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# Direct Inhibition of Pepsinogen Secretion from Rat Gastric Chief Cells by Somatostatin

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**Effects of somatostatin on pepsinogen secretion was investigated in the rat *in vivo* and *in vitro*. In the perfused rat stomach, somatostatin inhibited secretagogue-induced acid secretion in dose-dependent manner. However, effects of somatostatin on secretagogue-induced pepsinogen secretion were obscure. To clarify the effects of somatostatin on the chief cells, gastric mucosal cells were isolated by a proteolytic enzyme. Somatostatin inhibited carbachol- and cholecystokinin octapeptide-induced pepsinogen secretion from dispersed gastric mucosal cells in a dose-dependent manner. Histamine-induced pepsinogen secretion, which was recovered by culturing, was also inhibited by somatostatin. These results suggest that somatostatin inhibits secretagogue-induced pepsinogen secretion directly.**

**Keywords** rat; perfused stomach; pepsinogen secretion; isolated gastric mucosal cell; culture; somatostatin; histamine; pentagastrin; carbachol; cholecystokinin

Somatostatin, a cyclic tetradecapeptide, which is known to be a growth hormone secretion-inhibiting hormone, is recognized as a universal chalone, responsible for inhibition of hormone secretion, digestive juice secretion, and neural transmission.[1] Somatostatin was known to exist in D-cells, whose processes abutted many functional cells including chief cells.[2] In regard to the function of the stomach, somatostatin inhibited not only gastrin secretion[3] but also gastric acid[4–6] and pepsinogen secretion.[7] Other physiological roles of somatostatin in the stomach were reported: gastric motility,[8] mucosal blood flow[9] and prostaglandin production[10] were influenced by it. Although many experimental gastric-ulcer models had been developed using rat, there have been few reports on research using isolated rat gastric mucosal cells. Effects of somatostatin on rat pepsinogen secretion *in vivo* and *in vitro* are not yet clear. We developed a method in which the chief cells were isolated from rat gastric mucosa and cultured. The aim of this study was to examine whether or not somatostatin directly acts on chief cells.

## Materials and Methods

**Perfused Rat Stomach** A male Wistar rat weighing about 200 g was starved for 24 h and used for the perfused rat stomach according to the method described previously.[11] Peptic activity was measured using hemoglobin as a substrate.[12]

**Isolation of Gastric Mucosal Cells** The stomach was removed from a 24 h-starved Wistar rat weighing about 200 g under urethane anesthesia. Gastric mucosal cells were isolated by the modified method described in detail previously.[13] The stomach was incubated with 0.2% Dispase in medium A gassing with 95% $O_2$ and 5% $CO_2$ for 1 h at 37 °C. It was then transferred to medium B and incubated for 30 min at room temperature while gassing continued with 95% $O_2$ and 5% $CO_2$. The cell suspension in medium B was filtered through a nylon filter (150 mesh), and centrifuged at 50 × *g* for 2 min. The mucosal cells were resuspended in medium C. The pepsinogen content in the gastric mucosal cells was about 25 ng/$10^4$ cells.

Medium A contained $NaH_2PO_4$ 0.5, $Na_2HPO_4$ 1.0, $NaHCO_3$ 20, NaCl 70, KCl 5.0, glucose 11, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) 25 (mM) and bovine serum albumin 20 (mg/ml) (pH 7.4). Medium B contained EDTA (ethylenediaminetetraacetic acid, disodium salt) 2 (mM) in medium A (pH 7.4). Medium C contained $CaCl_2$ 0.1 and $MgCl_2$ 1.0 (mM) in medium A (pH 7.4).

**Culture of Gastric Mucosal Cells** Gastric mucosal cells were cultured according to the method reported previously.[13] The gastric mucosal cell suspension obtained by the above procedure with 0.7%. Dispase was added to a mixture of Dulbecco's modified MEM and Ham's nutrient mixture F-12 (1 : 1) containing 10% fetal calf serum in collagen-treated dishes. The cells were cultured for 24 h at 37 °C in a $CO_2$ incubator. For the measurement of pepsinogen release, after replacing the medium, gastric mucosal cells were incubated in medium C with a secretagogue for 30 min at 37 °C in a $CO_2$ incubator.

**Measurement of Pepsinogen Concentration** After centrifugation, pepsinogen release in an aliquot of the supernatant was measured by radioimmunoassay, as described previously,[14] and was expressed as a percentage of the total cellular pepsinogen estimated after freezing and thawing of residual cells.

Student's *t* test was used for the statistical analyses.

**Chemicals** Carbamylcholine (carbachol), and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St Louis, U.S.A.). Cholecystokinin octapeptide, sulfated form, (CCK-8) and somatostatin were from Peptide Institute Inc. (Osaka), pentagastrin from Sumitomo Seiyaku Co. (Osaka), histamine from Wako Pure Chemical Industries (Osaka) and Diapase® was from Godo Shusei Co. (Tokyo).

## Results

**Effects of Somatostatin on Gastric Acid and Pepsinogen Secretion Induced by Secretagogues in the Perfused Rat Stomach *in Vivo*** Figure 1 shows a typical profile of inhibition of CCK-8-(5.7 μg/kg)-induced acid and pepsinogen secretion by somatostatin infusion (10 μg/kg/h), indicating the time schedule and how to calculate the

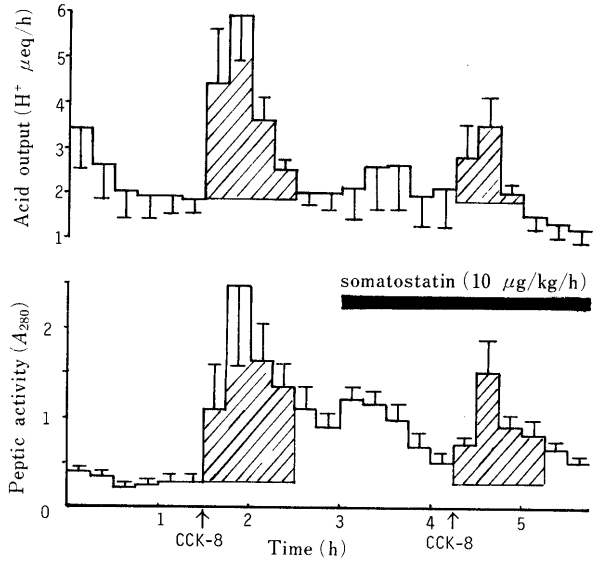


Fig. 1. Effects of Somatostatin on Gastric Acid and Pepsinogen Secretion Induced by CCK-8 in the Perfused Rat Stomach

Secretagogue and somatostatin were singly and continuously injected through the femoral vein, respectively. Hatched areas were used for calculation of the responses of acid and pepsinogen secretion to secretagogues before and after infusion of somatostatin (10 μg/kg/h) in Tables I and II.

TABLE I. Effects of Somatostatin on Gastric Acid Secretion Induced by Secretagogues

| | Somatostatin (μg/kg/h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 2.5 | | 5 | | 10 | |
| | Before | After | Before | After | Before | After | Before | After |
| Carbachol | 17.5 | 19.9 | 18.1 | 13.0 | 19.8 | 8.2 | 11.6 | 4.0 |
| 5 μg/kg | ±3.8 | ±5.2 | ±4.8 | ±5.0 | ±3.8 | ±5.7 | ±3.8 | ±1.3 |
| CCK-8 | 6.8 | 7.1 | 7.6 | 5.4 | 6.8 | 4.0 | 9.5 | 3.4 |
| 5.7 μg/kg | ±2.6 | ±1.3 | ±1.5 | ±1.3 | ±0.2 | ±1.1 | ±1.7 | ±1.0 |
| Histamine | 10.0 | 9.7 | 8.6 | 9.9 | 10.4 | 3.8 | 10.9 | 6.6 |
| 0.5 mg/kg | ±2.2 | ±2.7 | ±2.0 | ±2.0 | ±1.8 | ±1.4 | ±3.6 | ±2.6 |
| P.G.[a] | 8.9 | 10.7 | 9.8 | 9.6 | 13.0 | 4.3 | 8.9 | 7.8 |
| 10 μg/kg | ±1.5 | ±2.6 | ±2.9 | ±1.3 | ±5.8 | ±1.9 | ±1.0 | ±1.3 |

Each value is expressed as $H^+$ μeq/h and is the mean±S.E. of four experiments. *a*) Pentagastrin.

TABLE II. Effects of Somatostatin on Pepsinogen Secretion Induced by Secretagogues

| | Somatostatin (μg/kg/h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 2.5 | | 5 | | 10 | |
| | Before | After | Before | After | Before | After | Before | After |
| Carbachol | 5.73 | 3.94 | 5.55 | 4.35 | 5.16 | 1.97 | 6.29 | 3.53 |
| 5 μg/kg | ±0.68 | ±0.37 | ±1.25 | ±0.77 | ±0.85 | ±0.31 | ±0.96 | ±0.52 |
| CCK-8 | 2.89 | 2.62 | 4.89 | 3.71 | 2.91 | 3.30 | 5.51 | 2.91 |
| 5.7 μg/kg | ±1.44 | ±0.69 | ±0.33 | ±0.90 | ±0.22 | ±1.00 | ±2.09 | ±0.74 |
| Histamine | 2.90 | 1.87 | 2.33 | 2.40 | 1.03 | 1.14 | 2.52 | 1.39 |
| 0.5 mg/kg | ±0.37 | ±0.43 | ±0.94 | ±0.76 | ±0.26 | ±0.35 | ±1.12 | ±0.65 |
| P.G.[a] | 5.59 | 2.39 | 5.36 | 2.08 | 6.24 | 1.44 | 4.47 | 2.20 |
| 10 μg/kg | ±2.02 | ±0.67 | ±1.51 | ±0.63 | ±3.14 | ±0.49 | ±1.04 | ±0.40 |

Each value is expressed as U/h and is the mean±S.E. of four experiments. *a*) Pentagastrin.

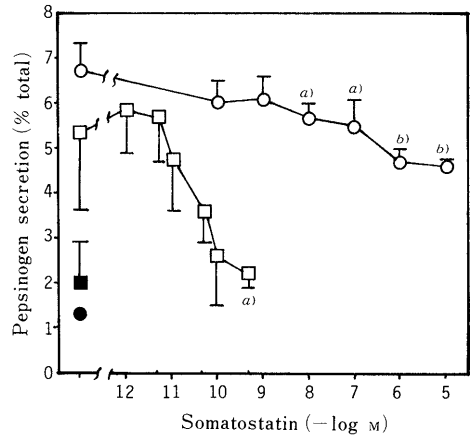


Fig. 2. Inhibition of Carbachol- and CCK-8-Induced Pepsinogen Secretion from Isolated Chief Cells by Somatostatin

○, carbachol ($1\times10^{-5}$ M); ●, basal; □, CCK-8 ($1\times10^{-9}$ M); ■, basal. Each value is the mean±S.E. from four separate experiments. *a*) $p<0.05$, *b*) $p<0.01$.

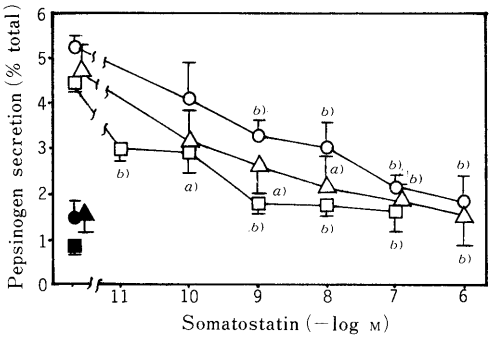


Fig. 3. Inhibition of Carbachol-, CCK-8- and Histamine-Induced Pepsinogen Secretion from Cultured Chief Cells by Somatostatin

○, carbachol ($1\times10^{-5}$ M); ●, basal; □, CCK-8 ($1\times10^{-7}$ M); ■, basal; △, histamine ($1\times10^{-6}$ M); ▲, basal. Each value is the mean±S.E. of five separate experiments. *a*) $p<0.05$, *b*) $p<0.01$.

percentage of inhibition. After measuring the basal secretion for 90 min, a rat was injected with a secretagogue through the femoral vein. Total outputs of acid and pepsinogen for 1 h after stimulation by a secretagogue during continuous infusion of somatostatin or saline were compared with control responses to a secretagogue; results are summarized in Tables I and II, respectively. The responses of acid secretion induced by secretagogues were inhibited by somatostatin. Carbachol- and CCK-8-induced gastric acid secretion were inhibited by somatostatin in a dose-dependent manner; but histamine- and pentagastrin-induced acid secretion were inhibited by somatostatin only at the dose of 5 μg/kg/h. The effects of somatostatin on pepsinogen secretions, however, were not clear. Pepsinogen secretion induced by CCK-8 or carbachol was inhibited by somatostatin at the dose of 10 or 5 μg/kg/h, respectively. Since responses of pepsinogen secretion to a certain dose of a secretagogue were scattered over a wide range, it was difficult to obtain a dose-dependent relation. Histamine- or pentagastrin-induced pepsinogen secretion was not inhibited by it significantly. Control response of pepsinogen secretion to pentagastrin, which was the response without somatostatin infusion, was about 55%. Thus, a lack of association was found between the responses of acid and pepsinogen secretion to pentagastrin.

**Effects of Somatostatin on Pepsinogen Secretion from Isolated Rat Gastric Mucosal Cells** As we reported earlier,[13] pepsinogen secretion from isolated gastric mucosal cells was stimulated by carbachol and CCK-8 in a dose-dependent manner, but was not by histamine or pentagastrin. Effects of somatostatin on pepsinogen secretion were investigated with stimulation by carbachol ($1\times10^{-5}$ M) or CCK-8 ($1\times10^{-9}$ M). Carbachol- or CCK-8-induced pepsinogen secretion was inhibited by somatostatin in a dose-dependent manner (Fig. 2). Significant inhibitions were observed at doses above $1\times10^{-8}$ M to carbachol and above $5\times10^{-9}$ M to CCK-8. Inhibition of CCK-8-induced pepsinogen secretion by somatostatin was stronger than that induced by carbachol.

**Effects of Somatostatin on Pepsinogen Secretion from Cultured Gastric Mucosal Cells** Based on our paper describing that primary culture cells responded not only to carbachol and CCK-8 but also to histamine and pentagastrin, we tested the effects of somatostatin using these cells. The pentagastrin-induced pepsinogen secretion was insufficient, however, to determine the action of the inhibitor. Inhibitory effects of somatostatin on pepsinogen secretion induced by carbachol, CCK-8 and histamine were measured (Fig. 3). Secretagogue-induced pepsinogen secretions were inhibited by somatostatin in a dose-dependent manner. Significant inhibitions were observed at

doses above $1 \times 10^{-9}$ M to carbachol and histamine, and above $1 \times 10^{-11}$ M to CCK-8.

## Discussion

Most of the mechanisms of gastric acid secretion were clarified in the past decade, and in the meantime, research on the mechanisms of pepsinogen secretion has progressed. The differences distinguishing gastric acid and pepsinogen secretions are that pepsinogen secretion follows the biosynthetic process of pepsinogen. This means that sufficient time is required for the recovery of pepsinogen storage after secretion in the gastric mucosa. We do not know the exact recovery time necessary under anesthesia after secretion induced by a certain secretagogue. In spite of there being 2.75 h between injections of a secretagogue, tachyphylaxis in pepsinogen secretion was observed when carbachol or pentagastrin was administered to a rat. This might indicate that a substance which stimulates pepsinogen secretion differs from one which stimulates pepsinogen biosynthesis.

Generally, somatostatin inhibits gastric acid and pepsinogen secretion *in vivo*[15]; however, species differences are known in histamine induced pepsinogen secretion.[16] High doses of histamine decreased it[17] and cholinergic pepsinogen secretion was also inhibited by histamine in the dog.[18] Albinus *et al.* reported that somatostatin inhibited pentagastrin-induced pepsinogen secretion, but not one histamine-induced in the conscious cat.[19] Although Raufman *et al.* described in detail the mechanisms of pepsinogen secretion, they refferred little to the action of histamine.[20] It seems likely that the histamine receptor in chief cells is sensitive to the isolating process. We demonstrated that histamine directly stimulated pepsinogen secretion from cultured chief cells in the rat,[8] and in this paper report that somatostatin directly inhibited pepsinogen secretion from chief cells. However, there are some possibilities that inhibition of pepsinogen secretion by somatostatin is the result of interaction between cells other than the chief cells and somatostatin. One theory is that prostaglandins mediates inhibition of gastric acid secretion by somatostatin.[21] There is thus a possibility that prostaglandins are released from other cells into the medium by somatostatin. Prostaglandins, however, increased pepsinogen secretion from dispersed chief cells.[22] Since the other possibilities still remain, we are developing a method which can obtain a respondent and enriched chief cell suspension. Park reported that somatostatin directly inhibited histamine-, carbachol- and pentagastrin-induced gastric acid secretion in canine parietal cells[23]; these results correspond with ours.

Some mechanisms for pepsinogen secretion have been reported.[24,25] Somatostatin inhibited carbachol-, CCK-8- and histamine-induced pepsinogen secretion, and the mechanisms involved may differ. Our results indicated that somatostatin inhibits a common pathway for pepsinogen secretion activated by these three main secretagogues.

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