Intra-acinar cell activation of trypsinogen during caerulein-induced pancreatitis in rats


Intra-acinar cell activation of trypsinogen during caerulein-induced pancreatitis in rats. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G352–G362, 1998.—Supramaximal stimulation of the pancreas with the CCK analog caerulein causes acute edematous pancreatitis. In this model, active trypsin can be detected in the pancreas shortly after the start of supramaximal stimulation. Incubation of pancreatic acini in vitro with a supramaximally stimulating caerulein concentration also results in rapid activation of trypsinogen. In the current study, we have used the techniques of subcellular fractionation and both light and electron microscopy immunolocalization to identify the site of trypsinogen activation and the subsequent fate of trypsin during caerulein-induced pancreatitis. We report that trypsin activity and trypsinogen-activation peptide (TAP), which is released on activation of trypsinogen, are first detectable in a heavy subcellular fraction. This fraction is enriched in digestive enzyme zymogens and lysosomal hydrolases. Subsequent to trypsinogen activation, both trypsin activity and TAP move to a soluble compartment. Immunolocalization studies indicate that trypsinogen activation occurs in cytoplasmic vacuoles that contain the lysosomal hydrolase cathepsin B. These observations suggest that, during the early stages of pancreatitis, trypsinogen is activated in subcellular organelles containing colocalized digestive enzyme zymogens and lysosomal hydrolases and that, subsequent to its activation, trypsin is released into the cytosol.

pancreas; immunolocalization; trypsinogen activation peptide; trypsin; subcellular fractionation; cathepsin B

Acute pancreatitis is generally believed to be an autodigestive disease in which the pancreas is injured by enzymes that are normally secreted by acinar cells. Under physiological conditions, most of these potentially harmful digestive enzymes are synthesized and secreted by acinar cells as inactive proenzymes or zymogens with activation occurring in the duodenum. There, the brush-border enzyme enteropeptidase cleaves the NH₂-terminal trypsinogen-activation peptide (TAP) from trypsinogen, releasing active trypsin that can then catalyze the activation of the other zymogens. In contrast, during the early stages of pancreatitis, digestive enzyme zymogens, including trypsinogen, are believed to be prematurely activated in the pancreas (1, 7, 17, 24, 25, 30, 35). The location in the pancreas of premature trypsinogen activation and the fate of activated trypsin during the early stages of pancreatitis are issues of considerable importance and interest, but a clear understanding of these phenomena has not yet been reached. Studies exploring these issues in clinical pancreatitis have not been possible, because pancreatic tissue is generally not available for examination during the early stages of pancreatitis in humans. To overcome this limitation, investigators have employed a number of experimental models of pancreatitis, which permit examination of pancreas samples during the very early stages of pancreatitis.

Secretagogue (caerulein)-induced pancreatitis is, perhaps, the most frequently employed model of experimental pancreatitis (6). Caerulein is an analog of the pancreatic secretagogue CCK. Rats infused with a dose of caerulein exceeding that required for maximal digestive enzyme secretion develop acute edematous pancreatitis (16). In time-dependence studies performed by our laboratory (7), we have recently noted that activated trypsinogen and elevated TAP levels can be detected in the pancreas within 15–30 min of supramaximal secretagogue stimulation. We have suggested (32) that the intrapancreatic activation of trypsinogen is catalyzed by the lysosomal hydrolase cathepsin B, which, during the early stages of secretagogue-induced pancreatitis, as well as in other models of pancreatitis, is colocalized with digestive enzyme zymogens.

In the present study, we report our findings using the model of secretagogue-induced pancreatitis to identify the intrapancreatic site of trypsinogen activation and the fate of activated trypsin. To address these questions, we have used the techniques of subcellular fractionation and both light and electron microscopy immunolocalization. Our studies were performed under both in vivo and in vitro conditions. We report that, in response to supramaximal secretagogue stimulation, trypsinogen is activated in a particulate intracellular compartment that is, most likely, composed of cytoplasmic vacuoles. Subsequent to its activation, trypsin moves from this compartment to a soluble intracellular compartment that, we suggest, is the acinar cell cytosol.

Materials and Methods

Male Wistar rats (125–200 g), obtained from Charles River Laboratories (Wilmington, MA), were housed in temperature-
controlled (23 ± 2°C) rooms with a 12:12-h light-dark cycle. They were fed standard laboratory chow and given water ad libitum. Caerulein was purchased from Research Plus (Bayonne, NJ), pentobarbital from Veterinary Laboratories (Lenexa, KS), V/3 tubing from Biolab Products (Lake Havasu, AZ), collagenase from Worthington Biochemical (Freehold, NJ), trypsin substrate (A-butyloxy carbonyl-L-glutaminyl-L-alanyl-L-arginine 4-methyl-coumaryl-7-amide) from Peptides International (Louisville, KY), Superfrost/Plus slides from Fisher Scientific (Pittsburgh, PA), Vectashield mounting medium, fluorescein anti-rabbit IgG, and Texas red anti-sheep IgG from Vector Laboratories (Burlingame, CA), and sheep anti-human cathepsin B from ICN Pharmaceuticals (Costa Mesa, CA). All other chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO). All experimental protocols were approved by the Institutional Animal Use Committee of the Beth Israel Deaconess Medical Center.

Animal and tissue preparation. For in vivo studies, animals were prepared and pancreatic tissue was processed as described previously (7). Similarly, for in vitro studies pancreatic acini were freshly prepared, using collagenase digestion according to the method of Powers et al. (23). The acini were incubated in 10 mM HEPES buffer (pH 7.4) containing 0.1 mM caerulein for 15–120 min. They were separated by extracellular fluid by centrifugation (60,000 × g for 30 s) and the pellet, containing the acini, was used for subcellular fractionation (see below).

Subcellular fractionation. For subcellular fractionation of in vivo-derived samples taken from whole pancreas, portions of the pancreas were minced in ice-cold 5 mM MOPS buffer (pH 6.5) containing 250 mM sucrose and 1 mM MgSO4. The minced pancreas was homogenized with a motorized glass-Teflon grinder (0.10- to 0.15-mm clearance) using three up and down strokes at a motor speed of 3,000 rpm. For subcellular fractionation of freshly prepared acini studied in vitro, the acini were washed twice by repeated centrifugation and resuspension in the same MOPS buffer and homogenized using the same tissue grinder but with seven up and down strokes. In both cases, a sample of the homogenate was used to measure DNA content. The remaining portion was subjected to low-speed centrifugation (150 g for 10 min) to remove nuclei and unbroken cells. The resulting supernatant (postnuclear supernatant) was used for subcellular fractionation, according to the method of Tartakoff and Jamieson (33) as slightly modified by Saluja et al. (28). Three subcellular fractions were obtained as follows: a zymogen granule-enriched fraction (1,300 g for 15 min pellet), a lysosome/mitochondria-enriched fraction (12,000 g for 13 min pellet), and a microsome/cytosol-soluble fraction (12,000 g for 13 min supernatant). The identity of these fractions was confirmed by assay of marker enzymes as previously described (28). In preliminary experiments, the possibility that either trypsin or TAP was located within microsomes as opposed to being in the unsedimentable soluble portion of the 12,000 g supernatant fraction was evaluated by subjecting the 12,000 g supernatant to high-speed centrifugation (105,000 × g for 60 min). All of the active trypsin and TAP was subsequently recovered in the resulting supernatant, indicating that they are soluble and not contained in microsomes.

Electron microscopy immunolocalization of TAP. For these studies, pancreatitis was induced by infusion of a supramaximally stimulating dose of caerulein, as described above, for 30 min. Pancreata were fixed in neutral-buffered Formalin for 12 h and after processing were embedded in paraffin wax. Immunocytochemical localization of cathepsin B and TAP, at the light microscopic level, was carried out on consecutive sections. Sections 4 µm thick were cut onto glass slides. They were deparaffinized by passing them through two changes of xylene and a graded series of alcohol followed by rinses in tap water and PBS (0.01 M phosphate buffer, pH 7.4, 0.154 M NaCl), respectively. Nonspecific binding was blocked by incubating sections in 1% BSA–PBS. Control sections were incubated in BSA–PBS. After rinsing in PBS, the sections were incubated for 30 min with the corresponding fluorescent secondary antibodies, i.e., FITC-conjugated anti-rabbit IgG and Texas red anti-sheep IgG. After three rinses in PBS, the sections were mounted in Vectashield mounting medium.

Assays. Amylase activity was determined according to the method of Pierre et al. (22), cathepsin B by the method of McDonald and Ellis (21) with modifications (27), and trypsin by the method of Kawabata et al. (12). In the trypsin assay, the slope of rising fluorescence emission was calculated as arbitrary units and expressed per milligram of DNA in the homogenate of that particular tissue or acini sample. To allow for pooling of data from different experiments, we expressed the trypsin activity in each fraction as a percentage of the total trypsin activity determined by combining the activity found in all of the fractions. We measured DNA, using Hoechst dye 33258, according to the method of Labarca and
increase in trypsin activity in the suspending medium from the acini can be accounted for by an activity from the acini, which declines with time (Fig. 2).

Exposure to supramaximal secretagogue stimulation (7), the total trypsin activity in acini increases with prolonged supramaximal stimulation and is, therefore, easier to detect in the lysosome/mitochondria-enriched fraction (27). This shift in cathepsin B distribution can be detected within 15–30 min of the start of supramaximal stimulation and is, therefore, believed to be accounted for by the formation of cytoplasmic vacuoles that contain colocalized digestive zymogens and lysosomal hydrolases (27, 29). We now report that a similar change in the subcellular distribution of cathepsin B is noted when freshly prepared acini are incubated in vitro with a supramaximally stimulating concentration of caerulein. The cathepsin B activity in the heavy fraction rises (Fig. 1).

Roughly 35% of the TAP can be recovered and measured, by ELISA, in the supernatant (soluble fraction) increases. In contrast to all other subcellular fractions, TAP can be detected in the supernatant (soluble fraction) within 10 min of supramaximal stimulation (30). A small portion of the activated trypsin is detected in the soluble (12,000 g) fraction rises (Fig. 1). With continued supramaximal stimulation for up to 210 min, trypsin activity gradually falls in the 1,300 g pellet and, during the same period, trypsin activity in the soluble (12,000 g) fraction rises (Fig. 1).

A similar pattern for the subcellular distribution of trypsin activity is detected when freshly prepared pancreatic acini are exposed to in vitro supramaximal stimulation with caerulein. Once again, trypsin activity in the acini is only detected when acini are exposed to a supramaximally stimulating concentration of the secretagogue, and this trypsin activity can be detected within 10 min of supramaximal stimulation (30). A small portion of the activated trypsin is detected in the lysosome/mitochondria fraction (not shown), but the greatest portion appears in the heavier, zymogen granule-enriched fraction (Fig. 1). With continued supramaximal stimulation, trypsin activity in the heavy particulate fraction declines, while that in the 12,000 g supernatant (soluble fraction) increases. In contrast to the in vivo situation in which intrapancreatic trypsin activity gradually increases with prolonged supramaximal stimulation (7), the total trypsin activity in acini exposed to supramaximal secretagogue stimulation declines with time (Fig. 2A).

Some of this loss of trypsin activity from the acini can be accounted for by an increase in trypsin activity in the suspending medium (Fig. 2B), suggesting that it reflects discharge of trypsin from the acini. Most of the fall in trypsin activity, however, is not accounted for by discharge into the medium, and this loss of total trypsin activity appears to be the result of either trypsin inhibition or autoinactivation.

Subcellular distribution of cathepsin B. We have previously noted that under in vivo conditions supramaximal secretagogue stimulation changes the sedimentation characteristics of lysosomal hydrolases such as cathepsin B and, as a result, a greater portion is recovered in the zymogen granule-enriched fraction and a lesser portion is recovered in the lysosomal/mitochondria-enriched fraction (27). Subcellular distribution of cathepsin B is noted when freshly prepared acini are incubated in vitro with a supramaximally stimulating concentration of caerulein. The cathepsin B activity in the heavy fraction rises (Fig. 1).

Subcellular distribution of TAP. TAP is the NH2-terminal peptide of trypsinogen that is cleaved from the zymogen during activation (9). Using the techniques of subcellular fractionation and light and electron microscopy immunolocalization, we have evaluated the distribution of TAP in the pancreas after in vivo supramaximal stimulation. In control samples, a small amount of TAP can be detected in the absence of supramaximal secretagogue stimulation, but this level of TAP increases markedly within 30 min of supramaximal stimulation (7). As shown in Fig. 4, roughly 50% of the TAP can be recovered and measured, by ELISA, in the zymogen granule-enriched 1,300 g pellet within 15 min of supramaximal stimulation. With more prolonged supramaximal stimulation, TAP levels in this heavy subcellular fraction fall. Roughly 35% of the TAP can also be detected in the soluble 12,000 g supernatant within 15 min of supramaximal stimulation. However, with more prolonged stimulation, this value gradually increases and by 210 min nearly all of the TAP measured by ELISA is in the soluble compartment (Fig. 4).

Immunolocalization of TAP at electron microscopic level. Immunogold labeling of pancreatic cryosections from control animals with anti-TAP antibody results in only a weak background gold decoration (Fig. 5A). This background label does not appear to indicate that TAP is present in acinar cells under physiological conditions, because it is also found on sections that are incubated without primary antibody (Fig. 5B). When the primary antibody is preincubated with a 50-fold excess of antigen (TAP peptide), no specific gold decoration beyond background levels results, regardless of whether or not the cryosections are from caerulein-infused or control animals. Even the cytoplasmic vacuoles that are characteristic of experimental pancreatitis remain negative under these conditions (Fig. 5C), but they are strongly TAP positive (Fig. 5D, inset) when the primary antibody is not preincubated with antigen.
After 30 min of supramaximal secretagogue stimulation, these vacuoles represent the principal TAP-containing compartment and neither zymogen granules nor the acinar lumen (Fig. 5D) is found to be significantly labeled with anti-TAP antibodies. Even the cytosol in the immediate vicinity of TAP-positive cytoplasmic vacuoles (Fig. 6A) and the adjacent endoplasmic reticulum (Fig. 6B) contain only background gold decoration at 30 min of supramaximal stimulation. After 3.5 h of caerulein infusion, the TAP-specific gold label becomes much less distinct. The surrounding cytoplasm near the cytoplasmic vacuoles becomes strongly TAP positive (Fig. 6C) and only the nucleus, mitochondria, and zymogen granules (Fig. 6C, inset) remain without gold decoration beyond background levels. At this time, the endoplasmic reticulum appears progressively dilated (Fig. 6D) and its TAP label is comparable to that of other cytoplasmic structures.

To quantitate the concentration of TAP in the respective subcellular compartments, we performed a morphometric evaluation of coded micrographs taken at a calibrated magnification. The results of these experiments (Fig. 7) confirm our suggestion that, after 30 min of supramaximal secretagogue stimulation, the cytoplas-
Mic vacuoles represent the only compartment in which the concentration of TAP-specific gold label is elevated above background levels. At this time, the TAP concentration does not appear to be increased in either the cytosol or zymogen granules and, in those areas, it is not found to be different from the label over the nuclei that were studied as an internal control. After 3.5 h of supramaximal stimulation with caerulein, cytoplasmic vacuoles still represent the principal TAP-containing compartment, but they are not the only compartment with significant TAP label. Although the density of gold decoration in vacuoles has increased above that noted at 30 min, the cytosol now contains an equally significant concentration of TAP. Because the volume of the cytosolic compartment greatly exceeds that of the vacuolar compartment, these observations indicate that most of the TAP is in the cytosolic space after 3.5 h of caerulein infusion. The nuclei and zymogen granules at this time point still remain at background levels.

Light microscopy immunolocalization of TAP and cathepsin B.

To determine whether the vacuoles that contain TAP shortly after the onset of supramaximal stimulation also contain the lysosomal hydrolase cathepsin B, we performed immunolocalization studies at the light microscopic level of resolution using antibody directed against either TAP or cathepsin B. As shown in Fig. 8, both TAP (Fig. 8A) and cathepsin B (Fig. 8B) are localized to cytoplasmic vacuoles. Figure 8C, which is the merged image obtained when both anti-TAP and anti-cathepsin B antibodies are used, indicates that both enzymes are localized in the same vacuoles.

DISCUSSION

The pathogenesis of acute pancreatitis has been the recent subject of intensive investigation and considerable controversy. Although most investigators agree that the disease is initiated by digestive enzyme activation, the location at which activation occurs and the fate of the enzymes, once activated, have not been unequivocally established. Some have suggested that enzyme activation occurs at an extracellular site in the gland parenchyma (2) and that pancreatic injury be-
gins in the periductal or perilobular areas (13, 25). We (32), on the other hand, have argued that digestive enzyme activation occurs in acinar cells as a result of the colocalization of digestive enzyme zymogens with lysosomal hydrolases. Evidence supporting this so-called "colocalization hypothesis" includes the following observations: 1) the earliest morphological changes in acute biliary pancreatitis are seen in acinar cells (18); 2) digestive enzyme zymogens and lysosomal hydrolases are colocalized in cytoplasmic vacuoles that appear, in several experimental models of pancreatitis, before other evidence of cell injury can be detected (14, 26, 27, 29); 3) under appropriate in vitro conditions, the lysosomal hydrolase cathepsin B can activate trypsinogen (3, 8, 19); 4) activated digestive zymogens, including trypsin, can be detected in the pancreas in clinical pancreatitis (5) and, in several models of experimental pancreatitis, activated trypsin can be detected before other evidence of cell injury (7, 24); 5) hereditary pancreatitis results from mutation of the trypsinogen gene with expression of trypsinogen that, once activated, is resistant to inactivation (34); and 6) supramaximal secretagogue stimulation, which is known to induce acute pancreatitis in animals, can cause activation of trypsinogen in isolated pancreatic acini studied in vitro and this activation can be prevented by the cell-permeant, specific cathepsin B inhibitor E64D (30).

The present study was undertaken to elucidate 1) the location of trypsinogen activation in acinar cells during the early stages of pancreatitis and 2) the fate of trypsin after its activation. We have chosen to explore these issues using the secretagogue model of pancreatitis, because it is a model that evolves in a rapid and highly reproducible fashion and, because we have recently noted (7) that activation of trypsinogen along with release of TAP can be detected in the pancreas as early as 15–30 min after the start of supramaximal stimulation. Bialek et al. (1) have already shown, using enzyme-blotting techniques, that activated zymogens can be found in an acinar cell particulate fraction 2 h after the onset of supramaximal secretagogue stimulation. We reasoned that measurement of trypsin activity in subcellular fractions prepared at varying but earlier times after the start of supramaximal stimulation would allow us to define the subcellular compartment in which activation occurs and to track the fate of trypsin after it becomes activated. Furthermore, we reasoned that immunolocalization of the activation peptide released during trypsinogen activation (i.e., TAP) would enhance our ability to define the location of trypsin activation. In designing these studies, we recognized the importance of making determinations at the earliest times possible after enzyme activation, because intracellular transport or organelle disruption could result in altered localization of both trypsin and TAP at later times.

As shown in Fig. 1, the trypsin activity detected in the pancreas after in vivo supramaximal stimulation and in acinar cells after in vitro supramaximal stimulation is first seen in a heavy subcellular fraction that sediments at 1,300 g. This fraction is normally enriched in zymogen granules and, after either in vivo or in vitro supramaximal stimulation, it is the fraction that is also enriched in the lysosomal hydrolase cathepsin B (32) (Fig. 3). This 1,300 g pellet is also the fraction that contains most of the measurable TAP after 30 min of supramaximal stimulation (Fig. 4). With prolonged supramaximal stimulation, both trypsin activity and measurable TAP decrease in this heavy subcellular fraction and a corresponding increase in both trypsin and TAP is noted in the soluble compartment (Figs. 1 and 4). With in vivo supramaximal stimulation, this shift in trypsin activity from the particulate to the soluble fraction occurs after 60 min, whereas it is first seen after 30 min of supramaximal stimulation in vitro (Fig. 1). This difference may reflect the influence of studying the same phenomenon under different experimental conditions. The value of the soluble compart-
ment may also differ between the in vivo and in vitro studies. In samples prepared from portions of whole pancreas (i.e., in vivo experiments), this soluble compartment would be expected to include both cytosol and extracellular interstitial edema fluid, but in samples prepared from suspended acini, the soluble compartment should include only the cytosol.

Taken together, these results suggest that trypsinogen activation during supramaximal secretagogue stimulation occurs in a particulate compartment that includes zymogen granules, and, during pancreatitis, also contains lysosomal hydrolases. Furthermore, these results suggest that, once activated, trypsin moves from this particulate location to the acinar cell cytosol.

In general, results of studies performed using the technique of subcellular fractionation must be interpreted with caution because of the possibility that enzyme redistribution during or after the time of tissue processing may alter the sedimentation characteristics of many components (31). Furthermore, subcellular fractionation experiments do not permit discrimination between different organelles that have similar sedimentation characteristics. Thus, using only subcellular fractionation, we would have been unable to establish whether trypsin is activated within zymogen granules, lysosomes, or cytoplasmic vacuoles, which cosediment along with the zymogen granules after supramaximal secretagogue stimulation.

We have used the techniques of light and electron microscopy immunolocalization to resolve these areas of uncertainty. At present, probes for pancreatic proteolytic activity at the ultrastructural level are not available and antibodies to digestive enzymes would not discriminate between the zymogens and their activated enzymes. For this reason, we have chosen to use purified antibodies raised against TAP. Those antibod-
ies are specific for TAP and have little or no cross reactivity with either trypsin or trypsinogen. It could be anticipated, therefore, that the site of highest TAP content at early times would correspond to the initial site of trypsinogen activation.

Our initial studies were performed using the technique of electron microscopy immunogold localization and anti-TAP antibodies. When we labeled the pancreas of saline-infused control animals for TAP, we found a sparse but even distribution of gold grains over the entire acinar cell. Although this could indicate that a small amount of trypsinogen activation takes place under physiological conditions, we reject this interpretation for two reasons. First, we would have expected to see a greater concentration of the TAP-specific label in compartments that are known to contain large amounts of trypsinogen (e.g., endoplasmic reticulum and zymogen granules), but these compartments were not labeled at a greater density than, for example, the nucleus. The second reason for rejecting this interpretation is the fact that the same background gold decoration was also found when no primary TAP antibody was used. Thus we conclude that trypsinogen activation either does not occur under physiological conditions or, alternatively, that it occurs at a level impossible to detect by immunogold labeling.

As early as 30 min after the start of caerulein infusion, the distribution of TAP-specific gold label

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1 It should be noted that morphometric studies quantitating immunogold anti-TAP labeling allow one to define the concentration of TAP in specified compartments. The actual content of TAP in a compartment would depend on the volume of that compartment. However, it is highly unlikely that a mechanism exists in acinar cells that results in the taking up of TAP against a concentration gradient. Thus the site of greatest TAP concentration is most likely to correspond with the site of initial TAP release (i.e., trypsinogen activation).
changes remarkably. At that time cytoplasmic vacuoles have formed and these pancreatitis-associated structures represent the principal and exclusive compartment in which significant concentrations of gold label are consistently found. Although occasional gold label is observed in other areas of the cell (zymogen granules, cytosol, nucleus), the density of that label is not significantly above the background level. This observation strongly suggests that the initial conversion of trypsinogen to active trypsin occurs in the cytoplasmic vacuoles. Furthermore, our findings all but exclude some other potential or additional sites of premature protease activation, such as zymogen granules, the acinar or ductal lumena, or the interstitial space between acinar cells. After 3.5 h of caerulein infusion, the density of gold label associated with cytoplasmic vacuoles has still further increased, but the vacuoles no longer represent the exclusive site of TAP localization. Large areas of the acinar cell cytosol, including the endoplasmic reticulum, are now found to be gold labeled, but this redistribution of TAP still excludes zymogen granules and nuclei. From these data we conclude that, very early after supramaximal stimulation, a premature and intra-pancreatic activation of proteases occurs and this event is initially confined to the cytoplasmic vacuoles in acinar cells. Later, TAP can be found over large areas of the cytosol in addition to the vacuoles. This subsequent redistribution of TAP could, theoretically, be explained in a number of ways. The explanation we consider most likely is that the cytoplasmic vacuoles, which are known to be unstable cellular organelles in vitro (27, 29), disintegrate in vivo and their content, which includes TAP and by inference active trypsin, is released into the cellular cytosol. An alternative explanation for our observed redistribution of TAP would be the passage of this small peptide, as opposed to the much larger trypsin, through the membrane of intact cytoplasmic vacuoles and, thus, into the cytosol. Unfortunately, probes that discriminate between activated digestive enzymes and their inactive zymogens are not currently available and, for this reason, we cannot unequivocally conclude that trypsin is released into the cytoplasm along with TAP.

The final issue we have addressed is the mechanism by which trypsinogen becomes activated during the early stages of pancreatitis. In previous reports (27), we have suggested that trypsinogen may be activated by the lysosomal hydrolase cathepsin B during pancreatitis. Indeed, cosedimentation of TAP, active trypsin, and cathepsin B in the same subcellular fraction during the early stages of caerulein-induced pancreatitis and after in vitro supramaximal stimulation with caerulein (Figs. 1, 3, and 4; Ref. 27) is compatible with this conclusion. To further evaluate this issue, we have performed light microscopy studies to immunolocalize cathepsin B and TAP 30 min after the start of in vivo supramaximal stimulation with caerulein. As shown in Fig. 8, cytoplasmic vacuoles located primarily on the luminal side of the nucleus are apparent under these conditions and these vacuoles contain both cathepsin B and TAP. Based on their location, one might conclude that these structures are generally not seen in control animals.
vacuoles are derived from the Golgi apparatus, but further studies will be needed to more clearly define their genesis. The colocalization of cathepsin B and TAP within these vacuoles is consistent with the conclusion that cathepsin B catalyzes trypsinogen activation in these organelles.

In summary, our findings lead us to conclude that intrapancreatic activation of trypsinogen occurs at a very early stage in the evolution of secretagogue-induced pancreatitis and that that activation occurs in cytosolic vacuoles containing both digestive enzymes and the lysosomal hydrolase cathepsin B. On the basis of these and previously reported observations (30), we conclude that intra-acinar cell activation of trypsinogen in the cytoplasmic vacuoles is catalyzed by cathepsin B. At later times, trypsin appears to be released from the cytoplasmic vacuoles into the cytoplasm. We suggest that the presence of activated trypsin in the cytoplasmic space is a critical event that eventually leads to cell injury, but further studies evaluating the relationship between cytoplasmic trypsin and cell injury will be needed to unequivocally establish this relationship. In the present study, we found that the increase in soluble (i.e., cytoplasmic) trypsin activity and TAP levels was not apparent before 60–120 min of caerulein infusion, yet, in earlier studies (6), we found that hyperamylasemia and pancreatic edema were present within 30–60 min of supramaximal stimulation. Although these time-dependent differences may merely reflect the inherent technical difficulty encountered in monitoring small but potentially critical changes in trypsin or TAP distribution, these differences may also indicate that other, as yet unidentified, phenomena contribute to cell injury in this model.

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