Inhibitory Action of Somatostatin on cAMP Dependent Pepsinogen Secretion from Rat Gastric Chief Cells: Involvement of Pertussis Toxin-Sensitive G-Protein

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We examined the inhibitory effect of somatostatin on pepsinogen secretion using isolated rat gastric chief cells. Secretin and forskolin significantly increased not only pepsinogen secretion from chief cells but also cellular cAMP accumulation in a dose-dependent fashion. Somatostatin significantly inhibited secretin- and forskolin-induced pepsinogen secretion and secretin-induced cellular cAMP accumulation. However, forskolin-induced cellular cAMP accumulation was not inhibited by somatostatin. The inhibitory effect of somatostatin on secretin- induced pepsinogen secretion was abolished by pretreatment with pertussis toxin, but inhibition of forskolin-, carbachol- and cholecystokinin octapeptide-induced pepsinogen secretion was not. These results suggest that somatostatin inhibits pepsinogen secretion in two ways, one is closely related to the pertussis toxin-sensitive G-protein and the other is not determined.

Keywords pepsinogen secretion; gastric chief cell; somatostatin; cAMP; G-protein; pertussis toxin

By recent advancements in radioimmunoassay and immunohistochemical techniques, it has been clarified that somatostatin is widely distributed in the nervous and digestive tissues. Actions of somatostatin are known to include the suppression of the secretion of a number of hormones, and inhibition of pancreatic and gastric secretion.[1,2] Therefore, somatostatin was proposed to be an important physiological regulator of a variety of secretory phenomena.

The second mediators of gastric acid secretion were made clear using isolated parietal cells; that is, histamine stimulates acid secretion *via* cellular cAMP production,[3] and carbachol and gastrin do it *via* an intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) increase.[4] Many investigators, including us, have confirmed that somatostatin inhibits histamine-, carbachol- and gastrin-induced acid secretion.[2,5,6] On the inhibitory mechanisms, somatostatin attenuates the stimulatory effects of such secretagogues as changes in cellular cAMP levels, similarly to histamine. It has been reported that the effects of somatostatin are abolished when cells are preincubated with pertussis toxin, which uncouples an inhibitory guanine nucleotide binding (Gi) protein.[5-7] So, the attenuation of acid secretion by somatostatin engages with the activation of Gi proteins.

During the past decade, signal-transduction mechanisms of pepsinogen secretion have been studied using isolated gastric chief cells from the guinea pig[8] and canine.[9] Two pathways of the signal-transduction mechanisms have now been established in the chief cells. Carbachol and cholecystokinin (CCK) induce a $[Ca^{2+}]i$ increase,[10] and secretin and vasoactive intestinal peptide induce cellular cAMP production.[11] Concerning the mechanism of action of somatostatin on pepsinogen secretion, there is a report that it inhibits pepsinogen secretion *via* a cAMP-independent pathway in frog esophageal mucosa peptic cells.[12] However, somatostatin receptors and its signal-transduction mechanism in chief cells from the mammalian stomach have not yet been characterized. Although rats have been used for various models of gastric ulceration and the evaluation of anti-ulcer drugs, investigations of pepsinogen secretion from rat chief cells are few. In our prior report, we described the effects of somatostatin on secretagogue-induced pepsinogen secretion and $[Ca^{2+}]i$ increase in enriched rat chief cells.[13] In this report, we examined the effects of somatostatin on secretagogues-induced pepsinogen secretion in the presence or absence of pertussis toxin and cellular cAMP production using rat gastric chief cells.

MATERIALS AND METHODS

Chemicals Carbamylcholine chloride (carbachol), bovine serum albumin (BSA) (fraction V), forskolin, 3-isobutyl-1-methylxanthine (IBMX) and pertussis toxin were purchased from Sigma Chem. Co. (St. Louis, U.S.A.). Cholecystokinin octapeptide (CCK-8), somatostatin and secretin were from Peptide Institute Inc. (Osaka). Histamine dihydrochloride, tetragastrin, Dispase I®, and Percoll® were from Wako Pure Chemical Industries (Osaka), MECT Co. (Tokyo), Godo Shusei Co. (Tokyo) and Pharmacia LKB Biotechnology (Uppsala, Sweden), respectively. All other reagents were of the best commercial quality available.

Isolation of Gastric Chief Cells Dispersed chief cells from the rat stomach were prepared by a Percoll gradient centrifugation after isolation from a reversed stomach with Dispase I as described previously.[13]

Culture of Gastric Chief Cells Obtained gastric chief cells were cultured with a mixture of Dulbecco's modified Eagle's minimum essential medium and Ham's F-12 (1 : 1) containing 10% fetal calf serum on collagen coated plastic dishes at 37 °C in a CO_2 incubator as described previously.[13]

Measurement of Released Pepsinogen and Cellular cAMP Pepsinogen concentration was measured by the avidin–biotin complex enzyme-linked immunosorbent assay (ELISA). Pepsinogen release was expressed as a percentage of total cellular pepsinogen which was es-

timated after the freezing and thawing of cultured cells. Cellular cAMP was extracted with 6% trichloroacetic acid from chief cells. cAMP was measured by a Yamasa cAMP assay kit.

RESULTS

Effects of Secretagogues on Pepsinogen Secretion and Cellular cAMP Accumulation Dose–response curves of pepsinogen secretion from cultured chief cells by stimulation of some secretagogues are shown in Fig. 1. Carbachol, CCK-8, secretin and forskolin significantly stimulated pepsinogen secretion in a dose-dependent fashion at doses above 1×10^{-7}, 1×10^{-10}, 1×10^{-10} and 1×10^{-5} M, respectively. However, the responses induced by histamine and gastrin were small.

Time courses of secretin and/or IBMX on cAMP accumulation in dispersed chief cells are shown in Fig. 2. The separate addition of IBMX (1×10^{-4} M) and secretin (1×10^{-8} M) did not induce cellular cAMP accumulation. However, the combined addition of both secretin and IBMX rapidly accumulated cellular cAMP, within 5 min, and the accumulation reached a plateau by 10 min. Next, we estimated the incubation condition as follows: cells were incubated with a secretagogue at 37 °C for 10 min in the presence of IBMX (1×10^{-4} M). The dose–response curves of some secretagogues on cellular cAMP accumulation in freshly dispersed chief cells are shown in Fig. 3. Secretin and forskolin significantly stimulated cellular cAMP accumulation in a dose-dependent fashion at doses above 1×10^{-10} and 1×10^{-6} M, respectively. The dose–response curves of cellular cAMP

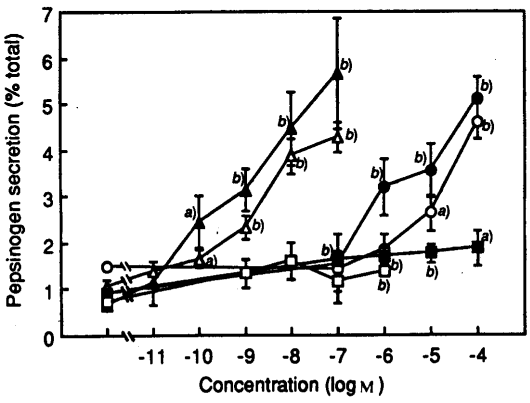

Fig. 1. Effects of Secretagogues on Pepsinogen Secretion from Chief Cells

Cells were incubated with carbachol (●), CCK-8 (▲), forskolin (○), secretin (△), histamine (■) and gastrin (□) for 30 min at 37 °C, and then pepsinogen in a medium was measured by ELISA. Each value is the mean ± S.E. of 5 separate experiments. *a*) $p<0.05$, *b*) $p<0.01$.

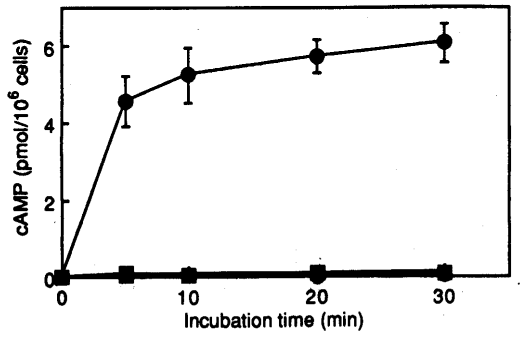

Fig. 2. Time Courses of cAMP Accumulation Induced by Secretin and/or IBMX in Chief Cells

Cells (2×10^6 cells/ml) were incubated with vehicle (○), IBMX (1×10^{-4} M) (▲), secretin (1×10^{-8} M) (■), or IBMX (1×10^{-4} M) plus secretin (1×10^{-8} M) (●) for 5, 10, 20 and 30 min at 37 °C. Cellular cAMP was measured by the method described in this text. Each value is the mean ± S.E. of 3 separate experiments.

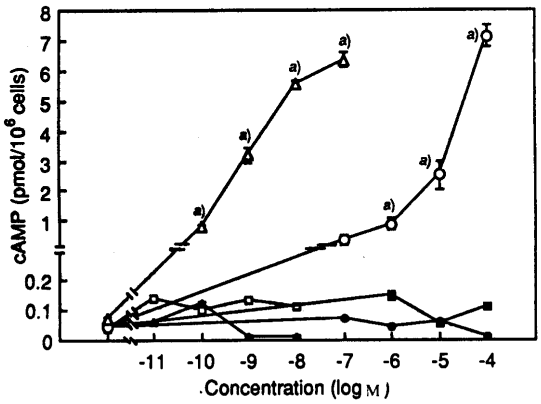

Fig. 3. Effects of Secretagogues on cAMP Accumulation in Chief Cells

Cells were incubated with carbachol (●), CCK-8 (▲), forskolin (○), secretin (△), histamine (■) and gastrin (□) for 10 min at 37 °C in the presence of IBMX (1×10^{-4} M). Each value is the mean ± S.E. of 4 separate experiments. *a*) $p<0.01$.

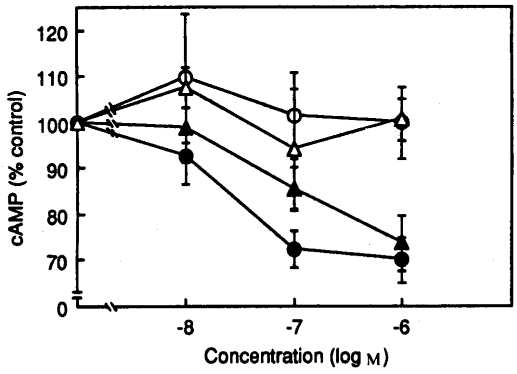

Fig. 4. Effects of Somatostatin on Secretin- and Forskolin-Induced cAMP Accumulation in Chief Cells

Cells were preincubated with somatostatin for 5 min, and then incubated with secretin (●, 1×10^{-9} M; ▲, 1×10^{-8} M) or forskolin (○, 1×10^{-5} M; △, 1×10^{-4} M) for 10 min at 37 °C. Each value is the mean ± S.E. of 4 separate experiments.

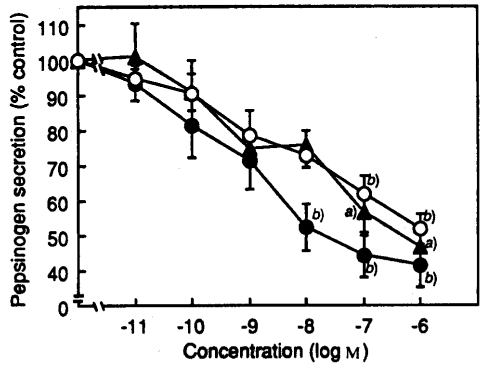

Fig. 5. Effects of Somatostatin on Secretin- and Forskolin-Induced Pepsinogen Secretion from Chief Cells

Cells were preincubated with indicated concentrations of somatostatin for 15 min, and then incubated with secretin (1×10^{-8} M) (●), forskolin (1×10^{-4} M) (▲) or secretin (1×10^{-8} M) plus IBMX (1×10^{-4} M) (○) for 30 min at 37 °C. Each value is the mean ± S.E. of 5 separate experiments. *a*) $p<0.05$, *b*) $p<0.01$.

TABLE I. Effects of Somatostatin on Secretagogue-Induced Pepsinogen Secretion from Chief Cells in the Presence or Absence of Pertussis Toxin

Secretagogue	Pepsinogen secretion (% total)			
	−Pertussis toxin		+Pertussis toxin	
	None	Somatostatin	None	Somatostatin
None	1.06±0.20	0.99±0.34	1.00±0.12	0.92±0.16
Secretin (1×10^{-8} M)	3.96±0.32	2.55±0.22[b]	3.70±0.41	4.47±0.38
Forskolin (1×10^{-4} M)	4.61±0.38	3.13±0.34[a]	4.49±0.31	3.41±0.25[a]
Carbachol (1×10^{-5} M)	3.99±0.28	3.11±0.27[a]	3.78±0.18	3.04±0.15[a]
CCK-8 (1×10^{-9} M)	3.94±0.27	3.15±0.21[a]	4.16±0.42	3.03±0.22[a]

After pretreatment with pertussis toxin (100 ng/ml) for 24 h, cells were preincubated with or without somatostatin (1×10^{-6} M) for 15 min and then incubated with secretagogues for 30 min at 37 °C. Each value is the mean±S.E. of 5 separate experiments. *a*) $p<0.05$, *b*) $p<0.01$.

accumulation induced by secretin or forskolin are paralleled pepsinogen secretion from cultured chief cells (Fig. 1). Other tested secretagogues did not stimulate cellular cAMP accumulation.

Effects of Somatostatin on Secretin- and Forskolin-Stimulated Pepsinogen Secretion and Cellular cAMP Accumulation The effects of somatostatin on cAMP accumulation induced by secretin (1×10^{-8} and 10^{-9} M) and forskolin (1×10^{-4} and 10^{-5} M) in chief cells are shown in Fig. 4. Chief cells were incubated with each secretagogue after preincubation with various concentrations of somatostatin for 5 min at 37 °C. Somatostatin at the concentration of 1×10^{-6} M decreased the stimulatory effect of secretin (1×10^{-8} and 10^{-9} M)-induced cellular cAMP accumulation to 73 and 70%, respectively. The stimulatory effect of forskolin-induced cellular cAMP accumulation was not influenced by somatostatin.

The inhibitory effects of somatostatin on pepsinogen secretion induced by secretin and forskolin were measured (Fig. 5). Induced pepsinogen secretions were inhibited by somatostatin in a dose-dependent manner. Significant inhibitions were observed at doses above 1×10^{-8} M of somatostatin to secretin and above 1×10^{-7} M of somatostatin to forskolin.

Effect of Pertussis Toxin on the Action of Somatostatin Effects of somatostatin on secretagogues-induced pepsinogen secretion were examined (Table I) after chief cells were cultured with pertussis toxin (100 ng/ml) for 24 h. Pepsinogen secretion induced by secretagogues was not affected by pretreatment with pertussis toxin. Also, the inhibitory effects of somatostatin on forskolin-, carbachol- and CCK-8-induced pepsinogen secretion were not affected by the pretreatment. However, the inhibitory effect of somatostatin on secretin-induced pepsinogen secretion was reversed by the pretreatment.

DISCUSSION

Since Soll *et al.* studied pepsinogen secretion using isolated gastric chief cells from the canine stomach,[9] and Raufman *et al.* developed it using the same kind of cells from the guinea pig,[8] many studies on pepsinogen secretion have been reported using these cells.[10,11,14] Although the rat has been used mostly for various models of experimental gastric ulceration, evaluation of anti-ulcer drugs, and *in vivo* experiments of gastric acid and pepsinogen secretion, studies on pepsinogen secretion using rat chief cells are few. Considering the above facts, we tried to study the mechanism of pepsinogen secretion using isolated chief cells from the rat stomach. After improving an isolation procedure, we succeeded in attaining enough gastric chief cells which responded to secretagogues. However, we were not able to obtain stably responsive cells on pepsinogen secretion. We used chief cells in two different ways: freshly isolated cells for study of the signal-transduction mechanisms, and cultured cells for those of pepsinogen secretion.[13]

Raufman reported that secretin and IBMX, an inhibitor of cyclic nucleotide phosphodiesterase, stimulated cellular cAMP production in guinea pig chief cells, even though they were added independently.[11,15] In the present paper, we showed that neither secretin nor IBMX alone increased cellular cAMP accumulation, but the combined use of both drugs did cause an increase. These results indicated that the phosphodiesterase activity in rat chief cells may be higher than that in the guinea pig cells.

Somatostatin is known to be a physiological inhibitor of gastric secretion. There are many reports which have dealt with the effects of somatostatin on cellular cAMP.[5,6,16] We showed that the second mediator of secretin-induced pepsinogen secretion from rat chief cells is cellular cAMP. Pepsinogen secretion was stimulated by forskolin, which is a direct activator of cAMP production and binds to the catalytic unit of adenylate cyclase. Somatostatin inhibited forskolin-induced pepsinogen secretion, although it did not decrease the production of cellular cAMP stimulated by forskolin. Somatostatin inhibited not only secretin-induced pepsinogen secretion but also the production of cellular cAMP in a dose-dependent fashion. Yet, the inhibitory effect of somatostatin on pepsinogen secretion was abolished after pretreatment with pertussis toxin. These data obviously indicate that the somatostatin receptor is related to the pertussis toxin-sensitive G-protein. The action of somatostatin on forskolin-induced pepsinogen secretion was not attenuated by pertussis toxin. Those results suggest that the inhibitory mechanisms of somatostatin may also be related to a route other than *via* the pertussis toxin-sensitive G-protein. In our previous report, we showed that somatostatin inhibited carbachol- and CCK-8-induced pepsinogen secretion, but did not inhibit carbachol- or CCK-8-induced $[Ca^{2+}]i$ increases.[13] Needless to say, the

inhibitory effects of somatostatin on carbachol- and CCK-8-induced pepsinogen secretion was not attenuated by pretreatment with pertussis toxin. Similar results were reported using isolated canine gastric parietal cells.[6] On the other hand, some reports were contradictory to ours, suggesting that somatostatin inhibited the basal and bombesin-stimulated phosphatidyl inositol turnover in isolated rat pancreatic acinar cells and also inhibited a norepinephrine-induced $[Ca^{2+}]i$ increase in rat medullary carcinoma cells, in which these actions of somatostatin were attenuated by pertussis toxin.[17,18] In this paper, we showed that somatostatin inhibits pepsinogen secretion *via* a pertussis toxin-sensitive G-protein and other unknown mechanisms.

REFERENCES

1) S. J. Konturek, "Gastrointestinal Hormones," ed. by G. B. J. Glass, Raven Press, New York, 1980, pp. 693—716.
2) S. Tani, T. Tanaka, *Chem. Pharm. Bull.*, **38**, 2246 (1990).
3) A. H. Soll, A. Wollin, *Am. J. Physiol.*, **237**, E444 (1979).
4) Y. Tsunoda, *Biochim. Biophys. Acta*, **855**, 186 (1986); C. S. Chew, M. R. Brown, *ibid.*, **888**, 116 (1986); P. A. Negulescu, T. E. Machen, *Am. J. Physiol.*, **254**, C130 (1988); J. L. Cabero, E. Grapegiesser, E. Gylfe, Z. Li, S. Mardh, *Biochim. Biophys. Res. Commun.*, **183**, 1097 (1992).
5) A. Zink, H. Scherubl, D. Kliemann, M. Hoflich, R. Ziegler, F. Raue, *Mol. Cell. Endocrinol.*, **86**, 213 (1992).
6) J. Park, T. Chiba, T. Yamada, *J. Biol. Chem.*, **262**, 14190 (1987).
7) M. M. Atwell, P. J. Hanson, *Biochim. Biophys. Acta*, **971**, 282 (1988).
8) J-P. Raufman, V. E. Sutliff, D. K. Kasbekar, R. T. Jensen, J. D. Gardner, *Am. J. Physiol.*, **247**, G95 (1984).
9) M. J. Sanders, D. A. Amirian, A. Ayalon, A. H. Soll, *Am. J. Physiol.*, **245**, G641 (1983).
10) J-P. Raufman, S. Berger, L. Cosowsky, E. Straus, *Biochim. Biophys. Res. Commun.*, **137**, 281 (1986); C. Sakamoto, T. Matozaki, M. Nagao, H. Nishizaki, S. Baba, *ibid.*, **142**, 865 (1987).
11) V. E. Sutliff, J. P. Raufman, R. T. Jensen, J. D. Gardner, *Am. J. Physiol.*, **251**, G96 (1986).
12) J. C. Fong, S-J. Hong, F-F. Wang, *Regul. Pept.*, **32**, 351 (1991).
13) T. Tanaka, K. Miyadera, S. Tani, *Biol. Pharm. Bull.*, **16**, 767 (1993).
14) J. Defize, R. H. Hunt, *Dig. Dis. Sci.*, **33**, 1583 (1988); J. Defize, R. H. Hunt, *Gut*, **30**, 774 (1989).
15) J-P. Raufman, L. Cosowsky, *Biochim. Biophys. Acta*, **970**, 318 (1988).
16) J. J. Segura, J. M. Guerrero, R. Goberna, J. R. Calvo, *Neuropeptides*, **23**, 39 (1992).
17) C. Linard, F. R. Desmars, M. J. M. Lewin, *Regul. Pept.*, **41**, 219 (1992).
18) A. Zink, F. Raue, *Acta Endocrinol.*, **127**, 378 (1992).