Pathobiology of acute pancreatitis: focus on intracellular calcium and calmodulin

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Abstract

The exocrine pancreas synthesizes all the enzymes needed for intestinal breakdown of proteins, fats, and carbohydrates in our diet. Unfortunately, the proteases needed for the digestion of the meat we eat, can, if inappropriately activated inside the acinar cells, also digest the pancreas itself as well as the surrounding tissues, which is what happens in the sometimes fatal human disease acute pancreatitis. The disease is currently untreatable, but significant progress has recently been made in understanding the fundamental processes initiating the pathological changes underlying pancreatic autodigestion. It is now clear that intracellular trypsin activation—a crucial step in pathogenesis—is due to excessive release of Ca\(^{2+}\) from intracellular stores, principally via two types of inositol trisphosphate receptor. The unexpected recent discovery of an intrinsic protective mechanism caused by intracellular calmodulin and, specifically, the finding that this protective effect can be boosted by a membrane-permeable Ca\(^{2+}\)-like peptide are promising.

Introduction and context

Acute pancreatitis is a human disease in which digestive proenzymes normally synthesized in the pancreatic acinar cells are activated inside the cells (rather than after they have been secreted), digesting the pancreatic tissue and its surroundings rather than food in the gut. Pancreatitis, mostly caused by gallstones or excessive alcohol intake, is found acutely in up to 100 per 100,000 people per year and causes severe disease in 20% of those with the condition. It is frequently complicated by agonizing pain, extensive pancreatic necrosis, multiple organ failure, and prolonged hospitalization. The overall mortality in patients with acute pancreatitis is about 5% [1]. It has become accepted that repeated attacks of acute pancreatitis may lead to chronic pancreatitis [1] and that chronic pancreatitis carries a markedly increased risk for development of pancreatic cancer [2], which is the fifth most common cause of death through cancer, with only about 3–4% of patients surviving beyond 5 years [3]. Heavy alcohol consumption has been known for years to be a major risk factor for the development of chronic pancreatitis, and smoking has now also been implicated as an independent risk factor [4,5]. There is currently no specific therapy for acute pancreatitis, but recently there has been progress in understanding the involvement of intracellular calcium in the initial pathobiological processes, and this may provide new opportunities for development of preventive and therapeutic measures.

There is general agreement that intracellular protease activation is the crucial initiating step and that this process depends on substantial release of Ca\(^{2+}\) from internal stores followed by Ca\(^{2+}\) entry from the extracellular space. It is also clear that the initial biochemical event, namely protease activation, occurs at the same time as—and is in some way linked to—intracellular vacuolization; that is, the transformation of zymogen granules (electron-dense proenzyme-containing secretory vesicles) into empty-looking vacuoles. Vacuolization, like protease activation, is a Ca\(^{2+}\)-dependent process. The demonstration that
trypsin activation was initiated in post-exocytotic, endocytic vacuoles was the crucial finding linking protease (trypsin) activation and vacuolization [6].

Physiological stimulus–secretion coupling and pathological stimulus–protease activation

We know that the initiation of acute pancreatitis is a Ca\(^{2+}\)-dependent process, but so is normal protease secretion. It is therefore important to differentiate between the physiological Ca\(^{2+}\) signal generation resulting in normal secretion and the abnormal (toxic) Ca\(^{2+}\) signal generation initiating pancreatitis. Exocytotic secretion of digestive proenzymes and the crucial fluid secretion needed to wash the secreted proteins out of the duct system into the gut are controlled by the neurotransmitter acetylcholine (released from parasympathetic nerve endings) and the hormone cholecystokinin. These agonists, at physiological concentrations, generate repetitive short-lasting elevations in the concentration of cytosolic Ca\(^{2+}\) (Ca\(^{2+}\) spikes) localized in the apical (granular) region of the cells. These local Ca\(^{2+}\) spikes are sufficient to activate exocytosis of digestive enzymes as well as fluid secretion [7]. These findings—specifically, the link between the presence of functional cholecystokinin receptors and Ca\(^{2+}\) signalling—originally made in studies on mouse pancreatic acinar cells, have been confirmed in a detailed study of normal human pancreatic acinar cells [8]. High (unphysiological) concentrations of acetylcholine or cholecystokinin, as well as various pathological stimuli causing acute pancreatitis, evoke sustained global elevations of cytosolic Ca\(^{2+}\). Such signals do not result in sustained secretion of proteases, but do—in contrast to physiological stimulation—cause intracellular trypsin activation [6].

Another very important aspect to consider with regard to pathogenesis is energy production. Repetitive cytosolic Ca\(^{2+}\) spikes cause repeated spikes of mitochondrial Ca\(^{2+}\) elevation that, in turn, activate Ca\(^{2+}\)-dependent Krebs-cycle dehydrogenases, generating mitochondrial ATP production. In contrast, a sustained elevation of cytosolic Ca\(^{2+}\) only gives rise to one initial burst of mitochondrial Ca\(^{2+}\) elevation and therefore only one transient period of ATP generation [7]. As ATP is required for the secretory process, this is undoubtedly one reason for the lack of protease secretion at high (unphysiological) levels of stimulation as well as in acute pancreatitis.

Bile acids induce acute pancreatitis

A frequent cause of acute pancreatitis is gallstones, which are thought to cause disease by blocking the pancreatic duct or obstructing a common (bile–pancreatic) channel. This latter mechanism would allow reflux of bile into the pancreas and cause pancreatic injury, although the importance of this particular mechanism has been debated [9]. In any case, it has been shown that transporter-mediated bile acid uptake causes Ca\(^{2+}\)-dependent cell death in pancreatic acinar cells in vitro [10]. The primary effect of intracellular bile acids is to release Ca\(^{2+}\) from both the endoplasmic reticulum and acid stores in the apical granular region through activation of inositol trisphosphate (IP\(_3\)) and ryanodine receptors (intracellular calcium channels) [11], inducing either apoptosis or necrosis. The intracellular ATP level seems to be crucial in determining which type of cell death occurs. This can be demonstrated in patch clamp whole-cell recording experiments (where the cell interior is in direct contact with a large volume of pipette solution), which show that the presence of ATP in the solution leads to bile acids causing apoptosis as opposed to necrosis [12].

Alcohol: Is it dangerous for the pancreas?

Although the risk of developing pancreatitis increases with increasing alcohol intake, it is nevertheless the case that only a minority (<10%) of those drinking excessive amounts of alcohol develop pancreatitis [5]. How can this be explained? Although alcohol usually has only modest effects on cellular Ca\(^{2+}\) homeostasis, even in very high concentrations, results of work on isolated normal pancreatic acinar cells show that a combination of alcohol and fatty acids (fatty acid ethyl esters) causes massive intracellular Ca\(^{2+}\) release and acute trypsin activation. Although Ca\(^{2+}\) release occurs from both the endoplasmic reticulum and acid stores in the granular part of the cells, it is the Ca\(^{2+}\) liberation from the acid stores that is principally responsible for the intracellular trypsin activation [13]. The mechanism involves specific intracellular Ca\(^{2+}\) release channels (IP\(_3\) receptors of types 2 and 3). In fact, deletion of the genes for these channels prevents the toxic action of fatty acid ethyl esters [13] (Figure 1). High concentrations of long-chain fatty acids in the plasma markedly increase the risk for development of pancreatitis, and slowly increase the global cytosolic Ca\(^{2+}\) [14,15]. This is mainly due to inhibition of mitochondrial energy production. The reduced level of intracellular ATP prevents full Ca\(^{2+}\) pump function (both in the endoplasmic reticulum membrane and in the plasma membrane), limiting the capacity for getting rid of Ca\(^{2+}\) accumulated in the cytosol [6]. From these studies on isolated cells, it would appear that the combination of alcohol and fat-rich meals would increase the risk of developing acute pancreatitis. It would be highly desirable to test this by conducting careful epidemiological studies.

To summarize, although alcohol (ethanol) itself mostly has only minor acute effects on the pancreatic acinar cells, there is a minority of cells that produce large sustained Ca\(^{2+}\) signals when exposed to ethanol [6]. Recent data
show that although ethanol has the capacity to elicit the release of substantial amounts of \( \text{Ca}^{2+} \) from intracellular stores, normal intact cells have an in-built protective mechanism, discussed below [16] (Figure 2).

The level of trypsin activity is correlated with the degree of \( \text{Ca}^{2+} \) release from acid stores in the granular apical pole through inositol trisphosphate (IP\(_3\)) receptors of types 2 and 3. (A) Transmitted light image showing two acinar cells. The left cell has been two-photon permeabilized. (B) and (C) Fluorescence images showing (in (C)) the initial localization of trypsin activity after stimulation with POAEE (the probe BZiPAR becomes fluorescent when trypsin cleaves the two oligopeptide side chains). (B) Before and (C) after start of stimulation with POAEE (100 \( \mu \text{M} \)). (D) The time course of the increase in intracellular trypsin activity following start of stimulation with 100 \( \mu \text{M} \) POAEE. (E) Results from experiments in which \( \text{Ca}^{2+} \) release from the acid granular pole of permeabilized cells and trypsin activation in wild-type (WT) mice were compared with results from mice in which type 2 IP\(_3\) receptors had been deleted (IP\(_3\)R\(_2\)\(^{-/-}\)) and from mice in which both types 2 and 3 IP\(_3\) receptors had been deleted (IP\(_3\)R\(_2\)\(^{-/-}\) IP\(_3\)R\(_3\)\(^{-/-}\)). Adapted from Gerasimenko et al., 2009 [13].

Activation of the ubiquitous calcium-binding protein calmodulin protects against alcohol-induced intracellular \( \text{Ca}^{2+} \) release and trypsin activation

Recent insights have shed light on how alcohol-induced \( \text{Ca}^{2+} \) release could be prevented. It has been found that when the \( \text{Ca}^{2+} \)-binding protein calmodulin is washed out of cells, alcohol itself has a strong and acute toxic effect on the acinar cells. Re-admission of calmodulin (at a normal intracellular concentration) has a protective effect. It is particularly exciting that recent data show that a membrane-permeable small peptide activator of calmodulin (the \( \text{Ca}^{2+} \)-like peptide known as CALP-3), when added to the outside of isolated cells, prevents the toxic actions of even very high alcohol concentrations [16] (Figure 2), as this suggests it may have potential as a therapeutic agent to reduce alcohol-induced pancreatic damage.
Cell death pathways
Pancreatitis is characterized by cell death, but the prognosis depends a great deal on which cell death process occurs [17,18]. Apoptosis is the “physiological” cell death mechanism and occurs without losing the integrity of the plasma membrane, whereas necrosis results in loss of cell constituents to the interstitial fluid, promoting inflammation. Apoptosis requires energy in the form of ATP, so if mitochondrial function is markedly impaired by complete depolarization of the inner mitochondrial membrane, the only cell death mechanism available is necrosis (Figure 3).

Potential therapeutic avenues
The experimental results demonstrating that the crucial intracellular trypsin activation is Ca\(^{2+}\)-dependent, and promoted by excessive Ca\(^{2+}\) release from internal stores as well as subsequent entry of Ca\(^{2+}\) from the interstitial fluid, suggest that inhibition of Ca\(^{2+}\) release from internal stores and/or inhibition of Ca\(^{2+}\) entry may be helpful in limiting the damage to pathological stimuli such as alcohol and alcohol metabolites, as well as bile acids. Indeed, caffeine, which has been shown to inhibit opening of IP\(_3\) receptor channels [6,7], reduces cytosolic Ca\(^{2+}\) signal generation in response to fatty acid ethyl esters and has also been shown to reduce the probability of ethanol-induced pancreatitis in a clinical study [19]. However, due to the relatively low affinity of caffeine for the IP\(_3\) receptors and its activating effect on ryanodine receptors, the therapeutic potential for caffeine is limited. The recent discovery that intracellular calmodulin has an intrinsic protective effect against alcohol-induced trypsin activation and, in particular, that this protection can be boosted by CALP-induced activation of calmodulin [16] deserves further study. Inhibition of Ca\(^{2+}\) entry channels of the CRAC (Ca\(^{2+}\) release-activated Ca\(^{2+}\)) type is another potentially interesting therapy [20] that has not yet been assessed in the pancreas, but could be powerful if Ca\(^{2+}\) entry through CRAC channels turns out to be the dominant pathway in the acinar cells.

Conclusions
In conclusion, the main points that have come out of the recent advances in the field are that: (a) excessive cytosolic Ca\(^{2+}\) loading initiates the intracellular protease activation that leads to acute pancreatitis; (b) the excessive entry of Ca\(^{2+}\) into the cytosol is primarily and principally due to release of Ca\(^{2+}\) from acid Ca\(^{2+}\) stores in the granular part of the acinar cells mediated via IP\(_3\) receptors of types 2 and 3; (c) an intracellular Ca\(^{2+}\)-binding protein, calmodulin, exerts a protective effect against alcohol-related pancreatitis by reducing the probability of opening of the IP\(_3\) receptor channels; and (d) activation of calmodulin by a membrane-permeable Ca\(^{2+}\)-like peptide boosts the protective effect of calmodulin against alcohol-induced intracellular protease activation. Hopefully by understanding more about the etiology of this disease, we will come closer to providing better preventative and therapeutic methods to relieve the suffering of those afflicted with this condition.

Abbreviations
CALP, calcium-like peptide; CRAC, Ca\(^{2+}\) release-activated Ca\(^{2+}\); IP\(_3\), inositol trisphosphate.

Competing interests
The authors declare that they have no competing interests.

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