Role of intracellular Ca^{2+} and the calmodulin messenger system in pepsinogen secretion from isolated rabbit gastric mucosa

T Miyamoto, M Itoh, Y Noguchi, K Yokochi

Abstract

Both carbachol (10^{-4} – 10^{-3} mol/l) and cholecystokinin octapeptide (CCK-8) (10^{-8} – 10^{-6} mol/l) significantly stimulated the release of pepsinogen from rabbit gastric mucosa maintained in organ culture (213–216% and 143–261% of control, respectively, $p < 0.05$ – 0.01). The secretion was not affected by removing Ca^{2+} from the culture medium with ethylene glycol tetra-acetic acid. Verapamil failed to inhibit the secretion of pepsinogen induced by the drugs in ordinary culture medium containing Ca^{2+} . In contrast, nicorandil (10^{-6} – 10^{-4} mol/l) attenuated the release of pepsinogen by the drugs in a dose dependent manner, regardless of the presence or absence of Ca^{2+} in the culture medium. W-7 (10^{-6} – 10^{-4} mol/l) and W-5 (10^{-5} and 10^{-4} , or 10^{-6} mol/l) reduced significantly the secretion of pepsinogen induced by carbachol (53–71% and 63–81% of control, respectively, $p < 0.05$ – 0.01) and that by CCK-8 (49–67% and 66–76% of control, respectively, $p < 0.01$) in the Ca^{2+} containing medium. However, W-7 did not show significant inhibition of cyclic adenosine monophosphate (cAMP) and forskolin induced pepsinogen secretion. These findings indicate that the calmodulin messenger branch that is activated by a rise of intracellular Ca^{2+} mobilised in cytosol from its intracellular, but not extracellular, source plays a critical role in pepsinogen secretion induced by carbachol and CCK-8. It seems likely that an increase in cAMP in cytosol does not provoke any calmodulin mediated pepsinogen secretion.

Gastric pepsin is an aggressive factor in the development of peptic ulceration as is gastric acid. The mechanism by which gastric acid is secreted by the parietal cells has been investigated extensively by methods based on the established stimulation-receptor coupling concept¹ but how pepsinogen is secreted by the gastric chief cells is less well defined. It was recently shown that carbachol and cholecystokinin octapeptide (CCK-8) are potent secretagogues for total pepsinogen secretion mediated by calcium ions (Ca^{2+}).^{2,3} Since it is unclear whether extracellular or intracellular Ca^{2+} is responsible for the pepsinogen secretion, we examined this using a calcium channel blocker and an intracellular calcium antagonist. In the intracellular calcium messenger systems, the calmodulin branch and the calcium activated protein kinase (C-kinase) branch have generally been recognised as modulators of cell responses.⁴ Calmodulin, a calcium receptor protein universally

present in eukaryotes, forms an active complex with Ca^{2+} , activating enzymes and culminating in physiological cell responses.^{4,5} The role of this messenger branch has been partially defined in pancreatic islet β cells, blood platelets, and smooth muscle,⁴ but its part in the secretion of pepsinogen by the gastric chief cells has not been fully investigated. We have therefore assessed the role of the calmodulin pathway in the calcium dependent secretion of pepsinogen using calmodulin antagonists.

Methods

ORGAN CULTURE TECHNIQUE

Organ culture was performed according to the method previously described.^{6,7} New Zealand albino rabbits weighing 1.5–2.0 kg were fasted overnight and anaesthetised by intravenous injection of 15–20 mg/kg of pentobarbital sodium. The abdomen was opened and the stomach was removed, opened along the greater curvature, and washed in cold saline (0.15 mol/l, pH 6.4). Gastric mucosal specimens (2 mm² including the submucosal layer) were excised from the corpus. Four specimens were placed with their mucosal surfaces upwards on a stainless steel grid in a plastic organ culture dish (Falcon Plastics, USA). The culture medium contained 80% Trowell T-8, 10% National Cancer Institute Tissue Culture (NCTC)-135, and 10% fetal calf serum. Penicillin G was added to the culture medium at a dose of 100 U/ml. In this 'ordinary' medium, the final concentration of total calcium (Ca^{2+}) measured was 1.5 mmol/l. Organ cultures were incubated at 37°C for 60 minutes in a sealed stainless steel jar in 95% O_2 –5% CO_2 at atmospheric pressure. Ca^{2+} free medium used in the drug studies was prepared by chelating Ca^{2+} from the ordinary medium with 0.2 mmol/l ethyleneglycol-bis-[b-aminoethylether]-N,N'-tetra-acetic acid (EGTA).

DRUGS

Drugs used were carbamylcholine chloride (carbachol); CCK-8; forskolin, an activator of adenylate cyclase; 5-[(3,4-dimethoxyphenethyl) methylamino]-2-[3,4-dimethoxyphenyl]-2-isopropylvaleronitrile hydrochloride (verapamil), a calcium channel blocker of cell membrane; N-2-[hydroxyethyl]-nicotinamide nitrate (nicorandil), an intracellular calcium antagonist; dibutyryl cyclic adenosine monophosphate (db-cAMP); N-[6-aminoethyl]-5-chloro-1-naphthalenesulfonamide (W-7), a specific calmodulin antagonist; and N-[6-aminoethyl]-1-naphtha-

First Department of
Medicine, Nagoya City
University Medical
School, Nagoya, Japan
T Miyamoto
M Itoh
Y Noguchi
K Yokochi

Correspondence to:
Dr Itoh, First Department of
Medicine, Nagoya City
University Medical School,
1-Kawasumi Mizuho, Nagoya
467, Japan.

Accepted for publication
13 March 1991

lenesulfonamide (W-5), a chlorine deficient derivative of W-7.

Forskolin and EGTA were dissolved in 0.5% ethanol and 1N NaOH, respectively, and other drugs in saline. The pH of all drug solutions was adjusted to 7.4 with 0.1 N HCl or 0.1 N NaOH. The drugs were then added alone or in combination to the medium of each experiment listed below. Saline served as control in each experiment.

(1) Carbachol (10^{-6} – 10^{-3} mol/l), CCK-8 (10^{-9} – 10^{-6} mol/l) or forskolin (10^{-7} – 10^{-4} mol/l);

(2) Carbachol (10^{-4} mol/l) or CCK-8 (10^{-8} mol/l) plus verapamil (10^{-6} – 10^{-4} mol/l);

(3) Carbachol (10^{-4} mol/l) or CCK-8 (10^{-8} mol/l) plus nicorandil (10^{-6} – 10^{-4} mol/l);

(4) Carbachol (10^{-4} mol/l) or CCK-8 (10^{-8} mol/l) plus W-7 (10^{-6} – 10^{-4} mol/l) or W-5 (10^{-6} – 10^{-4} mol/l);

(5) Forskolin (10^{-5} mol/l) or db-cAMP (10^{-3} mol/l) plus W-7 (10^{-6} – 10^{-4} mol/l).

The carbachol and CCK-8 studies in experiments 1 and 3 were performed in both the Ca^{2+} containing 'ordinary' and the Ca^{2+} free media. In the db-cAMP study the basal secretion of pepsinogen was also examined in adding saline to the medium in place of db-cAMP.

The drugs tested were obtained from the following sources: carbachol, EGTA, W-7, W-5, and db-cAMP – Sigma Chemical, USA; CCK-8 – Peptide Institute, Japan; forskolin – Calbiochemical Co, USA; verapamil – Eisai Pharmaceuticals, Japan; nicorandil – Chugai Pharmaceuticals, Japan; penicillin G – Banyu Pharmaceuticals, Japan; Trowell T-8, NCTC-135, and fetal calf serum – Grand Island Biologic Co, USA.

Dose response in relation to cholecystokinin octapeptide (CCK-8), carbachol, and forskolin stimulation of pepsinogen secretion. In each experiment each value was determined in duplicate and results are given as mean (SEM) from five separate experiments with CCK-8 and carbachol and from six with forskolin. Data are expressed as a percentage of the control value, which was calculated from total pepsinogen content from five to six untreated culture wells. Actual control values of CCK-8, forskolin, and carbachol were 1.34 (0.05), 1.40 (0.10), and 1.95 (0.17) μg tyrosine/mg tissue protein, respectively. Horizontal lines and vertical bars represent 1 SEH. Significantly different from the respective base lines: * $p<0.01$, † $p<0.05$.

MEASUREMENT OF PEPSINOGEN ACTIVITY

Pepsinogen secretion was determined as described previously^{6,7} and expressed as μg tyrosine per mg of tissue protein determined by the method of Lowry.⁸

STATISTICAL ANALYSIS

In each experiment, each value was determined in duplicate, and results were expressed as mean (SEM). The one way analysis of variance (ANOVA) and the Dunnett test were used for comparison of each value with its control value in each experiment except for the db-cAMP study in experiment 5. Multiple comparison of data from the db-cAMP study was performed using ANOVA and the Scheffe test. The Wilcoxon rank sum test was employed for comparison of actual values or increased rates of pepsinogen release obtained by the same concentration of drug from the Ca^{2+} containing medium with those from the Ca^{2+} free medium in the dose response studies for carbachol and CCK-8. Probability values of <0.05 were considered to be significant.

Results

STIMULATION OF PEPSINOGEN RELEASE BY CARBACHOL, CCK-8, AND FORSKOLIN

Carbachol significantly stimulated the secretion of pepsinogen at concentrations of 10^{-4} and 10^{-3} mol/l ($p<0.01$), and CCK-8 at 10^{-8} mol/l ($p<0.05$) and above ($p<0.01$) when compared with controls. Forskolin also significantly enhanced the secretion of pepsinogen at concentrations of 10^{-5} and 10^{-4} mol/l ($p<0.01$) (Figure). Even in the Ca^{2+} free medium containing EGTA, carbachol, and CCK-8, each enhanced pepsinogen secretion in a dose dependent manner. In particular, carbachol at 10^{-4} ($p<0.05$) and 10^{-3} mol/l ($p<0.01$) and CCK-8 at 10^{-8} mol/l and above ($p<0.01$) significantly enhanced the secretion of pepsinogen compared with controls. When the pepsinogen release from the Ca^{2+} containing and Ca^{2+} free media was compared there was no significant difference in the percentage increases induced by the same concentration of drug in either medium in the carbachol and CCK-8 studies (Table I). The actual releases induced by 10^{-4} mol/l and 10^{-3} mol/l of carbachol in the Ca^{2+} containing medium were, however, significantly greater ($p<0.05$ and <0.01 , respectively) than those in the Ca^{2+} free medium. The greater pepsinogen secretion induced by 10^{-4} mol/l of carbachol in the Ca^{2+} containing medium (Table I) compared with values in Tables II–IV may be attributable to the large basal value, which was probably a result of different experimental conditions.

EFFECTS OF VERAPAMIL AND NICORANDIL

Verapamil did not affect the pepsinogen secretion stimulated by either CCK-8 or carbachol (Table II). Nicorandil, however, inhibited the secretion of pepsinogen induced by the drugs in a dose dependent manner, both in the Ca^{2+} containing and Ca^{2+} free media (Table III). In

TABLE I Pepsinogen secretion induced by different concentrations of carbachol and cholecystokinin octapeptide (CCK-8) in Ca²⁺ containing (+) and Ca²⁺ free (-) media. Values, mean (SEM) µg tyrosine/mg tissue protein or % of control value

	Carbachol (mol/l)			
	0	10 ⁻⁵	10 ⁻⁴	10 ⁻³
Ca ²⁺ (+) (n=5)	1.95 (0.17) (100 (9)%)	2.90 (0.36) (149 (17)%)	4.16 (0.38)* (213 (19)%)	4.21 (0.49)* (216 (26)%)
Ca ²⁺ (-) (n=6)	1.44 (0.19) (100 (15)%)	2.18 (0.36) (151 (28)%)	2.72 (0.28)† (189 (21)%)	2.86 (0.28)* (199 (21)%)
	CCK-8 (mol/l)			
	0	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶
Ca ²⁺ (+) (n=5)	1.34 (0.05) (100 (8)%)	1.91 (0.21)† (143 (14)%)	2.96 (0.22)* (221 (15)%)	3.50 (0.32)* (261 (22)%)
Ca ²⁺ (-) (n=6)	1.47 (0.06) (100 (4)%)	2.28 (0.06)* (155 (4)%)	2.56 (0.12)* (174 (8)%)	3.05 (0.25)* (207 (16)%)

n=number of experiments performed to determine each value in each experiment.
*p<0.01, †p<0.05, significantly different from the respective (ANOVA and the Dunnett test). The actual value of pepsinogen secretion induced by 10⁻⁴ and 10⁻³ mol/l of carbachol in the Ca²⁺ (+) medium was significantly greater (p<0.05 and <0.01 respectively) than the value induced by the same concentration of the agent in Ca²⁺ (-) medium. When expressed as percentages, however, there were no significant differences between the increased rates of pepsinogen secretion induced by the same concentration of drug in the Ca²⁺ (+) and Ca²⁺ (-) media in either the carbachol or CCK-8 studies (Wilcoxon rank sum test).

TABLE II Effect of verapamil on pepsinogen secretion induced by carbachol and cholecystokinin octapeptide (CCK-8) in the Ca²⁺ containing (+) medium. Values, mean (SEM) µg tyrosine/mg tissue protein

Secretagogues	Verapamil (mol/l)			
	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
Carbachol (10 ⁻⁴ mol/l) (n=6)	2.13 (0.11) (100%)	1.66 (0.08) (78%)	2.06 (0.13) (97%)	1.08 (0.11) (85%)
CCK-8 (10 ⁻⁸ mol/l) (n=6)	1.89 (0.18) (100%)	1.83 (0.11) (97%)	1.90 (0.17) (101%)	1.85 (0.16) (98%)

n=number of experiments performed to determine each value in each experiment. Percentage of control value calculated from the mean value is given in parentheses. No value shows significant difference from the respective control (ANOVA and the Dunnett test).

TABLE III Effect of nicorandil on pepsinogen secretion induced by carbachol and CCK-8 in the Ca²⁺ containing (+) and Ca²⁺ free (-) media. Values, mean (SEM) µg tyrosine/mg tissue protein

Secretagogues	Medium	Nicorandil (mol/l)			
		0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
Carbachol (10 ⁻⁴ mol/l)	Ca ²⁺ (+) (n=6)	2.10 (0.07) (100%)	1.82 (0.24) (87%)	1.65 (0.13) (79%)	1.18 (0.14)* (56%)
	Ca ²⁺ (-) (n=6)	2.05 (0.19) (100%)	1.73 (0.11) (84%)	1.47 (0.08)* (72%)	1.11 (0.13)* (54%)
CCK-8 (10 ⁻⁸ mol/l)	Ca ²⁺ (+) (n=6)	1.60 (0.12) (100%)	1.15 (0.05)* (72%)	1.10 (0.04)* (69%)	0.88 (0.07)* (55%)
	Ca ²⁺ (-) (n=6)	2.13 (0.22) (100%)	1.89 (0.22) (89%)	1.47 (0.10)* (69%)	1.33 (0.04)* (62%)

n=number of experiments performed to determine each value in each experiment.
Percentage of control value calculated from the mean value is given in parentheses.
*p<0.01 significantly different from the respective controls (ANOVA and the Dunnett test).

TABLE IV Inhibition by calmodulin antagonists N-[6-aminohexyl]-5-chloro-1-naphthalenesulfonamide (W-7) and N-[6-aminohexyl]-1-naphthalenesulfonamide (W-5) of pepsinogen secretion induced by carbachol and CCK-8 in the Ca²⁺ containing ordinary medium. Values, mean (SEM) µg tyrosine/mg tissue

	Calmodulin antagonists (mol/l)			
	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
Carbachol (10 ⁻⁴ mol/l)				
+ W-7 (n=6)	2.33 (0.16) (100%)	1.66 (0.10)* (71%)	1.59 (0.10)* (68%)	1.23 (0.07)* (53%)
+ W-5 (n=6)	2.16 (0.12) (100%)	1.74 (0.14)† (81%)	1.72 (0.03)† (80%)	1.36 (0.10)* (63%)
CCK-8 (10 ⁻⁸ mol/l)				
+ W-7 (n=6)	2.69 (0.19) (100%)	1.79 (0.03)* (67%)	1.62 (0.12)* (60%)	1.33 (0.09)* (49%)
+ W-5 (n=6)	1.66 (0.09) (100%)	1.56 (0.13) (94%)	1.26 (0.06)* (76%)	1.10 (0.07)* (66%)

n=number of experiments performed to determine each value in each experiment.
Percentage of control value calculated from the mean value is given in parentheses.
*p<0.01, †p<0.05 significantly different from the respective controls (ANOVA and the Dunnett test).

the Ca²⁺ containing medium, 10⁻⁴ mol/l of nicorandil significantly reduced pepsinogen secretion induced by carbachol to 56% of control (p<0.01). Concentrations of 10⁻⁶, 10⁻⁵, and 10⁻⁴ mol/l of nicorandil also significantly attenuated the secretion of pepsinogen induced by CCK-8 to 72, 69, and 55% of control, respectively (p<0.01). In the Ca²⁺ free medium, nicorandil at concentrations of 10⁻⁵ and 10⁻⁴ mol/l significantly reduced the amount of pepsinogen secreted in response to carbachol to 72% and 54% of control respectively (p<0.01); the values in response to CCK-8 were 69% and 62% of control, respectively (p<0.01).

EFFECTS OF W-7 AND W-5

Concentrations of W-7 ranging from 10⁻⁶ to 10⁻⁴ mol/l significantly reduced the secretion of pepsinogen stimulated by carbachol and CCK-8 in a dose dependent manner from 71-53% and 67-49% of control values respectively (p<0.01) (Table IV). W-5 at concentrations of 10⁻⁶ (p<0.05), 10⁻⁵ (p<0.05), and 10⁻⁴ mol/l (p<0.01) significantly attenuated the secretion of pepsinogen stimulated by carbachol to 81%, 80%, and 63% of control values respectively. Concentrations of 10⁻⁵ and 10⁻⁴ mol/l of W-5 also reduced significantly the secretion of pepsinogen induced by CCK-8 to 76% and 66% respectively (p<0.01) (Table IV). However, W-7 at the concentrations tested did not show significant inhibition of pepsinogen secretion enhanced by forskolin (Table V). In the db-cAMP study, the basal value obtained by saline added to the medium in place of db-cAMP was 1.17 (0.02) µg tyrosine/mg tissue protein, which was significantly smaller than the value induced by db-cAMP alone (p<0.01) and db-cAMP plus 10⁻⁶ (p<0.01), 10⁻⁵ (p<0.05), and 10⁻⁴ mol/l (p<0.05) of W-7. There were, however, no significant differences between values induced by db-cAMP alone or db-cAMP plus the various concentrations of W-7 examined (Table V).

Discussion

These results show that both carbachol and CCK-8 stimulated the secretion of pepsinogen in the Ca²⁺ containing and Ca²⁺ free media and showed similarly increased rates of release. They indicate that intracellular Ca²⁺ may be primarily responsible for the secretion of pepsinogen induced by the secretagogues. To confirm this, we undertook studies with verapamil and nicorandil. Nicorandil is believed to be an antagonist of the mobilisation of Ca²⁺ bound to intracellular storage sites.⁹⁻¹² In the Ca²⁺ containing medium, verapamil showed no effect on pepsinogen secretion induced by either carbachol or CCK-8, consistent with the observation by Muallem *et al*¹³ that La³⁺ and nifedipine did not affect the release of pepsinogen induced by carbachol. They also reported that these calcium channel blockers did not affect the changes in cytosolic Ca²⁺ induced by carbachol and CCK-8. Thus, the observation in verapamil studies confirms that extracellular Ca²⁺ may not be involved in the carbachol and CCK-8 induced stimulation of pepsinogen release. On the other hand,

TABLE V Effect of N-[6-aminoethyl]-5-chloro-1-naphthalenesulfonamide (W-7) on pepsinogen secretion induced by forskolin and dibutyryl cyclic adenosine monophosphate (db-cAMP). Values, mean (SEM) μ g tyrosine/mg tissue protein

	W-7 (mol/l)			
	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
Forskolin (10 ⁻⁵ mol/l) + W-7 (n=6)	2.34 (0.07) (100%)	2.51 (0.16) (108%)	2.52 (0.13) (108%)	2.13 (0.04) (91%)
db-cAMP (10 ⁻³ mol/l) + W-7 (n=6)	2.15 (0.15) (100%)	2.06 (0.15) (96%)	1.85 (0.17) (86%)	1.76 (0.14) (82%)

n=number of experiments performed to determine each value in each experiment. Percentage of control value calculated from the mean value is given in parentheses. In the forskolin study, no value was significantly different from the control value (ANOVA and the Dunnett test). In the db-cAMP study, the values for db-cAMP alone and for db-cAMP plus 10⁻⁶ (p<0.01), 10⁻⁵, and 10⁻⁴ mol/l (p<0.05) of W-7 were each significantly greater than the basal value (1.17 (0.02) μ g tyrosine/mg tissue protein, (n=6) obtained by adding saline to the medium in place of db-cAMP. However, there were no significant differences between the values induced by db-cAMP alone or db-cAMP plus W-7 (ANOVA and the Scheffe test).

nicorandil significantly inhibited the release of pepsinogen induced by carbachol and CCK-8, regardless of the presence or absence of Ca²⁺ in the medium, suggesting that intracellular Ca²⁺ is primarily responsible for pepsinogen secretion. Carbachol and CCK-8 have been shown to increase the free cytosolic Ca²⁺ content in the chief cells.¹³ In preliminary studies using monolayers of canine chief cells, Sanders *et al*¹⁴ showed that N,N-(diethylamino) octyl 3,4,5-trimethoxybenzoate (TMB-8), a possible inhibitor of intracellular Ca²⁺ mobilisation, reduced the secretion of pepsinogen stimulated by carbachol. Furthermore, it has been shown that Ca²⁺ released from the endoplasmic reticulum⁴ may trigger the initial secretion of pepsinogen when isolated chief cells are stimulated by CCK-8.³ It is thus possible that the cytoplasmic Ca²⁺ mobilised from its intracellular stores may play a critical role in the release of pepsinogen induced by carbachol and CCK-8.

It has generally been recognised that the calcium dependent cell response is biphasic in many tissues, including the gastric chief cells.^{3,4} The initial response, which lasts for several minutes, is believed to be regulated by the calmodulin messenger branch and the subsequent sustained response by the C-kinase branch.⁴ Calmodulin, a biologically inactive receptor protein, is activated by a rapid but transient rise in cytosolic Ca²⁺ after the extracellular messenger interacts with its plasma membrane receptor, and forms an active calcium/calmodulin complex which in turn activates many enzymes resulting in the initial cell response.¹⁵ The calmodulin antagonists, W-7 and W-5, significantly reduced the secretion of pepsinogen induced by carbachol and CCK-8. The evidence on the role of calmodulin in pepsinogen secretion is both limited and controversial. In previous studies,^{14,16} putative calmodulin antagonists such as trifluoperazine (TFP) and chlorpromazine were studied. According to Modlin *et al*,¹⁶ TFP failed to inhibit CCK-8 stimulated pepsinogen release in isolated rabbit gastric glands and, conversely, TFP itself significantly enhanced the secretion of pepsinogen. In contrast, Sanders *et al*¹⁴ observed that chlorpromazine considerably impaired the release of pepsinogen in response to carbachol.

The role of intracellular Ca²⁺ in cholinergic and CCK-8 stimulation has been extensively studied in the secretion of amylase by pancreatic islet cells,¹⁷ whose intracellular control mechanism is similar to that of gastric chief cells.¹⁸ Calmodulin was also shown to mediate the response of pancreatic exocrine cells to secretagogues.¹⁹ W-7 is a potent antagonist which is more selective for calmodulin than such putative calmodulin antagonists as chlorpromazine and prenylamine.^{16,20} Hence, the present study using calmodulin antagonists clearly indicates that the calmodulin branch of the calcium messenger system is an important pathway for pepsinogen secretion in response to carbachol and CCK-8 stimulation. The C-kinase branch is another distinct mechanism responsible for cell response in the calcium messenger system. Cytosolic free Ca²⁺ was shown to be partly involved in this branch in association with raised diacylglycerol after coupling of extracellular stimuli with their receptors.⁴ This mechanism in pepsinogen secretion, however, still remains to be defined.

Forskolin (Figure) and db-cAMP (legend to Table V) each showed a significant stimulation of pepsinogen secretion. The concentration of db-cAMP used has previously been shown to be effective in stimulating pepsinogen release from the rabbit gastric chief cells.⁷ The present results agree with previous observations^{2,18,21} confirming that the cAMP messenger system is involved as another intracellular mechanism in pepsinogen secretion. On the other hand, the secretion of pepsinogen induced by forskolin and db-cAMP was not affected by W-7, suggesting that pepsinogen secretion stimulated by cAMP elevated in cytosol was not augmented by the calmodulin messenger branch. It has recently been indicated that a potentiating interaction for pepsinogen secretion between the calcium and cAMP messenger systems may exist.²² Forskolin was shown to cause large increases in cellular cAMP, stimulating a rise in cellular Ca²⁺ in isolated rabbit gastric glands.¹⁸ A rise in cellular cAMP was also shown to increase the plasma membrane influx of Ca²⁺ via voltage dependent Ca²⁺ channels of mouse pancreatic β cells.²³ In addition, a forskolin induced increase in cellular cAMP was reported to enhance the response of pancreatic islet cells to combined A23187-TPA.²⁴ These previous observations seem to contrast with our finding that cellular cAMP increased by forskolin or exogenous db-cAMP does not influence the calmodulin messenger branch. Since it is not yet clear whether the effect of cAMP on intracellular events can be explained solely by a cAMP dependent increase in cellular Ca²⁺ in the pancreatic islet cells,⁴ the mechanism of the interaction between the cAMP and calcium messenger systems for inducing pepsinogen release should be investigated further. From present results, we conclude that the calmodulin messenger branch, which is activated by a rise in intracellular Ca²⁺ mobilised in cytosol from its intracellular, but not extracellular source, plays a critical role in the secretion of pepsinogen induced by carbachol and CCK-8. It seems probable that an increase in cAMP in cytosol does not provoke any calmodulin mediated pepsinogen secretion.

This study was supported by Grants (Nos 62770485 and 63770465) from the Ministry of Education, Japan.

We thank Professor Toshihiko Takeuchi, Head of our department, for his valuable advice and encouragement.

- Soll AH. Potentiating interactions of gastric stimulants on (^{14}C) aminopyrine accumulation by isolated canine parietal cells. *Gastroenterology* 1982; 83: 216–33.
- Koelz HR, Hersey SJ, Sachs G, Chew CS. Pepsinogen release from isolated gastric glands. *Am J Physiol* 1982; 243: G218–25.
- Tsunoda Y. The cholecystokinin-induced Ca^{2+} shuttle from the inositol triphosphate-sensitive and ATP-dependent pool, and initial pepsinogen release connected with cytoskeleton of the chief cell. *Biochim Biophys Acta* 1987; 901: 35–51.
- Rasmussen H, Barrett PQ. Calcium messenger system: An integrated view. *Physiol Rev* 1984; 64: 938–84.
- Cheung WY. Calmodulin plays a pivotal role in cellular regulation. *Science* 1980; 207: 19–27.
- Itoh M, Noguchi Y, Yokochi K, Kishimoto T, Tomomatsu T, Katsumi K, *et al.* Effect of betazole hydrochloride and cyclic AMP on the pepsinogen secretion by rabbit gastric mucosa in organ culture. *Digestion* 1982; 23: 169–73.
- Noguchi Y, Itoh M, Katsumi K, Yokoyama Y, Yasue N, Miyamoto T, *et al.* Effects of cyclic nucleotides, betazole hydrochloride and acetylcholine on pepsinogen secretion from isolated rabbit gastric mucosa. *Gastroenterol Jpn* 1986; 21: 129–34.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–75.
- Hester RK. Effects of 2-nicotinamidoethyl nitrate on agonist-sensitive Ca^{2+} release and Ca^{2+} entry in rabbit aorta. *J Pharmacol Exp Ther* 1985; 233: 100–11.
- Morimoto S, Koh E, Fukuo K, Imanaka S, Hironaka T, Shiraishi T, *et al.* Effect of nicorandil on the cytosolic free calcium concentration and microsomal (Ca^{2+} + Mg^{2+})-ATPase activity of vascular smooth muscle cells. *J Cardiovasc Pharmacol* 1987; 10 (suppl 8): S31–7.
- Nabata H, Sakai K. Effect of a new antianginal agent, nicrodanil, on normoxic and anoxic contractions in isolated miniature pig coronary arteries exposed to 5-hydroxytryptamine and norepinephrine: comparison with nitroglycerin and diltiazem. *Eur J Pharmacol* 1983; 96: 37–44.
- Imai S, Kitagawa T. A comparison of the differential effects of nitroglycerin, nifedipine and papaverine on contractures induced in vascular and intestinal smooth muscle by potassium and lanthanum. *Jpn J Pharmacol* 1981; 31: 193–9.
- Muallem S, Fimmel CJ, Pandol SJ, Sachs G. Regulation of free cytosolic Ca^{2+} in the peptic and parietal cells of the rabbit gastric gland. *J Biol Chem* 1986; 261: 2660–7.
- Sanders MJ, Amiran DA, Soll AH. Stimulus-secretion coupling of pepsinogen release in canine chief cell monolayers. *Fed Proc* 1983; 42: 591.
- Haiech J, Klee CB, Demaille JG. Effects of cations on affinity of calmodulin for calcium: ordered bindings of calcium ions allows the specific activation of calmodulin-stimulated enzymes. *Biochemistry* 1981; 20: 3890–7.
- Modlin IM, Fratesi GR, Schafer DE, Zdon MJ, Ballantyne GH, Tyshkov M. Paradoxical effect of trifluoperazine, a calmodulin antagonist on pepsinogen secretion. *J Surg Res* 1986; 40: 624–31.
- William JA. Regulation of pancreatic acinar cell function by intracellular calcium. *Am J Physiol* 1980; 238: G269–79.
- Chew CS. Cholecystokinin, carbachol, gastrin, histamine and forskolin increase $[\text{Ca}^{2+}]_i$ in gastric glands. *Am J Physiol* 1986; 250: G814–23.
- Gorelick FS, Cohn JA, Freedman SD, Delahunt NG, Gershoni JM, Jamieson JD. Calmodulin-stimulated protein kinase activity from rat pancreas. *J Cell Biol* 1983; 97: 1294–8.
- Asano M, Suzuki Y, Hidaka H. Effects of various calmodulin antagonists on contraction of rabbit aortic strips. *J Pharmacol Exp Ther* 1982; 220: 191–6.
- Ballantyne G, Schafer DE, Tyshkov M, Zdon MJ, Fratesi GR, Modlin IM, *et al.* Evidence for dual modulation of pepsinogen secretion using isoproterenol, carbachol, CCK-8, forskolin, 8 bromo-cAMP and A23187 probes. *Ann Surg* 1986; 204: 559–65.
- Raufman J-P, Cosowsky L. Interaction between the calcium and adenylate cyclase messenger systems in dispersed chief cells from guinea pig stomach. *J Biol Chem* 1987; 262: 5957–62.
- Henquin JC, Schmeer W, Meissner HP. Forskolin, an activator of adenylyl cyclase, increases Ca^{2+} -dependent electrical activity induced by glucose in mouse pancreatic 3-cell. *Endocrinology* 1983; 112: 2218–20.
- Seamon KB, Daly JW. Forskolin: an unique diterpen activator of cyclic AMP-generating systems. *J Cyclic Nucleotide Res* 1981; 7: 201–24.