



Published in final edited form as:

Cell Calcium. 2008 July ; 44(1): 14–23. doi:10.1016/j.ceca.2007.11.013.

Mitochondrial injury in pancreatitis

R. Mukherjee^{a,b}, D.N. Criddle^b, A. Gukvoskaya^c, S Pandol^c, O.H. Petersen^b, R. Sutton^{a,b,*}

^aDivision of Surgery and Oncology, University of Liverpool, Royal Liverpool University Hospital, Daulby Street, Liverpool L69 3BX, United Kingdom

^bMRC Secretary Control Research Group, Physiological Laboratory, University of Liverpool, Liverpool L69 3BX, United Kingdom

^cUSC-UCLA Research Centre for Alcoholic Liver and Pancreatic Diseases, Veterans Affairs Greater Los Angeles Healthcare System and University of California, Los Angeles, CA, United Kingdom

Summary

Pancreatitis is an increasingly common disease that carries a significant mortality and which lacks specific therapy. Pathological calcium signalling is an important contributor to the initiating cell injury, caused by or acting through mitochondrial inhibition. A principal effect of disordered cell signalling and impaired mitochondrial function is cell death, either by apoptosis that is primarily protective, or by necrosis that is deleterious, both locally and systemically. Mitochondrial calcium overload is particularly important in necrotic injury, which may include damage mediated by the mitochondrial permeability transition pore. The role of reactive oxygen species remains controversial. Present understanding of the part played by disordered pancreatic acinar calcium signalling and mitochondrial inhibition offers several new potential therapeutic targets.

Keywords

Pancreatitis; Mitochondria; Calcium signaling; Apoptosis; Necrosis

Pancreatitis is a disease of increasing incidence caused by gallstones, ethanol excess, hyperlipidaemia and other, rarer precipitating factors that in severe cases induces pancreatic necrosis, multiple organ failure and death [1]. Pancreatic necrosis is a critical contributing event, in part through the local and systemic release of activated digestive enzymes, accounting for extreme effects despite the relatively small volume of the gland (<200 ml). Dysregulation of Ca²⁺ homeostasis was proposed over a decade ago [2] and since confirmed by a number of groups to be the key trigger of pancreatic acinar cell injury induced by hyperstimulation [3,4], bile salts [5,6], ethanol metabolites (fatty acid ethyl esters, FAEEs) [7,8] and fatty acids (FAs) [7,8]. These agents are implicated in the pathogenesis of pancreatitis [1], as bile salts may reflux into the pancreatic duct during passage of gallstones through the sphincter of Oddi [9,10], FAEEs are produced in the pancreas through non-oxidative metabolism of ethanol [11-14], and FAs accumulate in the pancreas after FAEE

*Corresponding author. Tel.: +44 151 706 4170; fax: +44 151 706 5826. r.sutton@liv.ac.uk (R. Sutton).

hydrolysis [11,12] or in hyperlipidaemia [1]. The central role of mitochondria in the transduction of intracellular Ca^{2+} signals [15], the supply of ATP and determination of cell fate [16,17] places mitochondrial injury centre stage in this scenario [18]. This review outlines current understanding of the place of mitochondrial injury in the pathogenesis of pancreatitis.

1. Pancreatitis and the lack of specific therapies

Acute pancreatitis was first described comprehensively by Fitz, a pathologist at Harvard who identified severe forms of the disease as haemorrhagic, suppurative or gangrenous [19] now recognised as various stages of acute necrotising pancreatitis [1]. During the first half of the last century, Moynihan described acute pancreatitis as ‘most terrible of all calamities that occur in connection with the abdominal viscera’ [20]. The disease occurs acutely in up to 100 per 100,000 people per year [21] and causes severe disease in 20% [22]. Although gallstones have accounted for almost half of all cases for several decades, alcohol abuse is the cause of an increasing number, in some countries the majority [21]. Rarer causes include hypercalcaemia, viral infections, drugs, endoscopic retrograde cholangiopancreatography, surgery, ductal tumours and autoimmune causes [1]. Whatever the cause, acute inflammation of the pancreas features necrosis (>30% of the gland) in up to 20% of patients, which may extend into peri-pancreatic tissues and be accompanied by injury of remote organ systems as well; up to 50% of deaths occur in the first week from multiple organ failure [1,22]. In subsequent weeks, secondary infection is the most significant factor-determining outcome [22]. In general, the more severe the pancreatic injury and the greater the extent of necrosis, the greater the accompanying systemic inflammatory response, subsequent organ failure and risk from superimposed infection [22]. Thus, in patients with necrosis compared to those without, organ failure is far more common (50% vs. 5–10%, respectively), as is mortality (17% vs. 3%) [1]. Although the mechanism by which necrosis leads to organ failure is unclear, experimental studies demonstrate that the severity and extent of pancreatic injury is an instrumental factor.

While digestive enzyme secretion is the primary function of the exocrine pancreas, premature intracellular digestive enzyme activation is the hallmark of, and a key component of tissue damage in, pancreatitis [23]. This is illustrated by hereditary pancreatitis, as is the continuum between acute and chronic pancreatitis, the result of cationic trypsinogen gene mutations that render trypsinogen more readily activated or once activated, less readily broken down [24]. Cationic trypsinogen gene mutations result in an autosomal dominant disease with 80% penetrance, beginning with recurrent attacks of acute pancreatitis in childhood and/or adolescence, developing into chronic pancreatitis during early adult life, and a 40% lifetime risk of pancreatic carcinoma [25]. In a similar manner continued alcohol excess and uncontrolled hyperlipidaemia can induce recurrent acute pancreatitis that is likely to become chronic pancreatitis, usually but not always at a later stage in adult life, the sporadic form of which increases the lifetime risk of pancreatic carcinoma by an order of $\times 15$ [26].

Despite the major burden of disease resulting from both acute and chronic pancreatitis, little specific treatment is available to halt their progression, let alone prevent their occurrence

[1,22]. Primarily for this reason research has been undertaken to understand the pathogenesis of pancreatitis, in the hope of identifying new prophylactic and therapeutic targets that may be altered to improve outcome. Many data now indicate that the initial site of injury is within pancreatic acinar cells, which make up the bulk of the organ [3-8,13,14,18,23]. Subsequently both intra- and extracellular factors contribute to the inflammation, oedema and cell death responses observed in pancreatitis as well as the progression to the systemic inflammatory response syndrome and multiple organ failure characteristic of severe disease [1], as summarised in Fig. 1. To understand what goes wrong in pancreatic acinar cells, however, normal mechanisms must first be outlined.

2. Pancreatic acinar cell Ca^{2+} signalling

The pancreatic acinar cell is the classical, polarised, secretory cell, with Ca^{2+} centre stage in the control of its activities (see Fig. 2) [23,27]. The copiously productive endoplasmic reticulum (ER) is densely packed around the nucleus in the basolateral region, and extends into the apical region, where the Golgi extends into a zymogen granule- (ZG-) rich and endo-lysosome- (EL-) rich secretory pole containing a panoply of inactive digestive enzymes [23,27-31]. The enzymes within ZGs that have a low pH (5.5—5.8) are held in a condensed matrix, tightly bound by high concentrations of Ca^{2+} [32,33]. Mitochondria surround the apical pole and nucleus, also lying beneath the basolateral plasma membrane, providing energy for the essential functions of these regions [28-30]. Pancreatic acinar cells normally synthesise inactive digestive enzymes, stored in apical ZGs which are subsequently secreted into the duodenum in response to stimulation by acetylcholine or cholecystokinin. These enzymes are triggered into an activation cascade through conversion of trypsinogen into active trypsin by duodenal enterokinase.

In response to physiological stimulation of G-proteinlinked receptors by the neurotransmitter acetylcholine (ACh) or hormone cholecystokinin (CCK), the second messengers inositol trisphosphate (IP3), cyclic ADP ribose (cADPr) and nicotinic acid adenine dinucleotide phosphate (NAADP) are generated then rapidly diffuse and bind to their receptors (IP3R, ryanodine receptors, RyR, and probably NAADPR), which are Ca^{2+} channels on the ER, ZGs and ELs [27,31]. As a result, repetitive local rises (spikes) in the free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) occur, buffered by peri-apical mitochondria that respond by increasing ATP production to drive secretion, and terminate the signal [28-30]. Ca^{2+} - induced Ca^{2+} release (CICR), notably via RyR, as well as the combinatorial action of two or more second messengers, gives rise to less frequent Ca^{2+} signals that spread beyond the peri-apical buffer zone into the basolateral region of the cell, again driving mitochondrial ATP production for transcription, translation and ATPase pump activity [27-31]. The sarcoendoplasmic reticulum Ca^{2+} -ATPase (SERCA) pump refills the ER Ca^{2+} store while the plasma membrane Ca^{2+} -ATPase (PMCA) pump assists to restore the normal basal $[\text{Ca}^{2+}]_i$ [34]. These two pumps also clear a continuous ER Ca^{2+} leak, the origin and function of which remains unclear [27,31,34].

Ca^{2+} entry into the cell is regulated at the plasma membrane by store-operated Ca^{2+} channels [35]. In non-excitatory cells such as acinar cells, extracellular Ca^{2+} entry does not typically initiate signalling, but does sustain continued signalling, and is required to replace

PMCA-mediated extrusion and following secretion, to replenish stores that will contribute to new ZGs [27]. In such cells, however, plasma membrane $\text{Na}^+/\text{Ca}^{2+}$ exchange activity is minimal, so the potential for Ca^{2+} clearance is restricted to high-affinity, low capacity PMCA pumps should cytosolic Ca^{2+} overload occur [23,27].

3. Pathological Ca^{2+} signalling

Abnormal, prolonged elevation of $[\text{Ca}^{2+}]_i$ was proposed to be the crucial trigger of pancreatic acinar cell injury in pancreatitis over a decade ago [2]. First tested by CCK octapeptide hyperstimulation (see Fig. 3) [3,4], an established means of inducing experimental acute pancreatitis, subsequent work with bile salts [5,6], FAs and FAEEs [7,8] confirmed the essential role of cytosolic Ca^{2+} overload in acinar cell injury. All these agents induce profound, global Ca^{2+} release from the ER, also likely from acid stores (ZGs and ELs), although the mechanisms and role of release from acid stores are less well characterised. Without sustained Ca^{2+} entry, cellular injury does not occur, the early signs of which include premature intracellular digestive enzyme activation, vacuolisation, secretory block, basolateral secretion and disorganisation of the cytoskeleton, all of which lead to cellular necrosis [3-8]. Nevertheless it is the sustained, global elevation of $[\text{Ca}^{2+}]_i$ and not ER Ca^{2+} depletion that induces the injury, since abrogation of elevated $[\text{Ca}^{2+}]_i$ with a Ca^{2+} chelator prevents these abnormal changes [3,6,8].

Three principal sites have been identified that are targets of toxins which induce excessively elevated $[\text{Ca}^{2+}]_i$, these being IP_3Rs , RyRs and Ca^{2+} -ATPase pumps [5,6,8,39]. Thus, bile salts induce Ca^{2+} release by increasing the open probability of IP_3Rs and RyRs [5,39], whereas FAEEs induce Ca^{2+} release via IP_3Rs [8]. FAs, however, do not induce significant release via ER Ca^{2+} channels at levels reached *in vivo* [8]. All these agents inhibit Ca^{2+} -ATPase pumps through inhibition of mitochondrial ATP production, but in the case of bile salts this may also occur via a direct action on SERCA pumps [6].

4. Normal acinar cell mitochondrial function

Peri-apical, peri-nuclear and sub-plasmalemmal mitochondria comprise the principal aerobic powerhouses for all ATP-dependent activities of the acinar cell (see Fig. 4) [28-30]. Much as elsewhere, mitochondria integrate local cytosolic Ca^{2+} signals that activate Krebs' cycle enzymes and drive ATP production, while maintaining a mitochondrial membrane potential (ψ_M) of 150–180 mV across the inner mitochondrial membrane, negative inside, by pumping protons into the inter-membrane space [15]. ψ_M concentrates Ca^{2+} within the matrix to effect Krebs' cycle metabolism [15]. Some reactive oxygen species (ROS) generation is normal, and their effect on ψ_M is unclear, but there are many mitochondrial targets of injury from excessive ROS generation, including Ca^{2+} channels, transporters and microdomains, as well as complexes and enzymes that produce ATP [16-18,36,37]. Should this occur, acinar cell mitochondria initiate the intrinsic apoptotic pathway [18], as in most other cell types [36]. Mitochondria within the acinar cell — unlike in some other cell types [40] — play a pivotal role in maintaining Ca^{2+} homeostasis, directly and indirectly [28-30]. This role may depend, in part, upon their close apposition to ER Ca^{2+} -release terminals [15,40]. Directly, the peri-apical mitochondria take up local cytosolic Ca^{2+} spikes through

the Ca^{2+} uniporter [38], preventing further spread of the signal, terminated via the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger [15]. Such signal termination prevents further emptying of the Ca^{2+} stores, prevents large, frequent Ca^{2+} fluxes, and reduces metabolic demands for ATP production and energy expenditure in the cell.

Within pancreatic acinar cells physiological Ca^{2+} signals always begin within the apical pole, but on occasions the combinatorial effects of multiple second messengers (IP_3 , cADPr and NAADP) and CICR exceed the immediate buffering capacity of peri-apical mitochondria, such that Ca^{2+} signals spread into the basolateral region to enter peri-nuclear and sub-plasmalemmal mitochondria and initiate cellular responses in that part of the cell [27,31]. These mitochondrial groups again respond to and assist in termination of the signal, supplying ATP for SERCA and PMCA activity that completes the process.

5. Abnormal mitochondrial function

The most significant mechanism of Ca^{2+} -ATPase inhibition by toxins that induce pancreatitis is likely to be that of direct impairment of mitochondrial ATP production [8,15-18,23,36,41]. FAEEs are formed non-oxidatively from ethanol and fatty acids by synthases that are relatively abundant in pancreatic acinar cells [11]. FAEEs induce experimental pancreatitis and are found in high concentrations within the pancreata of people who have died from alcohol intoxication [11,13,14]. FAEEs bind to and accumulate within the inner mitochondrial membrane, where hydrolases release locally high concentrations of FAs, which uncouple oxidative phosphorylation, deplete ψ_M , and impair ATP production [8,12]. Hyperlipidaemia, another cause of pancreatitis, also results in high concentrations of long-chain FAs within cells, may be transported into mitochondria by carnitine palmitoyltransferase and induce mitochondrial impairment similar to that caused by FAEEs [8]. Failure of ATP production reduces the capacity of the acinar cell to clear Ca^{2+} , whatever the cause, whether from Ca^{2+} signals, Ca^{2+} leak [15,18], or an abnormal elevation in $[\text{Ca}^{2+}]_i$ caused by toxins [39]. Under these circumstances, peri-granular mitochondria cannot buffer apical Ca^{2+} elevations. The resulting elevation of $[\text{Ca}^{2+}]_i$ increases the Ca^{2+} load on mitochondria, induces further collapse of ψ_M and impairment of ATP production, until the cell passes beyond any capacity to sustain sufficient ATP production for apoptosis, such that necrosis results [18]. Protection of cells from this sequence can be provided by excluding Ca^{2+} from the external medium [3,5,7,8], or providing supplementary intracellular ATP [8]. This has been demonstrated in patch clamp experiments where 4mM ATP has been supplied via the patch pipette, maintaining activity of SERCA and PMCA pumps to avoid global, sustained, toxic elevations of $[\text{Ca}^{2+}]_i$ [8].

One important effect of FAs on pancreatic acinar cell mitochondria is uncoupling of oxidative phosphorylation, an effect that does not depend on gross depolarisation of ψ_M *per se* [8]. It is, however, noteworthy that loss of ψ_M is an early event in experimental pancreatitis [48], in large measure attributable to toxic elevation of $[\text{Ca}^{2+}]_i$ [8]. Thus, in cells loaded with a cytosolic Ca^{2+} chelator and treated with FAs, diminished ATP production is seen (suggested by a fall in NADH auto-fluorescence, see Fig. 4) without obvious changes in ψ_M . Without Ca^{2+} chelation, large, global, sustained elevations in $[\text{Ca}^{2+}]_i$ occur that induce obvious loss of ψ_M and accelerate mitochondrial impairment [8,18,27]. Bile salts,

however, may induce a modest loss of ψ_M even in the presence of Ca^{2+} chelation, although detection of such a loss of ψ_M depends on high concentrations ($>100 \mu\text{M}$, which may have detergent effects) of bile salts and high ‘dequench’ concentrations of mitochondrial fluorescent dyes, since ‘classic’ concentrations of these dyes fail to demonstrate any loss of ψ_M [37].

6. Apoptosis versus necrosis in pancreatitis

For many years it has been accepted that in pancreatitis the major form of pancreatic acinar cell death is necrosis [42]. Necrosis is characterised by severe pathophysiological changes that include mitochondrial swelling, plasmalemmal disruption and leakage of activated digestive enzymes and other cellular contents, which trigger acute exudative inflammation of the surrounding tissue [1,43-45]. The process is largely uncontrolled but can be initiated by a variety of toxic insults, including bile salts, FAEEs and FAs, inducing cytosolic Ca^{2+} overload, excessive ROS generation, severe depletion of ψ_M and failure of ATP production, as described [3-8] (see Fig. 5). In contrast, apoptosis is genetically regulated, occurs via both caspase dependent and independent pathways [41] and requires ATP to proceed. Both intrinsic (classical, mitochondrial, see Fig. 5) and extrinsic (receptor-mediated) apoptotic pathways progress via a cascade of events that result in removal of cells from tissue which unlike necrosis occurs with minimal release of intracellular content, inflammation and collateral damage [16-18,41,45].

In vivo, cell death pathways are less distinct from one another, although apoptotic pathways are more evident in mild pancreatitis, whereas by definition, necrosis is more obvious in severe pancreatitis [18,46-48]. Recent data suggest that promotion of apoptosis is beneficial, whereas inhibition of apoptosis is harmful [46,48]. Thus, promotion of caspase activity, through reduction of endogenous caspase inhibition, has been found to protect from pancreatic necrosis in caerulein pancreatitis, whereas caspase inhibitors were found to increase pancreatic necrosis [48]. A further issue in understanding how cell death occurs in the pancreas is that stressed but surviving acinar cells produce and release cytokines that prime inflammatory responses, which by themselves contribute to further cell injury [1,49]. Promotion of apoptosis may reduce the capacity of acinar cells to produce such inflammatory responses. Infiltrating neutrophils are significant in this regard, since neutrophil depletion or genetic deletion of NADPH oxidase significantly lessens the severity of experimental acute pancreatitis [50], although how — and why — activated neutrophils exacerbate pancreatitis remains unclear. Nonetheless, moderate cell stresses are more likely to induce apoptosis, while severe stresses overcome apoptotic pathways and result in necrosis, especially when the protective functions of mitochondria are overwhelmed, as from massive Ca^{2+} overload or direct inhibition by FAEEs and/or FAs [8,16-18,36,46-48].

Elevations of $[\text{Ca}^{2+}]_i$ have been shown to be essential to the induction of apoptosis in isolated pancreatic acinar cells by the ROS-producing oxidant menadione [51,52]. Although global, these elevations are not prolonged ($>30\text{s}$), unlike those induced by supramaximal stimulation with CCK, bile salts, FAEEs or FAs. The combination, however, of a modest, global elevation of $[\text{Ca}^{2+}]_i$ plus abnormal ROS production induces mitochondrial release of cytochrome c and activation of caspase 9 ($\sim 2 \text{ min}$), in keeping with the mitochondrial,

apoptotic pathway [51,52]. When ROS production is augmented by inhibition of endogenous anti-oxidant defence mechanisms, pancreatic acinar cell apoptosis is potentiated [52]. Additionally, there is a slower (~30 min), 'fail-safe', Ca^{2+} -independent mechanism of caspase 8 activation, dependent on lysosomal release of cathepsins, evident in a minority of cells [53]. Both cytochrome c and caspase 3 have been shown to increase Ca^{2+} release via the IP_3R [54], but the changes in $[\text{Ca}^{2+}]_i$ that occur in the later stages of pancreatic acinar cell apoptosis have not yet been examined. Nevertheless, should apoptosis fail, necrosis is likely to supervene, as may follow inhibition of caspases or ATP production [18].

Some acinar cells appear more resistant to toxic stresses than others, although the determinants of relative vulnerability have yet to be defined [47]. Initiation of apoptosis prior to the induction of experimental pancreatitis reduces the severity of the disease [55], suggesting that such pretreatment removes cells that would otherwise become necrotic. Unlike acute infarction in other organs, pancreatic necrosis is a slow process, developing over 72 h or more [1,22,23], during which time cells may pass through various phases of apoptosis. Although the determinants, nature and extent of the switch from apoptosis to necrosis in pancreatitis remain to be identified [18,47], it would appear that mitochondria are likely to play a central role in this switch.

7. Role of the mitochondrial permeability transition pore

An evolving hypothesis is that the mitochondrial permeability transition pore (MPTP) determines cell fate from injury, notably in glutamate-induced neurotoxicity as well as ischaemia-reperfusion injury in the brain and heart [56]. Mitochondrial Ca^{2+} overload and ROS induce a 'permeability transition' across the membranes of mitochondria, causing the membrane to become permeable to any molecule of less than 1.5 kDa, including protons, leading to a loss of ψ_M [17,36,40,56]. Induction of the MPTP is a graded phenomenon, in that the fall in ψ_M may be partial and/or transient with continued mitochondrial function, or ψ_M may collapse fully and ATP production may fail [56]. In the former circumstance apoptosis is likely, although this is unlikely to be by the intrinsic apoptotic pathway via cytochrome c release [1,15-17,56-60] (see Fig. 5); without ATP, necrosis follows. Nevertheless, since Ca^{2+} overload with or without ROS generation and mitochondrial failure are features of pancreatic acinar cell injury from agents that induce pancreatitis [1,3-8], the MPTP could be a key factor in any switch from apoptosis to necrosis in the development of severe, necrotising pancreatitis.

The pore has at least three components, these being a voltage dependent anion channel (VDAC) in the outer membrane, adenine nucleotide translocase (ANT) in the inner mitochondrial membrane and CyP-D within the mitochondrial matrix [17,56]. VDAC is closely associated with ER IP_3Rs and localises Ca^{2+} signals between the ER and mitochondria [15], while ANT exchanges cytosolic ADP for mitochondrial ATP (and can act in reverse) [36,56]; CyP-D is within the mitochondrial matrix and exhibits peptidyl-prolyl cis—trans isomerise (PPIase) activity [56]. High concentrations of Ca^{2+} induce conformational changes that produce macro-molecular complexes of these proteins to form the MPTP [17,56]. Data implicate each molecule in both apoptotic and necrotic cell death, depending on the effect on ψ_M [15-18,36,41,56] (see Fig. 6). It has been shown in other

models that mitochondrial Ca^{2+} overload together with oxidative stress may lead to sustained opening of the MPTP, mitochondrial swelling and leakage, a profound failure of ATP production and cell necrosis [16,17,56]. In pancreatic acinar cells, blockade of the MPTP with bongkreikic acid inhibits the partial, transient loss of ψ_M and caspase activation typically induced by menadione, and so inhibits menadione-induced apoptosis [51], although any effect on necrotic cell death has not been studied. Recent data, however, bring the role of VDAC [57] and of ANT [58] in MPTP mediated-injury into question. Also, CyP-D knockout mice develop normally and show no protection from a range of stimuli of that induce apoptosis via the intrinsic pathway [59,60], although these mice are protected from Ca^{2+} overload and ROS in heart and brain ischaemia-reperfusion injury [59,60]. Surprisingly, cyclosporin A, which binds to CyP-D and inhibits the MPTP, does not ameliorate but exacerbates acute pancreatitis by an unclear mechanism [61]. These data are consistent with independent operation of the intrinsic apoptotic pathway by another mechanism, the most likely candidate for which is cytochrome c release via Bax/Bak channels in the outer mitochondrial membrane [1,15-17,58-60]. In the setting of chronic pancreatic injury, cyclosporin A prevents tissue remodelling, and exacerbates fibrosis, perhaps by altering cytokine expression [62]. Clearly, a logical next step would be to determine whether sustained opening of the MPTP has a major role in acute pancreatitis, and to explore relationships between the intrinsic apoptotic pathway, Bcl-2 proteins and the MPTP.

8. Therapeutic prospects

Both acute and chronic pancreatitis have no specific pharmacological therapy and a growing mountain of negative randomised control trials [1,63]. Several approaches could be adopted to ameliorate these diseases [23]:

- i. Prevent the disease entirely.
- ii. Modify the early course of severe acute pancreatitis.
- iii. Halt progression to chronic disease.

The increasing, worldwide abuse of alcohol is likely to be the most stubborn obstacle to the first approach [23], as well as the prevalence of silent gallstones and the diversity of less frequent aetiologies. Thus, a cure is required, most especially for severe, necrotising disease that is the major cause of morbidity and mortality. Acute infarction in the pancreas is very different to that in other organs such as the brain or heart, being a slower process that develops over 72 h or more [1,22,23]. Potentially, this is an important window during which compounds that limit acinar cell injury could abrogate severe disease. Similarly, those agents that limit acute injury could modify continuing injury that drives progression to chronic disease.

To date, all data indicate that cytosolic Ca^{2+} overload is common to all forms of pancreatic acinar cell injury from toxins that induce pancreatitis [3-8,23]. Attractive interventions would be to (1) reduce the effect of toxins prior to the induction of Ca^{2+} release, (2) inhibit Ca^{2+} entry into or accumulation within the cell, (3) enhance $[\text{Ca}^{2+}]_i$ clearance (see Fig. 7). Inhibition of FAEE synthesis and/or hydrolysis could lessen the toxicity of ethanol [8]. As

yet no agent specifically blocks Ca^{2+} entry into non-excitable cells, let alone into pancreatic acinar cells [15]; the nature and location of pancreatic acinar cell store-operated Ca^{2+} entry channels remain controversial [27,35]. Preservation of mitochondrial function to enhance $[\text{Ca}^{2+}]_i$ clearance may reduce the severity of pancreatitis, using strategies under development for other organs. Thus, enhancement of glucose oxidation in preference to β -oxidation may reduce toxicity from FAs [64], while anti-oxidants and/or chemical preconditioning could be beneficial.

Acknowledgements

This work was supported by Programme, Cooperative and Component Grants from the Medical Research Council, UK (OHP, RS) and by National Institutes of Health Grants DK59936 (ASG) and DK59508 (SP); OHP is a Medical Research Council Professor and RM is supported by an Amelie Waring Clinical Research Fellowship from CORE.

Abbreviations:

ACh	acetylcholine
ADP	adenosine diphosphate
ANT	adenine nucleotide transporter
ATP	adenosine triphosphate
$[\text{Ca}^{2+}]_i$	cytosolic free Ca^{2+} ion concentration
cADPr	cyclic ADP ribose
CCCP	carbonyl cyanide 3-chlorophenylhydrazone
CCK	cholecystokinin
CICR	Ca^{2+} -induced Ca^{2+} release
CyP-D	cyclophilin D
ψ_m	mitochondrial membrane potential
EL	endo-lysosome
ER	endoplasmic reticulum
FA	fatty acid
FAEE	fatty acid ethyl ester
IP_3	inositol trisphosphate
IP_3R	inositol trisphosphate receptor
MPTP	mitochondrial permeability transition pore
NAADP	nicotinic acid adenine dinucleotide phosphate
NAADPR	nicotinic acid adenine dinucleotide phosphate receptor

NADH	reduced nicotinamide adenine dinucleotide
PMCA	plasma membrane Ca ²⁺ ATPase pump
POA	palmitoleic acid
ROS	reactive oxygen species
RyR	ryanodine receptor
SERCA	sarcoplasmic/endoplasmic reticulum Ca ²⁺ ATPase pump
SOC	store-operated Ca ²⁺ entry channel
TMRM	tetramethyl rhodamine methyl ester
VDAC	voltage-dependent anion transporter
ZG	zymogen granule

References

- [1]. Pandol SJ, Saluja AK, Imrie CW, Banks PA, Acute pancreatitis: bench to bedside, *Gastroenterology* 132 (2007) 1127–1151. [PubMed: 17383433]
- [2]. Ward JB, Petersen OH, Jenkins SA, Sutton R, Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? *Lancet* 346 (1995) 1016–1019. [PubMed: 7475553]
- [3]. Raraty M, Ward J, Erdemli G, Vaillant C, Neoptolemos JP, Sutton R, Petersen OH, Calcium-dependent enzyme activation and vacuole formation in the apical granular region of pancreatic acinar cells, *Proc. Natl. Acad. Sci. U.S.A.* 97 (2000) 13126–13131. [PubMed: 11087863]
- [4]. Kruger B, Albrecht E, Lerch MM, The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis, *Am. J. Pathol.* 157 (2000) 43–50. [PubMed: 10880374]
- [5]. Voronina S, Longbottom R, Sutton R, Petersen OH, Tepikin A, Bile acids induce calcium signals in mouse pancreatic acinar cells. Implications for bile-induced pancreatic pathology, *J. Physiol.* 540 (2002) 49–55. [PubMed: 11927668]
- [6]. Kim JY, Kim KH, Lee JA, Namkung W, Sun AQ, Ananthanarayanan M, Suchy FJ, Shin DM, Muallem S, Lee MG, Transporter-mediated bile acid uptake causes Ca²⁺-dependent cell death in rat pancreatic acinar cells, *Gastroenterology* 122 (2002) 1941–1953. [PubMed: 12055600]
- [7]. Criddle DN, Raraty MG, Neoptolemos JP, Tepikin AV, Petersen OH, Sutton R, Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites, *Proc. Natl. Acad. Sci. U.S.A.* 101 (2004) 10738–10743. [PubMed: 15247419]
- [8]. Criddle DN, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP, Sutton R, Petersen OH, Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis, *Gastroenterology* 130 (2006) 781–793. [PubMed: 16530519]
- [9]. Armstrong CP, Taylor TV, Pancreatic-duct reflux and acute gallstone pancreatitis, *Ann. Surg.* 204 (1986) 59–64. [PubMed: 3729584]
- [10]. Tashiro S, et al., Pancreatobiliary maljunction: retrospective and nationwide survey in Japan, *J. Hepatobiliary Pancreat. Surg.* 10 (2003) 345–351. [PubMed: 14598134]
- [11]. Lange LG, Sobel BE, Mitochondrial dysfunction induced by fatty acid ethyl esters, myocardial metabolites of ethanol, *J. Clin. Invest.* 72 (1983) 724–731. [PubMed: 6308061]
- [12]. Laposata EA, Lange LG, Presence of nonoxidative ethanol metabolism in human organs commonly damaged by ethanol abuse, *Science* 231 (1986) 497–499. [PubMed: 3941913]

- [13]. Werner J, Laposata M, Fernandez-del Castillo C, Saghir M, Iozzo RV, Lewandrowski KB, Warshaw AL, Pancreatic injury in rats induced by fatty acid ethyl ester, a nonoxidative metabolite of alcohol, *Gastroenterology* 113 (1997) 286–294. [PubMed: 9207289]
- [14]. Werner J, Saghir M, Warshaw AL, Lewandrowski KB, Laposata M, Iozzo RV, Carter EA, Schatz RJ, Fernandez-Del Castillo C, Alcoholic pancreatitis in rats: injury from nonoxidative metabolites of ethanol, *Am. J. Physiol. Gastrointest. Liver Physiol.* 283 (2002) G65–G73. [PubMed: 12065293]
- [15]. Rizzuto R, Pozzan T, Microdomains of intracellular calcium: molecular determinants and functional consequences, *Physiol. Rev.* 86 (2006) 369–408. [PubMed: 16371601]
- [16]. Orrenius S, Gogvadze V, Zhivotovsky B, Mitochondrial oxidative stress: implications for cell death, *Annu. Rev. Pharmacol. Toxicol.* 47 (2007) 143–183. [PubMed: 17029566]
- [17]. Kroemer G, Galluzzi L, Brenner C, Mitochondrial membrane permeabilization in cell death, *Physiol. Rev.* 87 (2007) 99–163. [PubMed: 17237344]
- [18]. Criddle DN, Gerasimenko JV, Baumgartner HK, Jaffar M, Voronina S, Sutton R, Petersen OH, Gerasimenko OV, Calcium signalling and pancreatic cell death: apoptosis or necrosis? *Cell Death Differ.* 14 (2007) 1285–1294. [PubMed: 17431416]
- [19]. Fitz RH, Acute pancreatitis, a consideration of pancreatic hemorrhage, hemorrhagic, suppurative, and gangrenous pancreatitis, and of disseminated fat necrosis, *Boston Med. Surg. J.* 70 (1889) 181–235.
- [20]. Moynihan B, Acute pancreatitis, *Ann. Surg.* 81 (1925) 132–142. [PubMed: 17865162]
- [21]. Jaakkola M, Nordback I, Pancreatitis in Finland between 1970 and 1989, *Gut* 34 (1993) 1255–1260. [PubMed: 8406164]
- [22]. Swaroop VS, Chari ST, Clain JE, Severe acute pancreatitis, *JAMA* 291 (2004) 2865–2868. [PubMed: 15199038]
- [23]. Petersen OH, Sutton R, Ca²⁺ signalling and pancreatitis: effects of alcohol, bile and coffee, *TIPS* 27 (2006) 113–120. [PubMed: 16406087]
- [24]. Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD, Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene, *Nat. Genet.* 14 (1996) 141–145. [PubMed: 8841182]
- [25]. Howes N, Lerch MM, Greenhalf W, Stocken DD, Ellis I, Simon P, Truninger K, Ammann R, Cavallini G, Charnley RM, Uomo G, Delhay M, Spicak J, Drumm B, Jansen J, Mountford R, Whitcomb DC, Neoptolemos JP, European Registry of Hereditary Pancreatitis and Pancreatic Cancer (EUROPAC). Clinical and genetic characteristics of hereditary pancreatitis in Europe, *Clin. Gastroenterol. Hepatol* 2 (2004) 252–261.
- [26]. Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andren-Sandberg A, Domellof L, Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group, *N. Engl. J. Med.* 328 (1993) 1433–1437. [PubMed: 8479461]
- [27]. Petersen OH, Ca²⁺ signalling and Ca²⁺-activated ion channels in exocrine acinar cells, *Cell Calcium* 38 (2005) 171–200. [PubMed: 16107275]
- [28]. Tinel H, Cancela JM, Mogami H, Gerasimenko JV, Gerasimenko OV, Tepikin AV, Petersen OH, Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca(2+) signals, *EMBO J.* 18 (1999) 4999–5008. [PubMed: 10487752]
- [29]. Park MK, Ashby MC, Erdemli G, Petersen OH, Tepikin AV, Perinuclear, perigranular and sub-plasmalemmal mitochondria have distinct functions in the regulation of cellular calcium transport, *EMBO J.* 20 (2001) 1863–1874. [PubMed: 11296220]
- [30]. Voronina S, Sukhomlin T, Johnson PR, Erdemli G, Petersen OH, Tepikin A, Correlation of NADH and Ca²⁺ signals in mouse pancreatic acinar cells, *J. Physiol.* 539 (2002) 41–52. [PubMed: 11850500]
- [31]. Ashby M, Tepikin AV, Polarised calcium and calmodulin signalling in secretory epithelia, *Physiol. Rev.* 82 (2002) 701–734. [PubMed: 12087133]
- [32]. Nguyen T, Chin W-C, Verdugo P, Role of Ca²⁺/K⁺ ion exchange in intracellular storage and release of Ca²⁺, *Nature* 395 (1998) 908–912. [PubMed: 9804425]

- [33]. Gerasimenko OV, Gerasimenko JV, Belan PV, Petersen OH, Inositol trisphosphate and cyclic ADP-ribose-mediated release of Ca^{2+} from single isolated pancreatic zymogen granules, *Cell* 84 (1996)473–480. [PubMed: 8608601]
- [34]. Burdakov D, Petersen OH, Verkhatsky A, Intraluminal calcium as a primary regulator of endoplasmic reticulum function, *Cell Calcium* 38 (2005) 303–310. [PubMed: 16076486]
- [35]. Parekh AB, Putney JW, Store-operated calcium channels, *Physiol. Rev.* 85 (2005) 757–810. [PubMed: 15788710]
- [36]. Davidson SM, Duchen MR, Calcium microdomains and oxidative stress, *Cell Calcium* 40 (2006) 561–574. [PubMed: 17049598]
- [37]. Voronina SG, Barrow SL, Gerasimenko OV, Petersen OH, Tepikin AV, Effects of secretagogues and bile acids on mitochondrial membrane potential of pancreatic acinar cells: comparison of different modes of evaluating DeltaPsim, *J. Biol. Chem.* 279 (2004) 27327–27338. [PubMed: 15084611]
- [38]. Kirichok Y, Krapivinsky G, Clapham DE, The mitochondrial calcium uniporter is a highly selective ion channel, *Nature* 427 (2004) 360–364. [PubMed: 14737170]
- [39]. Gerasimenko JV, Flowerdew SE, Voronina SG, Sukhomlin TK, Tepikin AV, Petersen OH, Gerasimenko OV, Bile acids induce Ca^{2+} release from both the endoplasmic reticulum and acidic intracellular calcium stores through activation of inositol trisphosphate receptors and ryanodine receptors, *J. Biol. Chem.* 281 (2006) 40154–40163. [PubMed: 17074764]
- [40]. Franzini-Armstrong C, ER—mitochondrial communication. How privileged? *Physiology* 22 (2007) 261–268. [PubMed: 17699879]
- [41]. Orrenius S, Zhivotovsky B, Nicotera P, Regulation of cell death: the calcium-apoptosis link, *Nat. Rev. Mol. Cell Biol.* 4 (2003) 552–565. [PubMed: 12838338]
- [42]. Kloppel G, Mailliet B, Pathology of acute and chronic pancreatitis, *Pancreas* 8 (1993) 659–670. [PubMed: 8255882]
- [43]. Hartwig W, Jimenez RE, Werner J, Lewandrowski KB, Warshaw AL, Fernandez-del Castillo C, Interstitial trypsinogen release and its relevance to the transformation of mild into necrotizing pancreatitis in rats, *Gastroenterology* 117 (1999) 717–725. [PubMed: 10464149]
- [44]. Hartwig W, Werner J, Jimenez RE, Z'graggen K, Weimann J, Lewandrowski KB, Warshaw AL, Fernandez-del Castillo C, Trypsin and activation of circulating trypsinogen contribute to pancreatitis-associated lung injury, *Am. J. Physiol.* 277 (1999) G1008–G1016. [PubMed: 10564107]
- [45]. Kroemer G, et al., Classification of cell death: recommendations of the Nomenclature Committee on Cell Death, *Cell Death Differ.* 12 (Suppl. 2) (2005) 1463–1467. [PubMed: 16247491]
- [46]. Kaiser AM, Saluja AK, Sengupta A, Saluja M, Steer ML, Relationship between severity, necrosis, and apoptosis in five models of experimental acute pancreatitis, *Am. J. Physiol.* 269 (1995) C1295–C1304. [PubMed: 7491921]
- [47]. Bhatia M, Apoptosis versus necrosis in acute pancreatitis, *Am. J. Physiol. Gastrointest. Liver Physiol* 86 (2004) G189–G196.
- [48]. Mareninova OA, Sung KF, Hong P, Lugea A, Pandol SJ, Gukovsky I, Gukovskaya AS, Cell death in pancreatitis: caspases protect from necrotizing pancreatitis, *J. Biol. Chem.* 281 (2006) 3370–3381. [PubMed: 16339139]
- [49]. Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, Pandol SJ, Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis, *J. Clin Invest.* 100 (1997) 1853–1862. [PubMed: 9312187]
- [50]. Gukovskaya AS, Vaquero E, Zaninovic V, Gorelick FS, Lusic AJ, Brennan ML, Holland S, Pandol SJ, Neutrophils and NADPH oxidase mediate intrapancreatic trypsin activation in murine experimental acute pancreatitis, *Gastroenterology* 122 (2002) 974–984. [PubMed: 11910350]
- [51]. Gerasimenko JV, Gerasimenko OV, Palejwala A, Tepikin AV, Petersen OH, Watson AJ, Menadione-induced apoptosis: roles of cytosolic Ca^{2+} elevations and the mitochondrial permeability transition pore, *J. Cell Sci.* 115 (2002) 485–497. [PubMed: 11861756]
- [52]. Criddle DN, Gillies S, Baumgartner-Wilson HK, Jaffar M, Chinje EC, Passmore S, Chvanov M, Barrow S, Gerasimenko OV, Tepikin AV, Sutton R, Petersen OH, Menadione-induced reactive

- oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells, *J. Biol. Chem.* 281 (2006) 40485–40492. [PubMed: 17088248]
- [53]. Baumgartner HK, Gerasimenko JV, Thorne C, Ashurst LH, Barrow SL, Chvanov MA, Gillies SG, Criddle DN, Tepikin AV, Petersen OH, Sutton R, Watson AJ, Gerasimenko OV, Caspase-8 mediated apoptosis induced by oxidative stress is independent of the intrinsic pathway and dependent on cathepsins, *Am. J. Physiol. Gastrointest. Liver Physiol* (4) (2007) [Epub ahead of print].
- [54]. Hanson CJ, Bootman MD, Roderick HL, Cell signalling: IP₃ receptors channel calcium into cell death, *Curr. Biol.* 14 (2004) R933–R935. [PubMed: 15530388]
- [55]. Bhatia M, Wallig MA, Hofbauer B, Lee HS, Frossard JL, Steer ML, Saluja AK, Induction of apoptosis in pancreatic acinar cells reduces the severity of acute pancreatitis, *Biochem. Biophys. Res. Commun.* 246 (1998) 476–483. [PubMed: 9610387]
- [56]. Halestrap AP, Calcium, mitochondria and reperfusion: a pore way to die, *Biochem. Soc. Trans.* 34 (2006) 232–237. [PubMed: 16545083]
- [57]. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR, Wallace DC, The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore, *Nature* 427 (2004) 461–465. [PubMed: 14749836]
- [58]. Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD, Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death, *Nat. Cell Biol.* 9 (2007) 550–555. [PubMed: 17417626]
- [59]. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, Inohara H, Kubo T, Tsujimoto Y, Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death, *Nature* 434 (2005) 652–658. [PubMed: 15800626]
- [60]. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, Robbins J, Molkentin JD, Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, *Nature* 434 (2005) 658–662. [PubMed: 15800627]
- [61]. Ito T, Kimura T, Yamaguchi H, Kinjo M, Sumii T, Nakano I, H. Nawata, Acute pancreatitis induced by cyclosporin A under stimulation of pancreas by caerulein, *Pancreas* 8 (1993) 693–699. [PubMed: 7504818]
- [62]. Vaquero E, Molero X, Tian X, Salas A, Malagelada JR, Myofibroblast proliferation, fibrosis, and defective pancreatic repair induced by cyclosporin in rats, *Gut* 45 (1999) 269–277. [PubMed: 10403741]
- [63]. Heinrich S, Schafer M, Rousson V, Calvien PA, Evidence based treatment of acute pancreatitis: a look at established paradigms, *Ann. Surg.* 243 (2006) 154–168. [PubMed: 16432347]
- [64]. Morrow DA, Givertz MM, Modulation of myocardial energetics: emerging evidence for a therapeutic target in cardiovascular disease, *Circulation* 112 (2005) 3218–3221. [PubMed: 16301353]

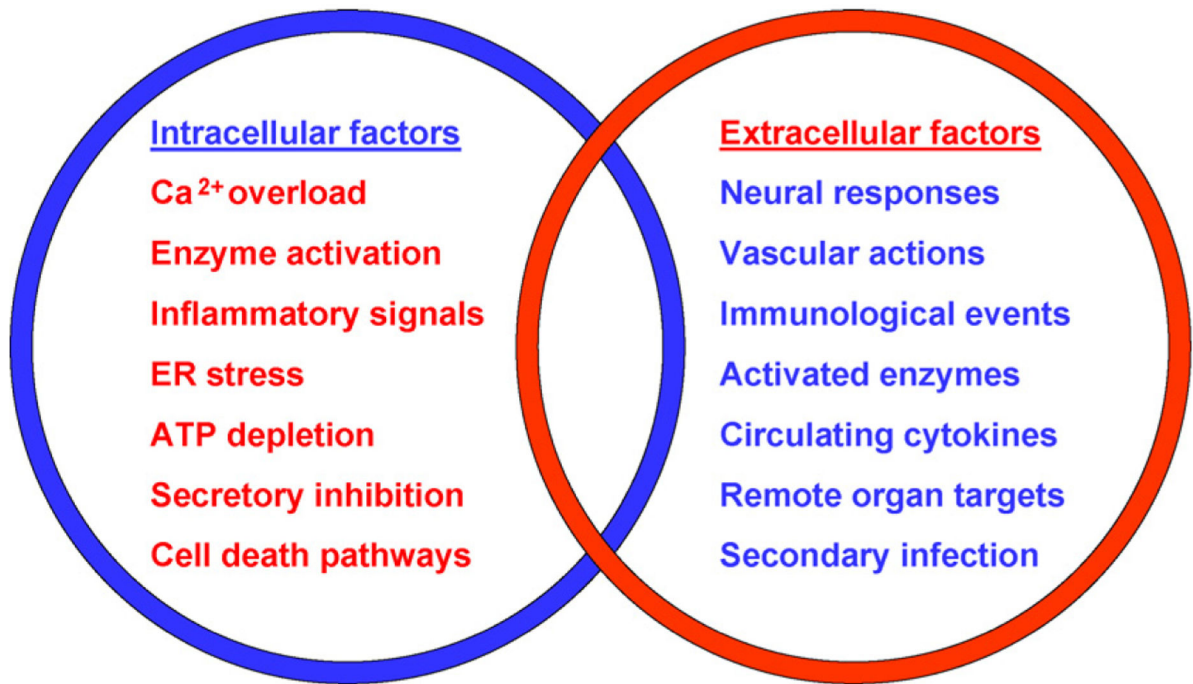


Figure 1.

Intra- and extracellular factors which contribute to the pathogenesis of pancreatitis. Acinar cell injury is the principal driving event, initially mediated via Ca²⁺ overload, leading to inflammatory signals that occur following cell stress. The loss of normal enzyme secretion and ATP production induce cell death pathways, which with severe stress are most likely to result in necrosis of cells. These factors contribute to and interact with extracellular factors, mediated initially via neural and vascular—endothelial mechanisms that accelerate inflammation. Activated resident and infiltrating monocyte-macrophages and/or neutrophils exacerbate the tissue injury, adding to the effects of activated interstitial and circulating digestive enzymes. Circulating cytokine responses increase the likelihood of remote organ damage, compromising recovery, while secondary infection adds to the likelihood of poor outcome.

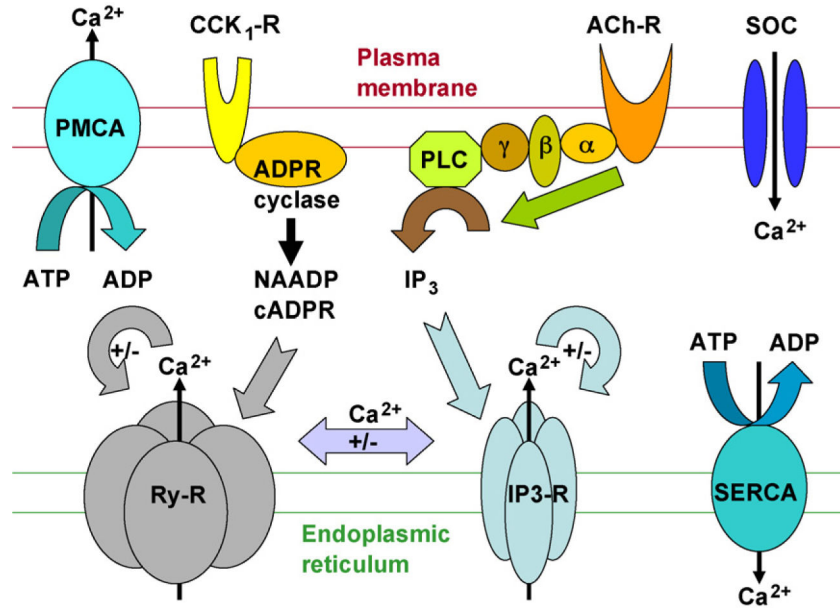


Figure 2.

Principal Ca²⁺ signalling machinery of the pancreatic acinar cell. Binding of cholecystokinin (CCK) and acetylcholine (ACh) to their respective receptors activates ADP ribose cyclase and phospholipase C, respectively to form the second messengers nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic ADP ribose (cADPr) and inositol trisphosphate (IP₃). Although not shown, CCK- and ACh-elicited Ca²⁺ signals also depend on the generation of IP₃ and cADPr, respectively, notably for Ca²⁺ signal amplification. The second messengers bind to their endoplasmic reticulum (ER) receptor Ca²⁺ release channels (RyRs and IP₃Rs), to elicit release of Ca²⁺ signals from this principal Ca²⁺ store (also from acid stores in the apical region). Ca²⁺ elicits further Ca²⁺ release (Ca²⁺-induced Ca²⁺ release, CICR) from these receptors (seen as '+' in the figure), which becomes inhibitory at higher concentrations (seen as '-' in the figure); there is also mutual interaction between RyRs and IP₃Rs through Ca²⁺. Ca²⁺ is then cleared from the cytosol by Ca²⁺-ATPase pumps (sarcoendoplasmic reticulum Ca²⁺-ATPase, SERCA; plasma membrane Ca²⁺-ATPase, PMCA), and any losses from cells stores are made up by Ca²⁺ entry via store-operated Ca²⁺ entry channels (SOCs).

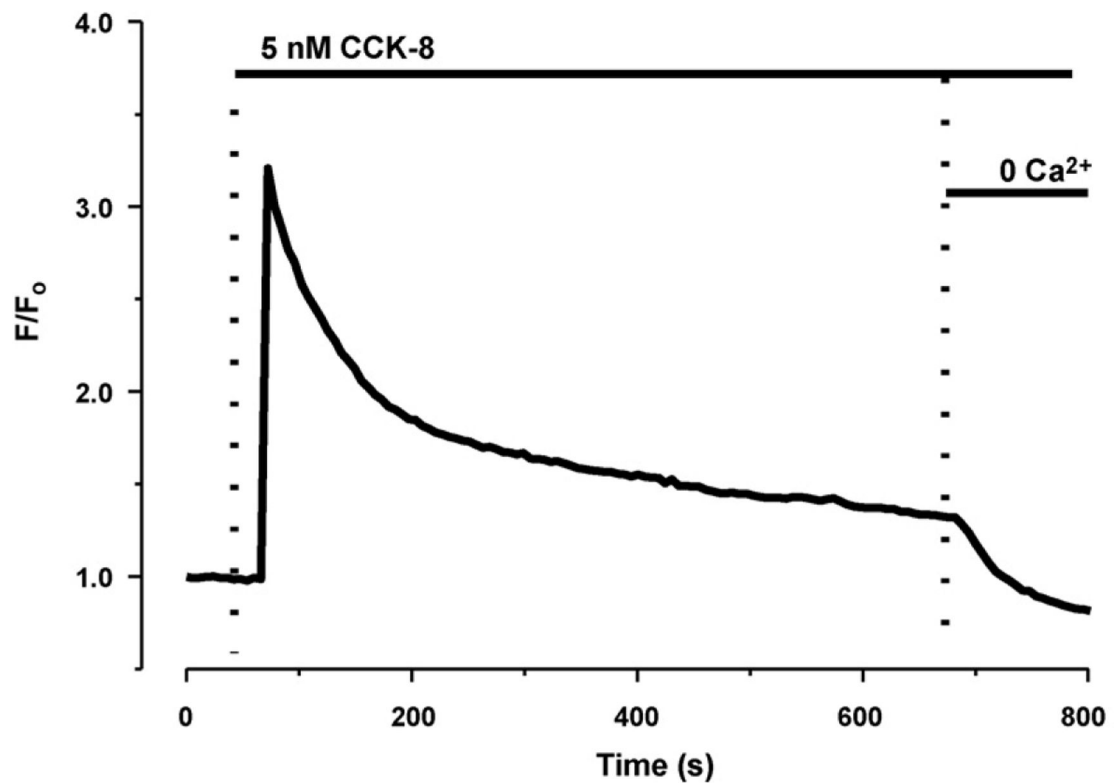
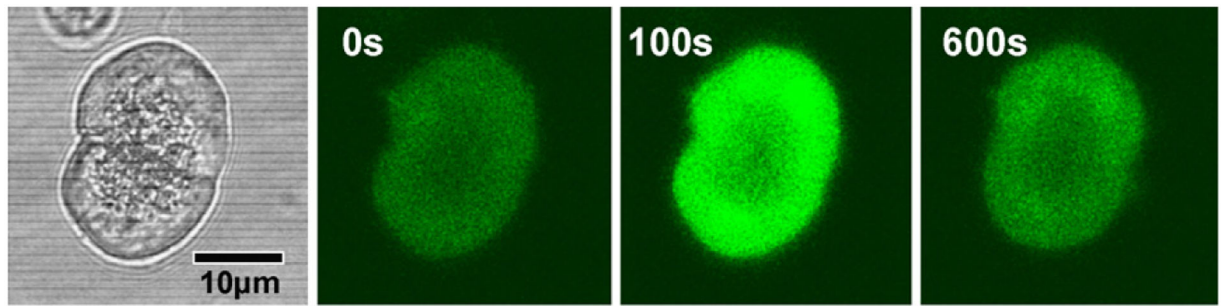


Figure 3. Changes in the free ionised cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) during hyperstimulation of a pancreatic acinar cell by CCK. Supra-maximal stimulation with 5 nM CCK induces a high peak and global, prolonged elevation of cytosolic Ca^{2+} , sustained by Ca^{2+} entry. Upon withdrawal of external Ca^{2+} , despite continuing hyperstimulation, $[\text{Ca}^{2+}]_i$ returns to basal levels. Continued hyperstimulation in the presence of external Ca^{2+} induces premature, intracellular digestive enzyme activation, vacuolisation, disruption of the cytoskeleton, mitochondrial impairment and necrosis.

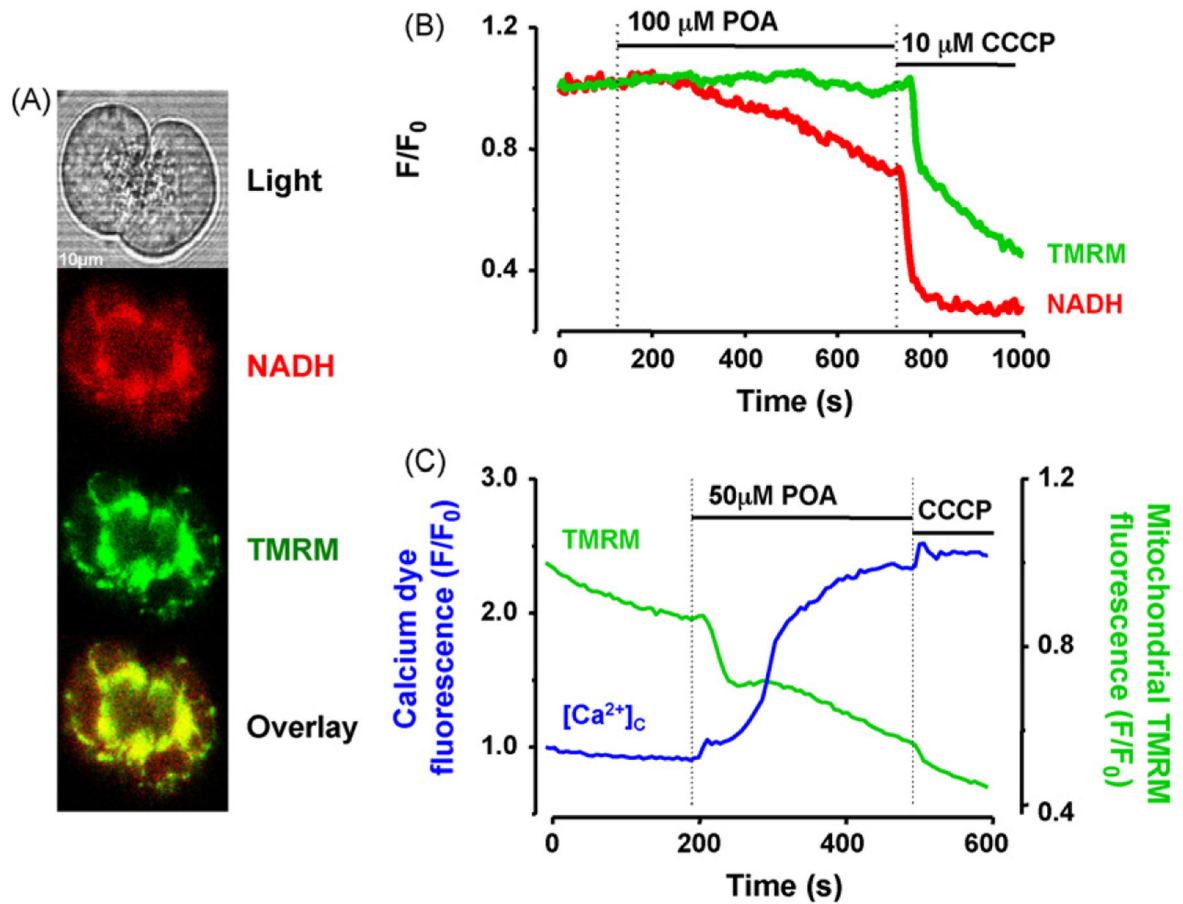


Figure 4.

Pancreatic acinar cell mitochondrial impairment induced by POA which is independent of, but exacerbated by, Ca^{2+} overload. (A) Typical light microscopic appearance of a doublet with NADH (auto-fluorescence) and tetramethyl rhodamine methyl ester (TMRM, ψ_{M} -dependent) fluorescence shown with overlay in the bottom panel. (B) Fall in NADH autofluorescence after POA administration in a cell pre-treated with a Ca^{2+} chelator that results in little if any change in $[\text{Ca}^{2+}]_{\text{i}}$, or fall in ψ_{M} , suggesting diminished ATP production without an immediate loss in electrochemical gradient, such as might occur with diminished β oxidation. (C) Large, sustained rise in $[\text{Ca}^{2+}]_{\text{i}}$ during POA administration to cell without pre-treatment with a Ca^{2+} chelator, preceding and accompanied by a fall in ψ_{M} , indicated by a drop in TMRM fluorescence. The drop in ψ_{M} is to such a level that the protonophore CCCP induces little further fall (reproduced in part with permission from reference [8], copyright Elsevier).

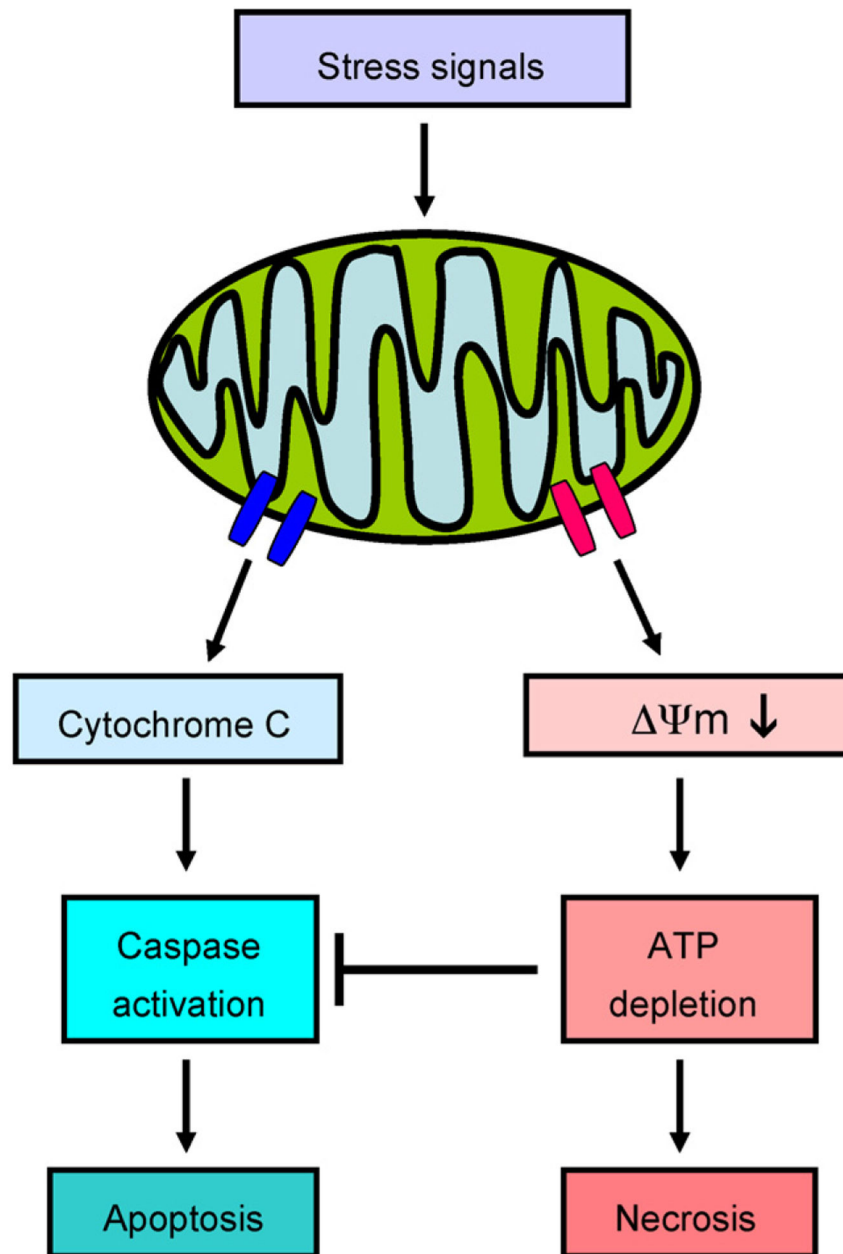


Figure 5. Alternative cell death pathways in pancreatic acinar cells associated with release of mitochondrial content. In response to Ca^{2+} loading of mitochondria and other stresses, cytochrome c is released from the inner mitochondrial membrane, activating caspases and inducing further Ca^{2+} release via IP_3Rs . These events may be controlled by Bax/Bak and Bcl-2 mediated channels (shown in blue). Caspase activation has a protective role in pancreatitis, inhibited by a lack of ATP. Marked loss of mitochondrial membrane potential, notably with induction of the MPTP (shown in red, see Fig. 6), inhibits ATP production, which in turn inhibits apoptosis and leads to necrosis.

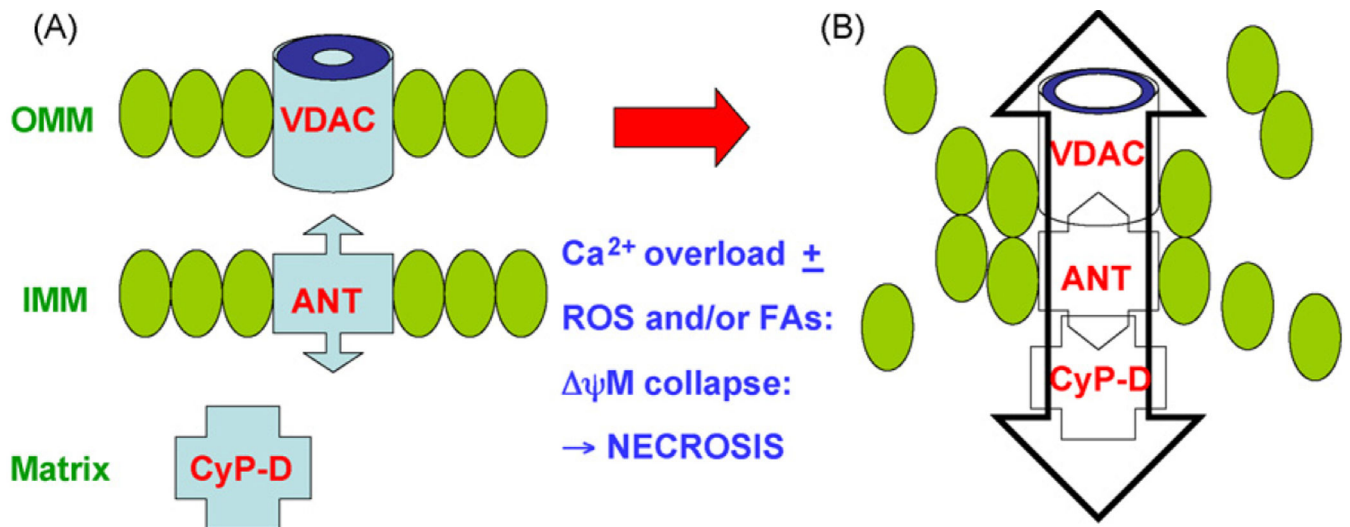


Figure 6.

Key components of the mitochondrial permeability transition pore (MPTP) contributing to partial or complete loss of ψ_M . (A) Normal mitochondrial components of the outer mitochondrial membrane (OMM; voltage-dependent anion transporter, VDAC), inner mitochondrial membrane (IMM; adenine nucleotide transporter, ANT) and mitochondrial matrix (cyclophilin-D, CyP-D) undergo conformational change following moderate or severe stress, resulting in transient or (B) sustained formation of the MPTP, the latter ending in necrosis. Ca₂₊ overload, FAs and reactive oxygen species (ROS) contribute to induction of the MPTP, which allows passage of all particles up to 1.5 kDa, contributing to mitochondrial swelling and rupture.

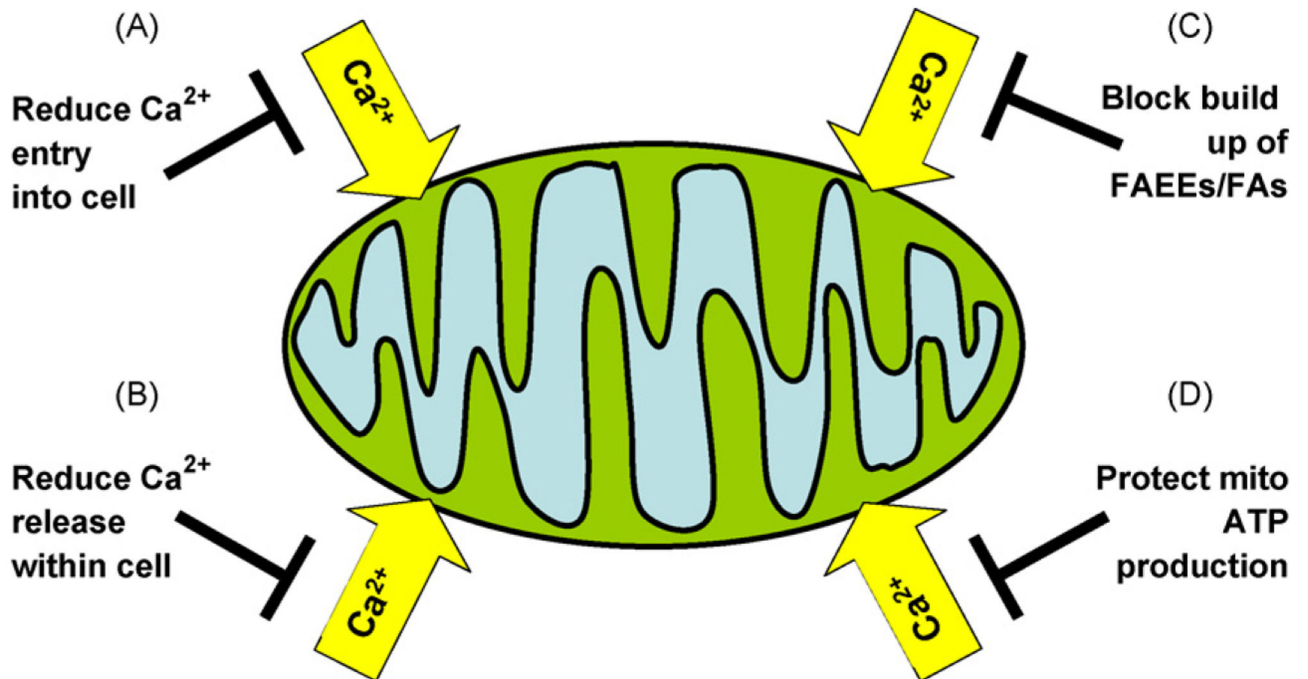


Figure 7.

Strategies to reduce mitochondrial injury and acinar cell damage in pancreatitis. (A) Blockage of Ca^{2+} entry will probably depend on identification of the SOC channel in the pancreatic acinar cell. (B) Prevention of intracellular Ca^{2+} release that awaits identification of specific antagonists of pancreatic acinar cell Ca^{2+} release mechanisms (see also Fig. 2). (C) Inhibition of fatty acid ethyl ester (FAEE) synthases combine ethanol with FAs, after which FAEEs bind to and accumulate within the inner mitochondrial membrane. Inhibition of hydrolases would prevent the release of FAs from FAEEs already accumulated within mitochondria. (D) Anti-oxidant and/or preconditioning strategies could preserve mitochondrial function to deliver adequate ATP for pancreatic acinar cells to survive intact.