Dysregulation of the calpain-calpastatin system plays a role in the development of cerulein-induced acute pancreatitis in the rat

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Submitted 16 September 2003; accepted in final form 9 January 2004

Weber, Heike, Ludwig Jonas, Saskia Hühns, and Peter Schuff-Werner. Dysregulation of the calpain-calpastatin system plays a role in the development of cerulein-induced acute pancreatic injury in the rat. Am J Physiol Gastrointest Liver Physiol 286: G932–G941, 2004; 10.1152/ajpgi.00406.2003.—Calpain, a calcium-dependent cytosolic protease, is implicated in a multitude of cellular functions but also plays a role in cell death. Recently, we have shown that two ubiquitous isoforms, termed μ-calpain and m-calpain, are expressed in rat pancreatic acinar cells and that calcium ionophore-induced calpain activation leads to acinar cell injury. On the basis of these observations, we have now investigated the role of both calpain forms and the endogenous calpain inhibitor calpastatin in acute pancreatitis. After treatment of rats either without or with calpain inhibitor Z-Val-Phe methyl ester (ZVP; 60 mg/kg ip), pancreatitis was induced by cerulein injections (10 μg/kg ip; 5 times at hourly intervals). Calpain activation and calpastatin expression in the pancreatic tissue were studied by Western blot analysis. Pancreatic injury was assessed by plasma amylase activity, pancreatic wet/dry weight ratio (edema), histological and electron-microscopic analyses, as well as fluorescence labeling of actin filaments. Cerulein caused an activation of both μ-calpain and m-calpain, accompanied by degradation of calpastatin. Prophylactic administration of ZVP reduced the cerulein-induced calpain activation but had no effect on calpastatin alterations. In correlation to the diminished calpain activity, the severity of pancreatitis decreased as indicated by a decline in amylase activity (P < 0.01), pancreatic edema formation (P < 0.05), histological score for eight parameters (P < 0.01), and actin filament alterations. Our findings support the hypothesis that dysregulation of the calpain-calpastatin system may play a role in the onset of acute pancreatitis.

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ally, our results revealed that ionomycin-induced cytosolic Ca\textsuperscript{2+} increase leads to calpain activation and acinar cell damage (43). On the basis of these findings, in the present study, we have investigated whether calpain activation plays a role in acute pancreatitis induced by cerulien hyperstimulation that is also known to cause an abnormal, sustained cytosolic Ca\textsuperscript{2+} increase in pancreatic acinar cells (4, 18, 30).

We provide evidence that cerulien induces a decrease in calpastatin, which may contribute to the activation of both calpain isoforms, observed in parallel. Prophylactic administration of the calpain inhibitor Z-Val-Phe methyl ester (ZVP) has no protective effect on the calpastatin alterations but significantly reduces the calpain activation. In correlation to the decreased calpain activity, the severity of cerulien pancreatitis is clearly diminished, thus suggesting a role of calpain in the onset of pancreatitis.

**MATERIALS AND METHODS**

**Antibodies and reagents.** For immunoblotting, rabbit polyclonal antibodies against \(\mu\)-calpain (domain I, pAB1) and m-calpain (domain III, pAB2) were purchased from Calbiochem-Novabiochem (San Diego, CA). Other rabbit polyclonal antibodies against \(\mu\)-calpain (domain I, NH\textsubscript{2} terminal, pAB3) and m-calpain (domain I, NH\textsubscript{2} terminal, pAB4) were from Sigma-Aldrich (St. Louis, MO). The anti-\(\mu\)-calpain antibodies showed no cross-reactivity with m-calpain, and the anti-m-calpain antibodies did not cross-react with \(\mu\)-calpain (data not shown). The mouse monoclonal anti-calpastatin antibody (clone 1F7E3D10; domain IV) was purchased from Sigma-Aldrich and did not cross-react neither with \(\mu\)-calpain nor m-calpain (data not shown). \(\mu\)-Calpain (porcine erythrocytes) and m-calpain (porcine kidney) were obtained from Calbiochem-Novabiochem. Horseradish peroxidase-conjugated secondary antibodies, SDS, polyvinylidene difluoride (PVDF) membranes, and Precision Plus protein standards were from Bio-Rad (Hercules, CA). Bodipy FL phallacidin was from Molecular Probes (Eugene, OR). All other reagents, including cerulien and ZVP, were purchased from Sigma.

**Animals.** Female rats of an inbred Lewis strain (LEW-1W, Department of Laboratory Animal Science, University of Greifswald, Germany) were used. Animals were starved for 18 h before starting the experiments but had free access to water.

**Induction of cerulien pancreatitis.** The experiments were approved by the ministry of agriculture of the federal state Mecklenburg-Vorpommern to be in accordance with the standards of care and use of laboratory animals.

Animals (180–200 g body wt) were injected intraperitoneally either with ZVP (60 mg/kg dissolved in 0.4 ml of DMSO) or with an equal volume of DMSO alone as previously described (4, 43). On the basis of these findings, in the present study, we have investigated whether calpain activation plays a role in acute pancreatitis induced by cerulien hyperstimulation that is also known to cause an abnormal, sustained cytosolic Ca\textsuperscript{2+} increase in pancreatic acinar cells (4, 18, 30).

The supernatant was again spun at 100,000 \(\times\) g for 10 min. After repeated rinsing with PBS and incubating with horseradish peroxidase-conjugated secondary antibody (1:3,000) for 60 min. The colorimetric Opti-4CN detection kit (Bio-Rad) or the Amplified Opti-4CN detection kit (Bio-Rad) was used to visualize the immunolabeling.

**Characterization of the calpain antibodies.** Ten microliters of purified m-procalpain (1 mg/ml) or \(\mu\)-calpain (0.7 mg/ml) were added to 170 \(\mu\)l buffer (60 mM imidazole, 5 mM cysteine, 2.5 mM 2-mercaptoethanol, pH 7.3, 37°C) and incubated without or with 10 \(\mu\)l casein (20 mg/ml). The calpain activation was induced by addition of 10 \(\mu\)l CaCl\textsubscript{2} (100 mM). After 5 and 30 min, the reaction was quenched by mixing 21-\(\mu\)l sample aliquots with 7 \(\mu\)l SDS-PAGE sample buffer. The proteins were analyzed by SDS-PAGE (10% gels) and immunoblotted as described above. The anti-calpain antibodies were used at 1:1,000 dilution, and immunoreactivity was visualized by the Opti-4CN detection kit.

**Pancreatic water content.** For quantification of pancreatic edema, a small piece of tissue was resected, trimmed of fat, blotted dry, and weighed (wet weight). Thereafter, the specimen was desiccated for 48 h at 105°C and weighed again (dry weight). Pancreatic water content was determined by calculating the wet weight/dry weight ratio.

**Morphology.** Cerulien-induced morphological pancreas damage was assessed by a pathologist who was not aware of the specimens identity.

**Histological evaluation.** For light microscopy, pancreatic tissue samples were fixed in 4% buffered neutral formaldehyde for 24 h, dehydrated, and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin and examined with a light microscope Jenamed (Carl Zeiss, Jena, Germany). The extent of acidophilia, vesiculation, and vacuolization of acinar cells as well as eosinophilia, hyperemia, edema, and granulocyte and round cell infiltration of the interstitial space was quantified using a previously described scoring system (45). According to the severity of tissue damage, a score from 0 to 3 was given for each parameter.

**Electron-microscopic evaluation.** For electron-microscopic investigations, pancreatic tissue was fixed for 1 h in 4% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.0), washed in the same buffer, and postfixed in 1% osmium tetroxide. Thereafter, it was embedded in 0.1% liquid agar. Small cubes of the samples were dehydrated in a graded series of ethanolic solutions and embedded in Durcupan ACM. Ultrathin sections were prepared, stained with uranyl acetate and lead citrate, and examined with an electron microscope EM 902 A (Zeiss, Oberkochen, Germany).

**Localization of F-actin in rat pancreatic tissue.** Cryosections were prepared with a cryostat CM 3000 (Leica) and fixed with acetone at 4°C for 10 min. After repeated rinsing with PBS and incubating with Dako Protein Block (Dako Diagnostika, Hamburg, Germany) for 30 min, the sections were stained with 100 \(\mu\)l bodipy FL phallacidin (5
U/ml) for another 30 min in the dark, following the manufacturer’s suggested protocol. Thereafter, the sections were washed twice with PBS and embedded in fluorescence mounting medium (Dako), which was followed by viewing and micrography on a fluorescence microscope Jenaluma (Carl Zeiss, Jena, Germany).

**Statistical analysis.** Means of normally distributed data were compared by Student’s t-test. In the case of significant differences in variance, means were compared by Welch’s t-test. P < 0.05 was considered significant.

**RESULTS**

**Cerulein-induced calpain activation in rat pancreatic tissue.** The autoproteolytic cleavage of the 80-kDa subunit at the NH2 terminus of calpain leading to a complex pattern of transient and stable autolysis products has been observed to be associated with calpain activation (10, 21, 24, 28). To investigate whether cerulein hyperstimulation induces activation of calpain in rat pancreatic tissue, we used a set of antibodies directed against the 80-kDa subunit of the corresponding calpain.

Fig. 1. In vitro autolysis of μ-calpain and m-calpain. Porcine μ-calpain (34 μg/ml) or m-calpain (50 μg/ml) was incubated without or with casein (1 mg/ml) at 37°C and activated by addition of Ca2+ (5 mM) as described in MATERIALS AND METHODS. Aliquots were withdrawn after the indicated times and analyzed by immunoblotting. Representative μ-calpain immunoblots using antibodies directed against the 80-kDa subunit of μ-calpain: antibody pAB1 (A) and antibody pAB3 (C; each with 26 μg protein on a 10% gel), and representative m-calpain immunoblots directed against the 80-kDa subunit of m-calpain: antibody pAB2 (B) and antibody pAB4 (D; each with 38 μg protein on a 10% gel). Ca2+ quickly induces autoproteolytical cleavage of the corresponding 80-kDa calpain subunit, leading to the formation of various autolysis products.

Fig. 2. Cerulein-induced activation of μ-calpain in rat pancreatic tissue. At 2.5 h after treatment either with Z-Val-Phe methyl ester (ZVP; 60 mg/kg ip in 0.4 ml DMSO) or with ZVP solvent DMSO, pancreatitis was induced by injections of 10 μg/kg ip cerulein (5 times at hourly intervals). One hour after the final injection, the rats were killed. Homogenized pancreatic tissue was subjected to Western blot analysis. Representative μ-calpain immunoblots using antibodies directed against the 80-kDa subunit of μ-calpain: antibody pAB1 (A; 40 μg protein on a 10% gel) and antibody pAB3 (B; 20 μg protein on a 7.5% gel). Cerulein hyperstimulation induces activation of μ-calpain as indicated by the decreased 80-kDa calpain subunit and the concomitant increased formation of a cleavage product corresponding to 55 kDa. ZVP preconditioning reduces the cerulein-mediated μ-calpain activation.

Fig. 3. Cerulein-induced activation of m-calpain in rat pancreatic tissue. Experimental design as in Fig. 2. Representative m-calpain immunoblots using antibodies directed against the 80-kDa subunit of m-calpain: antibody pAB4 (A) and antibody pAB2 (B; each with 40 μg protein on a 10% gel). Cerulein hyperstimulation induces activation of m-calpain as indicated by the decreased 80-kDa calpain subunit and the concomitant increased formation of different autolysis products including fragments corresponding to 50 and 28 kDa. ZVP preconditioning reduces the cerulein-mediated m-calpain activation.
pain form on Western blots. In pilot in vitro experiments, we tested whether the selected antibodies are capable of detecting the calpain activation. Immunoblot analyses using anti-μ-calpain antibody pAB1 and anti-m-calpain antibody pAB2 showed that both purified μ-calpain and m-calpain autolyzed rapidly when incubated with Ca\(^{2+}\) in the presence of the calpain substrate casein. After only 5 min, the 80-kDa band had significantly decreased, accompanied by the formation of immunologically reactive fragments (Fig. 1, A and B; each with lane 2). After 30 min, both antibodies clearly identified cleavage products corresponding to apparent molecular weights of 55, 50, and 40 kDa (Fig. 1, A and B; each with lane 4). In addition, pAB1 detected a further μ-calpain fragment of ~35 kDa, whereas pAB2 reacted with an m-calpain autolysis product of ~43 kDa. When the calpains were activated with Ca\(^{2+}\) in the absence of casein, the autolysis was still faster than in the presence of the substrate, leading to a complete disappearance of the 80-kDa band after 30 min (Fig. 1, A and B; each with lanes 3 and 5). The 55- and 50-kDa cleavage products were likewise disappeared, suggesting that they are merely transient products, whereas the other low molecular weight fragments observed seem to be more resistant to autolytic proteolysis.

Anti-μ-calpain antibody pAB3 and anti-m-calpain antibody pAB4 detected the Ca\(^{2+}\)-induced processing of the 80-kDa subunit as well but were not able to react with calpain fragments (Fig. 1, C and D; each with lanes 2–4). Thus our results indicate that all antibodies tested can be used for the immunochemical detection of calpain activation.

Western blot analysis of both calpain isoforms in homogenized pancreatic tissue of the control group clearly detected the 80-kDa subunit using these antibodies as demonstrated in Figs. 2, A and B, and 3, A and B (each with lanes 2 and 3). Stimulation of animals with supramaximal doses of cerulein caused a significant decrease in the 80-kDa subunit of both calpain forms compared with the respective control, which may indicate the activation of the proteases (Fig. 2A, lanes A and B).}

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**Fig. 4.** Effect of cerulein hyperstimulation on calpastatin. Experimental design as in Fig. 2. A: representative immunoblot for calpastatin (20 μg protein on an 8% gel) showing an altered calpastatin expression pattern in response to cerulein hyperstimulation that is not affected by ZVP preconditioning. B: calpastatin expression pattern in untreated isolated rat pancreatic acinar cells (20 μg protein on an 8% gel).

**Fig. 5.** Effect of ZVP on cerulein-induced macroscopic alterations of rat pancreas. Experimental design as in Fig. 2. Shown are control (A) and cerulein administration either without (B) or with ZVP preconditioning (C). Note that the macroscopically visible edema formation associated with cerulein hyperstimulation is obviously reduced in response to ZVP preconditioning.
4 and 5; Fig. 2B, lane 4; Fig. 3, A and B; each with lanes 4 and 5). A further indication for cerulein-induced calpain activation may be the concomitant appearance of cleavage products possessing the same molecular weights as the calpain autolysis fragments generated after calpain activation in vitro. Thus immunoblot analysis of μ-calpain using antibody pAB1 showed the increased formation of a ~55-kDa product compared with the control (Fig. 2B, lanes 4 and 5). With the use of anti-m-calpain antibody pAB2, enhanced concentrations of immunologically reactive cleavage products corresponding to ~50 and ~55 kDa were observed. In addition, both a minor fragment of ~60 kDa and a prominent fragment of ~28 kDa appeared (Fig. 3B, lanes 4 and 5). These fragments were not observed in the present activation experiment with purified m-calpain but have been described by others (10, 24).

Cerulein-induced decrease in the calpastatin protein expression in rat pancreatic tissue. The calpain activation as well as the calpain activity are regulated by the endogenous inhibitor calpastatin. Therefore, we investigated whether cerulein-mediated calpain activation may be associated with a decrease in the pancreatic calpastatin level. Western blot analysis of calpastatin in the pancreatic tissue of the control animals detected two minor high molecular weight proteins of ~130 and 100 kDa and two major low molecular weight proteins corresponding to ~80 kDa (Fig. 4A, lanes 1 and 2). In addition, several bands of lower molecular weights were also recognized by the antibody. To further confirm these findings, similar immunoblot analysis was performed with untreated isolated rat pancreatic acinar cells. A representative immunoblot is demonstrated in Fig. 4B and reveals a comparable band pattern.

Cerulein hyperstimulation led to clear changes in the calpastatin expression pattern (Fig. 4A, lanes 3 and 4). Thus both...
high molecular weight proteins became undetectable, accompanied by the appearance of immunologically reactive bands of ~85 and 65 kDa compared with the control, probably representing calpastatin cleavage products. In addition, a significant increase in the intensity of the ~50-kDa band was observed. The magnitude of both the 65- and the ~50-kDa band suggests that a fragmentation of the 80-kDa native calpastatin forms may occur as well.

Our results further suggest that treatment of rats with ZVP before cerulein hyperstimulation does not affect the cerulein-mediated alterations of calpastatin (Fig. 4A, lanes 5–7).

**Effect of ZVP on the severity of cerulein-induced acute pancreatitis.** To gain insight into the possibility that calpain is involved in cerulein-induced pancreatic damage, we investigated whether the attenuation of cerulein-induced pancreatic calpain activation in response to ZVP preconditioning may be associated with protection against cerulein-induced pancreatitis. Our results revealed that ZVP induced a protective effect that was already macroscopically apparent. Thus, whereas cerulein provoked an edematous pancreatitis with pancreata macroscopically appearing swollen and grayish, these changes were greatly diminished when cerulein was given after ZVP preconditioning (Fig. 5). Histologically, the extent of interstitial edema and acinar cell vacuolization, the most prominent histological features of the pancreas in rats suffering from cerulein pancreatitis, was less pronounced compared with the group treated with cerulein only (Fig. 6). Quantifying the histopathological changes by our scoring system, a decline of the average score for all morphological criteria investigated was found (data not shown). Altogether, the histological score decreased by 41% (P < 0.01) in the inhibitor-pretreated cerulein group compared with the only cerulein-treated group as demonstrated in Fig. 6D.

The beneficial effect of ZVP treatment before cerulein administration was also evident at the ultrastructural level. In particular, the large cytoplasmic vacuoles that also characterize cerulein pancreatitis were strongly reduced in size and number compared with the cerulein group without ZVP preconditioning (Fig. 7, A–C). Furthermore, the pronounced loss of microvilli and disappearance of the terminal microfilaments likewise seen with cerulein alone were clearly smaller (Fig. 7, D–F). Thus the apical region in acinar cells of ZVP-pretreated animals resembled, at least in part, that visible in control animals. Staining of the actin filaments with bodipy FL phallacidine also reflected the protective effect of ZVP on the actin cytoskeleton, consequently further supporting the electron-microscopical findings. Whereas cerulein hyperstimulation resulted in a nearly absent fluorescence along the luminal and lateral membrane of the acinar cells compared with the control, the loss of actin labeling was less vigorous when cerulein was injected after ZVP treatment (Fig. 8).

Finally, the protective effect of ZVP against cerulein pancreatitis noted in the morphological studies was also apparent on biochemical criteria that characterize this model, such as hyperamylasemia, hyperlipasemia, and increased tissue water...
content, which was considered to represent pancreatic edema. In fact, the activities of amylase and lipase decreased by 46 (P < 0.01) and 62% (P < 0.01), respectively, when cerulein was given after ZVP treatment (Fig. 9, A and B). The pancreatic edema was reduced by 60% (P < 0.05), thus confirming our morphological observations (Fig. 9C).

DISCUSSION

In the present study, we investigated the role of calpain activation in acute cerulein pancreatitis. To specifically explore which of the calpain isoforms is activated in pancreatic tissue in response to cerulein hyperstimulation, we performed Western blot analysis. In preliminary experiments with purified porcine μ-calpain and m-calpain, we found that the antibodies chosen for immunoblotting reacted specifically with the corresponding form of calpain and not with the other (data not shown). We also found that the antibodies can be used for the immunochemical detection of calpain activation. Specifically, anti-μ-calpain antibody pAB1 and anti-m-calpain antibody pAB2 have been observed to be good tools. Unlike pAB3 and pAB4, these antibodies identified not only the autolysis of the respective 80-kDa subunit associated with calpain activation but also recognized different autolysis products of the large subunit (10, 24). With the use of these antibodies, our results clearly indicate that both calpain isoforms are constitutively expressed in rat pancreatic tissue, thus reconfirming our recent immunohistological findings (43). In addition, our data indeed provide evidence that stimulation of rats with supramaximal doses of cerulein leads to activation of both μ-calpain and m-calpain. This is further supported by the observation that administration of calpain inhibitor ZVP before cerulein treatment clearly reduces the activation of both calpains. ZVP is a hydrophobic cell-permeable dipeptidyl methyl ester, containing a benzyloxy carbonyl residue at the NH2 terminus. Studies by others (8, 16, 17) using this low molecular weight inhibitor have shown that it is relatively specific for calpain and highly potent, causing sustained inactivation of the protease. The mechanism of action probably involves a covalent interaction between an electrophilic center of the inhibitor and the thiol group of the active site cysteine of calpain (31).
The activation as well as the activity of calpain are Ca^{2+}-dependently regulated by their endogenous inhibitor calpastatin (22). Thus calpain activation requires both increased cytosolic Ca^{2+} and inadequate calpastatin activity. A sustained cytosolic Ca^{2+} increase has previously been reported to occur in response to cerulein hyperstimulation (4, 30). Consequently, here we investigated whether cerulein-induced calpain activation may be associated with a calpastatin downregulation. Our immunoblot analyses reveal for the first time that rat pancreatic tissue contains multiple calpastatin forms. In isolated rat pancreatic acinar cells, we observed a similar protein pattern, further confirming the findings in the pancreatic tissue. Consistent with our observation, calpastatin has been found to be present in different multiple forms in different tissues and mammalian species, likely resulting from alternative splicing, mutations, or posttranslational modifications such as phosphorylation and dephosphorylation (19, 20, 38). In addition, low molecular weight calpastatins can be produced by conservative proteolytic degradation of a high molecular precursor (7). The expression of multiple calpastatin forms is supposed to be relevant for the specific regulation of different calpain isoforms normally present in a single cell type (20).

Cerulein hyperstimulation of rats caused marked alterations of the pancreatic calpastatin pattern, possibly leading to loss of inhibitory activity. Similarly, a downregulation of calpastatin accompanied by a decline in calpastatin activity has been observed in various injury models, such as in rat hearts and renal cortex during ischemia-reperfusion (35, 36) and in erythrocytes of hypertensive rats (7). There is evidence that the decrease in calpastatin is caused by nonspecific proteolytic degradation induced by different mechanisms. In erythrocytes of hypertensive rats, during rat cerebral hypoxia-ischemia, and TNF-induced apoptosis in U937 cells, calpain by itself was responsible for this finding (3, 7, 27). In contrast, during rat renal ischemia-reperfusion and in several cellular apoptotic systems, members of another family of cysteine proteases, the caspasas, were identified to cleave the calpain inhibitor (27, 35, 41). Our results showing a cerulein-mediated fragmentation of the pancreatic calpastatin may indicate a proteolytic process as well. However, on the basis of our finding that ZVP has no effect on cerulein-induced calpastatin alterations, we conclude that proteases other than calpain have to be responsible for this phenomenon. Whether caspases provoked the calpastatin degradation, as observed in other systems, or other proteases activated during acute pancreatitis remains to be elucidated.

The decrease in calpastatin may represent critical situations under which calpain activity cannot be regulated within physiological ranges, consequently leading to cell damage. Consistent with this assumption, our data reveal that cerulein-induced pancreatic injury requires activation of calpainlike protease. Thus inhibition of the calpain activity by treatment of rats with calpain inhibitor ZVP before cerulein administration indeed resulted in less-severe pancreatic damage. In particular, the pancreatic edema, the acinar cell vacuolization, as well as cytoskeletal alterations, characteristic histological features of cerulein-induced pancreatitis, were obviously reduced (11, 14). Certainly, we cannot rule out that proteases other than calpain account for some of the ZVP effects reported here. However, previous studies (8, 16, 17) showing that ZVP reduced the calpain activity to a great extent in hypoxic isolated renal cells and during ischemia-reperfusion in the liver, resulting in marked attenuation of cell damage, support our conclusion. Further support is provided by the observation that ZVP has only minimal inhibitory effects on other cysteine proteases such as cathepsin B and several caspases such as caspase-1, -2, -3, or -7 (8, 17). Finally, in our own in vitro preliminary experiments, ZVP was found to only minimally inhibit caspase-8 and the serine proteases trypsin and chymotrypsin (data not shown).

The fact that ZVP induced merely a partially protective effect suggests that, apart from calpainlike proteases, other enzymes or factors concomitantly or independently contribute
to morphological pancreas damage evoked by cerulein. In this regard, a similar protection was found by using the synthetic inhibitor E3123, which blocks trypsin, plasmin, elastase, and phospholipase A2 activities (25). Accordingly, the activation of digestivezymogens such as trypsinogen, procarboxypeptidase A1, procarboxypeptidase B, and chymotrypsinogen in pancreatic acini as a consequence of supramaximal secretagogestimulation has been demonstrated (12, 30, 34).

Regarding the mechanisms underlying calpain-mediated pancreatic damage, we can only speculate at this time. Some of the many calpain substrates include various actin-associated proteins such as α-actinin, spectrin, talin, and ankyrin that are involved in maintaining and regulating of cytoskeletal structures and functions (6, 33). There is increasing evidence that nonphysiological calpain activation is coincident with degradation of cytoskeletal proteins. In particular, calpain activated during ischemia or hypoxia in different systems was observed to cleave spectrin and ankyrin (2, 13, 35, 47). In isolated pancreatic acini as a consequence of supramaximal secretagoguestimulation was clearly diminished, implicating a role of calpain in morphological pancreas damage evoked by cerulein. In this study, the inhibitors were administered alone or in combination with 250 μM CaCl₂ to modulate calpain activation.

In summary, our study demonstrates that cerulein hyperstimulation causes a decrease in the calpastatin level, which may contribute to the activation of both calpain isoforms observed in parallel. Prophylactic administration of calpain inhibitor ZVP has no protective effect on calpain alterations but significantly reduces calpain activation. In correlation to the decreased calpain activity, the severity of cerulein pancreatitis was clearly diminished, implicating a role of calpain in the onset of acute pancreatitis. Consequently, prophylactic application of calpain inhibitors may be a promising strategy for alleviating the pancreatic damage occurring during pancreas transplantation including organ preservation. Whether ZVP exerts a beneficial effect on preestablished pancreatitis remains to be investigated. We believe that the inhibitor is likely to prove ineffective, because the protease activation has already occurred.

GRANTS

This work was supported by a grant from the Faculty of Medicine, University of Rostock.

REFERENCES


17. Kohli V, Madden JF, Bentley RC, and Clavien PA. Calpain mediates ischemic injury of the liver through modula-
21. Mellonl E, Michetti M, Salamino F, Minafra R, Pontremoli S. Modula-


