Secretin differentially sensitizes rat pancreatic acini to the effects of supramaximal stimulation with caerulein

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Perides, G., A. Sharma, A. Gopal, X. Tao, K. Dwyer, B. Ligon, and M. L. Steer. Secretin differentially sensitizes rat pancreatic acini to the effects of supramaximal stimulation with caerulein. Am J Physiol Gastrointest Liver Physiol 289: G713–G721, 2005.—Supramaximal stimulation of the rat pancreas with CCK, or its analog caerulein, triggers acute pancreatitis and a number of pancreatitis-associated acinar cell changes including intracellular activation of digestive enzymezymogens and acinar cell injury. It is generally believed that some of these various acinar cell responses to supramaximal secretagogue stimulation are interrelated and interdependent. In a recent report, Lu et al. (8) showed that secretin, by causing generation of cAMP and activation of PKA, sensitizes acinar cells to secretagogue-inducedzymogen activation, and, as a result, submaximally stimulating concentrations of caerulein can, in the presence of secretin, trigger intracellularzymogen activation. We found that secretin also sensitizes acinar cells to secretagogue-induced cell injury and to subapical F-actin redistribution but that it did not alter the caerulein concentration dependence of other pancreatitis-associated changes such as the induction of a peak plateau intracellular [Ca2+]i rise, inhibition of secretion, activation of ERK1/2, and activation of NF-κB. The finding that secretin sensitizes acinar cells to both intracellularzymogen activation and cell injury is consistent with the concept that these two early events in pancreatitis are closely interrelated and, possibly, interdependent. On the other hand, the finding that, in the presence of secretin, caerulein can trigger subapical F-actin redistribution without inhibiting secretion challenges the concept that disruption of the subapical F-actin web is causally related to high-dose secretagogue-induced inhibition of secretion in pancreatic acinar cells.

cAMP; actin; trypsin; zymogen activation; cell injury; pancreatitis; extracellular signal-regulated kinase 1/2; nuclear factor-κB

The most widely utilized and best-characterized experimental model of acute pancreatitis involves exposing rodents to concentrations of CCK, or its decapetide analog caerulein, that exceed those that elicit a maximal rate of digestive enzyme secretion from the pancreas (7). In addition to eliciting pancreatic parenchymal injury and inflammation, which are the hallmarks of acute pancreatitis, this supramaximal secretagogue stimulation elicits a number of additional pancreatic responses including redistribution of acinar cell subapical F-actin to the basolateral region of the cell (10), inhibition of digestive enzyme secretion (14), intracellular activation of digestive enzymezymogens (3), activation of stress-activated kinases such as ERK1/2 (2), and activation of proinflammatorytranscription factors such as NF-κB (4). With the exception of inflammation, each of these responses, including cell injury, can also be elicited by exposing freshly isolated rodent pancreatic acini to supramaximally stimulating concentrations of caerulein under in vitro conditions (15, 16), and, under in vitro conditions, supramaximal stimulation with caerulein is also associated with the induction of a “peak plateau” pattern of increased cytoplasmic [Ca2+]i (20).

Previous reports by several groups of investigators have suggested that some of these acinar cell responses to supramaximal secretagogue stimulation may be interrelated and interdependent. For example, the inhibition of digestive enzyme secretion is thought to be the consequence of the dissolution of the subapical F-actin web that is triggered by supramaximal secretagogue stimulation (10) and acinar cell injury is thought to be the consequence of intracellular digestivezymogen activation (13). In addition, each of these responses is thought to be closely related to the changed pattern of intracellular [Ca2+]i ([Ca2+]i) rise that follows exposure to a supramaximally stimulating secretagogue concentration (12, 18).

Rodent pancreatic acinar cells display receptors for a variety of secretagogues including CCK and secretin (19). CCK receptor occupancy regulates digestive enzyme secretion via the phospholipase C/inositol trisphosphate/Ca2+/PKC signal transduction pathway. Low concentrations of CCK or caerulein elicit an oscillatory rise and fall in [Ca2+]i, whereas high concentrations elicit a peak plateau [Ca2+]i response. In contrast to CCK or caerulein, occupancy of secretin receptors regulates fluid and electrolyte secretion via activation of adenylate cyclase, generation of cAMP, and activation of PKA. In a recent report, Lu and co-workers (8) noted that secretin, by increasing rat acinar cell cAMP levels, sensitized those cells to caerulein-induced intracellular digestivezymogen activation, i.e., in the presence of secretin, maximally or submaximally stimulating concentrations of caerulein could trigger intracellular activation of trypsinogen and chymotrypsinogen, whereas, in the absence of secretin, higher, supramaximally stimulatingcaerulein concentrations were required to elicit intracellularzymogen activation.

We examined the possibility that secretin might selectively and differentially sensitize acinar cells to the other responses that are known to follow supramaximal stimulation with caerulein. In addition to confirming the observations reported by Lu et al. (8), we found that secretin also sensitizes acinar cells to caerulein-induced acinar cell injury/death and to caerulein-induced cytoskeletal changes, i.e., redistribution of subapical F-actin to the basolateral region of the cell. On the other hand, secretin stimulation does not sensitize acinar cells to caerulein-
induced inhibition of digestive enzyme secretion, to induction of the peak plateau \([\text{Ca}^{2+}]_i\) response, or to activation of either ERK1/2 or NF-κB. Our findings led us to conclude that 1) supramaximal secretagogue-induced subapical F-actin redistribution and inhibition of enzyme secretion are not interdependent events and 2) secretin can differentially sensitize acinar cells to some, but not all, of the effects of supramaximal secretagogue stimulation.

**MATERIALS AND METHODS**

**Materials.** Collagenase was purchased from Worthington (Freehold, NJ). Caerulein was obtained from Bachem Bioscience (King of Prussia, PA). The fluorescent substrates for trypsin (Boc-Gln-Ala-Arg-MCA) and chymotrypsin (Suc-Ala-Ala-Pro-Phe-MCA) were purchased from Peptides International (Louisville, KY). Phalloidin conjugated to Alexa Fluor 546, DNase I conjugated to Texas red, fura-2 AM, Pluronic F-127, and Live/Dead Reagent were purchased from Invitrogen (Carlsbad, CA). The PKA inhibitors N-(2-guanidinoethyl)-5-isoxquinolinesulfonamide (H89), (9R,10S,12S)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-12-epoxy-1H-diindololo-(1,2-f;3′,2′-k)pyrrolo-(3,4-1)(1,6)benzodiazocine-10-carboxylic acy hexyl ester (KT-5720), and myristoylated protein kinase inhibitor peptide (14–22) (MPKI), the PKA activator dibutyryl-cAMP (db-cAMP), and the PKG activator dibutyryl-cGMP (db-cGMP) were obtained from BioMol Research Laboratories (Plymouth Meeting, PA). The amylase substrate 2-chloro-p-nitrophenyl-α-maltoside was obtained from Physicians Chemical (Oxford, CT). Antibodies to cAMP-reactive element binding protein (CREB), ERK1/2, and their phosphorylated forms were obtained from Cell Signaling (Beverly, MA). The NF-κB oligonucleotide consensus binding sequence was purchased from Promega (Madison, WI), and \([\gamma-33P]ATP\) was purchased from Perkin-Elmer (Boston, MA). All other chemicals were of analytical grade and purchased from Sigma Chemicals (St. Louis, MO).

**Preparation of acini.** All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of Tufts-New England Medical Center. Male Sprague-Dawley rats (Charles River Laboratories) weighing 80–100 g were housed in temperature-controlled (20 ± 2°C) rooms with a 12:12-h light-dark cycle and given standard laboratory chow ad libitum. They were fasted overnight but given water ad libitum before each experiment. Pancreatic acini were freshly isolated by collagenase digestion as previously described (11) and used immediately for each experiment. Cell viability, as measured by trypan blue exclusion, was >95% before the start of each experiment. Unless otherwise stated, the acini were suspended in 15 mM HEPES-Ringer buffer (pH 7.4) containing 130 mM NaCl, 5 mM KCl, 10 mM glucose, 9 mM Na pyruvate, 1 mM CaCl2, 1 mM MgCl2, and 0.1% BSA and incubated at 37°C.

**Zymogen activation.** Activation of trypsinogen and chymotrypsinogen was quantitated by measuring trypsin and chymotrypsin activity in acinar homogenates. For this purpose, trypsin and chymotrypsin activity were fluorometrically measured using their specific substrates Boc-Gln-Ala-Arg-MCA and Suc-Ala-Ala-Pro-Phe-MCA, respectively, as described previously by Lu et al. (8).

**Quantitation of acinar cell injury/death.** Acinar cell injury/death was quantitated using Live/Dead Reagent (Molecular Probes), to which viable cells are impermeant. This method of quantitating cell injury/death is based on the cytotoxicity assay reported earlier (6).

Under certain conditions, a mixed response pattern was observed in which the response appeared to consist of oscillations superimposed upon a peak plateau pattern.

**Activation of CREB, ERK1/2, and NF-κB.** Activation of the PKA-specific substrate CREB and the stress-activated kinase ERK1/2 were quantitated by immunoblot analysis using anti-CREB antibodies, anti-phospho-CREB antibodies, anti-ERK1/2 antibodies, and anti-phospho-ERK1/2 antibodies from Cell Signaling. Activation of NF-κB was quantitated by electrophoretic mobility shift assay using the NF-κB consensus sequence 5′-AGTGGAGGGACTTTCCCA-
GGC-3' as described previously (5). The resulting gels were dried and exposed to X-ray films. Autoradiograms were analyzed by densitometry using Kodak 1D image-analysis software (Eastman Kodak; Rochester, NY) to quantitate the extent of NF-κB activation.

Analysis of data. The results reported in this communication reflect the observations made in three or more independent experiments. Figures report mean values ± SE. The significance of changes was evaluated using Student’s t-test when two groups were compared or ANOVA when more than two groups were compared. Differences were considered significant when P values were <0.05.

RESULTS

Effects of secretin on caerulein-induced intracellular activation of zymogens. Supramaximally stimulating concentrations of caerulein (i.e., 1–10 nM) but not submaximally stimulating caerulein concentrations (i.e., 0.01–0.1 nM) caused intra-acinar cell activation of both trypsinogen (Fig. 1A) and chymotrypsinogen (not shown, see Ref. 8). In the presence of 100 nM secretin, the dose-response relationship for caerulein-induced zymogen activation was altered, and digestive enzyme zymogen activation was observed when the caerulein concentration was 0.1 nM (Fig. 1; see Ref. 8). In addition to shifting the dose dependence of caerulein-induced zymogen activation, secretin also increased the magnitude of caerulein-induced zymogen activation (Fig. 1A). The effect of secretin on caerulein-induced trypsinogen activation was reproduced when the cell-permeant form of cAMP (i.e., db-cAMP) but not db-cGMP was used in place of secretin, and the effect of secretin on caerulein-induced zymogen activation was inhibited when the cAMP-dependent PKA inhibitors H89, KT5720, or MPKI were present (Fig. 1B).

Fig. 1. Effect of caerulein (Caer) and secretin (Secr) on trypsinogen activation. A: freshly prepared rat pancreatic acini were incubated with varying concentrations of caerulein in the absence (solid bars) or presence (open bars) of 100 nM secretin. After 30 min at 37°C, acini were homogenized, and trypsin activity was determined. Note that trypsin activity, in the presence of 100 nM secretin, is increased when the concentration of caerulein is 10⁻¹¹ M, whereas increased trypsin activity, in the absence of secretin, is only noted when the caerulein concentration is 10⁻⁸ M. Results shown are mean ± SE values from 3 independent experiments, each performed in duplicate. *P < 0.01 when trypsin activity in the presence of caerulein ± secretin is compared with that in the absence of caerulein or secretin. B: freshly prepared rat pancreatic acini were incubated with combinations of 0.1 nM caerulein, 100 nM secretin, 100 μM dibutyryl (db)-cAMP, 100 μM db-cGMP, 10 μM H89, 10 μM KT5720, and 10 μM myrisolated protein kinase inhibitor (MPKI). After 30 min at 37°C, acini were homogenized, and trypsin activity was determined. Results shown are mean ± SE values from 3 independent experiments, each performed in duplicate.

Fig. 2. Effect of caerulein and secretin on cell injury. Acinar cells were incubated with varying concentrations of caerulein in the absence (solid bars) or presence (open bars) of 100 nM secretin. Fifteen minutes before the end of the experiment, Live/Dead Reagent was added, and cell injury/death was measured as described in the text. Results are expressed as the ratio of the sample value to the value noted for samples incubated without stimulants (0). Data shown reflect mean ± SE values obtained from 3 independent experiments, each performed in triplicate. *P < 0.01 compared with samples incubated in the absence of stimulants.

Fig. 3. Effect of caerulein and secretin on amylase secretion by acini. Acinar cells were incubated with varying concentrations of caerulein in the absence or presence of 100 nM secretin. After 30 min at 37°C, acini were homogenized, and amylase activity was determined. Note that secretin increases the rate of amylase secretion at each caerulein concentration but does not change the biphasic response pattern or the concentration of caerulein that elicits the maximal rate of secretion. Results shown are mean ± SE values from 3 independent experiments, each performed in duplicate.
Effects of secretin and caerulein on acinar cell injury/death. We utilized Live/Dead Reagent to quantitate the effects of secretin on the concentration dependence of caerulein-induced acinar cell injury/death. Although the results obtained roughly paralleled those noted in pilot studies using leakage of lactate dehydrogenase or permeability to trypan blue as indicators of cell injury/death, we noted a greater level of consistency between experiments and closer agreement between replicate samples using the Live/Dead Reagent method. As shown in Fig. 2, secretin concentrations of up to 100 nM did not elicit an increase in acinar cell injury/death. In contrast, caerulein elicited concentration-dependent acinar cell injury/death, and the threshold concentration of caerulein needed for the induction of injury/death in the absence of secretin was 1 nM. In the presence of 100 nM secretin, however, the dose dependence of caerulein-induced cell injury/death was altered, and the threshold concentration of caerulein needed for the induction of injury/death was reduced to 0.1 nM.

Effect of secretin and caerulein on acinar cell secretion of amylase. Both secretin and caerulein stimulated secretion of amylase from freshly isolated rat pancreatic acini (Fig. 3). The dose-response relationship for secretin-stimulated secretion was monophasic, and 100 nM secretin elicited a maximal rate of secretion (not shown). In contrast, the dose-response relationship for caerulein-stimulated secretion was biphasic, and 0.1 nM caerulein elicited a maximal rate of secretion (Fig. 3). Higher concentrations of caerulein led to inhibition of amylase secretion. Maximal secretin-stimulated secretion was smaller.

Fig. 4. Effect of caerulein and secretin on F-actin distribution. Pancreatic acini were incubated with 0.01, 0.1, and 1 nM caerulein in the presence or absence of 100 nM secretin for 30 min (A–E) or 5 min (F). They were then fixed, stained for F-actin as described in the text, and visualized using a Leica confocal microscope. Note the redistribution of F-actin from the apical to basolateral region in acini treated, in the absence of secretin, with 1 nM, but not 0.1 nM, caerulein for 30 min. In the presence of secretin, F-actin redistribution is observed in acini exposed to 0.1 nM caerulein for 30 min, and that redistribution is also observed when acini are exposed to secretin plus 0.1 nM caerulein for 5 min (F). db-cAMP at 100 μM had a similar effect as secretin on caerulein-induced F-actin redistribution (H). This effect could not be seen with the addition of db-cGMP (G) and could be inhibited with the addition of 10 μM MPKI (I).
in magnitude than maximal caerulein-stimulated secretion but, as noted many years ago by Williams and colleagues (1), the combination of secretin with caerulein elicited additive secretion, i.e., secretion occurred at a rate that roughly corresponded to the rate elicited by secretin combined with the rate achieved by the concentration of caerulein being used. As shown in Fig. 3, the dose-response relationship for secretion stimulated by the combination of secretin plus caerulein continued to be biphasic, and secretin failed to prevent the inhibition of secretion noted in the presence of high concentrations of caerulein. Furthermore, the presence of secretin did not alter the concentration dependence of caerulein-induced inhibition of secretion, and, as a result, inhibition of secretion was not observed when the concentration of caerulein was 0.1 nM or lower. Similar results were obtained when acinar cells were incubated with increasing amounts of caerulein in the presence of db-cAMP, i.e., 100 μM db-cAMP had an additive effect on caerulein-stimulated amylase secretion and did not alter either the biphasic character or the concentration dependence of caerulein-regulated amylase secretion (not shown).

Effect of secretin on caerulein-induced subapical F-actin distribution. We found that the F-actin-to-G-actin ratio in acinar cells was not altered by exposure of cells to a supramaximally stimulating concentration of caerulein. That ratio was found to be 0.28 ± 0.01 in the presence of 0.1 nM caerulein and 0.23 ± 0.03 in the presence of 1.0 nM caerulein (P > 0.05). In contrast, we found that the distribution of F-actin changes when acini are exposed to supramaximally stimulating concentrations of caerulein. In unstimulated cells and in cells exposed to submaximally stimulating concentrations of caerulein (0.1 nM), most of the F-actin was located in the subapical region (Figs. 4A and 5). As shown previously by O’Konski et al. (10), supramaximal stimulation with caerulein (1.0 nM) did not lead to F-actin distribution nor have any effect in caerulein-induced F-actin redistribution (Fig. 4H). As a negative control, 100 μM cGMP was added, which did not alter the F-actin distribution nor have any effect in caerulein-induced F-actin redistribution (Fig. 4H). The F-actin redistribution could be inhibited with 10 μM MPKi (Fig. 4I) and PKA inhibitors H89 (10 μM) or KT5720 (10 μM) (not shown).

Temporal relationship between F-actin redistribution and inhibition of amylase secretion. The potentially causal relationship between secretagogue-induced F-actin redistribution and the inhibition of amylase secretion was evaluated in time-dependent experiments that tracked both of these responses. The redistribution of subapical F-actin induced by supramaximal stimulation with caerulein (1.0 nM) or by a combination of secretin with a submaximally stimulating concentration of caerulein (0.1 nM) was found to be complete within 5 min of adding the secretagogues to the acini suspension (Fig. 4G). Secretion experiments were then undertaken to examine the effects of F-actin redistribution on amylase secretion. As shown in Fig. 6, left and middle, the percentage of total amylase content that is released per minute, over each 2.5-min interval, in response to either secretin or to secretin plus a submaximally stimulating concentration of caerulein (0.1 nM) rose and declined at a similar rate for at least the first 12.5 min after cells were exposed to the secretagogues despite the fact that, under these conditions, subapical F-actin has been redistributed to the basolateral region of acinar cells (Fig. 4). Similarly, the cumulative secretion of amylase elicited by 0.1 nM caerulein plus secretin continued to exceed that elicited by either secretagogue alone over the initial 12.5 min after cells were exposed to the secretagogues despite the fact that, under these conditions, subapical F-actin has been redistributed to the basolateral region of acinar cells (Fig. 4).

Similarly, the cumulative secretion of amylase elicited by 0.1 nM caerulein plus secretin continued to exceed that elicited by either secretagogue alone over the initial 12.5 min after cells were exposed to the secretagogues despite the fact that, under these conditions, subapical F-actin has been redistributed to the basolateral region of acinar cells (Fig. 4). Similarly, the cumulative secretion of amylase elicited by 0.1 nM caerulein plus secretin continued to exceed that elicited by either secretagogue alone over the initial 12.5 min after cells were exposed to the secretagogues (Fig. 6, right). Taken together, these observations indicate that subapical F-actin can be redistributed without altering the rate of amylase secretion.
higher elicited a peak plateau response. An intermediary pattern of response, in which oscillations were superimposed upon a peak plateau response, was observed with 0.1 nM caerulein. Inclusion of secretin along with caerulein did not alter the pattern of response noted with any of the caerulein concentrations. Although small changes in oscillation amplitude and frequency were occasionally observed, the concentration of caerulein required for eliciting an intermediate or peak plateau response was not altered by the inclusion of secretin (Fig. 8).

Effect of caerulein and secretin on NF-κB and ERK1/2 activation. NF-κB activation was noted when acini were exposed to 0.1 nM caerulein, and 0.01 nM caerulein elicited activation of ERK1/2 (Fig. 9). Secretin (100 nM) did not activate either NF-κB or ERK1/2, and secretin did not alter the caerulein concentrations needed for the activation of either NF-κB or ERK1/2.

DISCUSSION

Supramaximal stimulation of the rat pancreas with the calcium-mobilizing secretagogue caerulein is associated with the development of acute pancreatitis. Many of the pancreatitis-associated acinar cell changes, including intracellular digestive zymogen activation, acinar cell injury, subapical F-actin redistribution, activation of stress-activated kinases, and activation of the proinflammatory transcription factor NF-κB, are also observed when freshly isolated rat acini are exposed to supramaximally stimulating concentrations of caerulein under in vitro conditions (15, 16). When studied under in vitro conditions, supramaximal stimulation of acini with caerulein has also been shown to inhibit digestive enzyme secretion and to elicit a peak plateau change in [Ca²⁺], (14, 20).

It is generally believed that some of these various acinar cell responses to supramaximal secretagogue stimulation are interrelated and interdependent. For example, redistribution of subapical F-actin is believed to cause inhibition of digestive enzyme secretion (10) and inhibition of secretion and, combined with a pathological peak plateau rise in [Ca²⁺], is believed to cause intracellular activation of digestive enzymezymogens. Intracellular activation of digestive enzymezymogens is believed to cause acinar cell injury (13), and, in a recent report, Tando et al. (17) suggested that CCK-induced activation of trypsinogen within acinar cells caused induction of IkB kinase leading to activation of NF-κB.

Recently, Lu and co-workers (8) have shown that secretin, an acinar cell secretagogue that acts via cAMP and not via

![Fig. 6. Time-dependent effects of secretin on caerulein-induced amylase secretion. Freshly isolated acini were preincubated at 37°C for 5 min. Secretin and caerulein were added to the concentrations indicated, and aliquots of the suspending medium were taken for amylase measurement at 2.5-min intervals for 12.5 min. 0, Acinar cells incubated without stimulates; 0S, acinar cells incubated with only 100 μM secretin; 10, acinar cells incubated with 0.1 nM caerulein; 10S, acinar cells incubated with 0.1 nM caerulein and 100 μM secretin. At the completion of the experiment, total amylase content of the acini and suspending medium was measured, and amylase discharge at each time was calculated as a percentage of total content. Left and middle: rates of amylase secretion (i.e., %total content that was discharged/min) during each interval. This rate of secretion was calculated by subtracting the percentage of content discharged at the start of the interval from that discharged at the completion of the interval and then dividing that value by 2.5. Right: cumulative discharge of amylase (i.e., %total content) at each time. Results shown indicate mean ± SD values from 4 independent experiments, each performed in duplicate.](http://ajpgi.physiology.org/)

![Fig. 7. Effect of PKA inhibitor H89 on activation of cAMP-reactive element binding protein (CREB). Isolated rat pancreatic acini were incubated for 30 min at 37°C with 1 nM caerulein, 100 nM secretin, and 100 μM db-cAMP in the absence or presence of 10 μM of the PKA inhibitor H89. The cells were lysed, and proteins were subjected to immunoblot analysis with anti- phospho-CREB (p-CREB) monoclonal antibody.](http://ajpgi.physiology.org/)

**Table 1. Effect of PKA inhibitor H89 on F-actin redistribution**

<table>
<thead>
<tr>
<th>Addition</th>
<th>F-Actin (apical/basal)</th>
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<tbody>
<tr>
<td>None</td>
<td>11.6 ±/− 1.4</td>
</tr>
<tr>
<td>db-cAMP</td>
<td>12.3 ±/− 1.7</td>
</tr>
<tr>
<td>Secretin</td>
<td>11.0 ±/− 1.7</td>
</tr>
<tr>
<td>Caerulein (0.1 nM)</td>
<td>12.9 ±/− 1.2</td>
</tr>
<tr>
<td>Caerulein (0.1 nM) + db-cAMP</td>
<td>3.6 ±/− 0.7*</td>
</tr>
<tr>
<td>Caerulein (0.1 nM) + secretin</td>
<td>1.9 ±/− 0.5*</td>
</tr>
<tr>
<td>Caerulein (0.1 nM) + db-cAMP + H89</td>
<td>10.5 ±/− 2.0</td>
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<tr>
<td>Caerulein (0.1 nM) + secretin + H89</td>
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</tr>
<tr>
<td>Caerulein (1 nM)</td>
<td>2.7 ±/− 0.1*</td>
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<tr>
<td>Caerulein (1 nM) + H89</td>
<td>13.2 ±/− 1.9</td>
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Results shown are means ±/− SE obtained from examining 10–14 acini, group. Rat pancreatic acini were incubated with varying combinations of caerulein, dibutyryl (db)-cAMP (100 μM), secretin (100 nM), and H89 (10 μM). The ratio of apical to basal F-actin staining intensity was measured as described in the text. *P < 0.01 compared with the none group.
calcium mobilization, can sensitize acinar cells to caerulein-induced digestive zymogen activation. They found that secretin alters the concentration-response relationship between caerulein and zymogen activation such that, in the presence of secretin, low concentrations of caerulein, which would otherwise submaximally stimulate secretion, could trigger activation of trypsinogen and chymotrypsinogen. Furthermore, they noted that this sensitizing effect of secretin is mediated via cAMP.

The observations reported by Lu et al. (8) were confirmed in our preliminary studies, and, in the context of generally accepted concepts regarding the interrelatedness and interdependence of acinar cell events triggered by supramaximal secretagogue stimulation, we hypothesized that secretin would sensitize acinar cells to many, if not all, of the other so-called "pancreatitis-related" effects of supramaximal caerulein stimulation. Thus we expected that secretin would alter the concentration dependence of caerulein-induced changes such that

Fig. 8. Effects of secretin on caerulein-induced calcium responses. Isolated rat pancreatic acini were incubated with varying concentrations of caerulein in the presence or absence of 100 nM secretin. Note that no significant differences in the intracellular [Ca\(^{2+}\)] response were seen, regardless of the presence or absence of secretin in the incubation mixture.

The observations reported by Lu et al. (8) were confirmed in our preliminary studies, and, in the context of generally accepted concepts regarding the interrelatedness and interdependence of acinar cell events triggered by supramaximal secretagogue stimulation, we hypothesized that secretin would sensitize acinar cells to many, if not all, of the other so-called "pancreatitis-related" effects of supramaximal caerulein stimulation. Thus we expected that secretin would alter the concentration dependence of caerulein-induced changes such that
lower concentrations of caerulein would cause acinar cell injury, trigger subapical F-actin redistribution, and activate both ERK1/2 and NF-κB. Reports by others (1, 9), however, suggested that secretin might not alter the concentration dependence of caerulein-induced effects on digestive enzyme secretion or changes in [Ca\(^{2+}\)].

As noted in this communication, our expectations were largely but not completely met. Indeed, we found that secretin sensitizes acinar cells to caerulein-induced injury/death, and this observation is certainly compatible with the concept that intracellular zymogen activation and acinar cell injury/death are closely related events. On the other hand, the observation that secretin exerts these sensitizing effects without altering the concentration dependence of caerulein-induced changes in [Ca\(^{2+}\)], is not compatible with the concept that either zymogen activation or cell injury are critically dependent on a peak plateau change in [Ca\(^{2+}\)]. We (13) and others (9) have previously noted that a rise in [Ca\(^{2+}\)] is required but, by itself, insufficient to trigger either zymogen activation or cell injury. These findings combined with the currently reported observations would suggest that an oscillatory, rather than a peak plateau, rise in [Ca\(^{2+}\)] might fulfill this requirement.

We found that secretin sensitizes acinar cells to caerulein-induced redistribution of subapical F-actin to the basolateral region of the cell and that, like secretin sensitization of intracellular zymogen activation, the sensitizing effect of secretin on caerulein-induced cytoskeletal changes is I) mediated via cAMP and 2) involves activation of PKA, i.e., it is a phenomenon that can be induced by exposure to a cell-permeant form of cAMP and it can be aborted by inhibitors of PKA. Taken together, these observations suggest that F-actin redistribution is closely related to both intracellular zymogen activation and acinar cell injury, but whether the cytoskeletal change is the result or cause of zymogen activation/cell injury cannot be determined from our studies. In any case, however, F-actin redistribution is clearly not dependent on a peak plateau change in [Ca\(^{2+}\)].

Redistribution of subapical F-actin and inhibition of digestive enzyme secretion are observed when acini are exposed to 1.0 nM or higher concentrations of caerulein. This phenomenon has been generally interpreted as indicating that secretagogue-induced inhibition of secretion is, in fact, caused by secretagogue-induced disruption of the subapical F-actin web and redistribution of apical F-actin to the basolateral region of the cell (10). We were surprised, therefore, to find that, in experiments spanning 30 min, secretin could sensitize acinar cells to caerulein-induced F-actin redistribution without sensitizing those same cells to caerulein-induced inhibition of secretion. Although these observations might suggest that F-actin redistribution and inhibition of secretion may be independent events, they are also compatible with the conclusion that the secretin-induced sensitization of acinar cells to caerulein-induced F-actin redistribution occurs slowly and that, therefore, most of the secretion measured over 30 min is complete before F-actin redistribution. In that case, a causal relationship between F-actin redistribution and 30-min secretion of amylase might still be possible. To examine this possibility, time-dependent experiments evaluating the effects of secretin on caerulein-induced F-actin redistribution and amylase secretion over short intervals were performed. We found that secretin-induced sensitization of F-actin redistribution could be clearly observed within 5 min of exposing acini to the combination of secretin and a submaximally stimulating concentration of caerulein but that no inhibition of amylase secretion could be detected during the initial 12.5 min of incubation. Taken together, these findings clearly indicate that F-actin redistribution can occur without leading to a reduction in the rate of amylase secretion, and they cast considerable doubt on the
widely held belief that subapical F-actin redistribution is the proximate cause of secretagogue-induced inhibition of secretion.

Finally, our study has that secretin does not sensitize acinar cells to caerulein-induced activation of either ERK1/2 or NF-κB. We found that caerulein-induced activation of both ERK1/2 and NF-κB was observed in the presence of caerulein concentrations that are below those required to elicit intracellular activation of trypsinogen and that the dose dependence of caerulein-induced NF-κB and ERK1/2 activation was not altered by the presence of secretin. Our observations, particularly those regarding caerulein-induced activation of NF-κB, are at variance with those recently reported by Tando et al. (17), which suggested that CCK induction of IkB kinase and activation of NF-κB are mediated by intracellular activation of trypsinogen. Although NF-κB and ERK1/2 activation in response to caerulein may play important roles in the evolution of secretagogue-induced pancreatitis, and they may ultimately regulate the severity of that pathological process, the intracellular events leading to ERK1/2 and NF-κB activation clearly differ from those that lead tozymogen activation, F-actin redistribution, and acinar cell injury.

In summary, we have shown that, in addition to sensitizing acinar cells to caerulein-induced intracellularzymogen activation, secretin, acting via cAMP and PKA, also sensitizes acinar cells to caerulein-induced F-actin redistribution and acinar cell injury but does not alter the concentration dependence of caerulein-induced inhibition of enzyme secretion, peak plateau change in \([\text{Ca}^{2+}]_i\), levels, ERK1/2 activation, or NF-κB activation. These observations led us to the following conclusions: 1) secretagogue-inducedzymogen activation, cell injury, and F-actin redistribution are closely related and potentially interdependent events, but they are not dependent on inhibition of secretion or on a peak plateau change in \([\text{Ca}^{2+}]_i\); 2) secretagogue-inducedzymogen activation, cell injury, and F-actin redistribution can all occur in the absence of an inhibition of digestiveenzyme secretion from acinar cells; and 3) redistribution of subapical F-actin to the basolateral region of the cell is not, by itself, sufficient to inhibit digestive enzyme secretion.

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