Secretagogues differentially activate endoplasmic reticulum stress responses in pancreatic acinar cells

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Kubisch CH, Logsdon CD. Secretagogues differentially activate endoplasmic reticulum stress responses in pancreatic acinar cells. Am J Physiol Gastrointest Liver Physiol 293: G1804–G1812, 2007. First published April 12, 2007; doi:10.1152/ajpgi.00078.2007.—Endoplasmic reticulum (ER) stress leads to the accumulation of misfolded proteins in the ER lumen and initiates the unfolded protein response (UPR). Components of the UPR are important in pancreatic development, and recent studies have indicated that the UPR is activated in the arginine model of acute pancreatitis. However, the effects of secretagogues on UPR components in the pancreas are unknown. The present study aimed to examine the effects of different types and concentrations of secretagogues on acinar cell function and specific components of the UPR. Rat pancreatic acini were stimulated with the CCK analogs CCK8 (10 pM–10 nM) or JMV-180 (10 nM–10 µM) or with bombesin (1–100 nM). Components of the UPR, including chaperone BiP expression, PKR-like ER kinase (PERK) phosphorylation, X box-binding protein 1 (XBP1) splicing, and CCAAT/enhancer binding protein homologous protein (CHOP) expression, were measured, as were effects on amylase secretion and intracellular trypsin activity. All three secretagogues increased BiP levels and XBP1 splicing. However, only supraphysiological levels of intracellular trypsin activity. Curves were monophasic, and high concentrations did not increase intracellular active trypsin curves. The effects of CCK8 on UPR components were rapid, occurring within 5–20 min. In conclusion, ER stress response mechanisms appear to be involved in both pancreatic physiology and pathophysiology, and future efforts should be directed at understanding the roles of these mechanisms in the pancreas.

exocrine acini; pancreas

THE EXOCRINE PANCREAS is the main organ responsible for the digestion of ingested foodstuffs. To this end, it is highly specialized for the synthesis, storage, and release of digestive proenzymes. To match the dietary demand for digestive enzymes, pancreatic acinar cells have the highest rate of protein synthesis among all human tissues (6). This process involves the endoplasmic reticulum (ER), which is a multifunctional organelle mainly responsible for the synthesis and correct folding of proteins in the secretory pathway (2). Because of its prominent role in digestive enzyme synthesis, the pancreatic acinar cell has particularly abundant ER. It has recently been realized that disturbances of ER function lead to a series of ER stress response mechanisms (13, 35, 42). ER stress has come to be recognized as a central component of a number of common diseases, including diabetes mellitus, cystic fibrosis, and Alzheimer’s disease (1). The pancreatic acinar cell seems to be particularly susceptible to perturbations of ER homeostasis, and mechanisms that relieve this stress have been shown to be necessary for normal pancreatic development (14, 25). However, the roles of ER stress responses and their specific components in pancreatic physiology and pathophysiology are currently unknown.

One of the most important pathophysiological states of the exocrine pancreas is acute pancreatitis, which arises initially in pancreatic acinar cells by incompletely understood mechanisms (47). After its initiation, the disease spreads systemically through the development of an inflammatory response that is driven by acinar cell expression of proinflammatory molecules (4). Along with proinflammatory mechanisms, protective and restorative mechanisms are also activated in stressed acinar cells. Thus, the ultimate severity of acute pancreatitis depends on the balance of these opposing forces. Microarray-based profiling techniques have provided insights into the complex pattern of altered gene expression that occurs early in stressed pancreatic acinar cells (18). One novel finding was that known key regulators of the ER stress response were dramatically altered early during the course of acute pancreatitis. It was also found that ER stress key regulators are activated during the development of acute pancreatitis in the arginine model (22). However, it is not known whether these responses are protective or induce further damage or whether ER stress key regulators are activated in other models of acute pancreatitis.

Stresses that affect the homeostasis of the ER influence several cellular signaling mechanisms, including the unfolded protein response (UPR). The UPR couples the ER protein load with the ER protein folding capacity. It is sensitive to a variety of perturbations, including oxidative stress, energy depletion, and calcium imbalance, all of which lead to an accumulation of misfolded proteins in the ER lumen (37). Key components of the UPR include three stress sensor/transducers constitutively expressed in the ER membrane: PKR-like ER kinase (PERK), activation transcription factor 6 (ATF6), and inositol-required enzyme 1 (IRE1) (13, 35). During homeostasis, these molecules are kept inactive by their association with the chaperone BiP (also called glucose-related peptide 78). In the presence of excessive unfolded proteins, BiP equilibrium shifts away from these regulatory molecules and allows their activation. PERK activation leads to subsequent phosphorylation of eukaryotic initiation factor 2α (eIF-2α) and results in a general decrease in...
translation initiation (16, 27). Activation of ATF6 leads to increased transcription of ER chaperones, including BiP, and protein foldases (44, 50). Activation of IRE1 causes splicing and activation of X box-binding protein 1 (XBPl) to its active transcription factor form (sXBPI), which also regulates expression of various chaperones, foldases, and other protective molecules (5). Together, the UPR mechanisms clear misfolded proteins from the ER and alleviate stress by either shutting down translation to reduce the protein flow into the ER or upregulating molecules that protect the cell. However, if the ER stress is not resolved, prolonged and increased activation of the UPR leads to programmed cell death. This apoptotic effect is mediated largely by increased expression of the transcription factor CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP; also called growth arrest and DNA damage inducible gene 153), which leads finally to apoptosis via the intrinsic mitochondrial pathway (34).

Which, if any, of these mechanisms are activated during physiological and supraphysiological stimulation of secretion in pancreatic acinar cells remains unknown. One approach to understanding physiological and pathophysiological mechanisms in the pancreas is through the use of different secretagogues. CCK is a hormone that regulates physiological pancreatic secretion, but at supraphysiological doses, the CCK analogs CCK8 or caerulein damage acinar cells in vitro and cause edematous pancreatitis (24). In vitro, these full CCK agonists also have biphasic dose-response curves for amylase secretion, with inhibition occurring at supramaximal doses. In contrast, the CCK partial agonist analog JMV-180 (9, 30, 31) and the gastrin-releasing peptide analog bombesin (39), both of which stimulate amylase release from acinar cells to an extent equivalent to that of CCK8, do not show high-dose inhibition of secretion and do not have deleterious effects on pancreatic acinar cells, even at supramaximally stimulating concentrations. Thus, a comparison of the effects of different concentrations of pancreatic secretagogues could provide important information about the involvement of ER stress signaling pathways in these events.

The purpose of this study was to determine, using differences in the actions of the secretagogues CCK8, JMV-180, and bombesin, whether ER stress response mechanisms are activated during stimulation of isolated exocrine rat acini in vitro. Furthermore, we investigated the association between qualitative and quantitative differences in the ER stress response pattern and physiological and pathological states of secretory stimulation. Our results indicate that ER stress occurs under pattern and physiological and pathological states of secretory and quantitative differences in the ER stress response mechanisms. Furthermore, we investigated the association between qualitative and quantitative differences in the ER stress response pattern and physiological and pathological states of secretory stimulation. Thus, a comparison of the effects of different concentrations of pancreatic secretagogues could provide important information about the involvement of ER stress signaling pathways in these events.

**Preparation of isolated acini.** Rats were treated according to federal guidelines for animal care, and the Institutional Animal Care and Use Committee of The University of Texas M. D. Anderson Cancer Center approved all animal experimental protocols. Pancreatic acini were prepared in Krebs-Ringer buffer (KRB) supplemented with 0.1% BSA and 0.1% soybean trypsin inhibitor, as described by Williams and co-workers (48). Pancreata from male Sprague-Dawley rats (100–130 g, Charles River Laboratories, Wilmington, MA) were removed, digested by collagenase at 37°C, dispersed by pipetting, and passed though a mesh nylon cloth. Acini were then purified by an albumin gradient in KRB and resuspended in HEPES-Ringer buffer (HRB) supplemented with 0.1% BSA and 0.01% soybean trypsin inhibitor (pH 7.4) under an atmosphere of 100% O2. To allow acinar cells to recover after isolation, they were incubated at 37°C in HRB for 2 h. Dispersed acini (2-ml aliquots in duplicate) were then stimulated with CCK8 (10 pM–10 nM), bombesin (1 nM–100 nM), and JMV-180 (10 nM–10 pM) for 30 min or with 10 nM CCK8 for different times (2–90 min). All experiments were carried out at 37°C and repeated three times with acini isolated from different animals.

**Quantification of pancreatic acini amylase secretion.** After being stimulated with secretagogues, acini were pelleted, and the supernatant was used to measure amylase secretion using the *Phadebas* amylase test. The net stimulated secretion of amylase was calculated by subtracting the amount of amylase secreted in the absence of secretagogue (“basal” secretion) from the amount secreted in the presence of secretagogue (“stimulated” secretion). The amount of amylase secreted was expressed as a percentage of the total amount of amylase in acinar cells.

**Trypsin activity in pancreatic acini.** After stimulation, acini were spun down, and the cell pellet was resuspended in ice-cold MOPS buffer and homogenized by hand using a Teflon and glass homogenizer. The homogenate was centrifuged, and the supernatant was analyzed for trypsin activity using Z-Gly-Pro-Arg-p-nitroanilide acetate according to the method of Chen et al. (7). The concentration of active trypsin was calculated using standards generated by commercially purified trypsin. The protein concentration of each sample was determined, and trypsin activity was expressed as nanograms of active trypsin per milligram of protein.

**Western blot analysis of pancreatic acini proteins.** Whole cell lysates were prepared from pancreatic acini and centrifuged at 4°C, and the supernatant was assayed for protein concentration as previously described (21). Western blot analysis was performed using 20 μg protein/lane for BiP analysis and 30 μg protein/lane for PERK. Protein was mixed with sample loading buffer (23), and PAGE was performed using Tris-HCl ready gels and nitrocellulose membranes. After membranes were blocked with LI-COR blocking buffer, the following antibodies were used: anti-BiP, 1:1,000; anti-pPERK, 1:1,000; and anti-actin, 1:10,000 (as an internal loading control). Membranes were incubated with the appropriate IgG fluorescent Alexa Fluor secondary antibody. A LI-COR Odyssey infrared imager detected antibody binding. Membranes were scanned, recorded digitally, and processed using Quantity One (Bio-Rad).

**RNA isolation and RT.** Pelleted acini were washed with ice-cold 0.9% NaCl and homogenized in TRIZol reagent. Total RNA was
extracted and cleaned using a Qiagen RNeasy kit. Total RNA concentration was measured spectrophotometrically at 260 and 280 nm, and final RNA was dissolved in RNase-free water to a 1 μg/μl concentration. Standard RT was performed using total RNA from secretagogue-treated and control acini. RT was conducted for 45 min at 42°C from 1 μg RNA by avian myeloblastosis virus reverse transcriptase in a 20-μl reaction volume.

Quantitative PCR. Quantitative PCR (qPCR) was performed for 40 cycles in an iCycler iQ (Bio-Rad). PCR mastermix was used, and specific primers were used for CHOP (GenBank Accession No. NM024134, forward: 5’T-AGG AGC CAG GCC CAG CAG AGG T-3’ and reverse: 5’T-ATC AGA GCC CGT GTG GTC-3’) and for the selective amplification of sXBP1 (GenBank Accession No. AF443192, forward: 5’T-GAG TCC GCA GCA GGT G-3’ and reverse: 5’T-GTG TCA GAG TCC ATG GGA-3’). 18S rRNA served as the internal loading control (forward: 5’T-GAG CGG TCG GCG TCC CCC AAC-3’ and reverse: 5’T-GCG CGT GCA GCC CCG GAC ATC-3’). PCRs were supplemented with 10 nmol SYBR green and fluorescein. Real-time PCR was performed in duplicate. Results were analyzed using Optical System Software version 3.1 (Bio-Rad) and normalized to 18S rRNA. In addition, we prepared a 1% agarose gel stained with ethidium bromide to separate the PCR products according to their size, providing visual support for the qPCR data.

Statistical analysis. Secretagogue stimulation was always performed in duplicate, and all experiments were repeated three times using acini from a different rat each time. Student’s t-test was used to investigate the differences between each parameter. Results were regarded as significantly different when the P value was <0.05. Values are reported as means ± SE.

RESULTS

Effects of secretagogues on secretion and intracellular trypsin activation. To obtain correlation data for the effects of CCK8, JMV-180, and bombesin on the ER stress response, we measured their effects on amylase secretion from isolated rat pancreatic acini after 30 min of incubation at 37°C (Fig. 1, A–C). CCK8 caused dose-dependent stimulation of amylase secretion at lower concentrations and subsequent inhibition at higher concentrations. In contrast, monophasic patterns of stimulation of amylase secretion, with no high-dose inhibition, were observed for both JMV-180 and bombesin. Despite the differences at high concentrations, the maximal effects of the three secretagogues on amylase stimulation were not significantly different. These observations are similar to what has
The three secretagogues also had different effects on the pattern of intracellular trypsin activity observed in isolated rat acini after 30 min of incubation at 37°C (Fig. 1, D–F). CCK8 increased trypsin activity in a concentration-dependent manner. A significant increase was noted at 100 pM CCK8, and trypsin activity was further increased by higher concentrations to a maximum of 228.9 ± 34.8% of control noted at 10 nM, the highest concentration evaluated. In contrast, JMV-180 and bombesin did not significantly increase intracellular trypsin activity in isolated pancreatic acini at any concentration. These results are consistent with those of previous studies in which CCK8, but not JMV-180 and bombesin, induced acinar cell damage (9, 31, 39).

Effects of secretagogues on components of the UPR. BiP is one of the major ER chaperones and is sensitive to stress inside the organelle. We investigated the effects of different concentrations of the three secretagogues on the levels of BiP in acini after a stimulation period of 30 min (Fig. 2A). All three secretagogues increased BiP levels in acinar cells compared with control within this time frame. The effects of CCK8 were dose dependent, with effects apparent at 10 pM CCK8, statistical significance (P < 0.05) at 100 pM, and a maximum increase to 222.2 ± 13.9% of control at 10 nM (Fig. 2B). The effects of bombesin were also dose dependent, with a statistically significant (P < 0.05) maximal effect of 186.5 ± 27.9% of control observed with 100 nM bombesin. Similarly, JMV-180 significantly stimulated BiP expression (P < 0.05) to 194.0 ± 13.6% of control at the maximal concentration of 10 μM. Differences between the maximal effects of the three secretagogues on BiP levels were not significant. Therefore, BiP responses did not differ between secretagogues that do or do not cause acinar cell damage.

PERK is one of the major transducers of the ER stress response and is normally localized to the ER membrane and previously been reported for these three secretagogues (9, 31, 39).

PERK phosphorylation completely discriminated between secretagogues that do and do not cause acinar cell damage. PERK is one of the major transducers of the ER stress response and is normally localized to the ER membrane and

Fig. 2. Influence of secretagogues on acinar cell BiP levels. BiP protein levels were analyzed using the Western blot technique in isolated acini from whole acinar cell lysates after treatment with the concentrations of secretagogues indicated for 30 min at 37°C. A: representative Western blot indicating the levels of BiP with actin as a loading control. B: quantification of Western blots was conducted using the LI-COR Odyssey system. Data are shown as means ± SE; n = 3. *P < 0.05 vs. control.

kept inactive by the ER luminal binding of BiP. When ER stress occurs, BiP binding shifts to misfolded proteins in the ER lumen and leads to its dissociation from PERK, the subsequent formation of PERK dimers, and PERK autophosphorylation (49). Therefore, we used a phospho-specific antibody to PERK to determine its activation status after treatments with the three secretagogues (Fig. 3A). CCK8 caused a concentration-dependent increase in PERK phosphorylation, with significant effects noted using 100 pM CCK8 (Fig. 3B). The highest increase in PERK phosphorylation of 299.3 ± 34.9% of control was observed after stimulation with 10 nM CCK8. In contrast to the strong effects of CCK8, we did not observe any significant effects on PERK phosphorylation when cells were treated with either bombesin or JMV-180, even at the highest concentrations. Therefore, PERK phosphorylation completely discriminated between secretagogues that do and do not cause cell damage.

IRE1 is another ER stress response component located in the ER membrane that is bound to BiP under normal physiological conditions. Upon stress, BiP dissociates, and IRE1 is free to dimerize and become active as an endonuclease. Active IRE1 targets the mRNA for the molecule XBP1 and generates a splice variant that has a frameshift that converts XBP1 into an active transcription factor, sXBP1. sXBP1 is then translocated to the nucleus and induces the transcription of several ER stress-related genes. For our experiments, we used a PCR primer that only recognizes and amplifies the spliced form of XBP1 to analyze changes in IRE1 activity (sXBP1 mRNA levels). We observed that CCK8 stimulated a significant increase in XBP1 splicing in a concentration-dependent manner (Fig. 4A). A significant increase was observed with 1 nM CCK8, and the peak level of sXBP1 was reached at 10 nM CCK8, with an increase of 758.0 ± 89.7% of control (Fig. 4B). Bombesin and JMV-180 were also able to increase sXBP1 levels at their highest concentrations significantly, indicating that all three secretagogues were able to induce cytoplasmic splicing via IRE1 activation. However, the effects of bombesin

Fig. 3. Influence of secretagogues on acinar cell PKR-like endoplasmic reticulum (ER) kinase (PERK) phosphorylation. PERK phosphorylation was analyzed using a phospho-specific antibody in Western blots of whole acinar cell lysates after treatment with different secretagogues for 30 min at 37°C. A: representative Western blot indicating the levels of phospho-PERK with actin as a loading control. B: quantification of Western blots was conducted using the LI-COR Odyssey system. Data are shown as means ± SE; n = 3. *P < 0.05 vs. control.

The three secretagogues also had different effects on the pattern of intracellular trypsin activity observed in isolated rat acini after 30 min of incubation at 37°C (Fig. 1, D–F). CCK8 increased trypsin activity in a concentration-dependent manner. A significant increase was noted at 100 pM CCK8, and trypsin activity was further increased by higher concentrations to a maximum of 228.9 ± 34.8% of control noted at 10 nM, the highest concentration evaluated. In contrast, JMV-180 and bombesin did not significantly increase intracellular trypsin activity in isolated pancreatic acini at any concentration. These results are consistent with those of previous studies in which CCK8, but not JMV-180 and bombesin, induced acinar cell damage (9, 31, 39).
and JMV-180 were both >50% less than those of maximal CCK8.

CHOP is a transcription factor that is produced during prolonged or severe ER stress. It reduces the expression level of a number of important targets that normally would inhibit apoptosis (28, 36). Therefore, increased expression of CHOP is associated with increased cell death. We evaluated the expression of CHOP mRNA using qPCR after 30 min of stimulation at 37°C with the respective secretagogues. We observed that CCK8 induced a concentration-dependent increase in CHOP levels (Fig. 4A). A significant effect was observed with 1 nM CCK8, and a maximal effect that was more than fivefold that of the control was observed with 10 nM CCK8 (Fig. 4C). In contrast, neither bombesin nor JMV-180 induced the expression of CHOP at any concentration.

**Time course of effects of CCK8 on the UPR, secretion, and intracellular trypsin activation.** To understand the relationships between CCK8 induction of the ER stress responses and other aspects of acinar cell function, we stimulated acini with a maximal dose of CCK8 at 37°C and examined several parameters at time intervals between 2 and 90 min. Amylase secretion was induced by high doses of CCK8 within 5 min and continued to increase for at least 60 min (Fig. 5A). Maximum secretion was reached at 90 min, with 22.6 ± 2.9% of total amylase being released. Intracellular trypsin activity was also increased after CCK8 stimulation (Fig. 5B). Trypsin activity was increased within 20 min and became significant by 40 min; the maximum of 325% ± 61% of control was reached at 90 min. CCK8 also induced time-dependent changes in ER stress parameters. A significant increase in BiP expression was measured after 10 min, and the peak increase was observed after 60 min, with an increase to 256.6% ± 15.8% of control (Fig. 6, A and B). PERK phosphorylation was significantly elevated very early, at 5 min, and rose to 382.8% ± 43.9% of control within 40 min (Fig. 6, C and D). IRE1 activity, as indicated by increased levels of sXBP1 mRNA, was significant within 10 min and reached a maximum at 40 min (758.7% ± 58.9% of control; Fig. 6, E and F). CHOP expression was the last of the ER stress components to become elevated, as it became significant only after 20 min, and levels peaked after 60 min at nearly 10-fold those of the control (Fig. 6, G and H).

**DISCUSSION**

In the present study, we found qualitative as well as quantitative differences in the activation of several key regulators of the ER stress response and could link those to the degree of acinar damage associated with different secretagogues. It has been previously demonstrated that ER stress response mechanisms provide a protective response during pancreatic development, as deletion of XBP1 (25) or PERK (14) caused major disruption of exocrine cell function. Reduced activation of ER stress responses was also observed in animals lacking pancreatic Mist-1, associated with a more severe acute pancreatitis (20). It is also known that ER stress, if severe or prolonged, can lead to cell death responses. Previously, we observed that all components of the UPR were activated during arginine-in-
duced acute pancreatitis, which is associated with severe pancreatic injury (22). In the present study, we investigated the effects on the ER stress response of different secretagogues at physiological and supraphysiological concentrations. We observed that all secretagogues activated some UPR components while other UPR components were exclusively activated by supramaximal concentrations of CCK8. Therefore, these data provide new insights into the complexity of ER stress responses in the regulation of pancreatic acinar cells under conditions of health and disease.

The present study takes advantage of differences in the signaling properties of well-known pancreatic secretagogues. Acini secrete comparable amounts of amylase in response to CCK8, JMV-180, and bombesin (9, 31, 39). However, the high-dose inhibition of secretion observed in vitro with CCK8 was absent with bombesin (39) and JMV-180 (9, 31). Likewise, in vivo, full CCK agonists, including CCK8 and caerulein, induce acute pancreatitis at high doses, but neither bombesin (39) nor JMV-180 (40) has this effect. Because of differences in the abilities of the various secretagogues to cause pancreatitis, they have served as tools to identify differences in physiological and pathophysiological events. This approach has been used to investigate a variety of cellular signaling mechanisms, including phosphatidylinositol 1,4,5-trisphosphate hydrolysis (30), intracellular Ca2+ changes (38), the activation of the stress kinase JNK (8, 10), NF-κB (12), and intracellular trypsin (11). Therefore, use of the different secretagogues makes it possible to identify mechanisms associated with physiological or pathophysiological aspects of acinar cell signaling.

Here, we focused on the UPR and its primary controller, the chaperone BiP. Under normal conditions, BiP binds to the three critical regulatory molecules embedded in the ER membrane: PERK, ATF6, and IRE1. However, in the presence of ER stress, excessive unfolded or misfolded proteins accumulate and compete for BiP. As the equilibrium shifts from the UPR regulators, they become activated and one of their first targets is BiP itself (3). Thus, BiP levels are highly sensitive to changes in the status of the ER. In the present study, BiP was the most sensitive indicator of ER stress investigated and was
induced by physiological levels of all three secretagogues. This observation supports a previous study (26) indicating that BiP levels are a sensitive indication of stress that are regulated by several overlapping mechanisms. Also, this observation is in agreement with previous animal experiments in which BiP levels were observed to be induced by two experimental conditions associated with large but physiological increases in exocrine production: endogenous release of CCK following proteinase inhibitor feeding and infusion of physiological concentrations of caerulein (17).

One of the important regulators of BiP is XBP1. The transcription factor XBP1 is activated by IRE1 in response to the accumulation of unfolded proteins in the ER (45). IRE1, a type I ER transmembrane protein, cleaves XBP1 mRNA to remove 26 nucleotides by using endoribonuclease activity in its cytoplasmic domain. The processed XBP1 mRNA encodes a potent transcriptional transactivator, sXBP1. sXBP1 has been shown to induce multiple secretory pathway genes, expand the ER, and elevate total protein synthesis when ectopically expressed in vitro (43, 46). Thus, XBP1 enforces changes in cellular structure and function consistent with the requirements of secretory cells. We observed that XBP1 splicing occurred within 10 min of stimulation with CCK8 and was also elevated by both JMV-180 and bombesin. The effects of high concentrations of CCK8 were significantly greater than those of the other secretagogues; however, the fact that splicing was observed with all secretagogues and with concentrations of CCK8 that were submaximal for secretion suggests that this pathway is activated physiologically by secretagogues. Combined with the previous observation that deletion of XP1 leads to destruction of the pancreas (25), this finding supports the suggestion that this UPR component is critical for normal pancreatic function.

Unlike BiP and XBP1, PERK phosphorylation was activated exclusively by CCK8. A major target of activated PERK is eIF-2α, a regulator of translation initiation. Translation initiation is controlled by eIF-2, a heterotrimer composed of α, β, and γ-subunits that forms a complex with the initiator methionyl-tRNAi and GTP and promotes translation initiation (19). However, phosphorylation of eIF-2α turns it into an inhibitor of general protein translation. This reduces the protein load in the ER and allows for recovery. Deletion of PERK has been shown to disrupt the development of the pancreas (14, 15). A previous study (41) has indicated that at high concentrations, CCK8 causes phosphorylation of eIF-2α and also inhibits protein synthesis in rat pancreatic acini. Our data suggest that PERK activity is responsible for these changes. JMV-180 and bombesin also do not inhibit acinar cell protein synthesis (31, 32). Taken together, the data suggest that high concentrations of CCK8, but not JMV-180 or bombesin, activate PERK, leading to eIF-2α phosphorylation and the inhibition of acinar cell general protein synthesis. This effect of CCK8 could be related to its ability to cause a profound release of Ca^{2+} from the ER, which is not observed with the other secretagogues, but further studies will be needed to confirm this hypothesis.

The first responses to ER stress involve induction of protective mechanisms that both reduce protein synthesis to decrease the synthesis burden of the ER and increase the protein bio-synthesis capacity of the secretory pathway as well as the folding capability in the ER. However, if the stress is particularly severe or prolonged, programmed cell death will occur. This may also be considered protective, as it eliminates damaged cells in a manner that does not cause an inflammatory response. A key mechanism involved in the ER stress response regulating apoptosis is transcriptional activation of CHOP. CHOP is a 29-kDa protein first identified to be a member of the C/EBPs that serves as a dominant negative inhibitor of C/EBPs (36). CHOP transcription is regulated by several ER stress-related transcription factors, including ATF6 and sXBP1. However, full activation of CHOP seems to require PERK. Maximal effects on CHOP are only achieved if all of the major components of the UPR are activated (36). We observed that CHOP was induced by CCK8 at supramaximal concentrations that were also able to activate all of the major pathways of the UPR, including PERK. In contrast, JMV-180 and bombesin did not cause PERK activation and did not induce CHOP. CHOP expression leads to cell apoptosis through a mechanism related to its ability to decrease the levels of Bcl-2 protein, which would usually form, together with Bcl-X1, a group of apoptosis antagonists (33, 34). We previously observed that CHOP levels were increased by arginine treatments associated with acinar cell apoptosis in vivo (22). In rats, caerulein induction of acute pancreatitis is also associated with high levels of apoptosis (29). However, the mechanisms involved in the activation of apoptosis during caerulein-induced acute pancreatitis are not well understood. It is possible that CHOP plays a role in this capacity, but further experiments will be necessary to verify this.

In summary, secretagogue treatment of isolated rat pancreatic acini leads to activation of specific components of the UPR depending on the type and concentration of the secretagogue. Supramaximal CCK8 stimulation stimulates all components of the UPR, including the proapoptotic factor CHOP, and is associated with acinar cell damage as well as acute pancreatitis. The extensive activation during supramaximal stimulation in vitro may be of importance in the early pathogenesis of acute pancreatitis and/or may reflect a universal reaction of the cells responding to maximal stress. In contrast, submaximal concentrations of CCK8, or treatments with JMV-180 and bombesin, only activate specific components of the UPR that are likely involved in ER quality control and matching protein synthetic capacity with synthesis rates. More studies will be required to fully understand the roles of these signaling pathways in the regulation of the pancreatic acinar cell. Ultimately, dissection of the signaling pathways of the UPR during the onset of pancreatitis could provide new insights for the development of therapeutic approaches for acute and/or chronic pancreatitis.

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REFERENCES


