Tauroursodeoxycholic acid reduces endoplasmic reticulum stress, trypsin activation, and acinar cell apoptosis while increasing secretion in rat pancreatic acini

A. Malo,1 B. Krüger,2 E. Seyhun,1 C. Schäfer,1 R. T. Hoffmann,3 B. Göke,1 and C. H. Kubisch1

1Department of Internal Medicine II, Campus Großhadern, University of Munich; 2Institute of Pathology, University of Rostock, Rostock; and 3Institute of Clinical Radiology, University of Munich, Munich, Germany

Submitted 16 October 2009; accepted in final form 12 July 2010

Malo A, Krüger B, Seyhun E, Schäfer C, Hoffmann RT, Göke B, Kubisch CH. Tauroursodeoxycholic acid reduces endoplasmic reticulum stress, trypsin activation, and acinar cell apoptosis while increasing secretion in rat pancreatic acini. Am J Physiol Gastrointest Liver Physiol 299: G877–G886, 2010. First published July 29, 2010; doi:10.1152/ajpgi.00423.2009.—Endoplasmic reticulum (ER) stress leads to accumulation of un- or misfolded proteins inside the ER and initiates the unfolded protein response (UPR). Several UPR components are physiologically involved in pancreatic development and are pathophysiologically activated during acute pancreatitis. However, the exact role of ER stress in exocrine pancreatic acini is mainly unclear. The present study examined the effects of tauroursodeoxycholic acid (TUDCA), a known ER chaperone, on acinar function and UPR components. Isolated rat pancreatic acini were stimulated by increasing concentrations of cholecystokinin (CCK-8) with or without preincubation of TUDCA. UPR components were analyzed, including chaperone binding protein (BiP), protein kinase-like ER kinase (PERK), X-box binding protein (XBP)-1, c-Jun NH2-terminal kinase (JNK), CCAAT/enhancer binding protein homologues protein (CHOP), caspase 3 activation, and apoptosis. In addition, TUDCA effects were measured on amylase secretion, calcium signaling, trypsin, and cathepsin B activation. TUDCA preincubation led to a significant increase in amylase secretion after CCK-8 stimulation, a 50% reduction of intracellular trypsin activation, and reduced cathepsin B activity, although the effects for cathepsin B were not statistical significant. Furthermore, TUDCA prevented the CCK-8-induced BiP upregulation, diminished PERK and JNK phosphorylation, and prohibited the expression of CHOP, caspase 3 activation and apoptosis. XBP-1 splicing was not altered. ER stress response mechanisms are activated in pancreatic inflammation. Chemical chaperones enhance enzyme secretion of pancreatic acini, reduce ER stress responses, and attenuate ER stress-associated apoptosis. These data hint new perspectives for an employment of chemical chaperones in the therapy of acute pancreatitis.

exocrine pancreatic acini; endoplasmic reticulum stress; chemical chaperone; TUDCA

THE EXOCRINE PANCREAS IS HIGHLY specialized in the production, storage, and release of inactive digestive enzymes (zymogenes), as well as a bicarbonate-rich fluid. To match the demand for digestive proteins, pancreatic acinar cells physiologically have the highest rate of protein synthesis among all adult human tissues (6). To function adequately, the pancreatic digestive proteins must fold into a distinct 3D arrangement and have to remain folded. Thus the process of protein folding and maturation is a crucial step in the transmission of genetic information into a specific biological function. Folding and stabilization take place inside the endoplasmic reticulum (ER) lumen after synthesis on membrane-bound ribosomes for a wealth of secretory proteins. After a protein enters the ER, it begins its chaperone-assisted folding and stabilization by multiple posttranslational modifications (12). To support its prominent role in the synthesis of digestive enzymes the exocrine pancreatic acinar cell has particularly abundant ER (2, 6).

Heavy chain binding protein (BiP), also known as glucose-related protein 78 (GRP78), is one of the dominant folding assisting chaperones. It is an ER-specific member of the heat shock protein 70 family. BiP binds folding intermediates of many proteins (26, 31). If the folding is incomplete or incorrect, export is inhibited by an ER quality control system. The proteins are retained in the ER, bound to a chaperone, e.g., BiP, until the folding process is complete. If the correct folding cannot be achieved, proteins are targeted for the ER-associated degradation (ERAD) (5).

Chaperone-protein association provides an optimal protein folding environment. It avoids uncontrolled and potentially harmful protein aggregation in the ER lumen (39). After correct protein folding, chaperones will dissociate and the protein exits the ER to move further along the secretory pathway. An overly prolonged chaperone-protein association directs misfolded proteins to the ERAD (14). The capacity of the ER to fold proteins is presumably limited by chaperone resources and can be exceeded by a high cellular protein demand during growth and differentiation, protein over-expression, lack of energy and oxidative imbalance. These disturbances lead to the accumulation of misfolded proteins in the ER, provoking the ER stress response (40).

The unfolded protein response (UPR) is a well-studied part of the ER stress response. It balances the folding capacity and folding demand within the organelle. It is achieved through an upregulation of ER-resident chaperones, ER enlargement, downregulation of gene transcription, and increase in ERAD (9, 40, 48). The UPR contains at least three distinct signaling components located in the ER membrane appearing with a cytosolic and ER-luminal domain. It is activated in response to ER stress: the double-stranded RNA-activated protein kinase-like ER kinase (PERK); the activation transcription factor (ATF) 6; and the inositol requiring protein (IRE) 1. These molecules and stress sensors are associated with the luminal chaperone BiP. When proteins accumulate inside the ER, BiP preferentially associates with the unfolded proteins to assist folding. This results in the activation of the molecules and their downstream signaling partners (15).

Active PERK autophosphorylates, inhibits translation initiation, and prevents further influx of nascent proteins into an
already saturated ER (40, 47). BiP dissociation from IRE1 activates its ribonucleas activity. Once activated, IRE1 cleaves the exon-intron junctions of the bZIP transcription factor X-box binding protein (XBP)-1 mRNA. After ligation the spliced XBP-1 encodes a transcriptionally active sXBP-1 protein. sXBP-1 increases the size of the ER and elevates levels of ER chaperones and folding enzymes (24, 54).

However, if these complex adaptive mechanisms are not sufficient and cells are exposed to prolonged and strong ER stress, the stress-damaged cells are eliminated through induction of apoptosis. Reports have shown that several molecules, including CHOP, caspases, and molecules in the MAP kinase cascades, play a role in ER stress-induced apoptosis (30). CHOP (also known as GADD153, a member of the CAAT/enhancer binding protein family of bZIP transcription factors) transcription is induced by PERK in response to ER stress. CHOP regulates the expression of Bcl-2 family members, induces intracellular reactive oxygen species, and promotes cell-cycle arrest (32, 35). Caspase 12 leads to apoptosis selectively in response to ER stress via activation of caspase 3 (29).

Active IRE1 binds and clusters TNF receptor-associating factor 2, an adaptor protein that phosphorylates c-Jun NH2-terminal kinase (JNK). JNK, known to be phosphorylated in pancreatitis, activates downstream transcription factors, such as c-Jun, c-Fos, and Sap-1 or regulates RNA stability (51, 57).

Acute pancreatitis (AP) arises initially in acinar cells but by incompletely understood mechanisms (43). Along with a plateau-shaped increase of intracellular calcium, starting at the apical zymogen-containing cell pole, a possible colocalization between lysosomal hydrolase cathepsin B and zymogenes may lead to a premature intracellular activation of trypsin and a blockage of secretion of the digestive enzymes (20, 52). It occurs with local inflammation, followed by apoptosis, necrosis, and a loss of organ function. AP can spread systemically through the development of a systemic inflammatory response syndrome (4).

Solid evidence suggests that ER stress-responses are prompted by various stress mechanisms and are involved in the early stages of the AP. This includes dilatation or vacuolation of the ER and loss of membrane-bound ribosomes (1, 17, 25, 27). ER changes seem a common reaction of acini to the induction of ER stress (18, 23). Key regulators of the ER stress response are altered during AP and several ER-resident chaperones are upregulated.

This study aims to determine whether a bile salt derivative with ER-chaperone properties, tauroursodeoxycholic acid (TUDCA), can reduce the ER stress with its consequences in rat pancreatic acinar cells. Utilizing its ER stress-reducing potency, TUDCA has successfully been employed in the treatment of primary sclerosing cholangitis (59). To identify and characterize possible intracellular mechanisms, we investigated the results for TUDCA pretreatment on calcium signalization, amylase secretion and trypsin and cathepsin B activation under conditions of physiological and pathophysiological CCK-8 stimulation in isolated rat acini.

MATERIALS AND METHODS

Materials

Essential amino acids were from Gibco and Invitrogen (Carlsbad CA), chromatographically purified collagenase from Worthington Biochemical (LakeWood, NJ), CCK-8 from Research Plus (Manassuan, NJ), and TUDCA from Calbiochem (Darmstadt, Germany).

Acinar amylase secretion. Phadebas amylase test was purchased from Magle Life Sciences International (Sweden).

Acinar trypsin activity. Protein assay was from Bio-Rad Laboratory (Hercules, CA), Substrat Boc-Gln-Ala-AMC,HCl from Bachem (Bubendorf, Switzerland), and purified trypsin for standards from Sigma-Aldrich (St. Louis, MO).

Western blotting. Molecular weight marker and protein assay and blotting cells were purchased from Bio-Rad Laboratory; proteinase inhibitor from Merck Calbiochem (Darmstadt, Germany); nitrocellulose membrane Protein from Whatman (Maidstone, UK); anti-phospho PERK antibody (sc-32577-R), anti-XBP-1 antibody (sc-7160), and anti-caspase 3 antibody (sc-7148) from Santa Cruz Biotechnology (Santa Cruz, CA); anti-BiP antibody (no. SPA-826) from Stressgen (Ann Arbor, MI); anti-CHOP (ab11419) from Abcam (Cambridge, UK); anti-phospho JNK (no. 9251) from Cell Signaling (Danvers, MA); anti-actin antibody (A5441) from Sigma-Aldrich (St. Louis, MO); and secondary horseradish peroxidase (HRP)-conjugated antibodies from GE Healthcare (Bucks, UK).

Annexin V staining. The ApoAlert annexin V-FITC apoptosis kit (no. 630109) was purchased from Clonetworks (Mountain View, CA).

Intracellular calcium measurement. Fura 2-acetoxymethyl ester (fura 2-AM) was from Molecular Probes (Eugene, OR).

Cathepsin B activity. The cathepsin B activity fluorometric assay kit (no. ab65300) was from Abcam (Cambridge, UK).

Preparation of Isolated Acini

The preparation of pancreatic acini was carried out as previously described (22). Briefly, pancreata from male Wistar rats (150–200 g) were removed and immediately digested by collagenase at 37°C under an atmosphere of 95% O2 and 5% CO2, mechanically dispersed, and passed though a 150-μm mesh nylon cloth. Acini were purified by an albumin gradient containing 4% BSA and resuspended in an Erlenmeyer flask in HEPES-Ringer buffer (supplemented with 0.1% BSA, 0.01% trypsin inhibitor pH 7.4) under the atmosphere of 100% O2.

In control experiments, basal levels of stress kinases and other phosphorylation-dependent pathways are activated to a limited extent in isolated, dispersed acini. Therefore, the isolated acini were preincubated for 2 h in a shaking water bath at 37°C before the experiments. TUDCA was dissolved in aqua bidest. The isolated cells were split and preincubated with or without 250 μg/ml (500 mM) TUDCA. Dispersed acini were stimulated with different concentrations of CCK-8 (10 pm-10 nm) for 30 min in 2-ml aliquots in duplicates for each experiment. All experiments were carried out at 37°C and repeated at least four times with acini isolated from different animals.

Quantification of Pancreatic Acini Amylase Secretion

After CCK-8 stimulation acini were spun down and the supernatant was used to quantify amylase release as previously described (21). Net stimulated secretion of amylase in percent (%) was calculated by subtracting the secretion in the absence of secretagogue (“basal” secretion) from the secretion of amylase noted in the presence of CCK-8 (“stimulated” secretion). Additionally, the total amount of amylase in acinar cells was determined to express the amount of amylase secretion in % of total amylase.

Measurement of Intracellular Calcium

Intracellular calcium concentrations were determined by use of the calcium-sensitive fluorescent dye furap AM. Acini were loaded with 1 μM furap 2-AM for 25 min at room temperature. After washing, the cells were incubated with 500 μM TUDCA. Measurements were performed with a radio imaging system (TILL Photonics, Gräfeling, Germany) using excitation wavelengths of 340 and 380 nm, and emitted light was collected at 510 nm according to Mooren et al. (28).
Tryptsin Activity in Pancreatic Acini

After stimulation the cell pellet was resuspended in ice-cold MOPS buffer and homogenized with a Teflon and glass homogenizer. The homogenate was used to determine the trypsin activity as described (21). The concentration was calculated by using standards generated by purified trypsin. Protein concentrations of each sample were determined and trypsin activity was expressed as femtomoles per milligram (fmol/mg) protein.

Cathepsin B Activity in Acinar Cells

The extent of cathepsin B activation after CCK-8 stimulation with or without TUDCA pretreatment was measured using a commercially available kit.

Western Blotting of Pancreatic Acini Proteins

Whole cell lysates were prepared from acini and used for Western blotting as described previously (21). Antibodies were used in the following concentrations: anti-BiP 1:1,000, anti-phospho-PERK 1:250, anti-XBP-1 1:500, anti-caspase 3 1:500, anti-CHOP 1:500, anti-phospho-JNK 1:2,000, and anti-actin 1:5,000, as an internal loading control. Membranes were incubated with the appropriate IgG HRP-conjugated secondary antibody (1:10,000). Antibody binding was detected by chemiluminescence radiography. Membranes were scanned, recorded digitally, and processed with ImageJ software.

Early Stages of Apoptosis and Necrosis in Pancreatic Acini

The extent of early apoptosis and necrosis in acinar cells was determined by annexin V-FITC/propidium iodide (PI) staining according to the manufacturer’s instruction with 5 μl annexin V and 10 μl PI.

Statistical Analysis

CCK-8 stimulation was performed in duplicates and all experiments were repeated at least four times using a different acini preparation each time. The Student’s t-test was used to establish the difference of each parameter. Results were regarded as significantly different when P value was <0.05. Values are means ± SE.

The local Animal Care and Use Committees (University of Munich) approved all animal experimental protocols. Rats were treated according to the Guiding Principles in the Care and Use of Animals.

RESULTS

Dose Finding of TUDCA

TUDCA has been used mostly in liver cells, occasionally in endothelial cells (33, 45, 53) with concentrations from 100 nM up to 5 mM. So far, TUDCA had not been not employed in exocrine pancreatic acini. Therefore, we used TUDCA for preincubation in three different concentrations (250, 500, and 1,000 μg/ml), based upon in vitro experiments and stimulated the acini thereafter with different CCK-8 concentrations (100 pM or 10 nM). Subsequently, amylase secretion was measured in all groups and compared with controls (acini stimulated only with CCK-8) (Fig. 1). The lowest TUDCA concentration (250 μg/ml or 0.5 mM) was the most effective to enhance secretion, whereas higher concentrations were inhibitory, and therefore the 0.5 mM concentration was chosen for all subsequent experiments. Incubation of acinar cells with TUDCA alone without CCK-8 stimulation caused no amylase secretion (very left bars).

Fig. 1. Dose finding for tauroursodeoxycholic acid (TUDCA). Pancreatic acinar cells were first incubated with the 3 TUDCA concentrations or not (solid and open bars at far left). Thereafter, pancreatic acini were preincubated with 250 μg/ml, 500 μg/ml, or 1,000 μg/ml TUDCA at 37°C and thereafter stimulated with either 100 pM CCK-8 or 10 nM CCK-8 over 30 min. Control acini were stimulated only with CCK-8 without TUDCA preincubation. Amylase secretion was measured in the cell supernatant and expressed in % of total amylase. Each bar in the figure represent mean ± SE of 3 independent experiments. *p < 0.05 compared with CCK-8 stimulation alone.

Effects of TUDCA Preincubation on Secretion and Intracellular Calcium Concentration

First we evaluated the effects of TUDCA on amylase secretion from isolated pancreatic acini after 30 min of incubation with various concentrations of CCK-8 (Fig. 2A). CCK-8 without TUDCA preincubation caused a dose-dependent stimulation of amylase secretion at lower concentrations and subsequent inhibition at higher concentrations, according to previously reported observations with CCK-8 (11). In contrast, preincubation with TUDCA for 2 h led to an increased amylase secretion starting at 30 pM CCK-8 with maximal effects at a 300 pM CCK-8 stimulation (maximal 15.06% of total amylase ± 1.18 vs. 10.73% of total amylase ± 0.46). Basal secretion of acinar cells incubated with TUDCA did not differ between the groups.

An increase in intracellular calcium is the primary driver of enzyme secretion, e.g., amylase (56). Furthermore, it is known that low-dose CCK-8 causes spikes of calcium release from intracellular calcium stores at the apical cell pole and coincidentally stimulates secretion whereas high CCK-8 concentrations induce a large increase in intracellular calcium followed by a sustained elevation of cytoplasmic calcium, inhibition of secretion, and intracellular trypsinogen activation. It was tested whether TUDCA preincubation changes the calcium signal in acinar cells and whether this is responsible for an increase in amylase secretion. Therefore, isolated pancreatic acini were loaded with the calcium-sensitive fluorescent dye fura 2 in the presence of TUDCA. In accordance with data from hepatocytes (3), TUDCA incubation caused a large intracellular calcium mobilization in acinar cells ~1 min after TUDCA administration. Hereby, the cytoplasmic calcium increase was first noted at the basal cell pole (data not shown). That was different from CCK-8-induced calcium signals. The signal returned to baseline 3 min later (Fig. 2B).
Effects of TUDCA Preincubation on Intracellular Trypsin and Cathepsin B Activation

Preincubation with TUDCA had significant effects on the activation of intracellular trypsin in rat acini after CCK-8 stimulation (Fig. 3A). CCK-8 alone increased trypsin activity in a concentration-dependent manner. An increase of trypsin activity compared with unstimulated cells was noted at 100 pM CCK-8. Trypsin activity was further increased by higher concentrations to a maximum of 129.93 ± 12.34 fmol/mg protein (equals an 896% increase compared with unstimulated acini). Pretreatment with TUDCA caused a significant reduction of trypsin activation up to 66.38 ± 8.11 fmol/mg protein at supraphysiological CCK-8 stimulation (Fig. 3A).

Next, the cathepsin B activation within the acinar cells after CCK-8 stimulation with or without TUDCA pretreatment was investigated (Fig. 3B). As expected, cathepsin B activity increased after secretory levels of CCK-8 (37). In our experiments it initially rose at the 100 pM CCK-8 stimulation peaking at 300 pM CCK-8. Preincubation with TUDCA abolished the cathepsin B activity after CCK-8 stimulation.

Effects of TUDCA Preincubation on Components of the UPR

BiP is an abundant ER-specific chaperone and its level is very sensitive to ER stress. Therefore, we investigated the effects of TUDCA on CCK-8-induced BiP expression in acini by Western blotting. The effects of CCK-8 on BiP expression were dose dependent, with apparent effects at 100 pM CCK-8, and a maximum increase up to 138.45 ± 33.93% of unstimulated cells at 1 nM CCK-8 and a steep decline thereafter (Fig. 4, A and B). Acini preincubated with TUDCA showed no significant increase of BiP expression at any CCK-8 concentration. Therefore, BiP expression was significantly lower in TUDCA-treated cells at 300 pM and 1 nM CCK-8 concentrations compared with CCK-8 stimulation alone. BiP expression was diminished by TUDCA in response to physiological and supraphysiological CCK-8 stimulation.

PERK is one of the major sensors and transducers of the ER stress, localized to the ER membrane and kept inactive by the ER luminal binding to BiP. When ER stress occurs, BiP binding shifts to unfolded proteins and leads to its dissociation from PERK with subsequent PERK autophosphorylation (58). Therefore, we used a phospho-specific PERK antibody to determine its activation status after CCK-8 treatment (Fig. 5, A and B). CCK-8 alone...
caused an increase in acinar PERK phosphorylation, with effects starting at 30 pM CCK-8. The maximum increase in PERK phosphorylation was observed after a stimulation with 100 pM CCK-8 (350.69 ± 88.28% of unstimulated control cells). TUDCA-pretreated cells showed only a small but not significant increase in phospho-PERK after CCK-8 stimulation compared with controls. PERK phosphorylation was significantly reduced in the TUDCA-treated cells under CCK-8 stimulation.

IRE1 is a second ER stress sensor and transducer, bound to BiP under physiological conditions. Upon stress, IRE1 becomes active as an endonuclease. Active IRE1 targets cytoplasmic XBP-1 mRNA and generates a splice variant that converts XBP-1 into sXBP-1, an active transcription factor. Subsequently, sXBP-1 induces the transcription of several ER stress-related genes involved in the biogenesis of the organelle itself. For our experiments, we analyzed the 28-kDa small splice variant sXBP-1 to describe changes in IRE1 activity (Fig. 6, A and B). CCK-8 stimulation caused a significant increase in XBP-1 splicing compared with unstimulated acini. sXBP-1 expression increased with 30 pM CCK-8 and peaked at 100 pM, with an increase of 145.22 ± 95.09% of unstimulated acini. Preincubation with TUDCA led only tendentially to an inhibition of XBP-1 splicing. But at no CCK-8 concentration the results were significant different compared with CCK-8 stimulation alone. Taken together, XBP-1 splicing was not altered by TUDCA preincubation.

CHOP is a proapoptotic transcription factor that is produced during prolonged or severe ER stress (32). Hence an increase of CHOP is associated with increased cell death. We evaluated the expression of CHOP by Western blotting after the stimulation by various concentrations of CCK-8. CCK-8 induced concentration-dependent CHOP expression (Fig. 7, A and B). A significant effect was first observed at 10 pm CCK-8 compared with unstimulated acini, with a maximal effect at the 100 pM CCK-8 concentration (156.18 ± 37.28% of unstimulated control cells). In contrast, TUDCA preincubation inhibited completely the expression of CHOP at any concentration.

Another proapoptotic pathway emanating from the ER involves the ER initiator procaspase 12, which is activated by cleavage under prolonged ER stress conditions. It can activate the effector caspase 3, leading to apoptosis. Using Western blotting from CCK-8 stimulated acinar cells we investigated the expression of the 17-kDa splice variant of active caspase 3 (Fig. 8, A and B). Our results show a dose-dependent increase in activated caspase 3 after CCK-8 stimulation. It peaks at the 1 nM CCK-8 with a maximal increase up to 394.71 ± 276.49% compared with unstimulated acini and declines thereafter. With the TUDCA preincubation, caspase activation was not detected at any CCK-8 concentration. TUDCA treatment completely prevented caspase 3 activation in rat acini.
We also analyzed the phosphorylation of JNK. Activation of IRE1 can lead via binding and clustering of TRAF2 to phospho-JNK. Several isoforms are known with different molecular weights: JNK1 (46 kDa) and JNK2/3 (55 kDa). Using Western blotting with a phosho-specific JNK antibody, we investigated the phosphorylation status of JNK in pancreatic acini after CCK-8 stimulation with or without TUDCA pretreatment (Fig. 9A). For the quantification of our data we analyzed the
levels of exemplary phospho-JNK1 (Fig. 9B). As described earlier (21), JNK phosphorylation increases after CCK-8 stimulation with minimal effects observed at 30 pM CCK-8. We observed maximal effects at the supraphysiological concentration of 10 nM CCK-8 (up to 197.46 ± 17.64% of unstimulated cells). TUDCA pretreatment was not able to abolish JNK phosphorylation but reduced it significantly (68.84 ± 9.52% of unstimulated cells at 10 nM CCK-8 stimulation).

To measure early stages of apoptosis in acinar cells, we determined the phosphatidylserine translocation to the outer cell membrane by annexin-V staining. We used PI to determine necrosis in acinar cells. Acinar cell stimulation with CCK-8 lead to a dose-dependent increase in annexin-V staining with its maximum at 300 pM CCK-8 (88.55 ± 7.55 RFU/µg protein) and a decrease thereafter (Fig. 10A). If cells were preincubated with TUDCA and then stimulated by CCK-8, significantly fewer apoptotic signals were found (47.60 ± 1.47 RFU/µg protein at 300 pM CCK-8 stimulation). This was mirrored when measuring necrosis in acinar cells using PI. Maximal necrosis was measured after stimulation with 1 nM of CCK-8 (87.78 ± 22.23 RFU/µg protein) and was significantly reduced up to 50% after TUDCA pretreatment (44.07 ± 5.44 RFU/µg protein) (Fig. 10B).

**DISCUSSION**

ER stress and its responses are relevant in the development of acute pancreatitis. Histological examinations demonstrate early on morphological changes of the ER during the onset of AP. Gene-profiling studies show significant alterations in ER stress key regulators and in a model of necrotizing pancreatitis all ER stress sensors (PERK, IRE1, and ATF6) become activated, an initiation of downstream signal pathways occurs initiated from the ER (1, 17, 18, 23, 25, 27). It has also been observed that stimulation of isolated pancreatic acini by different secretagogues can generate an ER stress response (22).

Following the principle of ER function and stress, we assumed that CCK-8 in physiological concentrations stimulates and increases zymogen production, protein folding, and secretion in acini. Supraphysiological stimulation leads to protein accumulation due to the exceedance of the ER folding capacity. It prompts the activation of the ER stress sensors PERK, IRE1, and ATF6, finally resulting in a general stop of transcription and translation, induction of chaperones, and expansion of the ER itself, trying to match demands of protein folding and secretion from the acinar cell upon stimulation. In vitro several secretagogues were able to induce the ER-specific chaperone BiP and XBP-1 splicing in a concentration-dependent manner. CCK-8 in supraphysiological doses induces acinar cell damage, inhibits amylase secretion, activates trypsin, and induces proapoptotic signals (22). Here, we investigated ER function in response to CCK-8 treatment. We aimed to interfere with the pathophysiological toxic ER signaling introducing TUDCA pretreatment as an antistress principle.

The expression of mutated proteins can lead to ER stress and point mutations may result in the production of misfolded and disease-causing proteins that are transcribed and translated at normal levels but owing to the ER quality control system are unable to reach their functional designation. Misfolded proteins are retained inside the ER and induce ER stress. Exam-
ples for misfolding diseases are Alzheimer’s disease and cystic fibrosis (10, 55). In some cases, however, the mutations are minor and result in only a partial loss of the protein’s normal activity. Thus, even though still exhibiting some biological activity, the mutants are retained in the ER. Folding intermediates form fibrillar aggregates, react to harmful substances, or can induce apoptosis of the stressed cell. Therefore, extraordinary efforts have been made to design therapeutic interventions that prevent or correct the structural abnormality of disease-causing misfolded proteins. In this regard, rescue of misfolded “trafficking defective” proteins by pharmacological chaperones is emerging as one of the most promising therapeutic strategies for such disorders (7, 34, 42). It is thought that they do one or several of the following: stabilize a specific conformation of the misfolded protein, reduce aggregation, prevent nonproductive interactions, and/or modify the activity of endogenous chaperones (50). Such effects increase the efficiency of the ER export and promote a proper trafficking of proteins (7, 34, 36).

Özcan et al. (33) described TUDCA as a bile salt conjugate with chemical or pharmaceutical chaperone properties. Treatment of hepatoma cells with TUDCA suppressed ER stress-induced phosphorylation of PERK and XBP-1 splicing. In vivo effects of TUDCA were studied in a model of leptin-deficient (ob/ob) mice, as a model of severe obesity and insulin resistance. Oral administration of TUDCA exhibited a potent antidiabetic activity with normalization of blood glucose within 1 wk. TUDCA improved insulin response and impaired glucose tolerance and suppressed PERK, IRE1, and JNK activation in liver and adipose tissue. TUDCA improved systemic insulin resistance and reduced the obesity-induced lipid accumulation in liver. In another setting, De Almeida et al. (8) showed a protective effect of TUDCA on ER stress induced by mutant protein aggregates of the mutated HFE C282Y protein associated with hereditary hemochromatosis.

Because of such promising results with TUDCA as chaperone, we employed the compound in a model of isolated rat pancreatic acini during supraphysiological stimulation by CCK-8. TUDCA preincubation reduced the activation of several ER signal pathways. We demonstrate a highly significant reduction in PERK phosphorylation. The ER chaperone BiP, sensitive to ER stress, was not altered by TUDCA preincubation. Several proapoptotic pathways emitting from the ER, including CHOP expression, caspase activation, and JNK phosphorylation, were significantly downregulated after TUDCA pretreatment in CCK-8-stimulated pancreatic acinar cells. Likewise, annexin V and PI staining were reduced as true early events of apoptosis. XBP-1 splicing after CCK-8 stimulation showed a slight reduction with TUDCA treatment. A likely explanation of these effects is the excess of folding chaperones in response to TUDCA administration. In our experiments, TUDCA preincubation was able to influence significantly the hallmarks of CCK-8 overstimulation. Amylase secretion significantly increased after TUDCA treatment. Increases in enzyme secretion caused by bile salt ursodeoxycholic acid, its taurine conjugate TUDCA, and another ER chaperone 4-phenylbutyric acid may be explained by an optimization of ER function, folding capacity, or an increase of the efflux clearance across the apical cell membrane (16, 41, 44). Furthermore, TUDCA increases intracellular calcium concentrations a main prerequisite of stimulus secretion coupling in the acini (56). In our experiments TUDCA increased the cytoplasmic calcium concentra-

**ACKNOWLEDGMENTS**

We thank U. Naumann and S. Rackow for expert technical assistance.

**GRANTS**

This study was supported by the FoFoLe grant of the University of Munich Reg- Nr. 538 as well as the Deutscherforschungsgemeinschaft grant KU 2617/ 1-1 (both to C. H. Kubisch).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**

8. De Almeida SF, Picarote G, Fleming JV, Carmo-Fonseca M, Azavedo JE, de Sousa M. Chemical chaperones reduce endoplasmic reticulum...


