Intracellular Proteolysis of Pancreatic Zymogens

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Received July 15, 1992

Activation of pancreatic digestive zymogens within the pancreatic acinar cell may be an early event in the development of pancreatitis. To detect such activation, an immunoblot assay has been developed that measures the relative amounts of inactive zymogens and their respective active enzyme forms. Using this assay, high doses of cholecystokinin or carbachol were found to stimulate the intracellular conversion of at least three zymogens (procarboxypeptidase A1, procarboxypeptidase B, and chymotrypsinogen 2) to their active forms. Thus, this conversion may be a generalized phenomenon of pancreatic zymogens. The conversion is detected within ten minutes of treatment and is not associated with changes in acinar cell morphology; it has been predicted that the lysosomal thiol protease, cathepsin B, may initiate this conversion. Small amounts of cathepsin B are found in the secretory pathway, and cathepsin B can activate trypsinogen in vitro; however, exposure of acini to a thiol protease inhibitor (E64) did not block this conversion. Conversion was inhibited by the serine protease inhibitor, benzamidine, and by raising the intracellular pH, using chloroquine or monensin. This limited proteolytic conversion appears to require a low pH compartment and a serine protease activity. After long periods of treatment (60 minutes), the amounts of the active enzyme forms began to decrease; this observation suggested that the active enzyme forms were being degraded. Treatment of acini with E64 reduced this late decrease in active enzyme forms, suggesting that thiol proteases, including lysosomal hydrolases, may be involved in the degradation of the active enzyme forms. These findings indicate that pathways for zymogen activation as well as degradation of active enzyme forms are present within the pancreatic acinar cell.

INTRODUCTION

In studies performed almost 100 years ago, Mouret found that excessive cholinergic stimulation was associated with the development of pancreatic injury [1]. The reasons behind the development of such injury were unknown. Mouret, however, suggested that activation of trypsin within the pancreas might be an important event in this process. Subsequently, with some supporting evidence, it has been predicted that an important initial event in the development of pancreatitis is the intrapancreatic proteolytic conversion of digestive zymogens to their active forms [2–4]. Steer and Meldolesi have popularized the notion that this conversion may take place within the pancreatic acinar cell [5]. Two major mechanisms have been proposed to initiate the proteolytic conversion of zymogens within the acinar cell: (1) trypsinogen activation by the lysosomal hydrolase cathepsin B [6] or (2) trypsinogen autoactivation [5]. Direct evidence for either type of proteolytic event within the acinar cell has, however, been lacking.

Abbreviation: CCK: cholecystokinin

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TABLE 1
The pH of Intracellular Compartments within the Pancreatic Acinar Cell

| Compartment            | pH (from [5]) | pH (from [6]) |
|------------------------|---------------|---------------|
| Nucleus                | 7.0           | 7.0           |
| Golgi stack            | 7.0           | 6.8           |
| Condensing vacuoles    | 5.6           | 6.2           |
| Zymogen granules       | 6.5           | 6.6           |
| Lysosomes              | 5.3           | ND            |

Following incubation in the weak base DAMP, pancreatic acini were fixed in glutaraldehyde, embedded in LR gold, and processed for immune electron microscopy, using a gold-rabbit anti-mouse secondary antibody. The number of gold particles was counted over each compartment, and the relative pH was calculated as described [5].

The aim of our studies has been to develop a system in which intracellular proteolysis of pancreatic zymogens may be detected. The design and interpretation of these studies is based on a body of knowledge that has established the pancreatic acinar cell as a paradigm of a protein-synthesizing and -exporting system [7].

BACKGROUND

Pancreatic Proteins: Synthesis, Sorting, and Transport

Digestive Secretory Proteins  The vectorial pathway of protein synthesis, storage, and export within the pancreatic acinar cell has been reviewed [8]. Pancreatic digestive zymogens are synthesized on ribosomes attached to the endoplasmic reticulum and released into its lumen. Following transfer to the Golgi complex, digestive enzymes are sorted away from other proteins, such as lysosomal hydrolases, and directed into the secretory compartment. Most sorting of digestive zymogens into the secretory compartment is thought to take place within the Golgi complex. The estimated pH varies among the different compartments along the synthetic pathway [9,10] and is summarized in Table 1. In the pancreatic acinar cell, the pH is acidic within the Golgi complex and falls further in the condensing vacuoles. Although the pH increases in the zymogen granules, they are still slightly acidic. While the roles of these pH variations are not entirely clear, a body of evidence suggests that the pH in various intracellular compartments plays an important role in protein sorting in many systems. In the region adjacent to the trans-Golgi complex, small vesicles containing digestive zymogens appear to fuse and form larger vesicles, known as condensing vacuoles. As these condensing vacuoles move toward the apical pole, they become more electron-dense and smaller. The decrease in size is due in part to the pinching off of membrane, which presumably returns to the Golgi complex. These mature zymogen granules represent the final storage compartment for digestive zymogens.
**Lysosomal Hydrolases** Lysosomal hydrolases travel through the same synthetic compartments as that described for digestive zymogens until they reach the Golgi complex. At that site, lysosomal enzymes undergo a specific covalent modification that separates these enzymes from the digestive enzymes destined for export. During biosynthesis, mannose residues, which are phosphorylated on their 6 carbon atom, are added to the oligosaccharide chains of lysosomal enzymes by domain-specific enzymes, which recognize only lysosomal hydrolases. In the Golgi complex, these mannose 6-phosphate residues are recognized by mannose 6-phosphate receptors, which remove them from the secretory pathway and target them to lysosomes [11]. The receptors accomplish this sorting by specifically binding to the lysosomal enzymes in the Golgi complex and transporting them to an acidic pre-lysosomal compartment. The low pH releases the mannose 6-phosphate receptor from the lysosomal enzyme, and the receptor recycles back to the Golgi complex for another round of sorting. The hydrolases then reach the primary lysosome through a non-receptor-mediated pathway. Under normal conditions, this sorting mechanism efficiently sequesters lysosomal enzymes. In the pancreatic acinar cell, however, this separation appears not to occur with complete fidelity, even under normal conditions [12]. Several percent of lysosomal hydrolase activity may be found in the secretory compartment even under basal conditions [13]. The mechanism for entry of lysosomal enzymes into the secretory compartment is unknown. Furthermore, since most lysosomal enzymes exhibit an acidic pH optimum, it is unclear if the lysosomal enzymes are active in the zymogen granule.

**Effect of Neurohumoral Stimulation on Digestive Zymogen Release**

The major function of the pancreatic acinar cell is the release of digestive zymogens in response to a meal. The principal mediators of this secretion are cholecystokinin and acetylcholine. Both of these agents have a direct effect on the acinar cell. Treatment of pancreatic acinar cells with either cholecystokinin or acetylcholine results in exocytosis of zymogen granules and stimulation of protein secretion. Both of these natural ligand agonists generate a biphasic dose response for enzyme secretion. Following maximal stimulation with either ligand, there is a peak release of secretory protein. Treatment with higher doses, known as hyperstimulation, results in retention of secretory products within the acinar cell and diminished amounts of protein secretion [14]. The mechanism of this high-dose inhibition of secretion is unknown. Similarly, these secretagogues also exhibit a biphasic secretory dose-response curve in vivo and inhibit pancreatic secretion at very high doses [15,16]. It is notable that treatment of animals with these high doses of secretagogue also generates pancreatitis.

Cholecystokinin interacts with at least two major receptor forms on the pancreatic acinar cell. Its interaction with a high-affinity form of the receptor results in stimulation of acinar cell enzyme secretion. When cholecystokinin interacts with the low-affinity receptor form, secretion is diminished [17]. The interaction of cholecystokinin with its low-affinity receptor form is also associated with the development of experimental pancreatitis [18]. It has been predicted that the pancreatitis induced by cholecystokinin as well as other agents is initiated by the aberrant intracellular activation of pancreatic zymogens. This activation would require intracellular prote-
olysis and may require conditions now established for several intracellular proteolytic pathways.

**Pancreatic Proteins: Proteolytic Processing**

Proteolytic events may be divided into two major categories: (1) *limited proteolysis*, a mechanism often used for the generation of biologically active proteins or peptides from inactive precursor molecules, and (2) *proteolytic degradation*.

**Limited Proteolysis** Many proteins are synthesized in an inactive precursor form (zymogen) that is later converted to its active state by limited proteolytic cleavage. This type of proteolytic event may occur within the cell or extracellularly. For peptide hormones and neurotransmitters such as endorphins, this proteolysis takes place within the cell along the synthetic pathway. The intracellular processing events responsible for generating biologically active peptides utilize a complex proteolytic cascade, often involving specific endoproteases. The activity of some of the proteases along this pathway may be influenced by pH or divalent cations. Thus, some steps of limited intracellular proteolysis may be regulated.

Limited proteolysis is also a mechanism for activating a variety of enzymes by catalyzing the removal of internal inhibitory domains from enzyme precursors known aszymogens. The activation of many pancreatic digestive enzymes within the lumen of the small intestine is one of the best-known examples of this form of limited proteolysis. The first step in this activation occurs when pancreatic zymogens reach the small intestine. Enterokinase, a protease found only in the proximal small bowel, proteolytically cleaves trypsinogen to generate trypsin. In turn, trypsin activates an enzyme cascade by removing the inhibitory domain from other pancreatic zymogens. This elegant mechanism helps to assure that pancreatic digestivezymogens become active only within the small intestine.

Within the acinar cell, other mechanisms have the potential to allow increased trypsinogen to be activated and initiate a proteolytic cascade. These mechanisms include the activation of trypsinogen through either autocatalysis or cleavage by lysosomal hydrolases. Theoretically, these effects may be magnified by decreased pancreatic trypsin inhibitor activity.

Some zymogens have small, but measurable amounts of enzymatic activity. Thus, under the appropriate conditions, the catalytic activity present within trypsinogen alone is sufficient to support its autoactivation [19]. In the normal physiological state, this autoproteolysis occurs at a low rate; it is increased at an acidic pH and is dependent on divalent cations. Trypsinogen may also be activated by other intracellular hydrolases. The best example of this process utilizes cathepsin B, a lysosomal hydrolase that can convert trypsinogen to trypsin [20]. The optimum conditions for the activation of trypsinogen by cathepsin B require an acidic pH [19]. Thus, the major pathways having the potential to generate active trypsin within the acinar cell both exhibit an acidic pH.

Pancreatic trypsin inhibitor is co-packaged with trypsinogen in the secretory pathway. When trypsin is generated, pancreatic trypsin inhibitor blocks trypsin activity by binding to its active site. Thus, premature activation of trypsinogen may be quenched by the endogenous pancreatic trypsin inhibitor. There is, however, sufficient pancreatic trypsin inhibitor to block less than 5 percent of potential cellular trypsin activity. Furthermore, the interaction of pancreatic trypsin inhibitor with
trypsin is pH-dependent, diminishing at acidic pH. Thus, under low pH conditions or in the event that large amounts of trypsinogen were activated, the ability of pancreatic trypsin inhibitor to block trypsin activity could be easily overwhelmed. Under the appropriate conditions, any of these pathways could promote the premature activation of the zymogen cascade within the pancreatic duct or the acinar cell itself.

Proteolytic Degradation The intracellular degradation of proteins has several important functions: (1) degradation of endogenous or exogenous proteins that are potentially harmful to a cell, (2) limiting the amount of protein available for either secretion or intracellular functions, and (3) recycling amino acids for utilization in other proteins.

Protein degradation takes place both within membrane-bound compartments and within the cytoplasm. The major enzymes responsible for protein breakdown in membrane-bound compartments are lysosomal hydrolases. Proteins may be directed to lysosomal compartments in the pancreas by (1) traveling through the endocytic pathway, (2) fusion of random membrane compartments (autophagy) or mature zymogen granules (crinophagy) with lysosomes, and (3) specific targeting signals on cytoplasmic proteins that direct them to lysosomes.

Mixing of lysosomal hydrolases with digestive zymogens has been observed in the exocrine pancreas. For example, under basal conditions, some lysosomal hydrolases are targeted to the secretory compartment. Under conditions which induce experimental pancreatitis, the amount of lysosomal hydrolases increases within the secretory compartment [21]. This increase may occur through mis-sorting of lysosomal hydrolases into the secretory compartment at the level of the Golgi complex or through abnormal autophagic fusion events. In that respect, both crinophagic and autophagic figures are often described in experimental models of pancreatitis [5]. Some studies have found that these figures contain both digestive zymogens and lysosomal hydrolases [5].

This background information indicates that two general classes of proteolytic events have the potential to influence intracellular zymogen proteolysis. First, digestive zymogens may undergo limited proteolysis within the acinar cell and be converted to active enzyme forms. Second, additional proteolytic events may degrade active enzyme forms.

**IN VITRO EFFECT OF HIGH DOSES OF CHOLECYSTOKININ AND CARBACHOL ON ZYMOGEN CONVERSION**

Stimulation of Limited Proteolytic Conversion of Zymogens

To examine the ability of neurohumoral agents to regulate the intracellular proteolysis of pancreatic zymogens, isolated pancreatic acini were treated with various doses of either the fully active cholecystokinin octapeptide or the cholinergic agonist, carbachol. A sensitive immunoblot technique was developed for the purpose of measuring the conversion of pancreatic zymogens to forms of lower molecular weight [22]. This assay quantitatively detects differences in the molecular weight of a zymogen following its proteolysis. The antibodies used in this study recognize both the zymogen and its active enzyme form. As shown in Fig. 1, hyperstimulation of isolated acini by either cholecystokinin or carbachol for 30 minutes stimulated a
FIG. 1. Hyperstimulation of pancreatic acini is associated with the proteolytic processing of several zymogens. Following treatment with media alone (Cont), carbachol hyperstimulation (CARB, 10^{-3}M), or cholecystokinin octapeptide hyperstimulation (CCK, 10^{-7}M) for 30 minutes, acinar proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and processed for immunoblot analysis as described [22]. Immunoblots were incubated in antisera that specifically label zymogens as well as their active enzyme forms, and bound antibody was detected, using ^{125}I-goat anti-rabbit IgG followed by autoradiography. The darkly labeled bands surrounded by the heavy brackets indicate the zymogens. The more lightly labeled bands marked by the interrupted brackets are the proteolytic products generated by hyperstimulation.

dramatic increase in lower molecular forms of at least three zymogens. The electrophoretic mobility of these species of lower molecular weight each corresponds to that reported for their respective active enzyme forms.

Additional studies performed in our laboratory indicate that the species of lower molecular weight generated within acini are likely to represent active enzyme forms. Exposure of purified pancreatic zymogens to trypsin has been previously shown to result in the generation of active enzymes. After similar treatment and immunoblot analysis, a discrete band of 35 kD with carboxypeptidase A1 immunoreactivity is generated [22]. By the use of two-dimensional immunoblot analysis, this newly generated band was found to co-migrate with the 35 kD species detected in pancreatic acini. Using the same analysis, the 35 kD procarboxypeptidase immunoreactive species generated in acini was also found to co-migrate with purified recombinant carboxypeptidase A1 [22]. Together, these findings strongly suggest that the forms of lower molecular weight of these enzymes generated by hyperstimulation are likely to represent active forms of the respective zymogens.

To determine the site of this conversion, pancreatic acini were carefully separated from the incubation media following stimulation. The forms of lower molecular weight of the enzymes were detectable only within an acinar pellet and not in the media. Thus, this conversion appears to take place within isolated acini and is likely to be an intracellular event.

The amount of conversion to forms of lower molecular weight varied with the individual zymogen studied. In some experiments, as much as 10 percent of the cellular pool of carboxypeptidase A1 was converted to a form of lower molecular weight. In similar assays of carboxypeptidase B and chymotrypsinogen 2, the amount
of conversion was considerably less, rarely exceeding 2 percent of the cellular pool. Since the conversion was qualitatively similar for all the zymogens examined, our subsequent studies used only measurements of carboxypeptidase to characterize this event.

**Time Course of Conversion**

The enhanced conversion observed following hyperstimulation occurs rapidly and was often significantly above control values following as little as ten minutes of stimulation (Fig. 2). The peak amount of conversion occurs within 30 to 45 minutes; by 60 minutes, the amount of the proteins of lower molecular weight began to decline toward control levels. Since the forms of lower molecular weight of the enzyme were not released into the media, this decline at the later time periods suggests that they are being degraded. Thus, the time course depicted in Fig. 2 may reflect a dynamic process of both conversion of zymogens to forms of lower molecular weight and the subsequent degradation of the species of low molecular weight.

**Protease and pH Requirements for This Conversion**

The proteolytic activity and pH requirements for conversion of carboxypeptidase A were next examined (Fig. 3). The addition of soybean trypsin inhibitor, a 21 kD cell impermeant serine protease inhibitor, did not alter the stimulated conversion. Addition of the peptide benzamidine, a small-cell permeant serine protease inhibitor, completely blocked this conversion. The effects of benzamidine occurred without altering the secretory response of the acinar cell to cholecystokinin (CCK). Addition of E64, a cell-permeant thiol protease inhibitor [23], did not block conversion of procarboxypeptidase A1 to lower molecular forms. Thus, the conversion initiated by cholecystokinin appears to require a serine, but not a thiol protease activity.
FIG. 3. Effect of protease inhibitors on the conversion of carboxypeptidase A1 following CCK hyperstimulation for 30 minutes. Acini were pre-incubated for ten minutes with either the serine protease inhibitors benzamidine (Benz, $10^{-2}$M), soybean trypsin inhibitor (STI, $10^{-3}$M), or the thiol protease inhibitors E64 (100 mM) and leupeptin (Leup, $10^{-3}$M) prior to hyperstimulation. $n = 4$ to six experiments performed in duplicate. Mean ± SEM.

To examine the pH requirements for this conversion, acini were exposed to either monensin or chloroquine; both of these agents raise the pH of acidic intracellular compartments. Both treatments blocked the conversion stimulated by high doses of cholecystokinin (Fig. 4). These findings suggest that, in addition to a serine protease activity, this conversion requires a low pH compartment.

**Effect of Blocking Thiol Proteases on Zymogen Conversion**

The effect of inhibition of thiol proteases on the time course of procarboxypeptidase A1 conversion was examined by pre-incubating acini in E64. As shown in Fig. 5, this treatment resulted in greater recovery of carboxypeptidase A1 at later time points. This dose of E64 was sufficient to block virtually all thiol protease activity within acinar cells [22]. Pre-treatment of cells with E64 did not change the secretory response (amylase release) in response to carbachol, indicating that E64 was influencing a post-receptor event [data not shown].

FIG. 4. Effect of agents that increase intracellular pH on the conversion of procarboxypeptidase A1 following CCK hyperstimulation for 30 minutes. Acini were pre-incubated with either monensin (monen, $10^{-2}$M) or chloroquine (chloro, $4 \times 10^{-2}$M) for ten minutes prior to hyperstimulation.
Acinar Morphology Following Secretagogue Exposure

To examine the effects of hyperstimulation on acinar cell morphology, acini were hyperstimulated by carbachol for various periods of time, fixed, sectioned, and stained (Fig. 6). Examination of control tissue (Fig. 6a) demonstrates darkly stained, apically localized zymogen granules. In some cells, clear vacuoles of various sizes are evident in the region of the Golgi complex. The number or size of these vacuoles did not, however, change over the time course of treatment. Notably, blebbing of the basolateral membranes was observed following 30 minutes of hyperstimulation. Identical results were observed in acini following hyperstimulation by CCK.

DISCUSSION

An important feature of the pancreatic acinar cell is the safeguarding measures that are taken to prevent the intracellular activation and release of digestive enzymes. These protective mechanisms include: (1) synthesis of many digestive enzymes as inactive zymogens, (2) segregation of digestive enzymes into a distinct membrane-bound compartment, (3) condensation of secretory proteins, (4) co-packaging of protease inhibitors with digestive zymogens, and, from the present study, (5) co-packaging of enzymes that degrade active enzyme forms. It has been predicted that, under pathological conditions, limited proteolysis may lead to zymogen activation to a degree that overwhelms the protective mechanisms of the acinar cell. These active enzymes may then attack the acinar cell and initiate pancreatitis.

Our studies indicate that several classes of proteolytic events may influence the intracellular processing of pancreatic zymogens. The first event involves the limited proteolytic cleavage of at least several zymogens to active enzyme forms. This event has been designated zymogen conversion. Although no carboxypeptidase B or chymotrypsinogen 2 is present in the basal state, a small amount of carboxypeptidase A1 is present within acini even under control conditions. Carboxypeptidase A1 is distinct in its requirement for a secondary hydrolysis step prior to expressing
FIG. 6. Effect of hyperstimulation on acinar cell morphology. Following treatment with buffer control or carbachol hyperstimulation, acini were fixed in glutaraldehyde (3 percent in cacodylate buffer), dehydrated, and embedded in Epon. Sections were stained with toluidene blue. a. Control. b. Five minutes' hyperstimulation. c. 15 minutes' hyperstimulation. d. 30 minutes' hyperstimulation. No differences in acinar cell vacuolization were observed over this time course.

enzymatic activity [24]. Thus, the immunological detection of carboxypeptidase A1 within the acinar cell, even in the basal state, does not indicate that active enzyme is present. To state that active enzymes are being generated by this treatment will require direct measurements of enzymatic activities in our systems. These studies are under way.

The intracellular site of this enzyme is unknown. It has been suggested that a low pH compartment is required for such conversion. This requirement would favor an environment known to have a low pH, such as the Golgi complex or the condensing vacuole. Under pathologic conditions, however, pH changes within other components of the secretory pathway may also fall to levels sufficient to support zymogen conversion.

Another feature of this conversion is that only a limited amount of the zymogen pool is converted. There are several explanations for this observation, including: (1) the conversion takes place within a distinct and limited pool of zymogens, and (2) co-factors required for this conversion are depleted. Several distinct pools of zymogens are present within acinar cells. That found in the condensing vacuole exists under some of the lowest pH conditions in the cell and has not yet undergone the tight packing present in the secretory granule. Other candidate pools are those
destined for constitutive secretion or export in the basal state [25,26]. Although little is known about this pool, it appears that some of the secretory products from this pool are different from those contained in storage granules. Specific probes, such as monoclonal antibodies that distinguish between zymogens and active enzyme forms, are required to localize the site of this conversion.

**Limited Proteolysis of Zymogens to Active Enzyme Forms**

All three zymogens examined in this test system showed some conversion to active enzyme forms under conditions of hyperstimulation. Therefore, it is likely that this phenomenon occurs with other digestive zymogens and reflects the limited proteolytic conversion of the zymogen cascade.

The biochemical events regulating this conversion are unknown. The major events which have been predicted to mediate such a conversion are: (1) trypsinogen autoactivation, (2) trypsinogen activation by the lysosomal hydrolase cathepsin B, and (3) diminished activity of pancreatic trypsin inhibitor. Since the first two events proceed optimally at a low pH, the inhibitory effect of chloroquine and monensin does not distinguish between these mechanisms. The dramatic effect of benzamidine, a serine protease inhibitor, on this conversion favors a major role for a serine protease in this conversion, however. Conversely, the lack of inhibition by the thiol protease inhibitor, E64, makes it unlikely that the lysosomal thiol protease cathepsin B has a role in this conversion. Finally, this conversion occurs extremely rapidly, taking place prior to any of the events such as autophagy or crinophagy that are associated with the redistribution of lysosomal enzymes. Thus, this conversion is likely to take place in a pre-formed compartment along the secretory pathway.

The current findings favor a mechanism for the initiation of this conversion which involves the autoactivation of trypsinogen by a serine protease (Fig. 7). Since small amounts of the active enzyme forms are found within the acinar cell even in the basal state, it is possible that the conversion represents amplification of an ongoing event. For example, during zymogen granule condensation, the low pH of the condensing vacuole may allow for some zymogen conversion. If the normal process of condensation, including raising the vacuole pH, is inhibited by hyperstimulation, the nominal zymogen conversion found in this compartment may be amplified. A lower pH would also diminish the affinity of the pancreatic trypsin inhibitor for trypsin and would decrease the inhibition of active enzymes. Thus, the enhanced conversion may reflect a shift in dynamic equilibrium of this system to conditions which favor the generation of active enzyme forms. This model would help to explain the necessity for multiple safety mechanisms to eliminate enzymes which become active within the secretory compartment.

**Proteolytic Degradation of Active Enzyme Forms**

One of these safety mechanisms may be additional proteolytic events that specifically degrade active enzyme forms. Our preliminary studies indicate that, following long periods of hyperstimulation, the amounts of carboxypeptidase A1 decrease. The most plausible explanation for this decrease is the degradation of carboxypeptidase A1. The addition of the thiol protease inhibitor E64 to the incubation media dramatically reduces the disappearance of carboxypeptidase A1 following prolonged stimulation. These findings suggest that a thiol protease activity may play an
FIG. 7. Theoretical diagram for proteolytic zymogen conversion in pancreatic acinar cells. Hyperstimulation of the acinar cell by either cholecystokinin (CCK) or carbachol stimulates pathways that lead to zymogen conversion. Although these agents stimulate enzyme secretion in lower doses, hyperstimulation is associated with blocked secretion and retention of enzymes within the acinar cell. The expanded panels to the right reflect conclusions drawn from data presented in the text. Limited proteolytic zymogen conversion is dependent on a serine protease activity and a low pH compartment. Indirect evidence suggests that the first step in this conversion may be trypsinogen autoactivation. The serine protease and low pH have been assigned the same compartment as the conversion. These factors may, however, influence the conversion at any point along a signaling pathway. In a second proteolytic step, active enzyme forms are degraded and inactivated by a thiol protease. It is possible that the lysosomal hydrolases, many of which are thiol proteases, found within the zymogen granule are responsible for degradation of active enzyme forms.

important role in degrading zymogens that have been converted to an active form within the cell.

The identity of the thiol protease and the site of this enzyme degradation are unknown. Many lysosomal hydrolases as well as the cytoplasmic protease calpain are thiol proteases and are candidates for mediating this reaction. The lysosomal proteases that are present in the secretory compartment may act to degrade rapidly any enzymes that become active within that compartment. This role for lysosomal hydrolases contrasts with the central role they have been previously assigned in initiating zymogen activation. The calpain family of cytoplasmic thiol proteases provides a mechanism to degrade active enzymes that have escaped the limiting membrane of the zymogen granule. Thus, thiol proteases contained within the zymogen granule or the cytoplasm provide potential pathways for protecting the pancreatic acinar cell against the intracellular activation of zymogens.

Since it appears that lysosomal enzymes are present in the zymogen granule even in the basal state [12,13] an unsolved question regards their enzymatic activity in this compartment. One possibility is that the lysosomal enzymes are inactive in the basal state, but only become active under the same conditions required for zymogen conversion, such as a decrease in pH.
Relevance to Pancreatitis

It is attractive to speculate that the enhanced proteolysis detected within hyperstimulated acini is a mechanism responsible for some of the damage observed in hyperstimulation pancreatitis. The two experimental systems share some similarities. Both require high doses of neurohumoral agonists. With regard to cholecystokinin, both systems appear to utilize the same receptor subtype. For example, the hyperstimulation pancreatitis elicited in the rat requires cholecystokinin to interact with its low-affinity receptor form [18]. In preliminary studies, we observed that the OPE analog of cholecystokinin, a peptide that stimulates the high- but not the low-affinity form of the cholecystokinin receptor, does not stimulate the conversion of zymogens in isolated acini. Similar to the observations made in vivo, when the OPE peptide was co-administered with cholecystokinin to isolated acini, it blocked the proteolytic effects of hyperstimulation. Thus, both the conversion reaction detected in pancreatic acini in vitro and pancreatitis in vivo appear to require the interaction of cholecystokinin with its low-affinity receptor.

Therefore, the conversion reaction described in our studies provides an attractive potential mechanism for generating active enzymes within the pancreatic acinar cell. Further studies are needed, however, to clarify the relationship between the in vivo and in vitro systems. A prediction derived from the in vitro studies is that the serine protease inhibitor, benzamidine, might block hyperstimulation pancreatitis in intact animals. The same studies indicate that the thiol protease inhibitor, E64, should have little effect on this form of pancreatitis and may even make it worse. It would be useful to detect the conversion of zymogens to their lower molecular weight forms using in vivo models of pancreatitis. Our preliminary studies indicate, however, that this task may be difficult since within acini the converted forms are (1) generated transiently and (2) appear to be degraded immediately when acini are manipulated. An additional confounding factor is the variability in the relative amounts of basal zymogen conversion that have been observed among individual animals. Despite these technical difficulties, it will be important to pursue studies that attempt to detect zymogen conversion in vivo.

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