Cellular mechanism of sodium oleate-stimulated secretion of cholecystokinin and secretin

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Chang, Cecilia H., William Y. Chey, and Ta-Min Chang. Cellular mechanism of sodium oleate-stimulated secretion of cholecystokinin and secretin. Am J Physiol Gastrointest Liver Physiol 279: G295–G303, 2000.—Long-chain fatty acids are potent stimulants of secretin and CCK release. The cellular mechanisms of fatty acid-stimulated secretion of these two hormones are not clear. We studied the stimulatory effect and mechanism of sodium oleate (SO) on secretin- and CCK-producing cells. SO stimulated the release of secretin or CCK from isolated rat mucosal cell preparations enriched in either secretin- or CCK-produced cells, respectively. SO also time- and dose-dependently stimulated secretin and CCK release from STC-1 cells. In STC-1 cells, SO-stimulated secretin and CCK release was potentiated by IBMX and inhibited by a protein kinase A-selective inhibitor and a CAMPS-specific antagonist. SO-stimulated releases of the two hormones were also inhibited by downregulation or inhibitors of protein kinase C, a calmodulin antagonist and an inhibitor of calmodulin-dependent protein kinase II. Chelating of extracellular Ca$^{2+}$ or addition of an L-type calcium channel blocker diminished SO-stimulated hormone releases. SO caused an increase in intracellular Ca$^{2+}$ concentration that was reversed by diltiazem but had no effect on production of cAMP, cGMP, or inositol-1,4,5-trisphosphate. These results indicate that SO acts on secretin- and CCK-producing cells. Its stimulatory effect is potentiated by endogenous protein kinase A and mediated by activation of Ca$^{2+}$ influx through the L-type channels and of protein kinase C and Ca$^{2+}$/calmodulin-dependent protein kinase II.

CCK and secretin are two classic gut hormones that regulate pancreatic exocrine secretion, gastric acid secretion, and gastric emptying (10, 28, 46, 49). In addition, CCK also stimulates gallbladder contraction and suppresses food intake (46, 49). These two hormones are produced in the corresponding endocrine cells in the upper small intestinal mucosa. On entry of gastric chyme, the endocrine cells are stimulated to release the two hormones to exert their digestive function. The release of CCK is stimulated by protein and digested products of protein and fat, including amino acids and fatty acids (46, 49). The release of secretin is stimulated by gastric acid and digested products of protein and fat (10, 28). In addition, several pharmacological agents derived from the plant kingdom and camostate (a synthetic protease inhibitor) also stimulate the release of secretin (10, 28). Camostate also stimulates the release of CCK (7, 26).

Recent studies have demonstrated that the release of CCK and secretin are subject to feedback inhibition by pancreatic juice in both fasting (16, 35, 52, 58) and digestive states (15, 24, 25, 31, 34, 48). Indeed, two CCK-releasing factors, luminal CCK-releasing factor (LCRF) and diazepam-binding inhibitor (DBI), have been isolated and shown to stimulate the release of CCK in rats (21, 57). Secretin-releasing factor appears to be present in duodenal acid perfusates of both rats (30) and dogs (32) and in canine pancreatic juice (33). The secretin-releasing factor isolated from canine pancreatic juice appears to be related to pancreatic phospholipase A$_2$ (PLA$_2$) (8). Recently, we (29) have shown that pancreatic PLA$_2$ mediates secretin release in response to duodenal acidification.

It is not clear at present whether the releasing factors mediate the release of CCK or secretin stimulated by all luminal stimulants, particularly amino acids and fatty acids that are absorbed from the intestinal lumen. We undertook the current study to determine if fatty acid (sodium oleate; SO) can directly stimulate CCK- and secretin-producing cells. We discovered that SO exerted a direct stimulatory action on secretin- and CCK-producing cells and explored the cellular mechanism involved in the release of the two hormones.

MATERIALS AND METHODS

Oleic acid was obtained from Fisher Scientific (Pittsburgh, PA). SO solution was prepared fresh daily by dissolving oleic acid in an equivalent amount of diluted NaOH to make a stock solution of 100 mM with a final pH of 9.0. This solution was diluted in bioassay medium before use. Diltiazem, IBMX, and 4$
u$-12-O-tetradecanoylphorbol 13-acetate (8-TPA) were purchased from Sigma Chemical (St. Louis, MO). Stau...
SODIUM OLEATE-STIMULATED CCK AND SECRETIN RELEASE

Roserpine, 1-(N,O-bis(5-isouquinoline-sulfonyl)-N-methyl-L-tyrosyl)-4-phenyl-piperazine (KN-62), calmidazolium chloride, and bisindolylmaleimide I were obtained from Calbiochem (La Jolla, CA). Fura 2-AM was obtained from Molecular Probes (Eugene, OR). All tissue culture medium and ware were obtained as described previously (4). RIA kits for cAMP and cGMP were obtained from Biomedical Technologies (Stoughton, MA). The radioreceptor assay kit for determination of inositol 1,4,5-trisphosphate (IP₃) was purchased from DuPont-NEN (Boston, MA).

Cell culture and preparation of S and I cell-enriched fraction from rat duodenal mucosa. STC-1 cells were maintained in monolayer cultures as described previously (4, 6, 7). The contents of secretin-like immunoreactivity and CCK-like immunoreactivity in these cells were 12.5 ± 0.3 (n = 96) and 13.3 ± 0.5 pmol/mg cell protein (n = 96), respectively. Mucosal cells enriched in S cells or I (CCK) cells were isolated from rat duodenal mucosa by collagenase-EDTA digestion and Percoll density gradient centrifugation as described previously (4, 6, 7). Unless otherwise indicated, the data were calculated as femtomoles of secretin-like or CCK-like immunoreactivity released per milligram of cell protein and compared with the corresponding controls without addition of SO.

Study of secretion of secretin or CCK from STC-1 cells. The release of secretin or CCK was studied in monolayer cultures of STC-1 cells as described previously (4, 6, 7). Briefly, monolayers of STC-1 cells were incubated in the presence or absence of SO and other agents in Earle’s balanced salt solution containing 10 mM HEPES, pH 7.4, 5 mM sodium pyruvate, 2 mM l-glutamine, 0.01% soybean trypsin inhibitor, and 0.2% BSA under 95% air-5% CO₂ at 37°C for 60 min or various time periods as specified. After incubation, the plate was chilled on ice, and aliquots of 0.1 ml of the medium were removed for RIA of secretin or CCK as described previously (4, 6, 7). The cells were washed with Dulbecco’s PBS and extracted and assayed for cellular protein and contents of secretin and CCK as described previously (4, 6, 7). Unless otherwise indicated, the data were calculated as femtomoles of secretin-like or CCK-like immunoreactivity released per milligram of cell protein and compared with the corresponding controls.

Study of secretion of secretin or CCK from S or I cell-enriched preparations. The release of secretin and CCK from S and I cell-enriched mucosal cell preparations, respectively, was carried out as described previously (6). Briefly, rat S or I cell-enriched preparation obtained from Percoll density gradient was centrifuged at 500 g for 5 min at room temperature. The pelletted cells were washed once by resuspension in Hanks’ balanced salt solution containing 10 mM HEPES, pH 7.4, 5 mM sodium pyruvate, 2 mM l-glutamine, 0.01% soybean trypsin inhibitor, and 0.2% BSA and centrifuged again. The cells were resuspended at 0.5–1.0 × 10⁶ cells/ml in the Hanks’ balanced salt solution described above and used immediately for release study. The cell suspension was incubated in a water bath for 30 min. The cell suspension was then centrifuged at 500 g for 5 min at 4°C, and an aliquot of the supernatant solution was removed for assay of secretin or CCK. The cell pellet was extracted with 0.1 N HCl. The extract was lyophilized and assayed for cellular content of secretin or CCK. The data were calculated as the percentage of total secretin-like or CCK-like immunoreactivity (medium + cell content) being released to the medium and compared with the corresponding controls.

Measurement of intracellular cAMP and cGMP. Intracellular cAMP content was measured by a specific RIA after extraction and acetylation using a commercially available assay kit as described previously (4, 6). Intracellular cGMP content was measured, also after extraction and acetylation, by RIA using a commercial kit (Biomedical Technologies).

Measurement of intracellular IP₃. Production of IP₃ in STC-1 cells was determined as described previously (6), using a radioreceptor assay kit according to the manufacturer’s suggested procedure.

Measurement of intracellular Ca²⁺ concentration. Intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) was measured fluorometrically in fura 2-AM-preloaded STC-1 cells. Monolayer cultures of STC-1 cells in 162-cm² tissue culture flasks (Costar 3150) were rinsed twice with 10 ml of Dulbecco’s PBS without Ca²⁺ and Mg²⁺ (DPBS). The cells were incubated with 10 ml of nonenzymatic dissociation medium (Sigma Chemical) at room temperature for 10 min. The cells, while still attached to the flask, were gently rinsed twice with DPBS and then suspended in DMEM. The cells were incubated with 5 μM fura 2-AM in the presence of 0.03% pluronic F-127 at room temperature for 30 min in the dark. After being washed twice with DPBS, the cells were resuspended in Hanks’ balanced salt solution containing 10 mM HEPES, pH 7.4, and 5 mM CaCl₂ at a concentration of 10⁶ cells/ml and placed in a cuvette cell with continuous stirring. After baseline stabilization, SO (0.25 mM) was added, and the fluorescence change in response to SO was monitored in a Perkin-Elmer LS50 spectrophotofluorometer with excitation at 340 nm and emission at 510 nm. The level of [Ca²⁺]ᵢ was calculated according to the method of Grynkiewicz et al. (17): [Ca²⁺]ᵢ = Fᵢ − Fₘᵢₐₓ(Fᵢ − Fₘᵢₐₓ) in which Kᵢ is the effective dissociation constant of fura 2-Ca²⁺ complex, Fₘᵢₐₓ is the maximum fluorescence intensity of fura 2-preloaded cells, Fᵢ is the maximum fluorescence intensity measured after complete displacement of Ca²⁺ from fura 2 with 10 mM MgSO₄, Aᵢ is the value of 224 nM (17) was used without further calibration so that the calculated [Ca²⁺]ᵢ values represented the relative cellular concentrations of Ca²⁺ before and after SO stimulation.

Analysis of data. All data are expressed as means ± SE. Statistical analyses were performed by using ANOVA for single-factor experiments with multiple treatments, followed by Dunnett’s post hoc analysis for comparing treatment means with the same control, using a Systat software program (Evanston, IL) as described previously (4, 6). When only

Table 1. Distribution of CCK-containing cells in a discontinuous Percoll density gradient

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total No. of Cells</th>
<th>Viability, %</th>
<th>CCK Content, pmol/10⁶ cells</th>
<th>Enrichment Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction 1</td>
<td>75 ± 4 × 10⁶</td>
<td>94.6 ± 0.4</td>
<td>0.75 ± 0.09</td>
<td>1.8</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>3.7 ± 0.1 × 10⁶</td>
<td>68.2 ± 2.1</td>
<td>1.84 ± 0.18</td>
<td>4.2</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>5.8 ± 0.2 × 10⁶</td>
<td>87.2 ± 1.2</td>
<td>2.54 ± 0.18</td>
<td>5.8</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>2.0 ± 0.2 × 10⁶</td>
<td>95.0 ± 0.4</td>
<td>0.04 ± 0.02</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE from 4 experiments. The mucosal cells were isolated from rat duodenal mucosa and fractionated by centrifugation in a discontinuous Percoll density gradient as described in MATERIALS AND METHODS. Fractions 1–3 were collected at the interfaces of 9%/24%, 24%/34%, and 34%/55% Percoll, respectively. Fraction 4 was collected as a pellet in 55% Percoll. Cell viability was determined by the trypan blue exclusion method. The CCK content was determined by RIA after extraction with 0.1 N HCl and lyophilized.
two means were compared, the data were analyzed by Student’s paired t-test. \( P < 0.05 \) between two means was regarded as significant.

RESULTS

Time-dependent stimulation of secretin and CCK release by SO. SO time dependently stimulated the release of both CCK and secretin from STC-1 cells. As shown in Fig. 1, SO at 0.25 mM stimulated secretin release continuously for 60 min and then leveled off subsequently. At 60 min, SO-stimulated secretin release reached an average of 6.85 \( \pm 0.47\% \) \((n = 4)\) of total secretin-like immunoreactivity content that corresponded to a fivefold increase over the basal secretion \((1.35 \pm 0.11\% \) of the cellular content, \( n = 4)\). SO also stimulated the release of CCK for 30 min and then leveled off. A maximal stimulation of 4.90 \( \pm 0.47\% \) \((n = 4)\) of total CCK-like immunoreactivity content was attained at 60 min. However, SO-stimulated CCK release reached a modest average of a 2.5-fold increase over the basal secretion \((1.91 \pm 0.30\% \) of total CCK content, \( n = 4)\).

Dose-dependent stimulation of secretin and CCK release by SO. The stimulatory effect of SO on secretin and CCK release was also concentration dependent. As shown in Fig. 2, SO at 0.1 mM or lower did not stimulate the release of both hormones over a period of 1 h. However, SO at 0.2 mM or higher concentration significantly stimulated the release of both hormones. A maximal effect of a ninefold increase in secretin release and a twofold increase in CCK secretion was attained at the dose of 0.3 mM. There were some variations in SO-stimulated secretion, particularly in the release of secretin. For example, stimulation of secretin release by 0.25 mM SO varied between 400 and 600\%. This was probably due to variations in both the secretin content and the basal release among the cell cultures used. Because the stimulation of hormone release by SO at 0.3 mM was highly significant, we did not test its effect at a higher concentration. However, it should be noted that at the concentrations of SO tested there was no apparent change in cell integrity as monitored by trypan blue inclusion (data not shown).

Stimulation of secretin and CCK release from mucosal endocrine cell-enriched fractions. SO \((0.2 \text{ mM})\) significantly stimulated the release of secretin from S cell-enriched mucosal cell preparations \((1.41 \pm 0.04 \text{ pmol secretin-like immunoreactivity/}10^6 \text{ cells}, n = 5)\), increasing from 3.7 \( \pm 0.7\% \) of cellular secretin content in control cells to 6.3 \( \pm 1.3\% \) in SO-treated cells \((P < 0.01, n = 5)\). The same concentration of SO also stimulated CCK release from I cell-enriched preparation \((2.54 \pm 0.18 \text{ pmol CCK-like immunoreactivity/}10^6 \text{ cells}, \text{Table 1})\) with an increase from the control level of 3.1 \( \pm 0.6\% \) to 5.9 \( \pm 1.1\% \) \((P < 0.01, n = 5)\).

Effect of IBMX and H-89 on SO-stimulated hormone secretion. When STC-1 cells were incubated with SO \((0.25 \text{ mM})\) in the presence of IBMX \((0.25 \text{ mM})\), the release of both secretin and CCK was greatly enhanced. In both cases, SO and IBMX together produced a significantly greater effect than the sum of the effects of the two agents when they were incubated alone with the cells. However, SO or IBMX alone or together did not increase cellular cAMP or cGMP production (data not shown). On the other hand, cellular cAMP content was increased from a basal level of 17.6 \( \pm 0.1\) to 36.4 \( \pm 4.1\) and 172.6 \( \pm 30.2 \text{ pmol/mg}\)
cell protein in the absence and presence of IBMX, respectively, after incubation for 10 min with 30 μM forskolin \((n = 4)\).

The stimulatory effect of SO on the release of secretin (Fig. 4A) and CCK (Fig. 4B) was reduced by 27.8 ± 5.3% and 30.1 ± 3.5%, respectively, when the cells were incubated with SO in the presence of the protein kinase A (PKA)-selective inhibitor N-[2-(p-bromocinnamylamino)-ethyl]-5-isouquinolinesulfonamide (H-89) (2 μM). This appeared to be the maximum effect of H-89 because increasing the concentration of the inhibitor to 4 μM did not increase its inhibition of the release of the two hormones (data not shown). Similarly, SO-stimulated secretin and CCK release was inhibited by 26.9 ± 1.8% and 18.9 ± 7.3%, respectively \((n = 4)\), when the incubation was carried out in the presence of the cAMP-specific antagonist Rp-cAMPS (100 μM). The same concentration of Rp-cAMPS also inhibited forskolin (30 μM)-stimulated releases of secretin (62.7 ± 7.4% over basal, \(n = 4\)) and CCK (94.2 ± 11.4% over basal, \(n = 4\)) by 78.1 ± 2.8% and 57.8 ± 0.6%, respectively. As shown previously, Rp-cAMPS also inhibited pituitary adenylate cyclase-activating polypeptide (PACAP)-stimulated secretion of secretin from STC-1 cells by 62% at the same concentration (6).

**Effect of PKC downregulation and PKC inhibitors on SO-stimulated hormone release.** Downregulation of PKC by pretreatment of STC-1 cells with β-TPA (0.1 μM) for 10 h or incubation of STC-1 cells with SO in the presence of a PKC inhibitor also reduced SO-stimulated releases of secretin and CCK. As shown in Fig. 5, downregulation of PKC resulted in 53.6 ± 9.7% reduction in secretin (Fig. 5A) and 71.0 ± 4.3% in CCK (Fig. 5B) release stimulated by SO. It should be noted that downregulation of PKC reduced β-TPA (0.1 μM)-stimulated secretion of the two hormones by >90% (data not shown). The PKC inhibitors staurosporine (0.1 μM) and bisindolylmaleimide I (0.1 μM) also inhibited SO-stimulated secretin release by 68.2 ± 13.7% and 52.4 ± 5.1%, respectively (Fig. 5A). Similarly, SO-stimulated CCK release was inhibited 72.2 ± 7.6% and 39.5 ± 13.3% by staurosporine and bisindolylmaleimide I, respectively (Fig. 5B). It should be mentioned that staurosporine at 0.1 μM similarly inhibited TPA (0.1 μM)-stimulated release of secretin (789.5 ± 23.9% over basal, \(n = 4\)) and CCK (519.3 ± 36.3% over basal, \(n = 4\)) by 52.7 ± 5.1% and 50.3 ± 3.6%, respectively. Although the above results suggested that SO activated PKC, SO did not stimulate accumulation of IP3 in STC-1 cells up to 2 h (data not shown). On the other hand, we were able to detect bombesin (5 nM)-stimulated IP3 production in STC-1 cells from basal levels of 5.9 ± 1.8 and 7.5 ± 2.8 pmol/mg cell protein to 58.2 ± 4.8 and 64.9 ± 7.1 pmol/mg cell protein at 15 and 30 s, respectively \((n = 4)\).
Effect of EGTA and L-type Ca\(^{2+}\) channel blocker on SO-stimulated hormone release. Chelation of extracellular Ca\(^{2+}\) with EGTA (1 mM) or addition of an L-type Ca\(^{2+}\) channel blocker, diltiazem (10 μM), also inhibited SO-stimulated hormone secretion. As shown in Fig. 6, incubation of STC-1 cells with SO in the presence of EGTA resulted in 79.7 ± 3.2% and 90 ± 9.0% reduction, respectively, in the release of secretin (Fig. 6A) and CCK (Fig. 6B). Incubation of STC-1 cells with SO in the presence of diltiazem also reduced the release of secretin and CCK by 35.9 ± 4.2% and 64.5 ± 4.7%, respectively. On the other hand, incubation of the cells with a K\(^+\) channel opener, diazoxide (0.1 mM), resulted in only a small inhibition (~20%) of SO-stimulated secretin and CCK release (data not shown).

Effect of calmodulin antagonist and Ca\(^{2+}\)/calmodulin-dependent protein kinase II inhibitor on SO-stimulated hormone release. Incubation of STC-1 cells in the presence of a calmodulin antagonist, calmidazolium chloride, and an inhibitor of Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII), KN-62, also inhibited SO-stimulated release of both hormones. Thus as shown in Fig. 7, incubation of STC-1 cells with SO in the presence of 15 μM calmidazolium chloride inhibited SO-stimulated release of secretin (Fig. 7A) and CCK (Fig. 7B) by 72.9 ± 8.3% and 71.7 ± 9.3%, respectively. This effect appeared to be maximal, as this inhibitor at 1.5 μM produced similar results (data not shown). Similarly, incubation of the cells with SO in the presence of 1 μM KN-62 inhibited SO-stimulated release of secretin and CCK by 57.5 ± 6.2% and 45 ± 8.9%, respectively.

Effect of SO on [Ca\(^{2+}\)]\(_i\). SO significantly increased [Ca\(^{2+}\)]\(_i\), as indicated by an increase in fluorescence in...
fura 2-loaded STC-1 cells. As shown in Fig. 8, inset, addition of SO to STC-1 cells resulted in a time-dependent increase in \([\text{Ca}^{2+}]_i\) in fura 2-preloaded cells. The increase in \([\text{Ca}^{2+}]_i\) was partially reversed by addition of diltiazem (10 μM). As shown in Fig. 8, the calculated \([\text{Ca}^{2+}]_i\) was increased from a basal level of 157.7 ± 12.2 to 353.6 ± 36.0 nM 10 min after SO addition. The stimulated level of \([\text{Ca}^{2+}]_i\) was reduced by diltiazem to 230.8 ± 28.0 nM (n = 7), corresponding to an average decrease of 62.8%.

**DISCUSSION**

The results of the present study indicate that SO stimulates secretin and CCK release from endocrine-enriched mucosal cell preparation isolated from rat duodenum and STC-1 cells. The stimulatory effect of SO in STC-1 cells appeared to involve an increased entry of \(\text{Ca}^{2+}\) that was in part mediated by L-type \(\text{Ca}^{2+}\) channels, since SO-stimulated release of the two hormones was substantially inhibited by both EGTA and diltiazem. The observation that SO stimulated the increase of \([\text{Ca}^{2+}]_i\) that was partially abolished by diltiazem further supports this conclusion. McLaughlin et al. (41) have also reported that medium chain (C-12) fatty acid-stimulated CCK release from STC-1 cells is \(\text{Ca}^{2+}\) dependent. The effect of SO also appears to be mediated through activation of PKC and CaMKII as inhibitors or antagonists of these enzymes and downregulation of PKC both reduced the stimulatory effect of SO profoundly.

The mechanisms through which SO stimulated elevation of \([\text{Ca}^{2+}]_i\), and activation of PKC and CaMKII are not clear at present. Long-chain unsaturated fatty acids are known to stimulate \(\text{Ca}^{2+}\) influx in various cell types (22, 45, 54). Their effect in ventricular myocytes was not mediated through activation of PKA, PKC, orPKG (22). On the other hand, oleic acid-stimulated cell proliferation of rat aortic smooth cells in primary cultures was mediated through activation of PKC (36). In isolated rat hepatocytes, oleate was shown to stimulate translocation of PKC to cellular membranes and elevation of \([\text{Ca}^{2+}]_i\) (12). Thus activation of PKC and elevation of \([\text{Ca}^{2+}]_i\) by SO in STC-1 cells could occur concurrently or separately. It has been shown that unsaturated fatty acids are capable of stimulating cytosolic PKC (27) and activating PKC synergistically.
with diacylglycerol in the presence of phospholipid, leading to a Ca\(^{2+}\)-independent enzyme of high specific activity (51). Moreover, \(^3\)H-labeled SO added to platelets was found to partition between the cytosolic (30%) and the membrane (70%) compartments (27). In isolated rat hepatocytes (12), the increased membrane translocation of PKC elicited by oleate was dependent on extracellular Ca\(^{2+}\), formation of diacylglycerol from oleate, and oleate itself and was not associated with any increase in IP\(_3\) formation. If a similar partitioning of SO occurs in hepatocytes, then it is possible that oleate partitioned in the plasma membrane may activate Ca\(^{2+}\) channels and provide the source of diacylglycerol for translocation of PKC, whereas those partitioned in the cytosol would activate the enzyme or might act synergistically with diacylglycerol to activate the membrane-bound enzyme. A similar mechanism may occur in SO-stimulated STC-1 cells, as we were also unable to detect elevation of IP\(_3\) in these cells. It is also not clear how CaM_KII is activated by SO in STC-1 cells. It is possible that stimulation of Ca\(^{2+}\) influx by SO elicited binding of Ca\(^{2+}\) to calmodulin and hence activation of the enzyme. CaM_KII has been shown to be involved in insulin secretion from pancreatic islets (13, 47) and found to be associated with insulin secretion vesicles in insulinoma cell lines (44) and thus may be involved in modulation of the exocytotic machinery. Indeed, this enzyme appears to be involved in Ca\(^{2+}\) influx and catecholamine secretion from bovine adrenal chromaffin cells (40). On the other hand, CaM_KII has been reported to mediate IBMX-elicited activation of Ca\(^{2+}\) channels in the STC-1 cell (2). Because CaM_KII is activated by autophosphorylation after binding to Ca\(^{2+}\)-calmodulin and inactivated by dephosphorylation (11), the effect of IBMX on Ca\(^{2+}\) channels might be mediated through a cAMP-dependent inactivation of protein phosphatase 1 (PP1), which dephosphorylates and inactivates CaM_KII, as seen in the brain during long-term potentiation (3). The involvement of PP1 in regulation of CaM_KII activity has also been observed in pancreatic acini (23) and \(\beta\)-TC-3 insulinoma cells (14). It should be pointed out that the role of calmodulin in SO-stimulated hormone secretion might not be limited to activation of CaM_KII. For example, calmodulin and PKC were shown to stimulate the release of norepinephrine from mechanically permeabilized PC12 cells. Stimulation by calmodulin was Ca\(^{2+}\)-dependent but independent of ATP; whereas the effect of PKC was ATP dependent (9). Thus PKC and calmodulin appear to participate in Mg-ATP priming and Ca\(^{2+}\)-dependent secretion steps, respectively, during regulatory exocytosis (19, 20) and could participate in SO-stimulated secretion in a similar fashion. Nevertheless, the relationships among the two effector enzymes and [Ca\(^{2+}\)]\(_{cyt}\) during SO-stimulated hormone secretion remain to be elucidated.

SO-stimulated hormone secretion also appeared to be modulated by an endogenous cyclic nucleotide-dependent protein kinase(s). Although SO did not stimulate production of cAMP or cGMP, its stimulatory effect was enhanced by IBMX, thereby suggesting that a cyclic nucleotide might be involved. However, we were unable to detect elevation of cAMP by IBMX. The lack of elevation of cAMP by IBMX in STC-1 cells in the basal state was also found in previous studies (4, 6, 50, 59). As reported previously (4, 6), we were unable to observe elevation of cAMP by PACAP and vasoactive intestinal polypeptide (VIP) in STC-1 cells unless IBMX was present. It was possible that, due to a low basal adenylate cyclase activity and continuous secretion of cAMP, the cell in the basal state was unable to maintain a significant elevation of intracellular cAMP in the presence of IBMX. Thus the lack of elevation of intracellular cAMP in the presence of IBMX or SO could not be attributed to a lack of sensitivity of the cAMP RIA we employed. It should be noted that although IBMX did not elevate the global level of intracellular cAMP, the local concentration of the nucleotide might be elevated at or near the plasma membrane where the endogenous adenylate cyclase is located. Consequently, a plasma membrane-associated PKA might be activated and exert its effect on a downstream substrate such as PP1 mentioned above. The observation that SO-stimulated hormone secretion was partially inhibited by the specific cAMP antagonist Rp-cAMPS and the PKA-selective inhibitor H-89 appears to support this argument. Thus the observed potentiation of SO-stimulated secretion by IBMX suggested that an endogenous cyclic nucleotide-dependent protein kinase, probably PKA, may produce a synergistic effect with the kinases stimulated by SO. Such a synergism has been shown to occur between bombesin and PACAP in stimulation of CCK and secretin release from STC-1 cells (4, 6). However, it is not clear at present whether this synergism is produced through multiple phosphorylation of a common substrate or different kinase substrates that are involved in regulated exocytosis of the hormones. Alternatively, the endogenous PKA may inactivate PP1 to prolong activation of CaM_KII as mentioned above. These questions will require further investigation.

It has been reported (18) that SO-stimulated CCK release in rats is in part mediated through the release of the putative CCK-releasing factor DBI because luminal application of an anti-DBI serum reduced SO-stimulated CCK release. It is possible that SO-stimulated secretin release is also mediated in part through a secretin-releasing factor. It has been shown that both CCK-releasing factors, LCRF (42) and DBI (1), and the secretin-releasing factor DBI because luminal application of an anti-DBI serum reduced SO-stimulated CCK release. It is possible that SO-stimulated secretin release is also mediated in part through a secretin-releasing factor. It has been shown that both CCK-releasing factors, LCRF (42) and DBI (1), and the putative secretin-releasing factor type I PLA2 (39) are localized in the epithelial cells of upper small intestinal mucosa where both S and I cells are found (56). Therefore, if SO can penetrate the mucus gel and act on the releasing factor-containing cells, it is likely to act on S and I cells as well. We have observed that SO stimulated secretin and CCK release from isolated mucosal cells and STC-1 cells and that SO elicited signal cascades in STC-1 cells. These observations appear to support the contention that SO can also act directly on the endocrine cells. However, the physiological significance of this direct action of SO on mucosal endocrine cells remains to be determined.
STC-1 cells are useful as a model for studying cellular mechanisms of CCK and secretin release (2, 4–7, 38, 50, 55, 59). The results of the present study indicated that these cells produced a more effective secretin release than that of CCK release in response to SOI, whereas our previous results (4, 6, 7) indicated that the neuropeptides VIP and bombesin/gastrin-releasing polypeptide also stimulated the release of secretin more effectively than CCK in STC-1 cells. Thus it appears that STC-1 cells may serve as a better model for studying the release of secretin than CCK.

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