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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, endo-
cytic 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 concen-
trations, 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) concen-
trations 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 produc-
tion. 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 activa-
tion [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 endoplas-
mic 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 Ca\(^{2+}\) from intracellular stores, normal intact cells have an in-built protective mechanism, discussed below [16] (Figure 2).

**Figure 1.** Intracellular trypsin activation following stimulation with palmitoleic acid ethyl ester (POAEE) is initiated in the apical granular pole of an isolated permeabilized pancreatic acinar cell

The level of trypsin activity is correlated with the degree of 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 \(\mu\)M). (D) The time course of the increase in intracellular trypsin activity following start of stimulation with 100 \(\mu\)M POAEE. (E) Results from experiments in which 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\(_3\)R2\(^{-}\)) and from mice in which both types 2 and 3 IP\(_3\) receptors had been deleted (IP\(_3\)R2\(^{-}\) 3\(^{-}\)). Adapted from Gerasimenko et al., 2009 [13].

**Figure 2.** The inhibitory effect of intracellular calmodulin (CaM) on ethanol-induced intracellular Ca\(^{2+}\) release and trypsin activation in permeabilized pancreatic acinar cells

(A) Typical traces from two experiments in which changes in [Ca\(^{2+}\)]\(_{\text{store}}\) were assessed after stimulation with ethanol. In the absence of CaM, 10 mM ethanol (EtOH) evokes a clear decrease in [Ca\(^{2+}\)]\(_{\text{store}}\), whereas this ethanol concentration fails to elicit any release when CaM (2.5 \(\mu\)M) is added to the external solution (in direct contact with the intracellular solution through the pore generated by two-photon permeabilization). A much higher ethanol concentration (100 mM) can still evoke Ca\(^{2+}\) release. (B) CaM inhibits ethanol-induced trypsin activation in a manner very similar to its protective effect against intracellular Ca\(^{2+}\) release. In addition, the lowest trace demonstrates that the Ca\(^{2+}\)-like peptide CALP-3—in the presence of CaM—abolishes the trypsin-activating effect of even a very high ethanol concentration (100 mM). Adapted from Gerasimenko et al., 2011 [16].

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

Recent insights have shed light on how alcohol-induced Ca\(^{2+}\) release could be prevented. It has been found that when the 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 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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References


