Inhibitory Effects of Intrahypothalamic Injection of Calcitonin on TRH-Stimulated Gastric Acid Secretion in Rats

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Abstract—Effects of the peripheral and central administration of porcine (PCT) and salmon (SCT) calcitonin on the gastric acid secretion stimulated by various secretagogues were studied in the perfused stomach of anaesthetized rats. The intraperitoneal administration of PCT and SCT inhibited gastric acid secretion stimulated by thyrotropin-releasing hormone (TRH) and 2-deoxy-D-glucose, but neither bethanechol nor tetragastrin. The intracerebroventricular PCT and SCT blocked TRH-induced acid secretion. The intrahypothalamic injection of PCT and SCT reduced the acid secretion stimulated by the intrahypothalamic administration of TRH. The present study indicates that PCT and SCT may in part suppress gastric acid secretion due to an interaction with TRH in the hypothalamus.

About ten years after the discovery of calcitonin (1, 2), it was shown that systemic administration of calcitonin suppressed basal or meal- and basal or pentagastrin-stimulated gastric acid secretion in man (3, 4) and rats (5–7), respectively. These results did not directly indicate whether the effect of calcitonin is due to peripheral or central actions. Immunoreactive calcitonin has been found in the brain as well as in the gastrointestinal tract (8–10). Recently, much evidence is accumulating to suggest that calcitonin may inhibit gastric acid secretion by a central action: Intraventricular calcitonin inhibits gastric acid secretion (11) and subcutaneous calcitonin prevents centrally evoked hypersecretion, but not the peripheral one (12). However, little is known about the brain regions involved in the hyposecretory effect of the peptide. We have examined the effects of calcitonin on gastric acid secretion after intraperitoneal, intracerebroventricular and intrahypothalamic administration to clarify the action site of the peptide.

Materials and Methods

Assay of gastric acid secretion: Male Wistar rats (ST, substrain from Sankyo Lab. Co., Ltd.), weighing 230–270 g, were used after a 24 hr fast, but were allowed free access to water up to the beginning of the experiment. The gastric lumen of urethane (1.25 g/kg, i.p.) anesthetized rats was perfused with saline solution through a dual polyethylene gastric cannula which was inserted into the forestomach after the ligation of the pylorus and the esophagus, and the perfusate was automatically titrated in the reservoir with NaOH using a pH stat as described previously (13).

The total amount of acid secreted was expressed in terms of μmol HCl/60 or 90 min per animal. Basal secretion was low and almost constant for a measuring period under the present experimental condition. Therefore, the data indicated the value in which the corresponding basal secretion before treatment was deducted from the acid output due to the treatments.

Drug administration: The rat was placed in a stereotaxic apparatus. An intracerebroventricular drug administration was performed by the technique of Noble et al. (14). TRH was injected twice through the needle (0.35 mm, diam.) at about 2 hr intervals into the lateral ventricle in a volume of 10 μl/rat during approximately 2 min. In the adminis-
treatment of TRH into the brain, unilateral injection (left) of the needle was stereotaxically performed following the Brain Atlas of König and Klippel (15) and Paxinos and Watson (16): the lateral hypothalamus (LH): 5.7 anterior from the interaural line, 1.5 lateral from the midline, 7.0 below the surface of the duramater; the ventromedial hypothalamus: 4.3 anterior from the interaural line, 0.5 lateral from the midline, 8.0 below the surface of the duramater; the caudate putamen: 8.7 anterior from the interaural line, 3.0 lateral from the midline, 7.0 below the surface of the duramater. TRH was administered through the needle twice at about 2 hr intervals into the brain in a volume of 1 μl/rat. To minimize leakage of the drug, the needle was left in place for 30 min following the injection. At the end of each study in which drug was injected into the brain, an excess electrical current was applied to the needle by a Lesion Producing Device. The needle placement was verified after the frozen brain was cut in a cryostat.

All drugs were dissolved in saline solution. Gastric acid secretion was peripherally stimulated by the subcutaneous injection of bethanechol (0.5 mg/kg) or tetragastrin (0.125 mg/kg, twice at about 1.5 hr intervals) and centrally stimulated by the intravenous administration of 2-deoxy-D-glucose (2DG, 200 mg/kg) or by the intracerebroventricular TRH (10 μg, twice at about 2 hr intervals). Calcitonin and atropine were administered 15 min before the injection of bethanechol and 2DG and also 15 min prior to the 2nd injection of tetragastrin and TRH. Calcitonin was also given intracerebroventricularly 15 min before the 2nd TRH injection, and the intrahypothalamic administration of calcitonin mixed with TRH was performed at the 2nd intrahypothalamic injection of TRH.

Drugs: Atropine sulfate (Wako Pure Chem., Tokyo), bethanechol HCl (Eisai, Tokyo), 2-deoxy-D-glucose (Nakarai Chem., Tokyo), porcine calcitonin (Calcitar inj.,* Yamanouchi, Tokyo), salmon calcitonin (Sandoz, Basel), tetragastrin (Protein Res. Foundation, Osaka), thyrotropin-releasing hormone (Protein Res. Foundation, Osaka) and urethane (Wako Pure Chem., Tokyo).

Statistical analysis: All data are presented as means±S.E. The data were analyzed by Student’s t-test and the paired t-test.

Results

Treatment with 0.5 mg/kg, s.c., of bethanechol stimulated basal gastric acid secretion about 10 min after its administration, and the effect lasted for about 3 hr. Pretreatment with 5 and 10 units/kg, i.p., of PCT (50 and 100 μg/kg) or 5 and 10 units/kg, i.p., of SCT (1.25 and 2.5 μg/kg) did not affect the stimulated acid secretion during the 90 min after the bethanechol-injection, but 0.1 mg/kg, i.p., of atropine blocked potently the acid response to bethanechol. The repeated injection of 0.125 mg/kg, s.c., of tetragastrin caused a temporal (about 40–60 min) but reproducible increase in acid secretion. Pretreatment with PCT and SCT 15 min prior to the 2nd tetragastrin injection did not significantly affect the 2nd response. Atropine, 0.1 mg/kg, i.p., tended to reduce the response, but its effect was not statistically significant. These results are summarized in Fig. 1.

Figures 2 and 3 show the inhibitory effect of the intraperitoneal calcitonin concerning the 2DG- and TRH-stimulated acid secretion, respectively. PCT (5, 10 units/kg), which did not affect the bethanechol- or tetragastrin-evoked hypersecretion, inhibited the 2DG-induced response in a dose-related manner. SCT and atropine, 5 units/kg and 0.1 mg/kg, respectively, also blocked the 2DG-induced acid response. The intracerebroventricular TRH-induced acid secretion was also significantly reduced by the intraperitoneal administration of PCT (5 units/kg), SCT (5 units/kg) and atropine (0.1 mg/kg). Both calcitonins did not affect basal secretion themselves at this dose level.

Influences of the intracerebroventricular injection of calcitonin on TRH-stimulated acid secretion are shown in Table 1. Pretreatment with 0.02 and 0.1 unit/rat of PCT (0.2 and 1 μg) or 0.1 and 0.4 unit/rat of SCT (0.025 and 0.1 μg) 10 min before the 2nd administration of TRH (10 μg) caused a decrease in the TRH-response. PCT and SCT completely inhibited the TRH response below the basal line at the dose of 0.1 and 0.4 unit,
respectively. This high dose of PCT or SCT tended to reduce the basal acid secretion by itself.

The injection of TRH (1, 2 μg) into the LH caused a dose-dependent increase in gastric acid secretion, and the repeated injection of the drug induced reproducible stimulation of acid secretion as seen in Table 2. The intraventromedial hypothalamic injection of TRH (2 μg) caused a weaker increase (64.2±27.9 μmol HCl/90 min, n=6) than that of LH injection in the gastric acid secretion, but TRH injected into the caudate putamen did not cause any changes (5.2±3.0 μmol HCl/90 min, n=5). The concomitant injection of PCT (0.02 unit) or SCT (0.1 unit) with TRH (2 μg) into the LH significantly reduced the 2nd TRH-stimulated acid secretion. When calcitonin and TRH were administered separately into the hypothalamus, the inhibitory activity of calcitonin was not different from that of the concomitant injection. That is, PCT (0.02 unit) given 15 min before the 2nd TRH injection caused almost the same inhibition in acid secretion.
(22.5±12.2 μmol HCl/90 min, n=5) as that of concomitant injection (27.1±13.7 μmol HCl/90 min, n=6). The needle placement was verified after the experiments, and animals which had the needle placement outside correct position were ruled out.

**Discussion**

Bolus injection of SCT or PCT in a dose of 10 units/kg, i.p., failing to inhibit the bethanechol- as well as tetragastrin-stimulated acid secretion in the present study is in contrast with the results of Becker et al. (17) that intravenous infusion of SCT (20 units/kg/hr) strongly inhibited pentagastrin-induced acid secretion in conscious cats. Variations in the sensitivity of rats and cats and/or the schedule of drug administration might explain this discrepancy.

The intraperitoneal SCT or PCT being without effect on bethanechol- and tetragastrin-stimulated acid secretion to any significant degree prevented the gastric acid secretion induced by 2DG or TRH, centrally acting secretagogues (18, 19), in the present study. These results agree with the finding that subcutaneous injection of eel calcitonin has blocked gastric acid secretion; not the peripheral one, but the centrally evoked hypersecretion (12). Since it has been claimed that the parenterally administrated calcitonin can enter the hypothalamus (20, 21), it appears reasonable to conclude that mammalian calcitonin as well as fish calcitonin suppresses gastric acid secretion by a direct action on the central nervous system.

Although SCT has a considerably different amino acid sequence (20 out of 32 amino acids) from PCT (22), the hypocalcemic activity of SCT is about 40 times more potent than that of PCT (23). The efficacy of the two calcitonins on gastric acid secretion was relatively parallel to the activity on the basis

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**Table 1. Effect of intracerebroventricular calcitonin on TRH-induced gastric acid secretion**

| Drugs | Dose (μg) | No. of rats | Acid output (μmol HCl/90 min) |
|-------|-----------|-------------|-------------------------------|
| Saline | 1 ml      | 6           | 142.6±29.5                   |
|       |           |             | 152.8±27.1                   |
| PCT   | 0.02      | 6           | 126.3±26.9                   | **P<0.05** |
|       | 0.1       | 6           | 141.1±36.0                   | **P<0.01** |
| SCT   | 0.1       | 6           | 149.0±26.9                   | **P<0.001** |
|       | 0.4       | 6           | 132.3±29.2                   |

| TRH (10 μg/rat, i.c.v.) was injected twice. Drugs were administered intracerebroventricularly 10 min before the 2nd TRH injection. *P<0.05, **P<0.01 vs. 2nd TRH control (Student’s t-test). *P<0.05, **P<0.01 vs. corresponding 1st value (paired t-test). |

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**Table 2. Influence of intrahypothalamic injection of calcitonin and TRH on gastric acid secretion**

| Treatments | Dose (μg) | No. of rats | Acid output (μmol HCl/90 min) |
|------------|-----------|-------------|-------------------------------|
| TRH        | 1 μg      | 6           | 55.6±15.9                     |
|            |           |             | 83.0±12.3                     |
| TRH        | 2 μg      | 8           | 108.8±27.9                    |
|            |           |             | 112.5±25.0                    |
| TRH + PCT  | 0.004 unit | 6           | 91.7±21.9                     |
|            | 0.02 unit  | 6           | 96.3±17.5                     | **P<0.05** |
| TRH + SCT  | 0.02 unit  | 8           | 101.2±42.7                    |
|            | 0.1 unit   | 8           | 123.8±23.3                    | **P<0.01** |

Intrahypothalamic administration of calcitonin was performed mixed with TRH at 2nd TRH injection. *P<0.05, **P<0.01 vs. 2nd TRH (2 μg) injection value (Student’s t-test). *P<0.05, **P<0.01 vs. corresponding 1st value (paired t-test).
of molecular weight. Effects of these two calcitonins on calcium and gastric acid secretion might depend on the common amino acid sequence. Feeding can be produced by increasing the concentration of calcium in the brain (24) and calcium-induced feeding can be inhibited by the concomitant administration of SCT (25). However, it seems likely that the inhibition of gastric acid secretion by calcitonin takes place without any change in plasma calcium level (17). Further studies are required for clarification of the relationship between chemical structure and these actions of calcitonin.

Intracerebroventricular calcitonin reduced basal acid output while the intrahypothalamic injection did not decrease it (Table 1). The effect of intrahypothalamic injection of calcitonin was not so potent even at a high dose level in comparison with that of intracerebroventricular administration (Table 2). These two results show that unilateral injection of calcitonin into the hypothalamus might not usefully affect the cell in comparison with bilateral injection or that calcitonin given to the cerebrospinal fluid may also influence other areas as well as the LH in the control system of acid secretion. Our findings suggest that one of the pharmacological effects of calcitonin is suppression of the gastric acid secretion due to an interaction with TRH in LH.

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