined using eye piece reticule (× 400 magnification) on the HE stained sections in the ulcer center. Each field was 140 μm × 140 μm. At least four fields were examined on each section. Capillary density within the granulation tissues of the ulcers was expressed as the average capillary numbers in the fields.

Collagen content within the scar tissues of ulcers Collagen content in scar tissues displayed blue color in sections stained by Masson's trichrome stain and were determined by point count using 1 cm^2-eye piece reticule (× 100 magnification). Collagen content was expressed as the volume of collagen in the ulcer tissue. Volume of collagen was calculated using the formula:

$$\text{Volume} = \frac{\text{Total number of points falling on the tissue}}{\text{Total number of points in the reticule}}$$

Experimental duodenal ulcers

Cysteamine-induced duodenal ulcers[14] Duodenal ulcers were induced by administration of cysteamine hydrochloride (400 mg/kg, *po*) twice at an interval of 4 h. Oxytocin was given 30 min before each dose of cysteamine HCl and at 6 h interval after the second dose. All animals were killed 24 h after the first dose of cysteamine and the duodena were excised carefully and opened along the antimesentric side. The duodenal ulcer

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6)
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area was determined using a grid (mm^2). The duodenal ulcers were scored for intensity on a scale of 0 to 3. 0, no ulcer; 1, superficial mucosal lesion; 2, deep ulcer or transmural necrosis; 3, perforated or penetrated ulcer (into the pancreas or liver). The ulcer index was calculated from the equation:

Ulcer index = Arithmetic mean of the intensity in a group + (Number of ulcer positive animals/Total number of animals) × 2.

Statistics All values are expressed as $\bar{x} \pm s$. Statistical significance was determined by unpaired t-test for all parameters except ulcer score for which non-parametric Mann-Whitney test was used. The statistical analysis was done using Graphpad Instat software. Values of $P < 0.05$ were considered to indicate statistical significance.

RESULTS

Acute gastric ulcers

Pylorus ligated rats Oxytocin (10 μg/kg, icv) given thrice after pylorus ligation significantly decreased ulcer index, volume of gastric secretion, free and total acidity ($P < 0.01$) when compared with control (Tab 1). Oxytocin (1 μg/kg, icv) given thrice after pylorus ligation decreased total acidity ($P < 0.05$) and ulcer index ($P < 0.01$). A single dose of oxytocin (10 μg/kg, icv) immediately after pylorus ligation did not produce any significant effect on the above parameters (data not shown). The selective oxytocin receptor antagonist atosiban reversed the effect of oxytocin on the above parameters. Atosiban when given alone (10 μg/kg, icv) thrice after pylorus ligation produced significant increase in ulcer index ($P < 0.01$).

Indomethacin-induced gastric ulcers Animals treated with indomethacin at a dose of 20 mg/kg orally developed considerable ulcers in the glandular portion of the stomach. Oxytocin (10 μg/kg, icv) did not produce any significant effect on indomethacin-induced gastric ulcers (Tab 2), rather, it showed an insignificant increase in ulcer index ($P = 0.587$).

Ethanol-induced gastric ulcers Pretreatment with a single dose of oxytocin (10 μg/kg, icv) significantly increased the ulcer index in ethanol-induced gastric ulcer ($P < 0.05$) when compared with control (Tab 2).

Chronic gastric ulcers induced by acetic acid

Ulcer index Topical application of acetic acid produced penetrating ulcers at a low perforation rate. Oxytocin (10 μg/kg, icv, bid, 9 d) promoted the ulcer healing in acetic acid-induced gastric ulcers and produced a significant reduction in ulcer index ($P < 0.01$) when compared with control (Tab 3). Atosiban (10 μg/kg, icv, bid), when given 10 min before oxytocin, prevented the ulcer healing effect of oxytocin in acetic acid chronic gastric ulcers. Atosiban, when given alone aggravated the ulcers as shown by a significant increase in ulcer index ($P < 0.01$).

Regeneration of glandular epithelium Oxytocin significantly increased the regenerated glandular epithelium width around the ulcer craters when compared with control ($P < 0.01$). Atosiban prevented the effect of oxytocin and when given alone significantly decreased the regenerated glandular epithelium width when compared with control ($P < 0.01$, Tab 3).

Capillary density in granulation tissues of the ulcers Oxytocin produced a significant decrease in the capillary numbers within the granulation tissues of the ulcers ($P < 0.05$). Atosiban reversed the effect of oxytocin. Atosiban, when given alone produced significant increase in capillary density ($P < 0.01$) when compared with control (Tab 3).

Effect of oxytocin on collagen content within scar tissues Oxytocin produced significant increase in collagen content ($P < 0.05$) when compared with control.

Tab 1. Effect of oxytocin and atosiban on gastric secretion, free acidity, total acidity and ulcer index in pylorus ligated rats. $n = 6$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* oxytocin (10 μg/kg)-treated group.

Treatment	Volume of gastric contents/mL	Free acidity/ mol·L^{-1}	Total acidity/ mol·L^{-1}	Ulcer index
Control	8.7 ± 1.7	32 ± 7	70 ± 7	0.337 ± 0.010
Oxytocin (10 μg/kg)	5.8 ± 1.3^c	22.4 ± 2.7^c	58 ± 4^c	0.083 ± 0.007^c
Oxytocin (1 μg/kg)	7.1 ± 0.6	29 ± 5	61 ± 4^b	0.191 ± 0.022^c
Atosiban (10 μg/kg)	9.5 ± 1.2	36 ± 3	75.1 ± 1.0	0.420 ± 0.004^c
Oxytocin (10 μg/kg) + Atosiban	9.2 ± 1.5^f	35 ± 8	73.1 ± 1.6^f	0.382 ± 0.027^f

Tab 2. Effect of oxytocin (10 μg/kg, icv) on ulcer index in indomethacin and ethanol-induced gastric ulcers. Ulcer index = 10 × Total ulcerated area/Total mucosal area. $n = 6$ rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

Ulcerogen	Control	Oxytocin
Indomethacin	0.25 ± 0.04	0.31 ± 0.09
Ethanol	1.20 ± 0.17	2.05 ± 0.24[b]

This effect was reversed by atosiban. Atosiban given alone did not produce any significant effect on the collagen content in scar tissue (Tab 3).

Effect of oxytocin on cysteamine-induced duodenal ulcers Oxytocin (10 μg/kg, icv) significantly reduced the mean ulcer area ($P < 0.05$) when administered 30 min before each dose of cysteamine HCl and at 10 and 16 h after the first dose of cysteamine HCl (Tab 4). Administration of cysteamine HCl resulted in 20 % mortality within 24 h. The rats that died had perforated ulcers. No mortality was seen in oxytocin group.

DISCUSSION

The present study employing various acute and chronic models investigated the effect of oxytocin and its receptor antagonist on gastric and duodenal ulcers. The results indicated a significant gastric anti-secretory and anti-ulcer activity of oxytocin and a pro-ulcerogenic effect for oxytocin antagonist, atosiban.

About 0.2 % of peripherally released or administered oxytocin reaches brain[15]. Moreover, oxytocin is directly released in the brain through parvocellular neurons. The concentration of oxytocin in CSF is 5 – 10 folds higher than in the plasma[7]. We earlier demonstrated that oxytocin when injected by subcutaneous route could reduce gastric acid secretion and the development of gastric and duodenal ulcers (unpublished data). Several oxytocin binding sites have been identified in the brain including PVN and DMX[16]. Oxytocin is also known to increase the sensitivity of α_2 adrenoceptors in the locus coerulus. With the present study, it is difficult to specify the site of action of oxytocin in the brain.

The possibility of oxytocin exerting cytoprotective effect is not indicated. This is because oxytocin administration failed to prevent the formation of gastric lesions in the indomethacin-induced and ethanol-induced ulcer models. On the contrary, it showed a significant increase in the formation of gastric lesions. However, an increase of the regenerated glandular epithelial width, decrease in capillary density, and an increase in collagen content in tissues obtained from oxytocin-treated animals in acetic acid-induced chronic gastirc ulcers indicate that oxytocin has an ulcer healing effect. The anti-peptic ulcer effect of oxytocin was further confirmed in cysteamine induced duodenal ulcer model.

Oxytocin receptor(s) has been identified endogenously and selective oxytocin receptor antagonists are made available. In the present study, atosiban, an antagonist of oxytocin receptor was used to identify the role of oxytocin receptors in the anti-ulcer effect of oxytocin. The reversal of effect of oxytocin on the gastric acid secretion, ulcer index in pylorus ligated rats, and on regenerated glandular epithelium, capillary density and volume of collagen content in acetic acid-induced chronic gastric ulcer strongly suggests the involvement of oxytocin receptors in the anti-ulcer activity of oxytocin. Additionally, the enhanced ulcer index and inhibited regeneration of glandular epithelium and capillary density observed in atosiban (alone)-treated animals are suggestive of endogenous role for oxytocin in the gastric ulcer healing.

To conclude, centrally administered oxytocin possesses gastric anti-secretory and anti-ulcer effect in experimentally induced gastric and duodenal ulcers.

Tab 3. Effect of oxytocin and/or atosiban (10 μg/kg, icv, bid, 9 d alone or both) on chronic gastric ulcers induced by acetic acid. $n = 6$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* oxytocin-treated group.

Treatment	Ulcer index	Regenerated glandular epithelium width/μm	Capillary density (number in 1.96 mm²)	Volume of collagen content
Control	0.61 ± 0.06	3280 ± 773	4.2 ± 0.8	0.18 ± 0.08
Oxytocin	0.40 ± 0.08[c]	4613 ± 522[c]	2.8 ± 0.9[b]	0.34 ± 0.15[b]
Atosiban	0.75 ± 0.02[c]	1200 ± 772[c]	7.1 ± 0.4[c]	0.14 ± 0.01
Oxytocin + Atosiban	0.64 ± 0.06[f]	3018 ± 662[f]	5.9 ± 1.4[bf]	0.15 ± 0.17

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jun; 22 (6)
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Tab 4. Effect of oxytocin (10 μg/kg, icv, two doses) on cysteamine HCl-induced duodenal ulcers. $n = 6$ rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

Treatment	Ulcer area/ mm^2	Ulcer score	Ulcer index
Control	19 ± 12	1.8 ± 0.7	3.7
Oxytocin	4 ± 8[b]	0.7 ± 0.7[b]	2.0

Since oxytocin aggravates the formation of indomethacin-induced and ethanol-induced gastric lesions, the mechanism for anti-ulcer activity can be attributed to its anti-secretory activity to a major extent. The oxytocin antagonist possesses pro-ulcerogenic activity indicating that endogenous oxytocin may have a physiological role in gastric ulcer healing.

ACKNOWLEDGEMENT The authors wish to thank M/s Ferring Pharmaceuticals, Malmo, Sweden for their generous gift of atosiban.

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脑室注射催产素对大鼠胃和十二指肠溃疡的作用

关键词 催产素; 阿托西班; 溃疡; 吲哚美辛; 组胺; 半胱胺

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