Effect of somatostatin analogue octreotide injected into the third cerebral ventricle on pentagastrin-induced gastric acid secretion in rats

Feng Gao, Xiu-Fen Hu

Abstract

AIM: To investigate the effect of long-lasting somatostatin analogue octreotide (Oct) injected into the third cerebral ventricle (TCV) on gastric acid secretion in rats.

METHODS: TCVs were cannulated in male Wistar rats anesthetized with sodium pentobarbital. One week later acute gastric lumen perfusion was carried out and gastric acid was continuously washed with 37°C saline by a perfusion pump. Gastric perfusion samples were collected every 10 min and titrated by 0.01 moL/L NaOH to neutral. On the basis of subcutaneous (sc) injection of pentagastrin (G-5, 160 μg/kg), Oct (0.025 μg, 0.05 μg, 0.1 μg, n=12 in each group) or vehicle (pyrogen-free physiological saline, n=10) was injected into the TCV. Before and after the TCV injection, 1 h total acid output (TAO) was determined and experimental data were expressed in change rate (%) of TAO.

RESULTS: Oct (0.025, 0.05 and 0.1 μg) injected into the TCV resulted in change rate of 1.56% (P>0.05), 20.21% (P<0.01) and 37.82% of TAO (P<0.001), respectively. Moreover, comparison in change rate of TAO among these 3 doses showed P<0.05 between 0.025μg and 0.05 μg, P<0.01 between 0.025 μg and 0.1μg, and P<0.05 between 0.05μg and 0.1 μg. However, sc injection of 0.05 μg Oct had no effect on G-5 stimulated gastric acid secretion.

CONCLUSION: Octreotide injected into the third cerebral ventricle inhibits gastrin-induced gastric acid secretion in a dose-dependent manner.

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Key words: Octreotide; Somatostatin analogue; Third cerebral ventricle; Gastric acid; Gastrin

INTRODUCTION

Somatostatin, a brain-gut peptide, distributes widely in brain tissue, gastrointestinal tract, pancreatic islets, etc and plays a diversified role in biological activities[1]. Due to its short half life, its application in basic research and clinical work is limited. Octreotide (Oct) is a long-lasting somatostatin analogue with a plasma half life of 45 min[2]. Clinically it is used in the treatment of acromegaly[3], gastrointestinal hemorrhage[4] as well as in the diagnosis and management of some tumors[5]. In the aspect of basic research on gastric acid secretion there have been few reports concerning the central administration of somatostatin and its analogues. Some results are contradictory. Such discrepancy may result from differences in animal species, experimental models and methods, types, doses and injected route of the drugs used. Of note, previous studies[6,7] strongly suggested that the site of injection of Oct into the brain could largely influence its effect on gastric acid secretion. Up to now there is no report regarding the effect of somatostatin and its analogues into the third cerebral ventricle (TCV). In the present study Oct was injected into the TCV of the rats via a chronically implanted cannula and its effect on gastrin-induced gastric acid secretion was observed in the process of acute gastric lumen perfusion.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 180-280 g were used. The
animals were deprived of food for 24 h, but allowed free access to water prior to anesthesia.

**Animal models**

The rats were anesthetized with a single intraperitoneal injection of sodium pentobarbital (50 mg/kg). Intra-TCV implantation and acute gastric lumen perfusion were carried out as described previously. Gastric perfusion samples were collected every 10 min and titrated by 0.01 M NaOH to neutral (using phenol red as a volume marker). The acid output per 10 min was calculated as previously described. The anus temperature was kept at 37°C by electric light during the experiment. Sufficient pentobarbital was given sc before G-5 was injected. By referring to the previous report, G-5 (160 μg/kg) was chosen to achieve the maximal acid output. After basal gastric acid secretion was kept stable for 30 min, G-5 (Sigma Company, USA) was injected sc, followed by collection of gastric perfusion samples at 10 min intervals for 1 h. Gastric acid secretion increased 10 min after the injection, reached its peak at 20 min, then declined gradually to the basal level 1 h after the injection. After the gastric acid secretion was kept stable at the original basal level for 30 min, the same dose of G-5 as the first time was given sc and gastric perfusion samples were collected for another 60 min. The experimental drugs were administered into TCV 10 min before the second injection of G-5. In brief, 2.5 μL of pyrogen-free physiological saline or various doses of Oct (Sandoetatin Norvartis, Basel, Switzerland) were given by a syringe pump (with the constant speed of 2.5 μL/min) in a silicon tube, which was equally long and connected with the implanted cannula. In order to prove the cannula was in TCV, 5 μL gentian violet was injected via the implanted cannula at the end of the experiment, and the rat was immediately killed. The brain tissue was removed and cut to see whether TCV was stained by gentian violet. TCV staining signified that the cannula was correctly placed in TCV and the injected drug successfully reached TCV. Therefore, the result was accepted only when TCV was stained. In this experiment, only data arising from the two rats in saline-injected group were excluded because their TCVs were not shown on gentian violet staining.

**Statistical analysis**

Experimental data was expressed in percent change of TAO change, which was calculated as follows: change of TAO (%) = (E2-E1)/E1×100%, in which E1 and E2 represent 1 h TAO (μmol/L) after the first and the second sc injections of G-5, respectively. The data were expressed as mean ± SE. Significance was assessed by unpaired t test for comparison between two groups while paired t test for comparison between pre-treatment and post-treatment in each group.

### RESULTS

#### Effect of sc injection of G-5 or TCV injection of saline on gastric acid secretion

The effect of sc injection of G-5 (160 μg/kg) two times on basal gastric acid secretion was observed in the same rat without cannulization in TCV (Table 1). The result showed that there was no significant difference in G-5 stimulated TAO between the first and second injections (P > 0.05). The effect of pyrogen-free physiological saline (2.5 μL) given into TCV on G-5 induced gastric acid secretion in the rat cannulated in TCV, revealed that TAO was almost identical between pre- and post-treatment of TCV-injected saline (P > 0.05, Table 2). Moreover, comparison in change of TAO between these two groups demonstrated an insignificant difference (P > 0.05). This strongly suggested that the experimental animal model was so stable that it could be employed in study of the effect of TCV-administered drugs on gastric acid secretion in rats.

| Group | n  | TCV injection | Change rate of TAO (%) | P  |
|-------|----|---------------|------------------------|----|
| 1     | 10 | Saline 2.5 μL | 5.90 ± 5.47            | >0.05 |
| 2     | 12 | Oct 0.025 μg/2.5 μL | 1.56 ± 6.63 | >0.05 |
| 3     | 12 | Oct 0.05 μg/2.5 μL | 20.21 ± 6.49*     | <0.01 |
| 4     | 12 | Oct 0.1 μg/2.5 μL | 37.82 ± 3.97          | <0.001 |

*P < 0.05 vs group 2 or group 4.

#### Effect of sc injection of Oct on G-5 induced gastric acid secretion

In 10 rats without cannulization in TCV, each received two injections of sc G-5. Saline (0.20 mL) or Oct (0.05 μg/0.20 mL) was given sc 10 min before the first or the second G-5 stimulation, respectively. TAO was 34.41 ± 3.75 μmol/h after saline administration and 33.49 ± 3.98 μmol/h after Oct treatment, which had no significant difference between them (P > 0.05).

#### DISCUSSION

Injection of brain-gut peptides (eg, somatostatin, neuropeptide Y, corticotropin-releasing factor, bombesin) into the central nervous system can influence gastric acid secretion. As a long-lasting somatostatin analogue Oct, intracisternal injection (ic) induces a dose-related (100-300 ng) and long-lasting stimulation of gastric acid output in pylorus-ligated conscious rats. Four years later, the same researchers reported that Oct injected into the different regions of the brain displayed its effect on gastric acid secretion. Interestingly, they found that Oct (7, 15, 30 and 60 ng) injected into the dorsal vagal...
complex (DVC), increases pentagastrin-stimulated gastric acid secretion of urethane-anesthetized rats in a dose-dependent manner. Whereas, Oct injected into the lateral ventricle, paraventricular nucleus (PVN) or lateral hypothalamus (LH) also inhibits pentagastrin-stimulated gastric acid secretion in a dose-dependent manner. Oct (30 ng) injected into the area surrounding the PVN or LH does not modify the acid secretion response to pentagastrin. These results strongly suggest that the injection sites of Oct in the central nervous system influence their effect on gastric acid secretion. The current study, for the first time, explored the effect of intra-TCV injection of Oct on gastric acid secretion in rats and showed that Oct (0.025, 0.05, 0.1 μg) injected into TCV inhibited pentagastrin-induced gastric acid secretion of pentobarbital-anesthetized rats with chronically implanted cannula in a dose-dependent manner.

Without no doubt, somatostatin and its analogues peripherally inhibit gastric acid secretion. There is evidence that somatostatin-14 injected into the lateral cerebral ventricles at 3-6 nmol/L induces massive hypersomatostatinemia resulting from leakage into the peripheral circulation of rats. Our present study revealed that gastric acid output was suppressed by TCV-administrated Oct through a chronically implanted cannula. Whether Oct injected into TCV is absorbed into blood via the capillaries of subarachnoid space, choroid plexus and cerebral parenchyma to function peripherally is unknown, but Oct is unlikely to enter blood directly due to acute damage to the blood vessel. However, sc injected Oct at the dose of 0.05 μg in our study did not have any effect on G-5 induced gastric acid secretion, thus excluding the peripheral action of Oct. Moreover, it was reported that somatostatin could not pass through the intact blood brain barrier in humans.

Hypothalamus plays an important role in the central regulation of gastric acid secretion. Somatostatin distributes in hypothalamus with the highest concentration. The somatostatinergic nerve fibers arising from hypothalamus end in dorsal nuclei of vagus to inhibit their activities, thus regulating gastric acid secretion. TCV is adjacent to hypothalamus and has complicated uptake mechanisms on its wall. These morphological characteristics provide the evidence that TCV-injected Oct exerts central inhibitory effect on gastric acid secretion.

Oct injected into TCV of the rat could suppress gastric acid secretion induced by G-5 at the dose of 0.05 μg other than 0.025 μg (Table 2). As the dose increased to 0.1 μg, such inhibition was increased, suggesting that the inhibitory effect of TCV-administrated Oct on the gastric acid secretion is dose-dependent. Moreover, Pless et al. used [125]I-Tyr3-Oct to investigate the distribution pattern of the central somatostatin receptors and found that the results are similar to those observed using [125]I-Tyr3-somatostatin, suggesting that non-specific combination of Oct in the brain does not exist. Based on the above evidence, it is concluded that the central inhibition of gastric acid secretion by TCV-administered Oct is likely to be specific.

In conclusion, Oct injected into the TCV inhibits gastrin-induced gastric acid secretion of rats in a dose-dependent manner. The exact mechanism underlying this effect needs to be further investigated.

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