CHAPTER 24

Experimental Acute Pancreatitis: Studies of the Early Events that Lead to Cell Injury

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It is generally believed that the morphologic changes that characterize acute pancreatitis result from digestion of the gland by enzymes that are normally synthesized and secreted by pancreatic acinar cells. Evidence supporting this belief includes the observations that (a) the morphologic changes of severe pancreatitis resemble those that are typical of digestive necrosis; (b) pancreatic acinar cells synthesize digestive enzymes, which, if activated, could lead to digestive necrosis of the gland; and (c) activated pancreatic digestive enzymes have been detected within the gland during severe pancreatitis (7,8). The potentially harmful digestive enzymes synthesized by acinar cells are, for the most part, normally synthesized and secreted as inactive zymogens, which are activated in the duodenum where the brush border enzyme enteropeptidase activates trypsinogen and trypsin activates the remaining zymogens. Although autoactivation within either acinar cells or the ductal space might theoretically occur, the gland is protected against injury by proteolytic enzyme inhibitors and because digestive zymogens are segregated from the cytoplasm by being enclosed within membrane bounded organelles.

Passage of a gallstone into or through the terminal biliopancreatic ductal system is the most common triggering event in clinical acute pancreatitis (1). In this chapter, we summarize some of our recent studies, which have focused on the mechanisms and events that may couple gallstone passage with the onset of acute pancreatitis. We address the following three issues: (a) Does stone passage into or through the terminal biliopancreatic ductal system induce pancreatitis by obstructing the pancreatic duct or, as originally suggested by Opie (25), by creating a common channel that permits bile to reflux into the pancreatic ductal system? (b) Does the earliest evidence of pancreatic injury in pancreatitis appear in acinar cells or, as suggested by others (4,11), in peri ductal or perilobular areas? (c) What are the potential cellular mechanisms that might couple stone passage with digestive enzyme activation? This is a highly selective review since it includes only those studies carried out in the authors' laboratory. While a number of experimental models of pancreatitis have been used for these studies, we describe those models only briefly. Readers interested in the general subject of experimental models of acute

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pancreatitis or in the results obtained by others in studies using these and other models should consult Chapters 25 and 26 (this volume).

MODELS

Hemorrhagic Necrotizing Pancreatitis in the Opossum

Ligation of the pancreatic duct or of the combined pancreatic and biliary duct of most animal species results in pancreatic edema with very mild inflammation. After days or weeks of obstruction, atrophy of the exocrine pancreatic elements occurs (3,42). In contrast, ligation of those ducts in the opossum results in hemorrhagic pancreatitis, which is associated with a 14-day mortality rate of 100% (2,37). The extraduodenal common bile duct and the pancreatic duct are easily dissected in this animal and, as a result, studies evaluating the effects of ligating the bile ducts, pancreatic duct, and common bile-pancreatic duct either alone or in combination can easily be performed.

Diet-Induced Acute Hemorrhagic Pancreatic Necrosis in Mice

Young female mice fed a choline-deficient diet supplemented with 0.5% ethionine (the ethyl analogue of methionine) develop hemorrhagic necrosis of the pancreas, which, if the diet is fed ad libitum, is uniformly lethal within 5 days (18). We have modified this protocol to reduce the mortality rate to 50% to 70% by limiting diet consumption (3 g/mouse) and feeding the choline-deficient ethionine-supplemented (CDE) diet for only 24 hr (9). The mice are fasted for 24 hr before and after being given the CDE diet and, with the exception of studies evaluating mortality rates, experiments are performed within the first 1 to 2 days after diet administration and therefore before gross evidence of pancreatic injury is apparent.

Secretagogue-Induced Acute Edematous Pancreatitis in Rats

Infusion of cerulein, an analogue of CCK, at a concentration (5 μg/kg/hr) in excess of that which evokes a maximal rate of protein and digestive enzyme secretion, causes massive peripancreatic edema and mild to moderate evidence of interstitial pancreatic inflammation (14). This form of acute edematous pancreatitis evolves rapidly with maximal changes noted by 3 to 6 hr of cerulein infusion. Thereafter the changes resolve and complete recovery of the animal can be expected.

Pancreatic Duct Obstruction in Rats and Rabbits

The pancreatic duct of rats and rabbits is easily cannulated and, once cannulated, temporary obstruction can be produced by elevating the extraductal portion of the cannula to a vertical position and allowing the column of secreted juice to reach a level at which the hydrostatic pressure equals the secretory pressure. Within 4 to 7 hr of pancreatic duct obstruction, hyperamylasemia and mild interstitial pancreatic edema can be detected (24,33). While gross or microscopic evidence of pancreatic inflammation is not noted after rat or rabbit duct obstruction, this model is believed to be useful because it closely resembles the mechanical events that would be expected to follow pancreatic ductal outflow obstruction during or after gallstone passage.

STUDIES EVALUATING THE ROLE OF BILE REFLUX

Opie, in 1901, reported two patients who, at autopsy after death from acute pancreatitis, were found to have stones impacted in the ampulla of Vater (25). He suggested that obstructing stones created a common bilio-pancreatic channel proximally and that permitted bile to reflux into the pancreatic ductal system. Several objections to the "common channel" theory have been raised. Many individuals with gallstone pancreatitis have a common channel that is so short that the obstructing stone would block either one or both of the ducts and thus actually prevent reflux of bile into the pancreatic duct (19). In addition, Robinson and Dunphy (28) demonstrated that low pressure perfusion of the pancreatic duct with bile does not induce pancreatitis. In spite of these objections, the common channel theory is still accepted by many as a valid explanation for the triggering event in gallstone pancreatitis.

We evaluated the importance of bile reflux in the evolution of acute pancreatitis in the opossum duct-obstruction model (16). Opossums of either gender were divided into four groups. Group A underwent bile duct obstruction proximal to the pancreatic-bile duct junction (jaundiced control group). Group B underwent a similar bile duct ligation as well as pancreatic duct ligation. Although jaundiced, these animals had pancreatic duct obstruction but could not develop bile reflux into the pancreatic ductal system. Group C underwent only pancreatic duct ligation and, since their bile duct was not obstructed, they remained unjaundiced. Finally, group D animals underwent ligation of the combined bile-pancreatic duct close to the duodenal wall. These animals therefore had combined bile and pancreatic duct obstruction but were subject to bile reflux into the pancreatic ductal system.
All animals underwent cystic duct ligation to prevent the gallbladder from acting as a reservoir for bile. The animals were sacrificed at selected times and macroscopic as well as microscopic examinations of the pancreas were performed. Each animal with obstruction to the pancreatic juice flow (groups B, C, and D) developed hyperamylasemia during the first day after the induction of obstruction and the magnitude of the hyperamylasemia was similar in each of the three groups. The animals in these three groups also developed pancreatic edema and, again, the degree of pancreatic edema was similar in each of these three groups.

We were particularly interested in determining if the site of obstruction had an effect on the microscopic severity of the pancreatitis that developed. To evaluate this issue, samples of the pancreas were removed and examined at the light microscope level. In addition, morphometry was performed to quantitate the degree of acinar cell necrosis. As can be seen in Fig. 1, approximately 20% of the acinar cells appeared necrotic on day 1 after duct obstruction and, by day 7, roughly 55% of the acinar cells appeared necrotic. No significant differences were noted when the animals with isolated pancreatic duct obstruction (group C) were compared with those having either obstruction of the combined biliopancreatic duct (i.e., a reflux system, group D) or those with separate bile and pancreatic duct obstruction (i.e., a nonreflux system, group B). These studies indicated that the severity of pancreatitis was similar after isolated pancreatic duct obstruction, separate bile and pancreatic duct obstruction, and obstruction of the combined biliopancreatic duct.

We therefore concluded that bile reflux is not necessary for the induction of pancreatitis and that the presence of reflux does not worsen the severity of pancreatitis. It would therefore appear that pancreatic duct obstruction is the sole requirement for the induction of pancreatitis—at least in the opossum.

LOCATION OF THE EARLIEST LESION IN ACUTE PANCREATITIS

Although the cause–effect relationship between stone passage and pancreatic injury in gallstone pancreatitis is well established, the localization of the earliest lesion in this disease has been the subject of considerable controversy. Some have argued that disruption of the ductal integrity leads to extravasation of pancreatic juice into the periductal parenchyma and that, as a result, the earliest evidence of pancreatic injury appears in periductal areas (11). Others have suggested that the earliest changes appear in perilobular areas, perhaps as a result of leakage of digestive enzymes and/or alterations in vascular integrity (4).

To further evaluate this issue, we sampled the pancreas of opossums at selected times during the initial 24 hr after ligation of the combined biliopancreatic duct (15). Representative light micrographs are shown in Fig. 2. Within 3 hr of duct ligation, scattered acinar cells lost their basal/apical polarity and stained clear blue (panel A), acinar lumina became dilated (panel B), and single acinar cells or groups of cells appeared necrotic. By 6 hr after duct ligation, groups of necrotic acinar cells could be seen and small foci of hemorrhage could be detected (panel D). Within 12 hr of duct ligation, necrosis of lobules along with hemorrhage and early polymorphonuclear leukocyte infiltration were noted (panel E). Finally, 24 hr after duct ligation, a massive inflammatory reaction could be detected (panel F). These changes all appeared to involve acinar cells and acini.

In contrast, no evidence of periductal or perilobular inflammation was noted during the initial 12 hr after duct obstruction and, by 24 hr, only scattered but infrequent areas of periductal inflammation could be found. These observations led us to conclude that the earliest lesions of acute pancreatitis, at least in this opossum model, involve the acinar cells themselves and that those earliest lesions are neither periductal nor perilobular.
FIG. 2. Time course of cellular lesions after opossum duct ligation. Tissue specimens were obtained 3 hr (A, B, C), 6 hr (D), 12 hr (E), and 24 hr (F) after ligation of the common biliopancreatic duct. A: Single acinar cells (arrows) change their staining pattern to a clear blue and lose their basal/apical polarity. B: Mildly dilated acinar lumina (asterisks). C: Single acinar cells acquire a reticular staining pattern, lose their luminal confinement, and undergo necrosis. D: Groups of necrotic acini can be seen along with evidence of hemorrhage (arrow). E: Necrosis of an entire lobule along with hemorrhage and early leukocyte infiltration. F: Massive inflammatory reaction and replacement of a necrotic group of acini by leukocytes. (From ref. 15, with permission.)
CELLULAR MECHANISMS OF PANCREATITIS

A large number of studies have been undertaken that were designed to probe the early acinar cellular events that might be critical to the development of pancreatitis. These studies have characterized acinar cell biology during the early stages of experimental pancreatitis and evaluated the effects of various pharmacological manipulations on the course and severity of pancreatitis.

The studies to characterize alterations in acinar cell biology during pancreatitis have focused on the processes of amino acid uptake, protein and digestive enzyme synthesis, intracellular transport and sorting of newly synthesized proteins, digestive enzyme secretion, and lysosomal fragility. These processes were evaluated in three markedly different experimental models of pancreatitis—the diet-induced, the secretagogue-induced, and the duct obstruction models. For example, diet-induced pancreatitis evolves slowly, is characterized by hemorrhagic necrosis of the gland, and is highly lethal (9,18). On the other hand, secretagogue-induced pancreatitis evolves within hours, is characterized by interstitial edema of the gland, and all animals survive (14). Finally, duct obstruction in rats and rabbits causes only hyperamylasemia and mild, transient pancreatic edema over the initial several days; all animals survive, but later develop exocrine pancreatic atrophy (24,33). In spite of these major differences among the various models, a number of common cellular biological processes occurred, suggesting that similar events occur during the early stages of clinical pancreatitis regardless of the initial pathophysiological event.

Amino Acid Uptake and Protein Synthesis

The pulse-chase technique was used to evaluate amino acid uptake and protein synthesis during diet-induced (9), secretagogue-induced (32), and duct obstruction-induced (33) pancreatitis. In these studies, animals were given a pulse infusion of a radioactively labeled amino acid. Subsequently, the uptake of the labeled amino acid into the pancreas and its incorporation into acid precipitable material (protein) were quantitated.

The results obtained with the secretagogue-induced model of pancreatitis are shown in Fig. 3. Amino acid uptake (not shown) and incorporation of the label into protein (protein synthesis) in animals with secretagogue-induced pancreatitis were not different from that noted in control animals. Similar results were obtained when the processes of amino acid uptake and protein synthesis were evaluated during diet-induced pancreatitis and shortly after obstruction of either the rat or rabbit pancreatic duct. These observations led us to conclude that amino acid uptake and protein synthesis were not altered during the early stages of experimental pancreatitis.

![Fig. 3. Protein synthesis and discharge during secretagogue-induced pancreatitis. Rats were preinfused with saline alone (○-○) or saline containing sufficient cerulein to deliver 5 μg/kg/hr for 1 hr (○-○), given a pulse of [3H]phenylalanine followed by a bolus of nonradioactive phenylalanine, and continued on infusion for varying times. At selected times, rats were sacrificed and trichloracetic acid precipitable radioactively in the pancreas homogenate was measured. (From ref. 32, with permission.)](image)

Intracellular Transport and Protein Sorting

Digestive enzymes as well as other proteins synthesized by acinar cells are assembled on the rough endoplasmic reticulum and transported to the Golgi. As they traverse the Golgi stacks, hydrolases destined for transport to lysosomes are glycosylated, 6-mannose phosphorylated, and bound to mannose-6-phosphate receptors. As a result, they are sorted from other proteins and carried to the acidic prelysosomal compartment where, at acidic pH, the lysosomal hydrolases dissociate from their receptors, allowing the free receptors to shuttle back to the Golgi and bind additional mannose-6-phosphorylated lysosomal hydrolases (13). In contrast, digestive enzymes are not mannose-6-phosphorylated and, as a result, they fail to bind to mannose-6-phosphate receptors. Rather, they pass through the Golgi and are packaged in condensing vacuoles, which mature into zymogen granules as they migrate to the apical cell surface, where they discharge their contents into the luminal space after fusion—fission of the zymogen granule limiting membrane and the apical plasmalemma (26). In our studies of pancreatitis, the intracellular transport of newly synthesized proteins from the endoplasmic reticulum to the zymogen granule compartments was found to remain unaltered in both the diet-induced and secretagogue-induced models (12,32).

In contrast to our observation that trafficking of secretory proteins is normal in pancreatitis, the sorting of lysosomal hydrolases from digestivezymogens was found to be markedly altered in the early stages of both diet-induced and secretagogue-induced pancreatitis and shortly after obstruction of either the rabbit or rat pancre-
atric duct (22,24,31,33,41). We studied the sorting of lysosomal hydrolases from digestive zymogens in each of these models using two separate but complementary techniques—subcellular fractionation and immunolocalization. For subcellular fractionation experiments, the homogenized pancreas was subjected to differential centrifugation and zymogen granule-enriched as well as lysosome-mitochondria-enriched fractions were obtained. For immunolocalization studies, light and/or electron microscopic specimens were exposed to antibodies directed against either digestive zymogens or lysosomal hydrolases and the location of the antigen–antibody complex was determined using either fluorescence light microscopy or immunogold electron microscopy.

Subcellular fractionation of the homogenized pancreas of control animals indicated that 20% to 30% of the lysosomal hydrolase cathepsin B could be recovered from the zymogen granule-enriched pellet. Initially, we interpreted this finding as indicating that the pancreas contains a population of “heavy” lysosomes that pellet with low-speed centrifugation and this interpretation may, at least in part, remain valid. Recently, however, we have found evidence that a considerable fraction of cellular lysosomal enzymes escape sorting in the Golgi. This fraction of lysosomal enzymes apparently enters the secretory pathway and is packaged in secretory vesicles (i.e., zymogen granules) (10). At least part of the 20% to 30% of cellular cathepsin B recovered in the zymogen granule-enriched fraction may result from this “physiologic” missorting or incomplete sorting.

In the early stages of diet-induced as well as secretagogue-induced pancreatitis and shortly after either rabbit or rat pancreatic duct obstruction, the sorting of lysosomal hydrolases from digestive zymogens is further perturbed. Subcellular fractionation experiments indicated that approximately 50% of cellular lysosomal hydrolases such as cathepsin B was in the zymogen granule-enriched fraction and the amount in the lysosome-mitochondria-enriched fraction was reduced (Fig. 4).

Immunolocalization studies indicated that, in each of these models, large cytoplasmic vacuoles appear that contain both lysosomal hydrolases and digestive enzyme zymogens (Fig. 5). The mechanisms responsible for the genesis of these cytoplasmic vacuoles is incompletely understood. In the diet-induced model of pancreatitis, the vacuoles appear to result from crinophagy, that is, fusion of zymogen granules with lysosomes (12). Crinophagy as well as defective sorting may be responsible for the genesis of the vacuoles seen after secretagogue hyperstimulation (30,41).

**Digestive Enzyme Secretion**

We have used the pulse-chase method to evaluate the secretion of digestive enzymes from the pancreas in vivo. Animals were given a pulse injection of radioactively labeled amino acid. During the subsequent chase period, the label was incorporated into newly synthesized acinar cell protein and, as a consequence, acinar cell radioactivity increased. With time, however, the newly synthesized protein was discharged into the ductal space and transported to the duodenum. As a result, pancreatic radioactivity declined. Thus the decline of pancreatic radioactivity with time could be used in these studies as an indicator of in vivo digestive secretion (Fig. 3).

In vivo newly synthesized protein and digestive enzyme secretion from the pancreas was found to be markedly reduced during the early stages of diet-induced (9) and secretagogue-induced (32) pancreatitis. Secretion of newly synthesized protein was also found to be reduced shortly after pancreatic duct obstruction (33). With continued synthesis, in the face of reduced secretion, newly synthesized proteins were noted to accumulate in the pancreas under these conditions, particularly during the early stages of diet-induced pancreatitis (9). In that model, the accumulation of digestive enzymes can be correlated with an expected increase in the numbers of zymogen granules and with an increase in the pancreatic content of digestive zymogens (9,18). These changes are not nearly as impressive during secretagogue-induced pancreatitis or following duct obstruction, an observation that may indicate some loss of zymogens into the extracellular but intrapancreatic space.

We examined the mechanisms responsible for reduced protein and digestive enzyme secretion during the...
early stages of experimental pancreatitis in both the diet (27) and secretagogue (34) models. Pancreatic acini were prepared from mice fed the CDE diet. Subsequently, we evaluated the in vitro secretion of digestive enzymes from these acini. Interestingly, in response to CCK or carbachol, the acini did not secrete amylase. Acinar cells from CDE diet-fed mice contain normal numbers of cholinergic receptors and the receptors have unaltered affinity for antagonists. These observations suggest that the failure of these acini to secrete reflects a postreceptor defect in stimulus-secretion coupling. Secretagogue stimulation of these acini fails to bring about a rise in cytoplasmic calcium levels but amylase secretion can be restored if either the calcium ionophore A23187 or the diacylglycerol analogue TPA is used in place of the hormone secretagogue. These findings suggest that the defect induced by the CDE diet interrupts the hydrolysis of PIP$_2$ by phospholipase C and thus prevents the subsequent secretagogue-induced rise in IP$_3$ levels. Indeed, when studies directly addressing this point were performed, the secretagogue caused only a blunted rise in acinar cell IP$_3$ levels (Fig. 6) although PIP$_2$ levels were found to be normal.

Protein secretion from acinar cells of the pancreas also is inhibited during the early stages of secretagogue-induced pancreatitis but the mechanisms responsible for this inhibition appear to differ markedly from those involved in the CDE diet-induced model. Dose-dependence studies characterizing protein secretion from pancreatic acinar cells both in vivo and in vitro using increasing concentrations of the secretagogue cerulein showed that the secretory response is biphasic. Low cerulein concentrations stimulated secretion while high concentrations of cerulein inhibited secretion. We interpreted these findings as indicating that acinar cells possess high-affinity CCK receptors that mediate the stimulation of protein secretion and low-affinity CCK receptors that mediate the inhibition of secretion. We reasoned that the inhibition of in vivo protein secretion as well as the interstitial pancreatitis that follows infusion of a supramaximally stimulating dose of cerulein might result from the interaction of cerulein with these low-affinity inhibitory CCK receptors.

FIG. 5. Colocalization of digestive zymogens with lysosomal hydrolases during secretagogue-induced pancreatitis. Rats were infused with a supramaximally stimulating dose of cerulein for 1 hr, sacrificed, and light microscopic localization of enzymes by immunofluorescence using antizymogen (A) and antichymotrypsin D (B) sera was performed. Numbers (1–10) point to vacuoles in two adjacent sections that were labeled by both sera used. (From ref. 41, with permission.)

FIG. 6. Inositol trisphosphate generation stimulated by carbachol during diet-induced pancreatitis. Mice were fed the choline-deficient ethionine-supplemented (CDE) diet (striped bars) or regular diet (solid bars), sacrificed, and acini were prepared. The acini were loaded with [$^{3}H$]myoinositol and the generation of [$^{3}H$]IP$_3$ measured after incubation for 5 min with varying concentrations of carbachol. (From ref. 27, with permission.)
To test this hypothesis, we employed a recently synthesized analogue of CCK, CCK-JMV-180 (6). Dose-response studies to evaluate the in vitro protein secretion revealed that increasing concentrations of CCK-JMV-180 causes only a monophasic effect; that is, high doses of CCK-JMV-18 do not inhibit secretion. Furthermore, the high-dose inhibition seen with supramaximally stimulating doses of either CCK or cerulein is prevented by CCK-JMV-180. These observations suggest that CCK-JMV-180 acts as an agonist and mimics the effect of CCK at the high-affinity receptors, which stimulate protein secretion. However, CCK-JMV-180 prevents the inhibition of secretion caused by the effects of cerulein, which are mediated via the low-affinity receptors (5,38).

Based on these observations, we suggested that CCK-JMV-180 might be a useful tool to evaluate which receptors were involved in the early stages of secretagogue-induced pancreatitis. If the high-affinity receptors were involved, CCK-JMV-180 should induce pancreatitis, while, in contrast, if the low-affinity receptors were involved, CCK-JMV-180 should prevent cerulein-induced pancreatitis. When experiments were performed to address this issue, infusion of high doses of CCK-JMV-180 did not induce pancreatitis and prevented cerulein-induced pancreatitis (Fig. 7). These results led us to conclude that cerulein-induced pancreatitis and the inhibition of protein secretion from the pancreas during that model of pancreatitis are both mediated by the low-affinity state of pancreatic acinar cell CCK receptors (34).

**Lysosomal Frangility**

When the homogenized pancreas is subjected to centrifugation at 12,000g for 10 min, a pellet containing roughly 80% of lysosomal enzyme content of the pancreas is obtained. Presumably, most of the pellet consists of lysosomal enzymes contained within lysosomes and, as noted above, the small fraction of lysosomal enzymes that normally are packaged within zymogen granules. During diet-induced as well as secretagogue-induced pancreatitis and after rabbit or rat pancreatic duct ligation, the 12,000g × 10 min pellet also should include the organelles that contain colocalized lysosomal and digestive enzymes.

We evaluated the fragility of the structures that contain lysosomal hydrolases under normal conditions and during the various models of pancreatitis. To accomplish these studies, the 12,000g × 10 min pellet was resuspended in buffer and incubated for varying periods. The suspension was then recentrifuged and lysosomal enzyme activity was measured in the particulate as well as the soluble fractions. As lysosomal enzyme-containing structures rupture during in vitro incubation, they release lysosomal hydrolases and the activity would move from the particulate to the soluble fraction. Thus the rate at which lysosomal hydrolases appeared in the soluble and disappeared from the particulate fractions could be used as an index of lysosomal fragility. As shown in Fig. 8, fragility increased markedly during the early stages of secretagogue-induced pancreatitis (31). Similar changes occurred during diet-induced pancreatitis and after obstruction of the rabbit or rat pancreatic duct (33).

**Plasma Factors**

The observation that infusion of a supramaximal stimulating dose of cerulein into rats caused changes resembling acute interstitial pancreatitis within 1 hr suggested that using pancreas fragments exposed to a supramaximally stimulating concentration of cerulein might be a satisfactory in vitro model of pancreatitis. Obviously, this system would not develop either hyperamylasemia or the morphological changes of pancreatitis (e.g., edema, inflammatory reaction). Therefore to verify that pancreatitis had developed, we reasoned that subcellular
redistribution of lysosomal hydrolases from the lysosome-enriched to the zymogen-granule-enriched fraction, which we had observed in all our experimental models of pancreatitis, might be used for this purpose (36).

Surprisingly, however, we found that incubation of pancreas fragments in buffer containing a supramaximally stimulating concentration of cerulein for up to 4 hr did not result in the redistribution of lysosomal enzymes. Similarly, no redistribution was noted when the fragments were incubated in normal rat plasma containing a supramaximally stimulating concentration of cerulein. The subcellular redistribution of lysosomal hydrolases was, however, observed when fragments were incubated with a supramaximally stimulating concentration of cerulein along with plasma obtained from a rat that had been infused with a supramaximally stimulating dose of cerulein (Fig. 9).

This observation led us to conclude that infusing a supramaximally stimulating dose of cerulein elaborated a plasma factor, which, when combined with a supramaximally stimulating dose of cerulein, caused the redistribution of lysosomal hydrolases.
maximally stimulating concentration of cerulein, resulted in in vitro subcellular redistribution of lysosomal hydrolases. Subsequently, we found the plasma factor to be a protein with protease activity and a molecular weight of 10 to 30 kDa. Obstruction of the opossum pancreatic duct leads to generation of a similar circulating plasma factor. The ultimate characterization of this factor will require its purification and identification.

**Pharmacological Manipulations**

The ability of various pharmacological manipulations to alter the course or severity of the various models of experimental pancreatitis has been examined because these manipulations might shed light on the pathophysiology of pancreatitis. PGE₂, at a dose of 0.05 to 0.20 µg/g body weight, protects against diet-induced pancreatitis, which indicates that these doses might reduce the lysosomal fragility that characterizes the diet-induced model (21). Higher or lower doses of the prostaglandin did not have this protective effect.

Agents that either scavenge oxygen-derived free radicals (superoxide dismutase and catalase) or inhibit their generation (allopurinol) reduce pancreatic edema, which occurs in both diet- and secretagogue-induced pancreatitis, but those agents do not reduce the mortality rate or the biochemical and morphological features that characterize these models (29,40). These observations suggested that oxygen-derived free radicals may play an important role in the pathogenesis of pancreatitis-related pancreatic edema but that free radicals have little if any role in the pathogenesis of acinar cell injury.

The effects of several esterase inhibitors were evaluated. Neither trasylol (aprotinin) nor chlorophyll-a had a protective effect against diet-induced pancreatitis (20). On the other hand, the low molecular weight esterase inhibitors gabexate mesilate and camostate prevented the hyperamylasemia pancreatic edema and acinar cell vacuolization that characterize secretagogue-induced pancreatitis and the hyperamylasemia and mortality that characterize diet-induced pancreatitis (22). In addition, both of these compounds decreased lysosomal enzyme redistribution in both models. These observations suggested that esterases might play an important role in the subcellular redistribution phenomenon seen in these two models.

The role of cholecystokinin in the evolution of diet-induced pancreatitis has been examined using the highly potent CCK receptor antagonist L-364,718. This antagonist failed to reduce the hyperamylasemia, acinar cell necrosis, and mortality that are associated with diet-induced pancreatitis, suggesting that CCK has little or no role in the pathogenesis of diet-induced pancreatitis (23).

Finally, to investigate our hypothesis that the colocalization of the lysosomal hydrolase cathepsin B with digestive zymogens causes acid-dependent activation of trypsinogen by cathepsin B within acinar cells we examined the effects of chloroquine and of E-64 on both diet- and secretagogue-induced pancreatitis. Chloroquine reduces the acidity of intracellular acidic compartments and E-64 is a potent cathepsin B antagonist. Thus we anticipated that each of these agents would prevent pancreatitis in these models. We were surprised to find that neither agent had a significant protective effect against either form of pancreatitis (17,35).

These negative results might indicate that cathepsin B activation of trypsinogen is not important to the genesis of diet- or secretagogue-induced pancreatitis but other interpretations remain possible. For example, neutralization of acidic compartments by chloroquine might have been inadequate to prevent cathepsin B activation of trypsinogen and the inhibition of cathepsin B by E-64 may have been incomplete. Alternatively, digestive enzyme zymogens may be activated by lysosomal hydrolases other than cathepsin B.

**OVERVIEW AND CONCLUSION**

The experiments described in this chapter have led to the following conclusions regarding the pathogenesis of acute pancreatitis. Gallstone or biliary tract related pancreatitis appears to result from pancreatic duct obstruction and neither bile duct obstruction nor bile reflux into the pancreatic duct is an important factor. Following ductal obstruction, the earliest changes in the pancreas occur in acinar cells and not in either the periductal parenchyma or the peribular spaces. Amino acid uptake, protein synthesis, and intracellular transport of newly synthesized proteins are not altered during the early stages of pancreatitis, but digestive enzyme secretion from acinar cells is blocked. The mechanisms underlying the block in secretion appear to vary from model to model and, in some, plasma factors generated during the initiation of pancreatitis may play an important role.

In any case, the reduced digestive enzyme secretion leads to a derangement in the intracellular sorting events that segregate lysosomal hydrolases from digestive zymogens. As a result, lysosomal hydrolases such as cathepsin B become colocalized, within acinar cells, along with digestive zymogens such as trypsinogen and, under these conditions, activation of trypsinogen by cathepsin B may occur (39).

The fragility of lysosomal hydrolase-containing organelles is increased and, as a result, in vivo rupture of those organelles may occur and lead to the release, within the cytoplasm, of activated digestive enzymes. The release of activated digestive enzymes within the acinar cell could,
under these conditions, result in acinar cell injury and/or necrosis.

It should clearly be understood that the evidence supporting most of these conclusions is circumstantial and incomplete. Obviously, there must be additional phenomena that play critical roles in the evolution of pancreatitis because most of the events we defined are similar but the course and severity of the pancreatitis differ markedly among the models we have examined. For example, diet-induced pancreatitis evolves over days, is characterized by acinar cell necrosis, and is highly lethal, while secretagogue-induced pancreatitis evolves over hours, is characterized by pancreatic edema, and is reversible. Finally, duct obstruction in all animals except the opossum results in only mild pancreatic inflammation and eventually leads to exocrine atrophy. Thus the search for additional events that, when superimposed on those elaborated in this chapter, define the course and severity of the disease should be encouraged. Those events may be subject to manipulation by pharmacological or other means, and therefore their identification may provide clues to methods whereby the clinical disease can be either prevented or treated.

It should also be clearly understood that our observations were made using animal models of pancreatitis and the relevance of animal models to human pancreatitis can always be questioned. Overall, the ultimate contribution of animal studies will depend on whether they identify mechanisms that lead to the prevention or treatment of human acute pancreatitis.

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