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Interaction between Cholecystokinin and Secretin in Isolated Rat Pancreatic Acini

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=ABSTRACT=

A possible potentiation between cholecystokinin (CCK) and secretin in amylase secretion from isolated rat pancreatic acini was investigated. Combined treatment of acini with secretin and CCK at low concentrations, which are known to be physiological, resulted in enzyme secretion larger than the arithmetic sum of their separate effects. Such a potentiating effect also occurred between secretin and A23187 (Ca ionophore), between forskolin (adenylate cyclase activator) and CCK, and between forskolin and A23187. Staurosporin (protein kinase C inhibitor) and W7 (calmodulin antagonist) inhibited markedly the potentiated amylase release induced by the agonists, but KT5720 (protein kinase A inhibitor) did not affect the potentiated amylase release.

Therefore, we concluded that the action of CCK in a physiological concentration is potentiated by secretin in a physiological concentration range and vice versa, and that the intracellular mechanism necessary for the potentiation is associated with Ca^{2+}. However, it is uncertain what mechanisms are involved in potentiation of amylase release after cAMP and Ca^{2+}.

Key Words: Calcium, cAMP, Cholecystokinin, Pancreatic amylase, Potentiation, Secretin

INTRODUCTION

The potentiating effect of the combination of secretin and cholecystokinin (CCK) on pancreatic exocrine secretion has been well known. To demonstrate the interaction between these two peptides on protein secretion, rat has been a good model for study (Folsch & Wormsley, 1973; Peterson & Grossman, 1977; Harrstard & Petersen, 1988; Moriyoshi et al, 1991). However, in many earlier rat studies, the doses of these peptides used were within a pharmacological range (Folsch & Wormsley, 1973; Peterson & Grossman, 1977; Harrstad & Petersen, 1988). Recently, the potentiation of amylase secretion induced by a combination of secretin and CCK in a physiological dose range has been reported in anesthetized rats, and this potentiating action appears to be cholinergic dependent (Moriyoshi et al, 1991). Although Lee (1979) found that these two hormones could potentiate each other's action on amylase secretion in isolated rat pancreatic acinar cells devoid of neural components, it does not seems to be physiological, because of a high concentration, which was nanomolar in range, of secretin used.

Since secretin and cholecystokinin, in a physio-

logical concentration comparable to postprandial plasma level, potentiated exocrine pancreatic secretion in vivo (You et al, 1983; Chey et al, 1984; Moriyoshi et al, 1991). It is important to evaluate whether these peptides in a physiological range that mimics the postprandial plasma level are responsible for the potentiated amylase release from the isolated pancreatic acini without cholinergic influence.

It has been known that the potentiation of enzyme secretion is observed when both intracellular Ca^{2+} and cyclic AMP (cAMP) are increased (Yule & Williams, 1994). Accordingly, the potentiation of pancreatic enzyme secretion has been suggested to occur at later step than these intracellular messengers (Williams et al, 1989; Yule & Williams, 1994). Whereas high doses of secretin and CCK are generally believed to act through cAMP and Ca^{2+} pathway in pancreatic acinar cells respectively, there is no direct evidence that these peptides in a physiological dose affect the cAMP pathway.

In the subsequent study, therefore, we investigated the involvement of protein phosphorylation on the potentiated pancreatic amylase secretion induced by a physiological dose of secretin and CCK.

MATERIALS AND METHODS

Materials

Male Sprague-Dawley rats (average 215 g) were used. The following substances were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.: CCK-8, secretin, A23187, forskolin, KT5720, staurosporin, W7, purified collagenase (type II), and soybean trypsin inhibitor (type IIs)

Tissue preparation

Pancreatic acini were prepared according to the modified method of Peikin et al (1978) and Williams et al (1978). Briefly, a rat was killed by cervical dislocation. The pancreas was removed immediately and trimmed of fat and mesentery, then treated by injection of collagenase solution into the parenchyme. Collagenase solution was prepared by dissolving 60 U/ml pure collagenase and 0.03 mg/ml chymotrypsin in modified Kreb's-Henseleit bicarbonate (KHB) solution containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 14 mM glucose, and 0.1 mg/ml soybean trypsin inhibitor. The pancreatic tissue was incubated in a 50 ml polycarbonate Erlenmeyer flask at 37℃ shaking 120 times per min. After 10 min, the enzyme solution was replaced with 5 ml fresh collagenase solution and incubated for another 40 min. The tissue was then mechanically dissociated by mild shaking and filtered through a 150 μm mesh nylon cloth. The flask was rinsed with an extra 10 ml of KHB solution containing 1% bovine serum albumin (BSA). The suspension of dispersed acini was purified through 4% BSA dissolved in KHB solution by centrifugation (50 g, 4 min). Acini were then resuspended in HEPES solution containing 20 mM HEPES, 120 mM NaCl, 1.2 mM KH_2PO_4, 5.0 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5.0 mM glucose, 0.01% soybean trypsin inhibitor, and 0.2% BSA. The pH of incubation solution was adjusted to 7.4, and equilibrated with 100% O_2. All incubations were performed with 95% O_2-5% CO_2 as the gas phase. The viability of acini was examined with tryphan blue exclusion under the light microscope (Sidel & Johnson, 1983).

Amylase release

One-milliliter aliquot of acini suspension was added to the stoppered polypropylene tube (3.5 ml capacity) containing test agent, flushed with O_2, and incubated for 30 min in a shaking water bath at 37 ℃. The tube was then removed from the bath, and an aliquot of the suspension was centrifuged at 10,000 g for 15 seconds in the microcentrifuge. The supernatant was assayed for amylase activity. When two or three test agents were added to one tube, they were put together into the tube just before acini was added. In each series of experiment, one sample of

the suspension was taken at the beginning of the incubation, centrifuged and the supernatant was collected for determination of 0 time amylase activity. In addition, another sample of the suspension, in which pancreatic acini had been lysed by sonication, was prepared for estimating total amylase activity present in the assay tube. The amount of amylase released from the acini was taken as the difference between the activity present in the supernatant at 0 time and after the incubation, and was expressed as a percentage of the total acinar amylase content. Amylase activity was determined with the procion yellow starch method (Jung, 1980). Absorbance of the dye released into the media was measured using microelisa reader (UV MR 700, Dynatech, Chantilly, VA, U.S.A.) at 410 nm. Dimethyl sulfoxide (DMSO) was used as a solvent for staurosporin, A23187 and KT5720. DMSO alone at the concentration used in the present study had no effect on amylase secretion.

Statistical Analysis

Analysis of variance with repeated measure, regression analysis, and non-paired Student's t test were employed to test for statistical significance of difference. A probability value less than 0.05 was considered statistically significant.

RESULTS

Interaction between pancreatic secretagogues in amylase secretion

Interaction between CCK and secretin: Fig. 1 illustrates the amylase secretory responses to CCK and/or secretin. CCK-8 alone at concentrations of 3, 10, 30 pM stimulated amylase secretion significantly and in a concentration dependent way ($r=0.928$, $P<0.01$). However, secretin at a concentration of 10 pM did not significantly stimulate amylase secretion. Amylase increase induced by secretin and CCK at each concentration was greater than the sum of

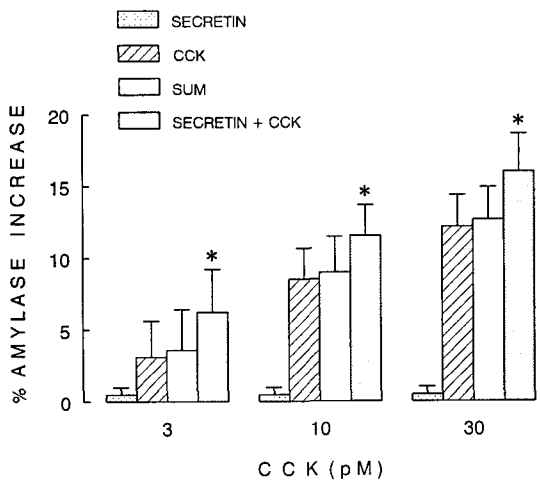

*Fig. 1. Potentiating effects of secretin (10 pM) and CCK (3, 10, 30 pM) on amylase release in rat pancreatic acini (SUM, sum of values obtained by each of two hormones). Data was expressed as mean±SD (n=7). *Comparison with SUM (P<0.01).*

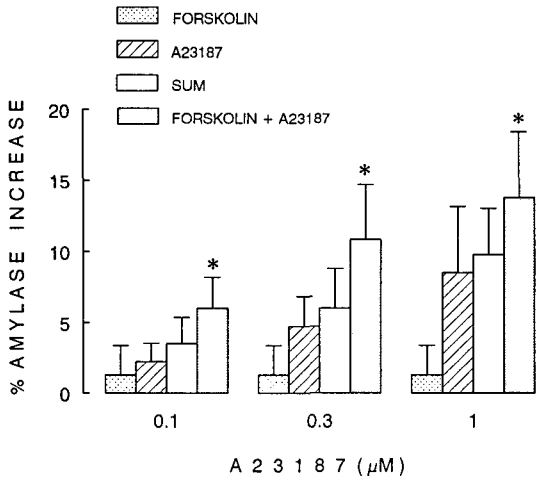

*Fig. 2. Potentiating effects of forskolin (1 μM) and A23187 (0.1, 0.3, 1 μM) on amylase release in rat pancreatic acini (SUM, sum of values obtained by each of forskolin and A23187). Data was expressed as mean ±SD (n=7). *Comparison with SUM (P<0.01).*

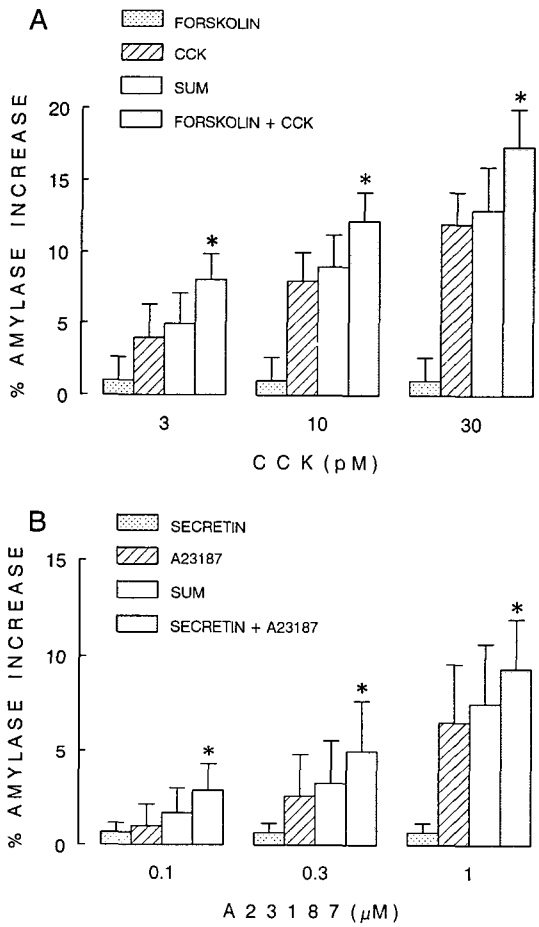

*Fig. 3. A: Potentiating effects of forskolin (1 μM) and CCK (3, 10, 30 pM) on amylase release in rat pancreatic acini (SUM, sum of values obtained by each of forskolin and CCK). B: Potentiating effects of secretin (10 pM) and A23187 (0.1, 0.3, 1 μM) (SUM, sum of values obtained by each of secretin and A23187). Data was expressed as mean±SD (A, n=7: B, n=8). *Comparison with SUM (P <0.01).*

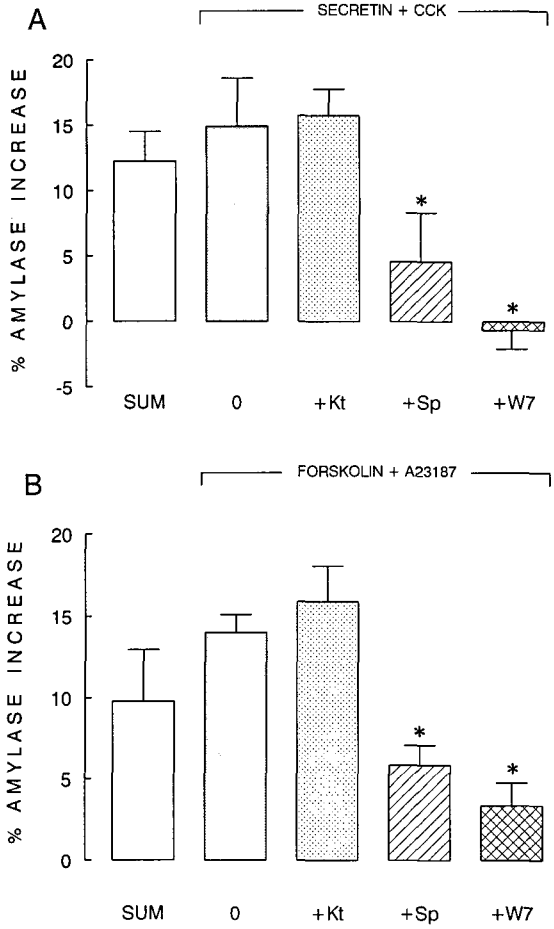

*Fig. 4. Amylase release in response to combination of (A) secretin (10 pM) and CCK (30 pM) or (B) forskolin (1 μM) and A23187 (1 μM). The addition of 1 μM KT5720 (+KT) was without effect while 1 μM staurosporin (+SP) or 100 μM W7 (+W7) significantly inhibited amylase release in response to combinations of the above secretagogues (O). Data was expressed as mean±SD (A, n=5: B, n=4). *Significantly different from the coresponding value induced by the combination of secretin and CCK or forskolin and A23187, respectively (P <0.01).*

amylase increase produced by the two individual hormones.

Interaction between A23187 and forskolin: As shown in Fig. 2, A23187 increased amylase secretion in a concentration-dependently manner (P <0.01). Forskolin (1 μM) alone failed to stimulate amylase secretion significantly. However, amylase increase in response to combination of A23187 and forskolin was greater than the sum of the response to forskolin alone and A23187 alone at each concentration.

Interaction between hormones and intracellular messengers: Potentiating effects for amylase

Table 1. Effects of protein kinase inhibitors on % amylase(mean±SD) increase in response to various secretagogues

	O	KT5720	SP	W7
CCK	14.87±2.08	13.53±2.42	5.27±0.88*	4.25±1.00*
Secretin	1.02±0.55	0.64±0.57	0.67±0.24*	- 0.99±1.06*
A23187	5.23±0.90	3.28±1.20*	1.76±0.84*	2.55±1.13*
Forskolin	3.57±1.10	3.17±1.62	1.77±1.03*	0.08±0.36*

*P<0.05 vs. the corresponding value without protein kinase inhibitors (O). CCK (30 pM), secretin (10 pM), A23187 (1 μM), forskolin (1 μM), KT5720 (1 μM), staurosporin (1 μM, SP) and W7 (100 μM) were used.

secretion were observed between CCK and forskolin as well as between A23187 and secretin (Fig. 3).

Effects of protein kinase inhibitors on amylase secretion in response to combinations of two secretagogues

In Fig. 4, KT5720, a protein kinase A inhibitor (Kase et al, 1987) did not influence the amylase secretion stimulated by either combination of secretin and CCK or combination of forskolin and A23187. However, staurosporin, a protein kinase C inhibitor, and W7, a calmodulin antagonist, significantly inhibited the amylase increase stimulated by either combination of secretin and CCK or combination of forskolin and A23187, respectively ($P < 0.01$).

Effects of protein kinase inhibitors on amylase secretion in response to various secretagogues

Since staurosporin and W7 inhibited the amylase secretion for more than the amount that is attributable to the potentiation between secretin and CCK as well as between forskolin and A23187 in Fig. 4, we tested the effects of these antagonists on each secretagogue-stimulated amylase secretion. As shown in table 1, staurosporin and W7 also inhibited the amylase secretion induced by each secretagogue.

DISCUSSION

Physiologically, pancreatic secretion is stimulated by gastrointestinal hormones including CCK and secretin, as well as by neurotransmitters. Based on the intracellular coupling mechanisms, CCK appears to utilize intracellular Ca^{2+} and diacylglycerol as intracellular messengers and secretin appears to function through alterations in intracellular cAMP levels (Williams et al, 1989). It has been known that when a Ca^{2+}-mediated secretagogue is combined with a cyclic AMP-mediated secretagogue, the enzyme secretory response is greater than the additive response to the two individual secretagogues in the pancreas (Yule & Williams, 1994). The potentiation of enzyme secretion has been believed to occur through postreceptor modulation of the actions of the secretagogues, because agents such as 8 Br-cAMP and A23187 that bypass receptors could potentiate each other's action (Collen et al, 1982). However, the doses of CCK or secretin used in these earlier studies were of the supraphysiological range. Therefore, it has not been known if secretin and CCK in a physiological range could potentiate each other's secretory activity. As shown in Fig. 1, secretin and CCK in low concentrations clearly potentiated each other's action on amylase secretion of the isolated rat pancreatic acini. And the concentrations of secretin and CCK used in this study were comparable to the postprandial plasma concentrations (Chey et al, 1978; Kim et al, 1979; Byrnes et al, 1981). Even though we have not measured cAMP level, it is unlikely that cAMP level was changed in response to secretin used, since secretin at a

concentration of 10 pM failed to increase amylase release in this study and it has been already reported that minimal secretin concentration for a rise in cAMP was 100 pM in rat pancreatic acini (Trimble et al, 1986).

CCK stimulates enzyme secretion by stimulating the breakdown of polyphosphoinositides, increasing intracellular diacylglycerol, and mobilizing intracellular cellular calcium (Matozaki & Williams, 1989). However, it has been reported that CCK at a concentration of 10 pM stimulates enzyme secretion with minimal calcium mobilization in pancreatic acinar cells (Rowley et al, 1990). Accordingly, doses of CCK we employed in the present experiment might have increased the intracellular calcium. The results are consistent with other's report that low concentration of CCK was reported to increase intracellular Ca^{2+} (Tsunda et al, 1990). Thus, it appears that the mechanism by which the low concentrations of secretin and CCK potentiate the pancreatic enzyme secretion involves an intracellular Ca^{2+} pathway.

In the present study, there has been a potentiation in the enzyme secretion induced by combination of forskolin and A23187. Since forskolin is an activator of adenylate cyclase (Heisler, 1983) and A23187 is a divalent cation ionophore (Reed & Lardy, 1972; Eimerl et al, 1974), the potentiated amylase secretion seems to be attributable to the actions of cAMP and calcium in the pancreatic acinar cells. Furthermore, the potentiation also occurred between secretin and A23187 as well as between forskolin and CCK in amylase secretion in this study. These results suggest that both cAMP and Ca^{2+} pathway can interact to potentiate the enzyme secretion. However, in the potentiation induced by low concentrations of secretin and CCK, involvement of cAMP pathway is uncertain. Nevertheless, the possible involvement of cAMP can not be ruled out, since current methods for measuring cAMP are not known to be sensitive enough to detect physiological small increases in cells (Marino et al, 1993) and the potentiation that occurred between A23187 and secretin. The involvement of cAMP in the potentiation remains to be verified.

KT5720 is one of K-252 compounds and a selective cAMP-dependent protein kinase inhibitor (Kase et al, 1987). As shown in Fig. 4, KT5720 had no effect on enzyme secretion in response to a combination of secretin and CCK or of forskolin and A23187. However, A23187-induced amylase secretion was inhibited significantly by KT5720, and the amylase release, which induced by combination of A23187 and forskolin, was not affected. These results point to a certain role of Protein Kinase A in pancreatic enzyme secretion potentiated by the combination of forskolin and A23187. The results that amylase secretion induced by secretin and/or CCK was not influenced by KT5720 in this study indicate that the potentiating mechanism of secretin and CCK may be different from that of forskolin and A23187.

On the other hand, both staurosporin and W7 inhibited the enzyme secretion potentiated by the combination of secretin and CCK or forskolin and A23187. Staurosporin and W7 inhibited the enzyme secretion stimulated by 4 secretagogues as well. Although staurosporin and W7 have been known to be Protein Kinase C inhibitors (Tamaoki et al, 1986) and calmodulin antagonists (Hidaka et al, 1981) respectively, nonspecific properties of these protein kinase inhibitors have hampered interpreting the biological effects of these agents (Ruegg & Burgess, 1989; Ederveen et al, 1990). Therefore, it is unclear at the present time if Protein Kinase C and Ca^{2+}-calmodulin dependent kinase is involved in potentiation of pancreatic enzyme secretion. Participation of the protein kinases in potentiation of pancreatic enzyme secretion should be illucidated proved by measuring intracellular levels of the enzyme in future studies.

Thus, from the results of our present study, it is concluded that secretin and CCK at a physiological concentration could interact to potentiate each

other's action on pancreatic enzyme secretion, and the intracellular mechanism for the potentiation is associated with intracellular Ca^{2+} change.

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