Somatostatin Inhibits Pepsinogen Secretion without Influencing Cytosolic Free Ca^{2+} Increase Induced by Carbachol and Cholecystokinin Octapeptide in Rat Chief Cells

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Gastric chief cells were isolated from the rat stomach in an attempt to identify those involved in the mechanism of action of somatostatin on pepsinogen secretion. The effects of several kinds of secretagogues on cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) were examined in the rat chief cells. Carbachol and cholecystokinin octapeptide (CCK-8) markedly induced $[Ca^{2+}]i$ increase, while histamine, gastrin I and secretin did not. Carbachol and CCK-8 also stimulated pepsinogen secretion. A similar dose–response relationship was seen in carbachol- and CCK-8-induced $[Ca^{2+}]i$ increase and pepsinogen secretion. Somatostatin did not inhibit carbachol- or CCK-8-induced $[Ca^{2+}]i$ increase, but did inhibit carbachol- and CCK-8-induced pepsinogen secretion by 30 and 50%, respectively.

Keywords gastric chief cell; pepsinogen secretion; somatostatin; cholecystokinin; carbachol; cytosolic free calcium

Three kinds of exocrine cells are known in the gastric mucosa; parietal cells, chief cells and mucous cells, which secrete gastric acid, pepsinogen and mucus, respectively. An outline of the excitation–secretion coupling in acid secretion from parietal cells has been made, though not in detail,[1] while the pepsinogen secretion from chief cells is obscure. Since Soll succeeded in the isolation of gastric mucosal cells,[2] studies on second mediators have used isolated gastric cells to exclude the influence or products of other cells. Cytosolic free Ca^{2+} and cAMP have already been established as second mediators in acid secretion; histamine stimulates gastric acid secretion *via* cAMP production,[3] and gastrin and cholinergic agonists stimulate it *via* cytosolic free Ca^{2+} concentration ($[Ca^{2+}]i$) increase after phosphatidyl inositide turnover.[4] Studies on second mediators of pepsinogen secretion from chief cells are not yet complete. Sutliff *et al.* showed the second mediator of secretin and vasoactive intestinal polypeptide was cAMP,[5] and Raufman *et al.* reported that of cholinergic agonists and cholecystokinin (CCK) to be cytosolic free Ca^{2+} in chief cells from the guinea pig stomach.[6] Though many antiulcer drugs had been developed using rat experimental models, reports dealing with pepsinogen secretion from rat chief cells are few. To learn the action mechanism of antiulcer drugs in rat experimental models required study of the effects of drugs not only on acid secretion but also on pepsinogen secretion. We therefore isolated chief cells from the rat stomach to determine whether or not the pepsinogen secretion process in these cells occurred through the same mechanism as that operating in guinea pig chief cells.

Somatostatin is generally known to be an inhibitor of several kinds of secretion: growth hormone and gastrin secretion, and gastric acid and pepsinogen secretion.[7] We showed earlier that somatostatin inhibited pepsinogen secretion from rat chief cells induced by carbachol and cholecystokinin octapeptide (CCK-8).[8] The purpose of this paper is to clarify the site of action of somatostatin on cytosolic free Ca^{2+} mediated pepsinogen secretion from these cells.

Materials and Methods

Chemicals Carbamylcholine chloride (carbachol) and bovine serum albumin (BSA, fraction V) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). CCK-8, human gastrin I and somatostatin were from Peptide Institute Inc. (Osaka). Histamine dihydrochloride, fura-2 acetoxymethyl (fura-2 AM) and EGTA, and Dispase I® were from Wako Pure Chemical Industries (Osaka), Dojin Laboratories (Kumamoto) and Godo Shusei Co. (Tokyo), respectively. Percoll® from Pharmacia LKB Biotechnology (Uppsala, Sweden), was diluted to 30 and 45% with saline and adjusted at pH 7.4 with 1 N HCl. All other reagents were of the best commercial quality available.

Preparation of Gastric Mucosal Cells After isolation of the stomach from a 24 h-starved Wistar rat weighing about 250 g under urethane anesthesia, gastric mucosal cells were dispersed by a modification of the method described previously.[8] Briefly, the stomach, which was everted and filled with medium C, was incubated with Dispase I (1000 PU/ml) in medium A gassing with 95% O_2 and 5% CO_2 for 1 h at 37 °C. The stomach was placed in medium B and incubated at room temperature gassing with 95% O_2 and 5% CO_2. Then, cells were gently isolated from the gastric mucosa with a pipette. The cell suspension in medium B was filtered through a nylon filter (150 mesh), and centrifuged at $50 \times g$ for 2 min. The precipitated mucosal cells were resuspended in medium C.

Medium A contains NaH_2PO_4 0.5, Na_2HPO_4 1.0, $NaHCO_3$ 20, NaCl 70, KCl 5.0, glucose 11, HEPES 25 (mM) and BSA 10 (mg/ml) (pH 7.4). Medium B contains EDTA disodium 2 (mM) in medium A. Medium C contains $CaCl_2$ 1.0 and $MgCl_2$ 1.0 (mM) in medium A.

Isolation of Gastric Chief Cells Chief cells were prepared using Percoll density gradient centrifugation. Mucosal cell suspension in 30% Percoll solution was gently poured onto 45% Percoll solution, and centrifuged at $1200 \times g$ for 5 min. The precipitated fraction which mainly contained chief cells was washed by centrifugation with medium C. Purity of chief cells was estimated by an immunofluorescence technique using rabbit anti-rat pepsinogen antiserum and fluorescein isothiocyanate conjugated goat anti-rabbit IgG; in this paper we used a chief cell fraction with a purity over 80% (average $83.43 \pm 1.91\%$ $n=4$) as chief cells.

Measurement of $[Ca^{2+}]i$ Fura-2 AM ($0.1\,\mu M/1 \times 10^6$ cells/ml) was loaded into isolated chief cells by incubating in medium C for 37 °C at 15 min while gassing with 95% O_2 and 5% CO_2. The fura-2 loaded chief cells were washed twice by centrifugation with medium C at $50 \times g$ for 2 min and resuspended with the same medium to obtain 2×10^6 cells/ml. The fluorescence of cell suspension was measured by a Shimadzu RF-5000 spectrofluorophotometer (Osaka). The excitation and emission wavelengths were 340 and 380 nm with a 5.0 nm bandwidth and 500 nm with a 5.0 nm bandwidth, respectively. The charts were recorded with fluorescence ratio (340/380). A Ca^{2+} free medium was prepared by omitting Ca^{2+} from medium C and adding 1 mM EGTA to this medium.

Culture of Gastric Chief Cells Gastric chief cells were cultured by the method described previously.[8] Briefly, chief cells were suspended in a mixture of Dulbecco's modified Eagle's minimum essential medium and Ham's F-12 (1 : 1) containing 10% fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin, and cultured on collagen coated plastic dishes at 37 °C in a CO_2 incubator.

Measurement of Pepsinogen Secretion Pepsinogen concentrations in

medium were measured by the avidin–biotin complex enzyme-linked immunosorbent assay. Pepsinogen secretion from cells to medium was expressed as a percentage to total cellular pepsinogen which was estimated after freezing and thawing of cells.

Results

Effects of Carbachol and CCK-8 on $[Ca^{2+}]i$ and Pepsinogen Secretion After fura-2 was loaded into freshly isolated chief cells, the effects of several kinds of secretagogues on $[Ca^{2+}]i$ were examined (Fig. 1). Carbachol and CCK-8 markedly induced $[Ca^{2+}]i$ increase in chief cells from the rat stomach, but histamine (1×10^{-4} M), gastrin I (1×10^{-7} M) and secretin (1×10^{-8} M) did not. In a Ca^{2+} free medium carbachol and CCK-8 also induced $[Ca^{2+}]i$ increase, even though they induced lower Ca^{2+} transient. Carbachol- and CCK-8-induced pepsinogen secretion from

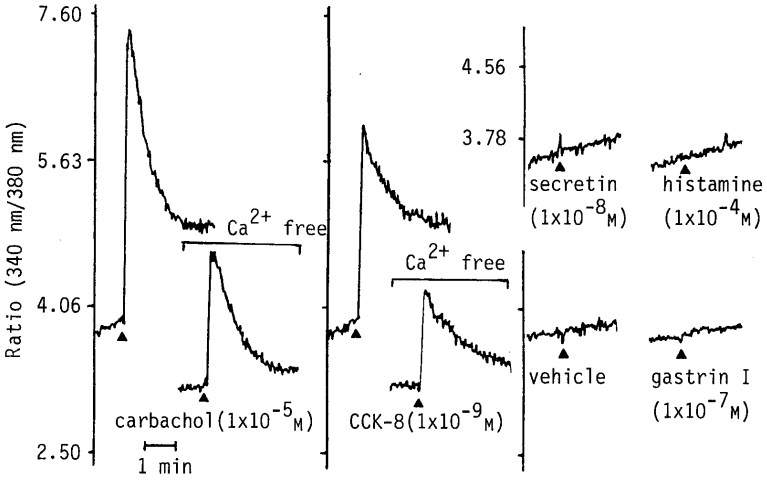

Fig. 1. Effects of Secretagogues on $[Ca^{2+}]i$ in Chief Cells

Fluorescence of fura-2 loaded cells (2×10^6 cells/ml) at 500 nm was measured by excitation them at 340 and 380 nm alternately. The fluorescence changes induced by carbachol and CCK-8 were measured in medium with or without Ca^{2+}.

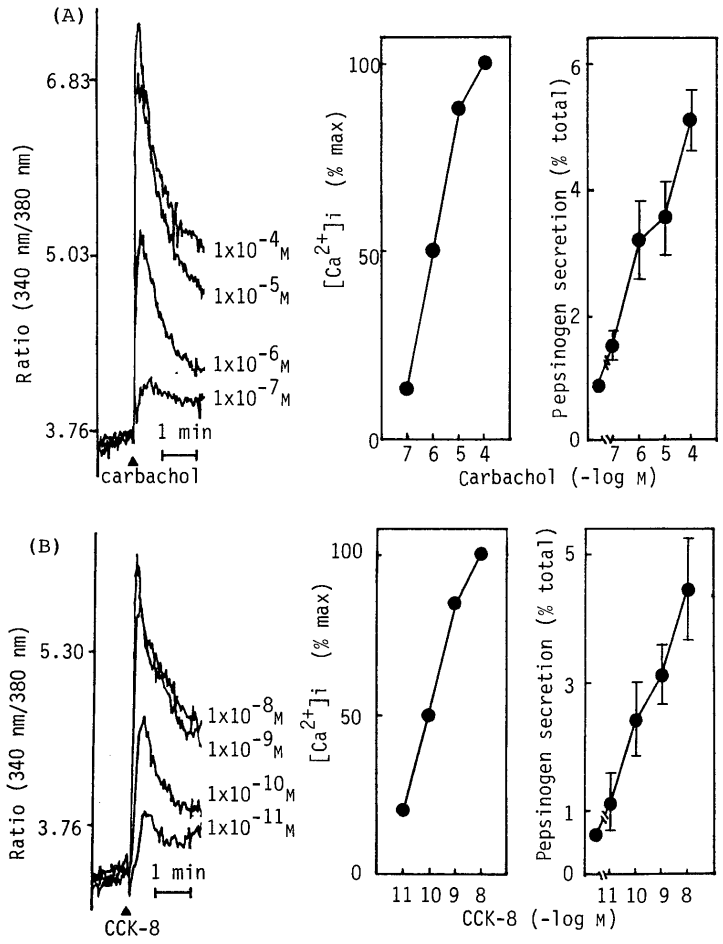

Fig. 2. Dose–Response Curves of $[Ca^{2+}]i$ Increase and Pepsinogen Secretion Induced by Carbachol (A) and CCK-8 (B)

Maximum responses in $[Ca^{2+}]i$ increase were obtained with a concentration of 1×10^{-4} M carbachol or 1×10^{-8} M CCK-8. $[Ca^{2+}]i$ increase is expressed as percentage of the maximum response. For the measurement of pepsinogen secretion, cells were incubated with carbachol for 30 min and separated from a medium by decantation and centrifugation. Each value of pepsinogen secretion is the mean ± S.E. from 5 separate experiments.

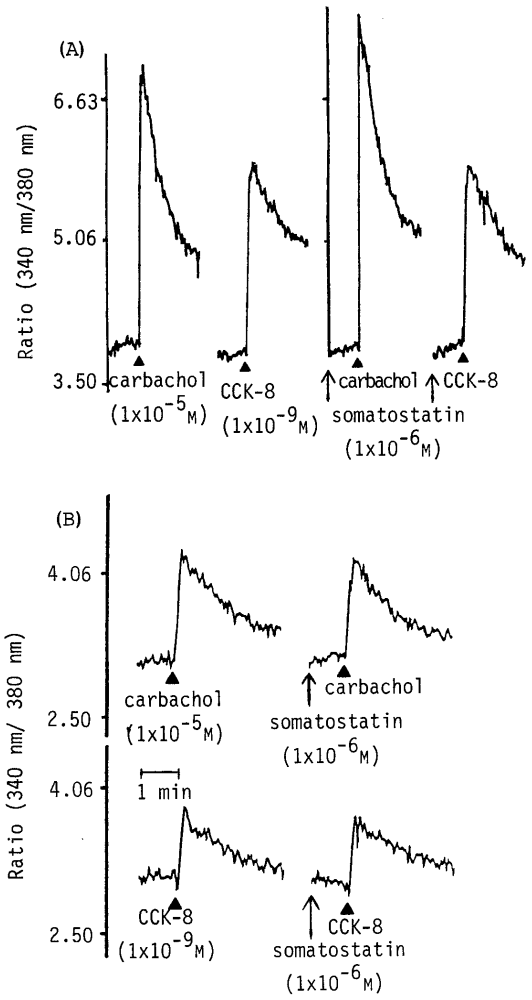

Fig. 3. Effects of Somatostatin on Carbachol- and CCK-8-Induced $[Ca^{2+}]i$ Increase in Chief Cells in the Medium with (A) or without (B) Ca^{2+}

After preincubation with somatostatin for 1 min in medium with or without Ca^{2+}, the fluorescence changes of chief cells induced by carbachol or CCK-8 were measured.

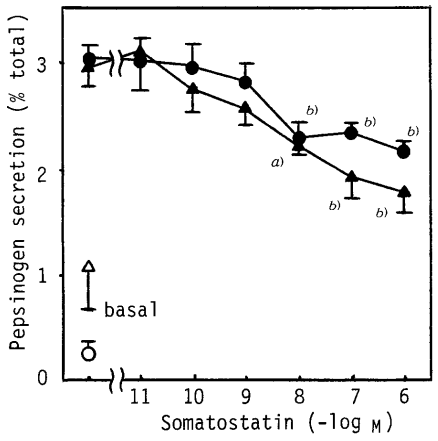

Fig. 4. Effects of Somatostatin on Carbachol- and CCK-8-Induced Pepsinogen Secretion

Cells were preincubated with indicated concentrations of somatostatin for 15 min and incubated with or without carbachol (1×10^{-5} M) (●, ○) and with or without CCK-8 (1×10^{-9} M) (▲, △) for 30 min. The values for carbachol and CCK-8 are the mean ± S.E. from 5 and 4 separate experiments, respectively. *a)* $p<0.05$, *b)* $p<0.01$.

cultured chief cells and $[Ca^{2+}]i$ increase in the freshly isolated chief cells were compared (Fig. 2). Carbachol and CCK-8 induced $[Ca^{2+}]i$ increase and pepsinogen secretion at doses above 1×10^{-7} and 1×10^{-11} M, respectively, in a dose-dependent manner. Although the experimental conditions of the chief cells differed, the dose–response curves of carbachol- and CCK-8-induced pepsinogen secretion were similar to those of $[Ca^{2+}]i$ increase.

Effects of Somatostatin on Carbachol- and CCK-8-Induced $[Ca^{2+}]i$ Increase and Pepsinogen Secretion Carbachol- and CCK-8-induced $[Ca^{2+}]i$ increases in freshly isolated chief cells were not affected by treatment with somatostatin whether or not Ca^{2+} was present in the medium (Fig. 3). However, somatostatin inhibited carbachol- and CCK-8-induced pepsinogen secretion from cultured chief cells in a dose-dependent manner (Fig. 4). Somatostain at the concentration of 1×10^{-5} M inhibited CCK-8 (1×10^{-9} M)-induced pepsinogen secretion by 50%, and that induced by carbachol (1×10^{-5} M) by 30%.

Discussion

It has already been reported that carbachol and CCK induced $[Ca^{2+}]i$ increase in chief cells from the guinea pig or rabbit stomach.[6,9] We wished to confirm whether or not the same process occurred in mechanism was present in chief cells from the rat stomach. As shown here, among the tested pepsinogen secretagogues carbachol and CCK-8 induced $[Ca^{2+}]i$ increase in the rat chief cells, so that we recognized there was no essential difference between chief cells from the rat and the guinea pig, rabbit or canine stomachs. Carbachol and CCK-8 would stimulate the production of inositol phosphates,[10] because they caused $[Ca^{2+}]i$ increase even in a Ca^{2+} free medium. Both carbachol and CCK-8 induced the Ca^{2+} transient followed by the sustained phase in a Ca^{2+} containing medium, but they induced only the Ca^{2+} transient in a Ca^{2+} free medium. It was also known that carbachol and CCK-8 stimulated pepsinogen secretion in a Ca^{2+} free medium,[11] and we confirmed this fact (not shown). The second mediator of pepsinogen secretion induced by cholinergic agonists and CCK-8 is thus cytosolic free Ca^{2+}.

Cherner *et al.* showed that there were two kinds of gastrin receptor in the guinea pig chief cells, one for gastrin and the other for CCK, and that the response of pepsinogen secretion to gastrin was about 60% of that to CCK.[12] They did not, however, mention the second mediator of these receptors. Structural similarity between CCK and gastrin suggests that they induce similar signal-transduction. In contrast with CCK, gastrin I did not induce $[Ca^{2+}]i$ increase, even though in our previous paper tetragastrin slightly stimulated pepsinogen secretion.[13] Therefore, the signal-transduction mechanism of gastrin I might be completely different from that of CCK.

It was reported that CCK did not exist in the gastric mucosa[14] but was secreted from the intestinal mucosa after the digestion of ingested food in the stomach and transfer of the gastric contents into the duodenum. Therefore, it is doubtful that CCK stimulates pepsinogen secretion in a physiological state. Actually, after gastric contents are emptied into the duodenum, somatostatin would be dominant in the gastric mucosa.[15] The potential action of CCK on pepsinogen secretion would be inhibited by somatostatin. However, CCK caused so strong a $[Ca^{2+}]i$ increase in chief cells even in the presence of somatostatin that it raised a question about what the physiological role

is of cytosolic free Ca^{2+} induced by CCK in chief cells. CCK may stimulate pepsinogen biosynthesis in these cells, because depletion of peptic granules occurs during digestion in the stomach.

Somatostatin is contained in the mucosa and nerve fibers in the stomach. Its inhibitory effects in gastric acid[16] and pepsinogen secretion[17] were reported a few years after its structure had been identified. Studies on the mechanism of action of somatostatin in pepsinogen secretion were reported in only a few papers. In the bullfrog, somatostatin inhibited pepsinogen secretion *via* a cAMP-independent pathway.[18] In the human gastric mucosa, somatostatin inhibited histamine-sensitive adenylate cyclase activities.[19] In this paper we show that somatostatin inhibited pepsinogen secretion without altering $[Ca^{2+}]$i increase in chief cells in the presence or absence of Ca^{2+} in a medium. We may conclude that somatostatin inhibits carbachol- and CCK-8-induced pepsinogen secretion by at some point depressing the pathway between $[Ca^{2+}]$i increase and exocytosis of peptic granules.

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