Interaction among Secretagogues on Pepsinogen Secretion from Rat Gastric Chief Cells

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Received December 22, 1994; accepted February 24, 1995

We examined the interaction among secretagogues that stimulate pepsinogen secretion through different pathways *in vivo* and *in vitro*. In *in vitro* study, a combined administration of secretin and carbachol or cholecystokinin octapeptide (CCK-8) to the culture medium of chief cells potentiated pepsinogen secretion. Moreover, the response induced by carbachol or CCK-8 with forskolin was greater than that with secretin. We examined the interaction among receptor-related second mediators, and found that carbachol- or CCK-8-induced intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) increase was not affected by secretin or forskolin. Both these substances, however, significantly reduced secretin-induced cAMP production. On the contrary, CCK-8 significantly increased forskolin-induced cAMP production, while carbachol increased it slightly. Calcium ionophore, A23187, or protein kinase C activator, phorbol 12-myristate 13-acetate (PMA), did not alter secretin- or forskolin-induced cellular cAMP production; and the reductive effect of carbachol or CCK-8 on secretin-induced cAMP production was restored by their competitive antagonists, atropine or lorglumide. EC_{50} of those antagonists was almost the same value as IC_{50} on pepsinogen secretion and $[Ca^{2+}]i$ increase. These results indicate that secretin-induced cAMP production is interfered with by receptor related agonists like CCK-8 and carbachol. It may be suggested that there is a kind of "cross-talk," between the adenylate cyclase system, that is, the secretin receptor, and carbachol or CCK-8 receptor.

The interactions between secretin and other secretagogues (carbachol, CCK-8, tetragastrin and histamine) were examined using the perfused rat stomach. The infusion of CCK-8 induced high pepsin secretion initially, which gradually decreased; with a simultaneous injection of secretin, however, high pepsin secretion was maintained. High pepsin secretion induced by an infusion of carbachol was obtained when secretin was injected simultaneously. Although tetragastrin or histamine did not stimulate significant pepsin secretion, pepsinogen secretion was stimulated if secretin injected simultaneously. These results suggest that pepsinogen secretion in the rat is not controlled only by pepsinogen secretagogues but by its combination with acid secretagogues.

Key words interaction; pepsinogen; rat; cAMP; $[Ca^{2+}]i$; secretin

Raufman *et al.* estimated the mechanism of pepsinogen secretion using isolated chief cells from the guinea pig stomach,[1] and identified the existence of receptors of the various physiological secretagogues on these cells. The relationships between the receptors and their second mediators on pepsinogen secretion were also investigated.[2] Two pathways among intracellular mechanisms on pepsinogen secretion from gastric chief cells have been identified. One is the pathway *via* the phosphatidyl inositol turnover, that is, inositol trisphosphate (IP_3) and diacylglyceride (DG) are produced by the phospholipase C activated by secretagogues such as carbachol or cholecystokinin octapeptide (CCK-8). IP_3 transiently increases intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and DG activates the protein kinase C.[3] The other is one *via* intracellular cAMP activated by secretin, vasoactive intestinal peptide and prostaglandins.[4,5] In our previous studies, similar results were obtained using isolated chief cells from the rat stomach.[6,7]

Studies of the potentiation of pepsinogen secretion from dispersed rat gastric glands and the interaction between $[Ca^{2+}]i$ and the adenylate cyclase system in guinea pig chief cells were reported by Raufman *et al.*[8,9]; however, they did not refer to the interaction between each second mediator. In the isolated guinea pig chief cells, they just reported that calmodulin which is activated by carbachol, CCK-8 and calcium ionophore, A23187, augments cellular cAMP production after pretreatment of chief cells with cholera toxin.[9] In this paper, we examined the interaction among secretagogues on pepsinogen secretion.

MATERIALS AND METHODS

Chemicals Carbamylcholine chloride (carbachol), bovine serum albumin (BSA) (fraction V), forskolin and 3-isobutyl-1-methyl-xanthine (IBMX) were purchased from Sigma Chem. Co. (St. Louis, U.S.A.). CCK-8 and secretin were from Peptide Institute Inc. (Osaka). Phorbol 12-myristate 13-acetate (PMA), atropine sulfate monohydrate and dibutyryl adenosine 3′,5′-cyclic monophosphate (dbcAMP) were from Wako Pure Chemical Industries (Osaka). Lorglumide sodium salt was from Research Biochem. Inc. (M.A., U.S.A.). A23187 was from Calbiochem-Behring Co. Dispase® was from Godo Shusei Co. (Tokyo). Fura-2 acetoxymethyl (fura-2 AM) was from Dojin Laboratories (Kumamoto). Percoll® was from Pharmacia LKB Biotechnology (Uppsala, Sweden). All other reagents were of the best commercial quality available.

Isolation of Rat Gastric Chief Cells Chief cells from the rat stomach were prepared by a Percoll gradient centrifugation after dispersing from a reversed stomach with Dispase I as described.[6]

Culture of Gastric Chief Cells Obtained gastric chief cells were cultured in 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.[6]

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Measurement of Released Pepsinogen and Cellular cAMP Pepsinogen concentration was measured by the avidin–biotin complex enzyme-linked immunosorbent assay (abcELISA). Pepsinogen release was expressed as a percentage of total cellular pepsinogen which was estimated after freezing and thawing of cultured cells. cAMP was measured by a cAMP enzymeimmunoassay system purchased from Amersham (Buckingham, England).

Measurement of $[Ca^{2+}]i$ The $[Ca^{2+}]i$ in chief cells was measured by the method described previously.[6] Briefly, fura-2 AM (0.1 μM/10^6 cells/ml) was loaded into isolated chief cells by incubating for 37 °C at 15 min. The fluorescence of cell suspension (2×10^6 cells/ml) 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 as a fluorescence ratio (340/380).

Perfused Rat Stomach A male Wistar rat weighing about 200 g was starved for 24 h under free access to water and used for the perfused rat stomach according to the method described.[10] Peptic activity was measured using hemoglobin as a substrate.[11]

Statistical Analyses The results were expressed as mean ± S.E. Differences between two groups were analyzed by Student's *t*-test.

RESULTS

Interactions among Secretagogues on Pepsinogen Secretion from Chief Cells Effects of secretin on carbachol- or CCK-8-induced pepsinogen secretion from chief cells are shown in Fig. 1. Carbachol or CCK-8 potentiated secretin-induced pepsinogen secretion. Carbachol potentiated secretin-induced pepsinogen secretion in a dose-dependent fashion at a dose above 1×10^{-5} M (Fig. 1A). CCK-8 also potentiated secretin-induced pepsinogen secretion, but not significantly (Fig. 1B). Carbachol or CCK-8 strongly and significantly potentiated forskolin-induced pepsinogen secretion (Fig. 2).

Effects of Secretagogues on $[Ca^{2+}]i$ Increase in Chief Cells After loading fura-2 into freshly dispersed chief cells, effects of secretin and forskolin on carbachol- or CCK-8-induced $[Ca^{2+}]i$ increase were tested (Fig. 3). Carbachol (1×10^{-4} M)- and CCK-8 (1×10^{-8} M)-induced $[Ca^{2+}]i$ increases were not affected by secretin (1×10^{-8} M) or forskolin (1×10^{-4} M) in the presence or

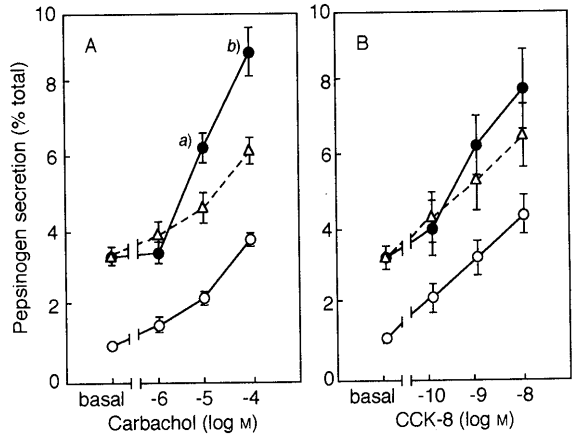

Fig. 1. Effects of Secretin with Carbachol or CCK-8 on Pepsinogen Secretion from Rat Chief Cells

Cells were incubated with the indicated concentrations of carbachol (○) or secretin (1×10^{-8} M) plus carbachol (●) (A) and CCK-8 (○) or secretin (1×10^{-8} M) plus CCK-8 (●) (B) for 30 min at 37 °C, and then pepsinogen in a medium was measured by ELISA. Dashed lines (△) represent calculated values of pepsinogen concentration with the addition of secretin (1×10^{-8} M) and dose–response curves of carbachol or CCK-8. Each value is the mean ± S.E. of 5 separate experiments. Significant differences between calculated values of carbachol plus secretin and those obtained by experiment (A) and those for CCK-8 plus secretin (B): *a*) $p < 0.05$, *b*) $p < 0.01$.

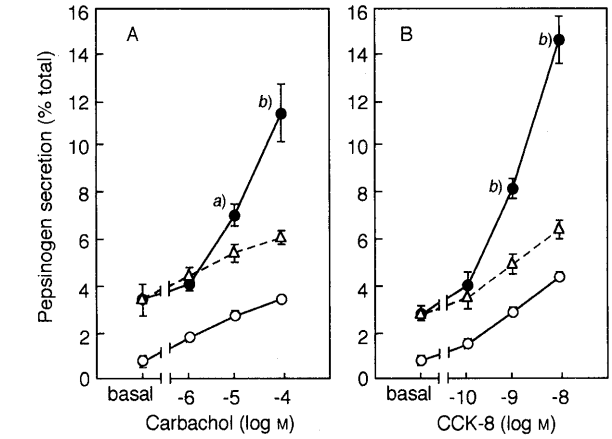

Fig. 2. Effects of Forskolin with Carbachol or CCK-8 on Pepsinogen Secretion from Rat Chief Cells

Cells were incubated with the indicated concentrations of carbachol (○) or forskolin (1×10^{-4} M) plus carbachol (●) (A) and CCK-8 (○) or forskolin (1×10^{-4} M) plus CCK-8 (●) (B) for 30 min at 37 °C, and then pepsinogen in a medium was measured by ELISA. Dashed lines (△) represent calculated values of pepsinogen concentration with the addition of forskolin (1×10^{-4} M) and dose–response curves of carbachol or CCK-8. Each value is the mean ± S.E. of 5 separate experiments. Significant differences between calculated values of carbachol plus forskolin and those obtained by experiment (A) and those for CCK-8 plus forskolin (B): *a*) $p < 0.05$, *b*) $p < 0.01$.

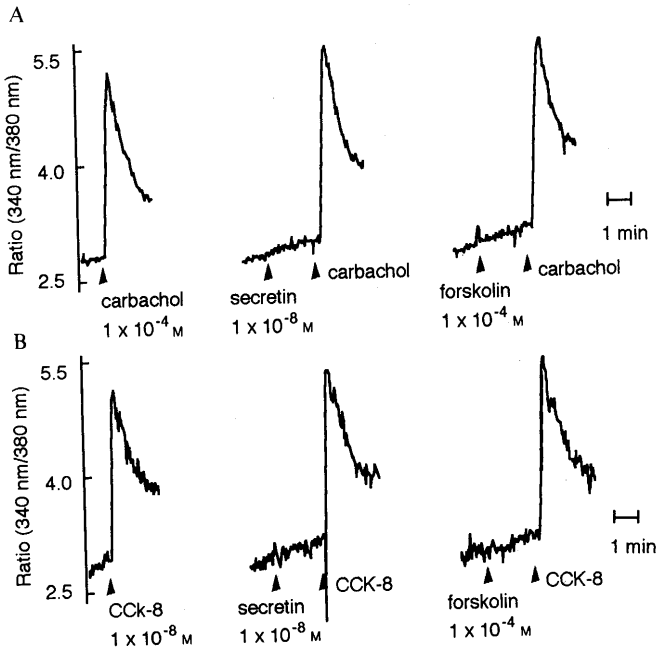

Fig. 3. Effects of Secretin or Forskolin on $[Ca^{2+}]i$ Increase Induced by Carbachol or CCK-8 in the Presence of IBMX

After fura 2-loaded cells (2×10^6 cells/ml) were preincubated with IBMX for 1 min, the changes of fluorescence ratio (340 nm/380 nm) in chief cells induced by carbachol (A) or CCK-8 (B) were recorded. Chemicals were added at times indicated by arrows.

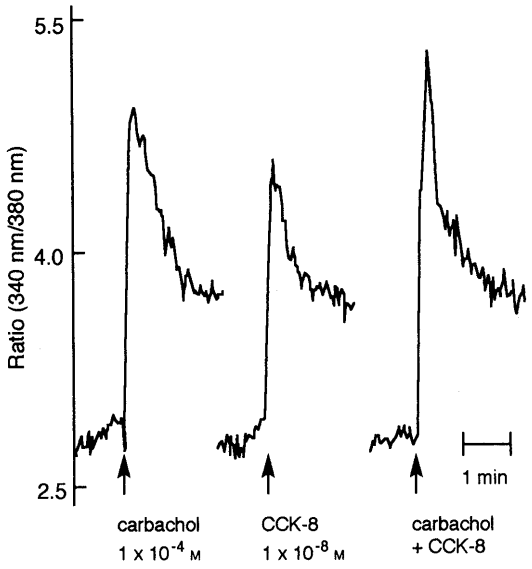

Fig. 4. Effects of Carbachol and CCK-8 on $[Ca^{2+}]i$ Increase

After fura 2-loaded cells (2×10^6 cells/ml) were preincubated for 1 min, the changes of fluorescence ratio (340 nm/380 nm) in chief cells induced by carbachol, CCK-8 or carbachol plus CCK-8 were recorded.

Table 1. Effects of Carbachol, CCK-8, Secretin and Combinations of Each Chemical on Pepsinogen Secretion

Secretagogue	Pepsinogen secretion (% total)
None	1.08±0.08
Carbachol (1×10^{-4} M)	4.11±0.76
CCK-8 (1×10^{-8} M)	3.20±0.11
Carbachol+CCK-8	5.46±0.50
Secretin (1×10^{-8} M)	3.30±0.12
Carbachol+secretin	9.31±1.09
CCK-8+secretin	7.64±0.37

Each value is the mean±S.E. of 4 separate experiments.

absence (data not shown) of IBMX (1×10^{-4} M). A combined addition of carbachol and CCK-8 stimulated $[Ca^{2+}]i$ increase and pepsinogen secretion more than carbachol or CCK-8 alone (Fig. 4, Table 1).

Effects of A23187, PMA and dbcAMP on Pepsinogen Secretion Dose–response curves of pepsinogen secretion from chief cells by the stimulation of A23187, PMA and dbcAMP are shown in Fig. 5. A23187, PMA and dbcAMP significantly stimulated pepsinogen secretion in a dose-dependent fashion at doses above 1×10^{-7}, 1×10^{-10} and 1×10^{-5} M, respectively. Any combination of these three secretagogues strongly potentiated pepsinogen secretion (Table 2).

Effects of Carbachol, CCK-8, A23187 and PMA on Secretin- or Forskolin-Induced cAMP Production Effects of carbachol or CCK-8 on cellular cAMP production induced by secretin (1×10^{-8} M) or forskolin (1×10^{-4} M) are shown in Fig. 6. Secretin-induced cAMP production was reduced by carbachol or CCK-8 in a dose-dependent manner. Significant reductions were observed at doses above 1×10^{-4} M of carbachol and 1×10^{-9} M of CCK-8, respectively. Carbachol at the concentrations of 1×10^{-5} and 10^{-4} M decreased secretin-induced cellular cAMP production to 79.7 and 63.4%, respectively, while CCK-8

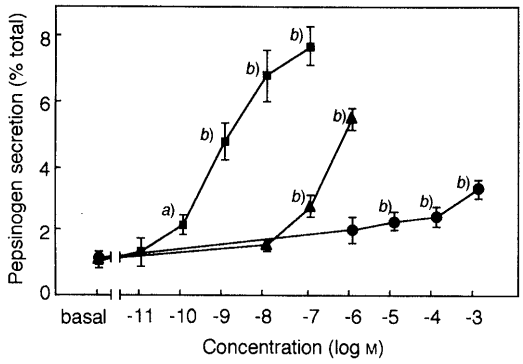

Fig. 5. Effects of A23187, PMA and dbcAMP on Pepsinogen Secretion from Chief Cells

Cells were incubated with the indicated concentrations of A23187 (▲), PMA (■) and dbcAMP (●) for 30 min at 37 °C. Each value is the mean±S.E. of 5 separate experiments. *a)* $p<0.05$, *b)* $p<0.01$, compared with the basal values.

Table 2. Effects of A23187, PMA, dbcAMP and Combinations of Each Chemical on Pepsinogen Secretion

Secretagogue	Pepsinogen secretion (% total)
None	0.42±0.06
A23187 (1×10^{-7} M)	3.34±0.35
PMA (1×10^{-10} M)	2.69±0.38
dbcAMP (1×10^{-3} M)	2.62±0.17
A23187+PMA	9.47±1.35
A23187+dbcAMP	10.12±1.02
dbcAMP+PMA	8.80±0.52
A23187+PMA+dbcAMP	17.78±1.34

Each value is the mean±S.E. of 5 separate experiments.

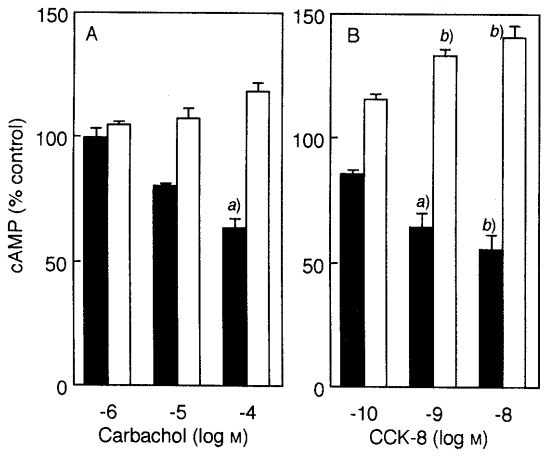

Fig. 6. Effects of Carbachol or CCK-8 on cAMP Production Induced by Secretin or Forskolin

Cells (1×10^6 cells/ml) were incubated with the indicated concentrations of carbachol plus secretin (1×10^{-8} M) (■) or forskolin (1×10^{-4} M) (□) (A) and CCK-8 plus secretin (1×10^{-8} M) (■) or forskolin (1×10^{-4} M) (□) (B) for 10 min at 37 °C in the presence of IBMX (1×10^{-4} M). Concentrations of cellular cAMP were measured by the method described in the text. The cAMP concentration induced by secretin (1×10^{-8} M) was about 6.98±0.66 pmol/10^6 cells. Each value is the mean±S.E. of 5 separate experiments. *a)* $p<0.05$, *b)* $p<0.01$, compared with the cAMP production induced by secretin alone.

at the concentrations of 1×10^{-9} and 10^{-8} M decreased it to 64.2 and 55.0%, respectively. However, forskolin-induced cAMP production was increased by CCK-8 in a dose-dependent manner, and significant augmentation was observed at a dose above 1×10^{-9} M. CCK-8 at the

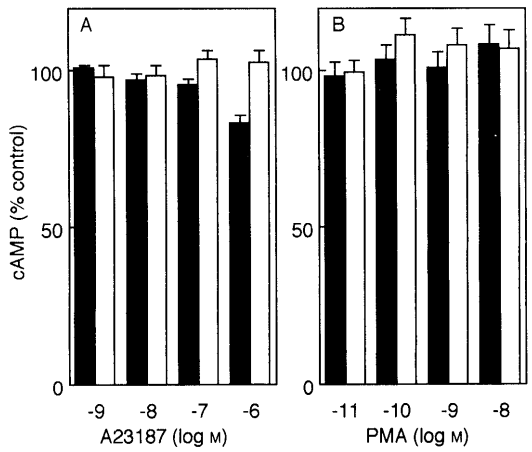

Fig. 7. Effects of A23187 or PMA on cAMP Production Induced by Secretin or Forskolin

Cells (1×10^6 cells/ml) were incubated with the indicated concentrations of A23187 plus secretin (1×10^{-8} M) (■) or forskolin (1×10^{-4} M) (□) (A) and PMA plus secretin (1×10^{-8} M) (■) or forskolin (1×10^{-4} M) (□) (B) for 10 min at 37 °C in the presence of IBMX (1×10^{-4} M). The cAMP concentration induced by secretin (1×10^{-8} M) was about 6.20 ± 0.66 pmol/10^6 cells. Each value is the mean ± S.E. of 5 separate experiments.

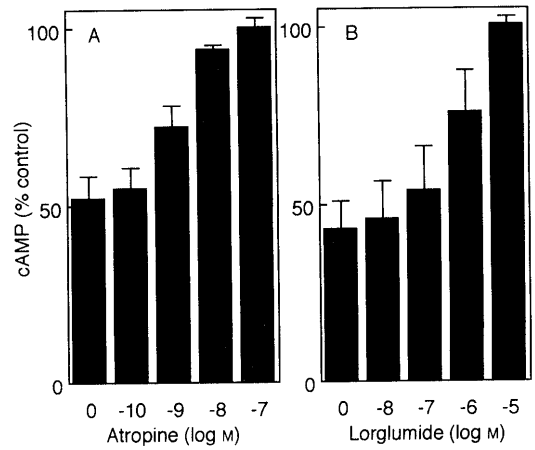

Fig. 8. Effects of Atropine or Lorglumide on Secretin-Induced cAMP Production Reduced by Carbachol or CCK-8

Cells (1×10^6 cells/ml) were incubated with the indicated concentrations of atropine to the medium containing carbachol (1×10^{-4} M) and secretin (1×10^{-8} M) (A) and lorglumide to the medium containing CCK-8 (1×10^{-8} M) and secretin (1×10^{-8} M) (B) for 10 min at 37 °C in the presence of IBMX (1×10^{-4} M). Each value is the mean ± S.E. of 5 separate experiments.

concentrations of 1×10^{-9} and 10^{-8} M increased forskolin-induced cAMP production to 133.0 and 140.4%, respectively (Fig. 6B), but carbachol at the concentration of 1×10^{-4} M increased it only to 118.7% (Fig. 6A). On the other hand, neither A23187 nor PMA affected the secretin- or forskolin-induced cellular cAMP production (Fig. 7A, B).

Effects of Atropine and Lorglumide on Secretin-Produced Cellular cAMP Reduction and Carbachol- and CCK-8-Induced Pepsinogen Secretion and $[Ca^{2+}]i$ Increase Reductions of secretin-induced cellular cAMP production by carbachol or CCK-8 were eliminated by their antagonists, atropine or lorglumide (Fig. 8). The dose–response curves of atropine and lorglumide on carbachol- or CCK-8-induced pepsinogen secretion and $[Ca^{2+}]i$ increase are shown in Fig. 9. IC_{50} of atropine to inhibit carbachol-induced pepsinogen secretion and

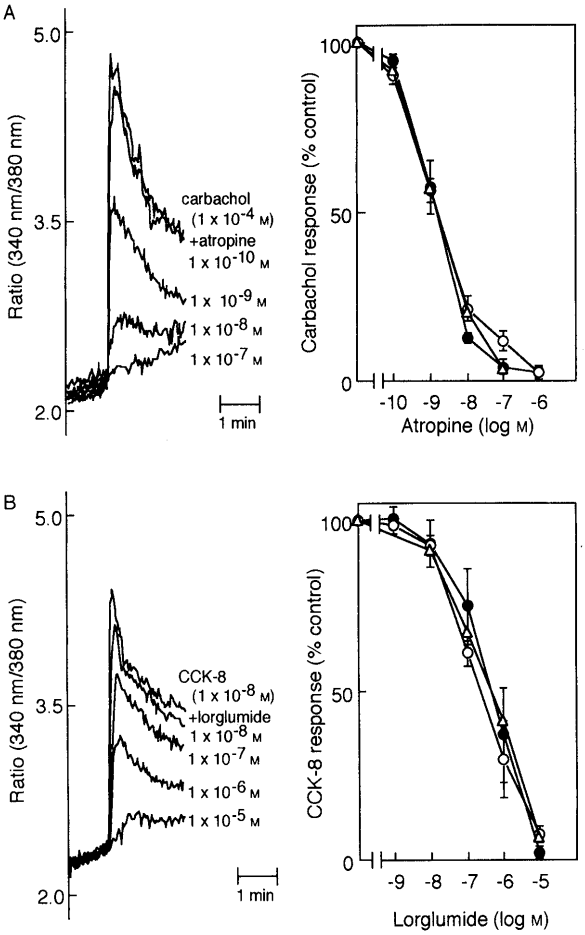

Fig. 9. Effects of Atropine or Lorglumide on Pepsinogen Secretion, $[Ca^{2+}]i$ Increase and Secretin-Induced cAMP Production Reduced by Carbachol or CCK-8

$[Ca^{2+}]i$ increase (△), pepsinogen secretion (○) and cellular cAMP decrease (●) are expressed as percentages of the response to carbachol (1×10^{-4} M) (A) or CCK-8 (1×10^{-8} M) (B). Values of pepsinogen secretion and cellular cAMP decrease are the mean ± S.E. of 4 separate experiments.

$[Ca^{2+}]i$ increase, and its 50% effective concentration (EC_{50}) to eliminate carbachol-induced reduction of secretin-induced cAMP production were about 1.5, 1.5 and 1.3×10^{-9} M, respectively (Fig. 9 A). Lorglumide also inhibited CCK-8-induced pepsinogen secretion and $[Ca^{2+}]i$ increase, and eliminated CCK-8-induced reduction of secretin-induced cAMP production; the IC_{50} and EC_{50} values were about 2.4, 4.0 and 3.5×10^{-7} M, respectively (Fig. 9B).

Pepsinogen Secretion in the Rat Gastric Perfusion *in Vivo* Effects of single injections of a few graded concentrations of secretin were investigated in a rat gastric perfusion. Secretin slightly decreased the basal secretion of acid, and increased the basal secretion of pepsinogen (Fig. 10A). Significant pepsinogen secretion was observed after a secretin injection during infusion of tetragastrin or histamine (Fig. 10B, C). Secretin induced high pepsinogen secretion under infusion of carbachol (Fig. 10D). An infusion of CCK-8 induced high pepsinogen secretion initially, which gradually decreased. But high pepsin secretion occurred by following secretin injection (Fig. 10E).

Effects of CCK-8 and Somatostatin on Secretin-Induced cAMP Production Somatostatin or somatostatin with

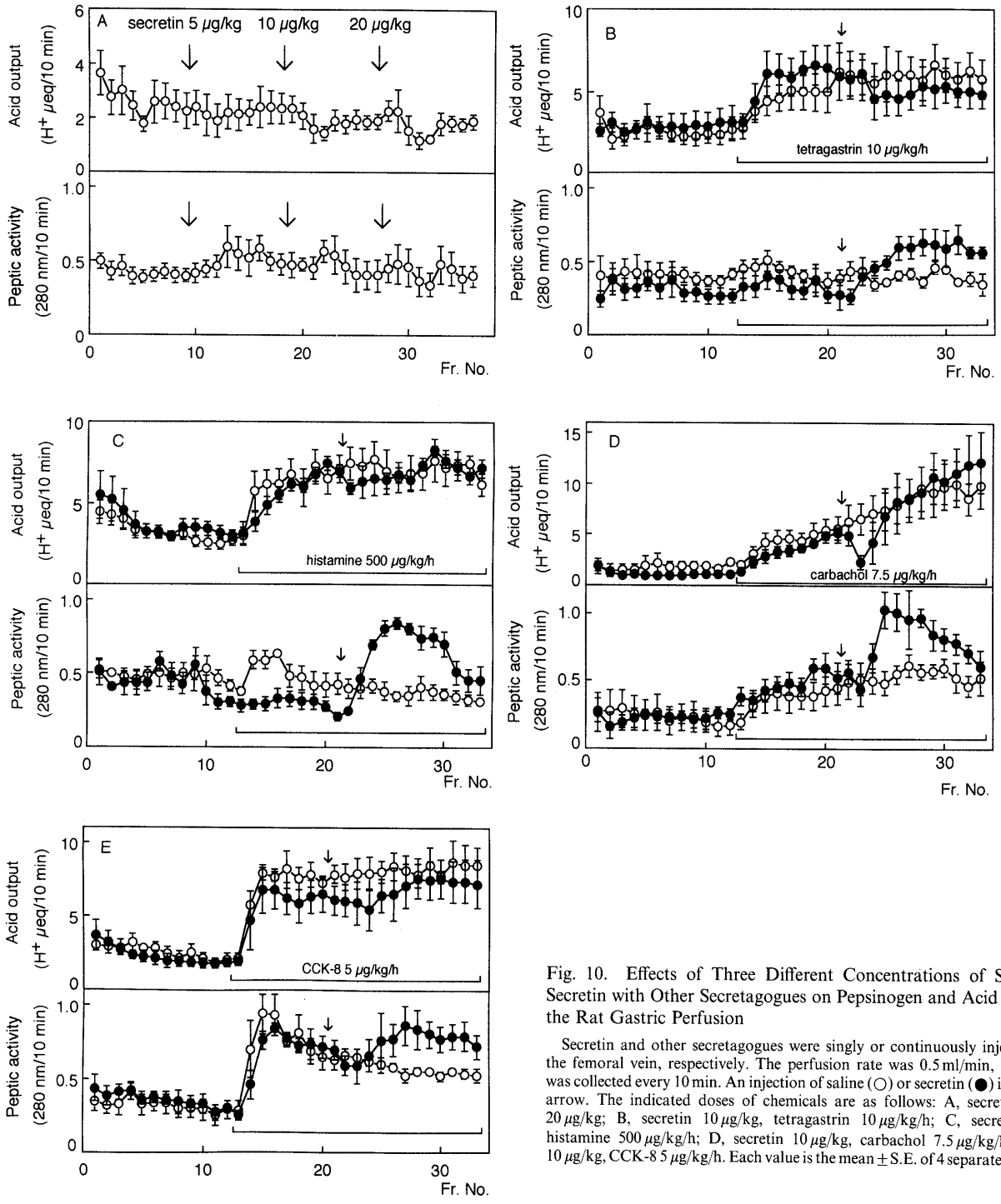

Fig. 10. Effects of Three Different Concentrations of Secretin and Secretin with Other Secretagogues on Pepsinogen and Acid Secretion in the Rat Gastric Perfusion

Secretin and other secretagogues were singly or continuously injected through the femoral vein, respectively. The perfusion rate was 0.5 ml/min, and perfusate was collected every 10 min. An injection of saline (○) or secretin (●) is shown by an arrow. The indicated doses of chemicals are as follows: A, secretin 5, 10 and 20 μg/kg; B, secretin 10 μg/kg, tetragastrin 10 μg/kg/h; C, secretin 10 μg/kg, histamine 500 μg/kg/h; D, secretin 10 μg/kg, carbachol 7.5 μg/kg/h; E, secretin 10 μg/kg, CCK-8 5 μg/kg/h. Each value is the mean ± S.E. of 4 separate experiments.

CCK-8 (1×10^{-8} M) decreased the stimulatory effect of secretin (1×10^{-8} M)-induced cAMP production in a dose-dependent fashion (Fig. 11).

DISCUSSION

We have already shown that carbachol, CCK-8 and secretin are secretagogues for pepsinogen secretion in the rat.[6,7] We indicated that carbachol- and CCK-8-induced pepsinogen secretions are mediated by $[Ca^{2+}]i$ increase, and that secretin-induced pepsinogen secretion is mediated by cellular cAMP production.[6,7] These findings are the same as those reported by Sutliff *et al.*,[4] and other investigators[3] using guinea pig chief cells. In parietal cells, it has been established that carbachol- and gastrin-induced acid secretions are mediated by $[Ca^{2+}]i$ increase, and that histamine-stimulated acid secretion is mediated by cellular cAMP production.[12] A potentiation phenomenon of acid secretion by histamine and carbachol or gastrin is known.[13] In this paper, we attempted to clarify the interaction among those secretagogues possessing different second mediators on pepsinogen secretion.

A combined administration of carbachol and CCK-8, both stimulants of pepsinogen secretion through the $[Ca^{2+}]i$ increase, actually augmented pepsinogen secretion. The rate of increase of this secretion by their combination was similar to that of $[Ca^{2+}]i$ increase, but smaller than the additive values of each secretagogue alone.

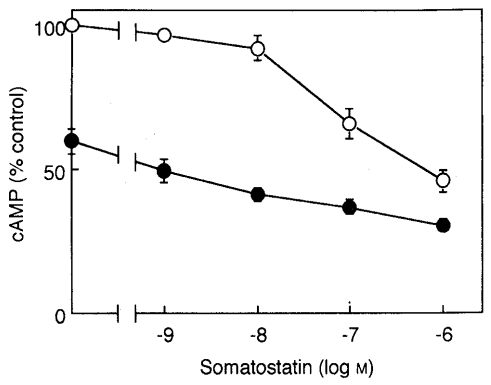

Fig. 11. Effects of Somatostatin and CCK-8 on Secretin-Induced cAMP Production

Cells (1×10^6 cells/ml) were incubated with the indicated concentrations of somatostatin (○) or somatostatin plus CCK-8 (1×10^{-8} M) (●) for 10 min at 37 °C in the presence of secretin (1×10^{-8} M) and IBMX (1×10^{-4} M). A concentration of cellular cAMP was measured by the method described in the text. Each value is the mean ± S.E. of 5 separate experiments.

A combined administration of secretin and carbachol or CCK-8, in contrast, induced potentiation of pepsinogen secretion. Combined administration of carbachol and secretin potentiated pepsinogen secretion much more than did CCK-8 and secretin. Then, we examined the mechanism of the potentiation of pepsinogen secretion using forskolin instead of secretin to estimate whether or not the potentiation was concerned with the receptor mediated cAMP production. Combined administration of forskolin and carbachol or CCK-8 augmented the potentiation of pepsinogen secretion more than did secretin and carbachol or CCK-8. Combined administration of CCK-8 and forskolin potentiated pepsinogen secretion much more than did carbachol and forskolin. It seems likely that the receptor associated cAMP production is controlled by other secretagogues whose second mediator is $[Ca^{2+}]i$.

We examined the effects of secretin and forskolin on carbachol- or CCK-8-induced $[Ca^{2+}]i$ increase and found that neither substance influenced carbachol- or CCK-8-induced $[Ca^{2+}]i$ increase in the presence or absence of IBMX, a phosphodiesterase inhibitor. These results suggest that the $[Ca^{2+}]i$ increase in chief cells is not controlled by the presence of cAMP. Next, we examined the effects of carbachol and CCK-8 on secretin- or forskolin-induced cAMP production. Carbachol and CCK-8 reduced secretin-induced cAMP production, but they did not reduce forskolin-induced production. These results give the logical conclusion that the potentiation of carbachol or CCK-8 to forskolin-induced pepsinogen secretion is greater than that to one secretin-induced. Actually, this is the result we obtained.

CCK-8 and carbachol are known to activate the phospholipase C which induces inositol phospholipid turnover, consequently generating IP_3 and DG. IP_3 causes $[Ca^{2+}]i$ increase from the store in the cells, and DG activates the protein kinase C. Carbachol- or CCK-8-induced pepsinogen secretion must be the result of the synergism between $[Ca^{2+}]i$ and DG.[14] In this paper, we confirmed that a combined administration of A23187 and PMA induces the potentiation of pepsinogen secretion. However, there is a report that the activated protein kinase C reduced histamine-induced cellular cAMP production in parietal cells.[15] There is also a report that PMA inhibited prostaglandin-induced cellular cAMP production, but did not alter the increase of cellular cAMP production caused by cholera toxin, forskolin, secretin, or vasoactive intestinal peptide in guinea pig chief cells.[16] Then, the effect of A23187 and PMA on secretin- or forskolin-induced cellular cAMP production was examined; neither substance altered secretin- or forskolin-induced cellular cAMP production in rat chief cells. Our results are consistent with those in the report obtained from guinea pig chief cells.[16] They suggest that PMA-activated protein kinase C and $[Ca^{2+}]i$ increase do not affect the stimulatory or inhibitory guanine nucleotide binding proteins or the catalytic component of the adenylate cyclase system. Although a high concentration of A23187 (1×10^{-6} M) slightly inhibited secretin-induced cAMP production, whether or not $[Ca^{2+}]i$ increase affects cellular cAMP production is doubtful.

An acetylcholine receptor antagonist, atropine, completely reversed the reductive effect of carbachol on secretin-induced cAMP production. A CCK_A receptor antagonist, lorglumide,[17] also completely reversed the reductive effect of CCK-8 on secretin-induced cAMP production. Each EC_{50} was almost the same as IC_{50} to pepsinogen secretion and $[Ca^{2+}]i$ increase. Therefore, we conclude that reductions of secretin-induced cAMP production by carbachol and CCK-8 are under a physiological regulation. We were not able to determine the reductive mechanism of carbachol or CCK-8 on secretin-induced cAMP production in this paper. However, we can say at least that there is cross-talk between the CCK-8 or carbachol receptor and the adenylate cyclase system in the chief cells.

We also confirmed the above results in *in vivo* study. Although a single injection of secretin decreased acid secretion, it slightly increased pepsin secretion. Usually, high acid secretion *in vivo* is accompanied by pepsin secretion. Especially, secretin-induced pepsinogen secretion was apparently observed when it was injected with the infusion of histamine which did not stimulate pepsin secretion. Therefore, washing out of pepsin by acid may be an important factor *in vivo*.

Secretin and CCK are known to be released from the duodenal mucosa when digested porridge-like meal is moved from the stomach to the duodenum. They stimulate secretion of various enzymes and HCO_3^- from the pancreas, and inhibit acid secretion.[18] In the physiological state, it seems hardly that both secretin and CCK are related to pepsin secretion. However, thinking about the digestive process in the stomach, the fluid part of ingested food may easily pass to the duodenum, and this would cause secretion of secretin and CCK. It is not so curious that secretin and CCK stimulate pepsin secretion; because pepsin works following the acidification of ingested food, it is not necessary for gastric acid and pepsinogen to be secreted simultaneously. In addition, cholinergic stimulation inhibits somatostatin release from D-cell.[19] Therefore, it is conceivable that secretin may work as a pepsinogen secretagogue during digestion of ingested food

in the stomach. Actually, suppression of acid secretion by secretin was weak. However, binding of secretin and CCK-8 to their own receptors on the chief cells at the same time may restrain them from excessive secretion rather than induce potentiation of pepsinogen secretion. Chiba *et al.* reported the effect of secretin on somatostatin and gastrin release from the isolated perfused rat stomach, and found that secretin evoked dose-dependent increase of somatostatin secretion and dose-related decrease of gastrin release simultaneously.[20] Soll *et al.* reported that CCK-8 potently released somatostatin from the canine fundic mucosal D-cells.[21] Buchan *et al.* reported that a combined administration of secretin and CCK-8 induced potentiation of somatostatin release from a primary culture of human antral mucosal cells.[22] Taking the above observations into consideration, after emptying the digested food from the stomach, secretin and CCK may stimulate somatostatin release followed by inhibition of acid secretion. We reported that somatostatin inhibited pepsinogen secretion stimulated by CCK-8 or secretin.[6,7] Therefore, in the intestinal phase, we believe that secretin-induced cellular cAMP production may be regulated by not only CCK-8 but also somatostatin (Fig. 11), and that acid secretion is also suppressed by secretin, CCK and somatostatin. There is a report that cAMP directly stimulated pepsinogen synthesis in cultured canine gastric chief cells.[23] Secretin-induced cAMP production was not completely inhibited by CCK-8 and somatostatin in chief cells. In conclusion, secretin works as a pepsinogen secretagogue during digestion of food in the stomach, and after food is transferred to the duodenum it may act as a stimulator of pepsinogen biosynthesis and a suppressor of acid secretion.

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