Cell Death and Differentiation (2007) 14, 1285–1294
© 2007 Nature Publishing Group All rights reserved 1350-9047/07 $30.00
www.nature.com/cdd

Review

Calcium signalling and pancreatic cell death: apoptosis or necrosis?

DN Criddle*[,1], JV Gerasimenko[1], HK Baumgartner[1], M Jaffar[2], S Voronina[1], R Sutton[3], OH Petersen*[,1] and OV Gerasimenko[1]

Secretagogues, such as cholecystokinin and acetylcholine, utilise a variety of second messengers (inositol trisphosphate, cADPR and nicotinic acid adenine dinucleotide phosphate) to induce specific oscillatory patterns of calcium (Ca^{2+}) signals in pancreatic acinar cells. These are tightly controlled in a spatiotemporal manner, and are coupled to mitochondrial metabolism necessary to fuel secretion. When Ca^{2+} homeostasis is disrupted by known precipitants of acute pancreatitis, for example, hyperstimulation or non-oxidative ethanol metabolites, Ca^{2+} stores (endoplasmic reticulum and acidic pool) become depleted and sustained cytosolic [Ca^{2+}] elevations replace transient signals, leading to severe consequences. Sustained mitochondrial depolarisation, possibly via opening of the mitochondrial permeability transition pore (MPTP), elicits cellular ATP depletion that paralyses energy-dependent Ca^{2+} pumps causing cytosolic Ca^{2+} overload, while digestive enzymes are activated prematurely within the cell; Ca^{2+}-dependent cellular necrosis ensues. However, when stress to the acinar cell is milder, for example, by application of the oxidant menadione, release of Ca^{2+} from stores leads to oscillatory global waves, associated with partial mitochondrial depolarisation and transient MPTP opening; apoptotic cell death is promoted via the intrinsic pathway, when associated with generation of reactive oxygen species. Apoptosis, induced by menadione or bile acids, is potentiated by inhibition of an endogenous detoxifying enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1), suggesting its importance as a defence mechanism that may influence cell fate.
Cell Death and Differentiation (2007) **14,** 1285–1294; doi:10.1038/sj.cdd.4402150; published online 13 April 2007

Acute inflammation of the pancreas, triggered by such major precipitants as alcohol and gallstones, currently affects approximately 50 per 100 000 people per year, with an increasing incidence.[1] It is characterised by autodigestion of the organ involving premature, calcium (Ca^{2+})-dependent activation of digestive enzymes, vacuole formation in the apical, granular secretory pole and colocalisation of lysosomes and zymogen granules.[2,3] Attacks of acute pancreatitis resolve spontaneously in a majority of individuals with no further episodes; however, approximately 20–30% of patients go on to develop more severe pancreatic injury, often with extensive pancreatic necrosis and the development of a systemic inflammatory response syndrome leading to multiple organ failure, which may prove fatal.[4]

Cell death, defined as an irreversible loss of membrane integrity,[5] has been categorised in up to 11 types [6] and is effected in the pancreatic acinar cell by two principal processes, apoptosis and necrosis. Apoptosis is genetically regulated and occurs via both caspase-dependent and independent pathways,[7] while necrosis is thought to be largely non-programmed and uncontrolled, although this view has recently been questioned.[8] Perturbation of Ca^{2+} signalling has been linked to both apoptotic and necrotic cell death;[9] in the pancreatic acinar cell, oscillatory global rises of cytosolic Ca^{2+} may induce apoptosis,[10] while sustained elevations promote necrosis.[11,12] These differences may relate to effects on mitochondrial function associated with the respective Ca^{2+} signals. Under physiological conditions these organelles respond to localised increases in cytosolic Ca^{2+} by stimulus-metabolism coupling that generates NAD(P)H, reductive intermediates that fuel the respiratory transport chain with consequent production of ATP required for secretion.[13–16] In contrast, global, sustained Ca^{2+} rises can drastically reduce ATP production in pancreatic acinar cells.[12]

For many years it has been accepted that the major form of pancreatic acinar cell death is necrosis[17] and current evidence suggests that the balance between apoptosis and necrosis may influence the severity of acute pancreatitis.[18,19] For example, induction of pancreatic acinar cell apoptosis

[1]MRC Secretory Research Group, Department of Physiology, University of Liverpool, Liverpool, L69 3BX, UK; [2]Morvus Technology Ltd, Porton Down Science Park, Salisbury, Wiltshire, UK and [3]Division of Surgery and Oncology, University of Liverpool, Liverpool, L69 3BX, UK
*Corresponding authors: DN Criddle and OH Petersen, MRC Secretory Research Group, Department of Physiology, University of Liverpool, Liverpool L69 3BX, UK. Tel: +44 1517945323; Fax: +44 1517945327;
E-mails: criddle@liv.ac.uk and o.h.petersen@liv.ac.uk
Keywords: calcium; apoptosis; necrosis; pancreatitis; ROS; NQO1
Abbreviations: ACh, acetylcholine; ANT, adenine nucleotide translocator; Ca^{2+}, calcium; cADPR, cyclic ADP-ribose; CCK, cholecystokinin; DMN, 2,4-dimethoxy-2-methylnaphthalene; FAEE, fatty acid ethyl ester; IP_3, inositol trisphosphate; MPTP, mitochondrial permeability transition pore; NQO1, NAD(P)H:quinone oxidoreductase 1; NAADP, nicotinic acid adenine dinucleotide phosphate; PI3K, phosphotidylinositol 3-kinase; ROS, reactive oxygen species; SERCA, sarco-endoplasmic reticulum Ca^{2+} ATPase; TMRM, tetramethyl rhodamine methyl ester
Received 30.1.07; revised 12.3.07; accepted 19.3.07; Edited by P Nicotera; published online 13.4.07

protected mice against cerulein-induced pancreatitis,[20] while a recent study has indicated that suppression of the apoptotic cascade in pancreatic acinar cells, via inhibition of caspases, leads to necrotising pancreatitis.[21] This review will focus on the role of Ca^{2+} signalling in the determination of pancreatic acinar cell fate.

Necrosis. Necrosis is characterised by severe pathophysiological changes including mitochondrial swelling, plasmalemmal disruption and ultimately leakage of cellular contents. The latter triggers acute exudative inflammation of the surrounding tissue; subsequent activation and infiltration of neutrophils is thought to increase intracellular digestive enzyme activation and exacerbate pancreatitis.[22]

Over a decade ago we proposed that abnormal, prolonged elevation of cytosolic Ca^{2+} is the crucial trigger of pancreatitis.[23] Under physiological conditions, Ca^{2+} is tightly regulated in discrete cellular microdomains, which when disrupted may result in severe pathological consequences.[24] Ca^{2+} signals in the pancreatic acinar cell, generated by the second messengers inositol trisphosphate (IP_3), nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic ADP-ribose (cADPR), are modulated in a precise spatiotemporal manner necessary for normal secretory function, and are generally confined to the apical pole.[16] A superficial perigranular mitochondrial buffer barrier restricts the spread of Ca^{2+} signals to the basolateral area of the cell,[25] an action that may be shared by the closely situated Golgi apparatus.[26]

Hyperstimulation with cholecystokinin (CCK) causes acute pancreatitis in all species so far examined,[1] and in pancreatic acinar cells obtained from mice repeatedly injected with the CCK analogue cerulein, there is a loss of apical to basolateral progression of secretagogue-induced Ca^{2+} signals, suggesting that normal homeostatic control becomes compromised.[27] Furthermore, supra-maximal concentrations of CCK-induced elevation of baseline Ca^{2+} in isolated pancreatic acinar cells which was strongly linked to premature intracellular activation of trypsinogen, the hallmark of acute pancreatitis,[2,3] an effect that may involve activation of the vacuolar ATPase.[28] This action to raise cytosolic Ca^{2+} is shared by other diverse pathological stimuli that cause pancreatic injury, including non-oxidative ethanol metabolites,[11,12] duct ligation[29] and bile acids.[30–32] In addition, recent evidence has shown that the enzyme phosphatidylinositol 3-kinase (PI3K) mediates prolonged elevations of cytosolic Ca^{2+}, under conditions of agonist stimulation, via an inhibitory action on the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump;[33] the PI3K system has been implicated in the development of acute pancreatitis since pharmacological inhibition or knockout of this enzyme was protective in experimental animal models.[34,35] Importantly, abrogation of sustained rises of Ca^{2+}, using the intracellular Ca^{2+} chelator 1,2-bis(*O*-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), prevents zymogen activation induced by CCK hyperstimulation,[2] bile acid-induced cell damage and death[30] and necrosis caused by non-oxidative ethanol metabolites.[11]

Apoptosis. Apoptosis is a genetically regulated, programmed form of cell death that can occur by two principal routes: extrinsic (receptor-mediated) and intrinsic (classical: mitochondrial) pathways.[9] It is characterised by distinct features that involve a cascade of events, and which ultimately lead to removal of the dead cell from the tissue; unlike necrosis, apoptosis does not involve release of intracellular contents and thus does not elicit inflammation.[6] Cell and organellar shrinkage, membrane blebbing, condensation of nuclear chromatin and DNA cleavage, and flipping of phosphatidylserine moieties from the inner to the outer side of the plasmalemmal membrane are typical events, the latter permitting detection of acinar cell apoptosis experimentally via annexin V staining using fluorescence microscopy.[10,36] Apoptotic pathways employ caspases, aspartate-specific cysteine proteases, as the key elements of the initiation and execution of programmed cell death, with at least 14 isoforms currently resolved.[37] Caspases are highly conserved through human evolution and are currently divided into ‘initiator’ (caspases 8 and 9), which induce the proteolytic cascade that results in activation of ‘executioner’ (caspases 3, 6 and 7), that cleave numerous target proteins. Cellular apoptosis may also occur independently of caspases via the action of lysosomal cathepsins, such as cathepsin D, which has been shown to translocate from lysosomes to the cytosol in response to apoptotic stimuli.[38] Furthermore, the scenario appears even more complex, since activation of the intrinsic route by the extrinsic pathway has been recently demonstrated in pancreatic acinar cells.[21]

The role of Ca^{2+} in the induction of apoptosis has been recognised for several decades. For example, early studies have shown that the Ca^{2+} ionophore A23187[39] and the SERCA pump inhibitor thapsigargin,[40] which raise cytosolic Ca^{2+}, both induced apoptosis, an effect prevented by Ca^{2+} chelation with BAPTA. Ca^{2+} is known to regulate the intrinsic apoptotic pathway,[41] and Ca^{2+}-dependent opening of the mitochondrial permeability transition pore (MPTP) is considered to be a critical feature that leads to cytochrome *c* release into the cytosol.[42] Once released, cytochrome *c*, apoptotic peptidase activating factor-1 and pro-caspase 9 combine to form an apoptosome that activates caspase 9, with subsequent activation of caspase 3 that cleaves specific apoptotic targets causing cell death.[43] However, recent evidence has suggested that mitochondria may undergo MPTP formation and cytochrome *c* release in cells that lack isoforms of the adenine nucleotide translocator (ANT); the pore was no longer regulated by ANT ligands, while higher Ca^{2+} was required for permeability transition.[44] Importantly, hepatocytes without ANT remained responsive to various initiators of cell death, questioning the importance of the MPTP in induction of apoptosis. Furthermore, cells from cyclophilin-D-deficient mice did not undergo cyclosporin A-sensitive MPTP formation but died normally in response to various apoptotic stimuli. These animals showed resistance to necrotic cell death induced by reactive oxygen species (ROS) and Ca^{2+} overload, suggesting the importance of the MPTP in necrosis, but not apoptosis.[45]

The features of the endoplasmic reticulum (ER) Ca^{2+} store in relation to secretagogue signalling have been extensively characterised in the pancreatic acinar cell.[16] The ER and mitochondria possess a close spatial relationship and recent

evidence has pointed to the potential importance of ER-mitochondrial Ca^{2+} transfer to cell death,[46] with the proposition that localised Ca^{2+} elevations, triggered via release from the ER, might induce or facilitate mitochondrial features of apoptosis. In particular, activation of IP_3 receptors may exert a pro-apoptotic role,[47] while complex effects of anti-apoptotic Bcl-2/Bcl-xL and pro-apoptotic Bax/Bak proteins on ER membrane Ca^{2+} permeability and mitochondrial function point to an important relationship between ER stress and cellular apoptosis.[46,48]

Bile acid effects on cell fate. In 1901, Opie[49] proposed the common duct theory, which suggested that gallstones that become impacted at the ampulla of Vater cause bile reflux into the pancreas, which in turn causes acute pancreatitis. The detrimental effects of bile on the pancreas have subsequently been confirmed in experimental animal models.[50,51] In isolated pancreatic acinar cells, bile acids induce elevations of cytosolic Ca^{2+} that are oscillatory or sustained in nature (Figure 1a)[31] and which result in Ca^{2+}-dependent cell death.[30] The effects of bile are associated with partial mitochondrial membrane depolarisation, when assessed using the sensitive 'dequench' mode of tetramethyl rhodamine methyl ester (TMRM) measurement but not by the classical mode (Figure 1a), an effect that is inhibited by chelation of intracellular Ca^{2+} with BAPTA.[32]

Recently, we described an acidic, thapsigargin-insensitive and bafilomycin A-sensitive Ca^{2+} store located in the secretory granule area of pancreatic acinar cells, which is responsive to the second messengers IP_3, cADPR and NAADP (Figure 1b).[52] While IP_3 and cADPR cause Ca^{2+} release by activation of IP_3-dependent receptors (IP_3Rs) and ryanodine receptors (RyRs), respectively, NAADP may activate a novel Ca^{2+} channel in the acidic compartment[53] or open RyRs via a mechanism distinct from that of cADPR.[54] The exact location of the acidic store is still uncertain, with endosomes and lysosomes as possible candidates;[53] however, the most likely association has been made with zymogen granules (ZGs), which store the inactive digestive enzyme precursors.[52,55] Our recent results show that bile acids, in addition to inducing Ca^{2+} release from the endoplasmic reticulum (ER) store, are able to stimulate the acidic store in the apical ZG area.[56] Using a novel two-photon permeabilisation technique, we have found that the bile acid taurolithocholic acid sulphate (TLC-S) specifically activated RyRs, via an NAADP-dependent mechanism, to release stored Ca^{2+} (Figure 1b).

It is generally recognised that the crucial step in the development of pancreatitis is activation of precursor enzymes in the zymogen granules.[1] A high intra-granular concentration of Ca^{2+} is required for stability of granule contents,[57] most of which is tightly bound together with H^+ ions within the granular matrix. IP_3 and cADPR induce Ca^{2+} release from the zymogen granules,[55] a feature common to other types of secretory granules,[58,59] and it is feasible that a local perigranular rise of Ca^{2+} induced by these second messengers, or by bile acids acting at RyRs,[56] would in turn activate Ca^{2+}-dependent K^+ channels present in the granular membrane, permitting the uptake of K^+ into the granule. Since the matrix behaves as an ion exchanger,[59] Ca^{2+} and H^+ would be replaced by K^+, causing disaggregation of the matrix that may facilitate activation of trypsinogen to trypsin. While disaggregation is necessary for normal secretion, excessive cytosolic Ca^{2+} concentrations may induce premature disaggregation and pathological intracellular digestive enzyme activation, a hypothesis that awaits thorough evaluation in pancreatic acinar cells.

Recently, we have directly studied the effects of acute application of TLC-S on pancreatic acinar cell fate, and have shown that it causes caspase activation, consistent with induction of the apoptotic death pathway (Figure 1c).[36] Interestingly, this action is markedly potentiated when an endogenous detoxifying enzyme NAD(P)H:quinone oxidoreductase (NQO1; DT-diaphorase) is inhibited, and our results suggest that acute generation of ROS by bile acids may be important for the promotion of pancreatic acinar cell death. Bile acids are recognised precipitants of acute pancreatitis the detrimental actions of which may involve oxidant stress *in vivo*,[51,60] and our results are in accordance with a recent detailed study in hepatocytes, showing that TLC-S activated caspases 8, 9 and 3 via NADPH oxidase-mediated ROS production.[61]

Oxidant stress: Ca^{2+}, ROS and apoptosis. Oxidative stress has been implicated in the development of acute pancreatitis in diverse animal experimental models, including fatty acid infusion, ischaemia, pancreatic duct obstruction, gallstone pancreatitis and alcohol ingestion,[60,62–64] and measured in patients with mild and severe acute pancreatitis.[65] Previously, we have demonstrated the vital role of Ca^{2+} on MPTP opening in pancreatic acinar cell apoptosis induced by the oxidant menadione.[10] Crucially, the Ca^{2+} chelator BAPTA prevented both menadione-induced repetitive cytosolic Ca^{2+} spikes and apoptosis, instigated via the intrinsic apoptotic pathway. Our study suggested that the characteristics of Ca^{2+} signals generated by menadione and physiological secretagogues might underlie differences in their effects on cell fate. For example, physiological concentrations of CCK and acetylcholine (Ach) induce oscillatory cytosolic Ca^{2+} rises, which were initiated in the apical secretory granular pole, and the spread of which to the basolateral area was substantially delayed and diminished by the mitochondrial perigranular and perinuclear buffer barriers (Figure 2a);[25,66] such stimulation did not cause apoptosis. In contrast, menadione, which elicited apoptosis, produced Ca^{2+} transients that were also generated in the apical pole, but which rapidly spread to the basal and nuclear regions of the acinar cell, indicating essential differences between the oxidant and the physiological secretagogues. Menadione was found to elicit partial, transient mitochondrial depolarisation that was inhibited by BAPTA, and by bongkrekic acid, an inhibitor of the MPTP,[67] suggesting that Ca^{2+}-dependent MPTP opening in pancreatic acinar cells and consequent mitochondrial inhibition might explain the rapid spread of menadione-induced Ca^{2+} waves. Interestingly, when inhibition of mitochondria with antimycin A was imposed, ACh-induced Ca^{2+} reponses effectively mimicked those of menadione; however, apoptosis did not occur[10] suggesting that other factors may contribute to menadione-induced pancreatic acinar cell death (Figure 2a).

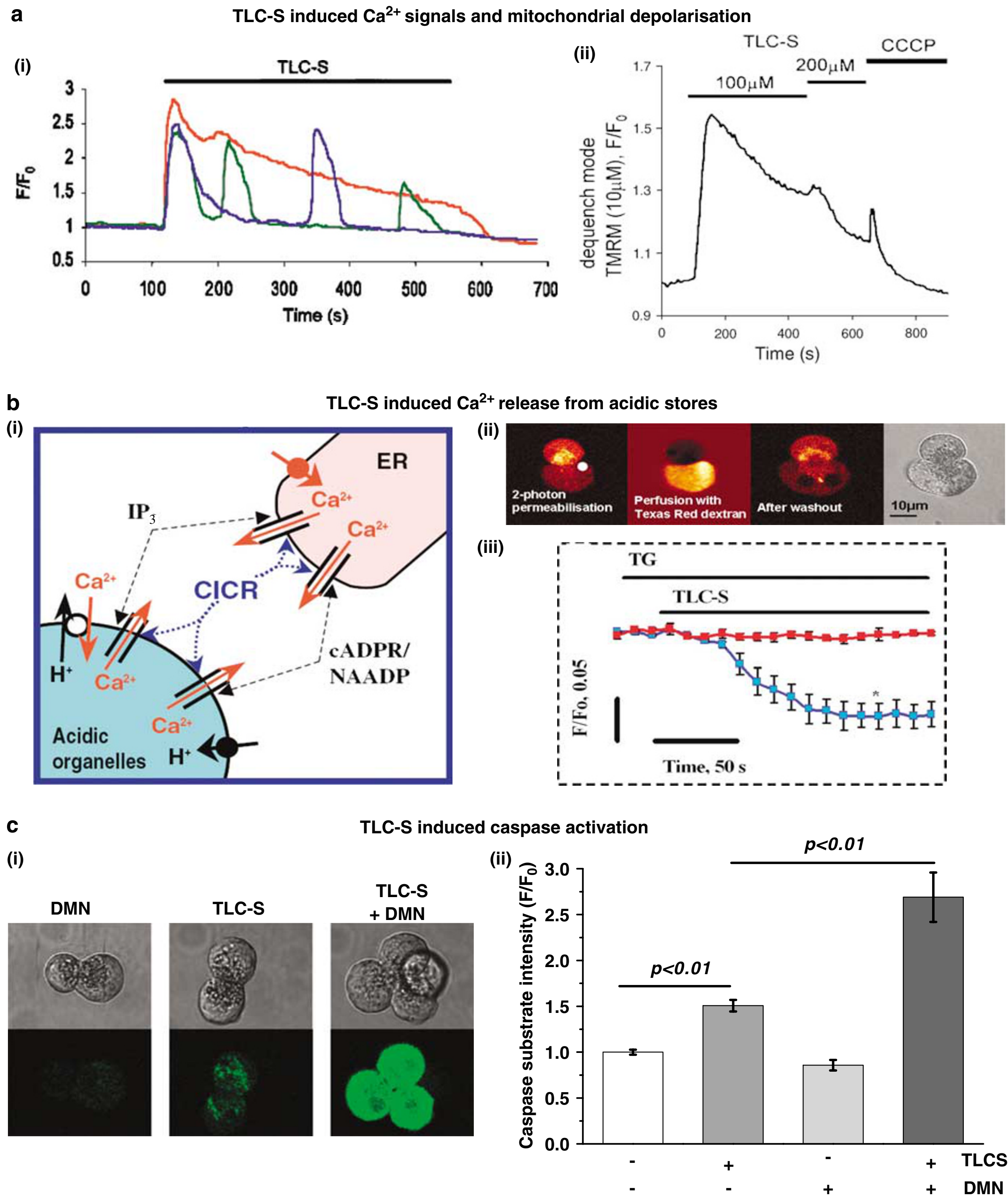

Figure 1 (**a**) TLC-S (100 μM) induces (i) variable patterns of cytosolic Ca^{2+} elevations in three individual pancreatic acinar cells from the same acinar triplet[31] and (ii) partial mitochondrial depolarisation, seen as changes of TMRM fluorescence ('dequench' mode); full depolarisation produced by subsequent application of the protonophore CCCP (10 μM).[32] (**b**) (i) Schematic model of second-messenger interactions with acidic and ER Ca^{2+} stores. IP_3 activates IP_3Rs in both stores, whereas cADPR and NAADP activate RyRs in both stores, but via separate binding sites and/or activation mechanisms.[52] (ii) Images showing the two-photon permeabilisation technique of a doublet of pancreatic acinar cells (loaded with Fluo-5N AM; white dot shows region of permeabilisation by two-photon laser beam, as described by Gerasimenko *et al.*[52]). Note that only the lower cell has been permeabilised and is initially bright, due to diffusion of Texas Red dextran into the cytosol, but becomes paler on washout. (iii) TLC-S (200 μM), added in the presence of thapsigargin (10 μM), induced further additional Ca^{2+} release from an acidic store located in the secretory granule (apical) area (blue), whereas no changes were detected in the basolateral area (red).[56] (**c**) Effects of bile acids on cell fate. (i) Typical light-transmitted and R110-aspartic acid amide fluorescent images and (ii) mean data showing that TLC-S (300 μM) induced caspase activation in pancreatic acinar cells that was greatly potentiated when the detoxifying enzyme NQO1 was inhibited by DMN (30 μM), whereas DMN alone had no effect[36]

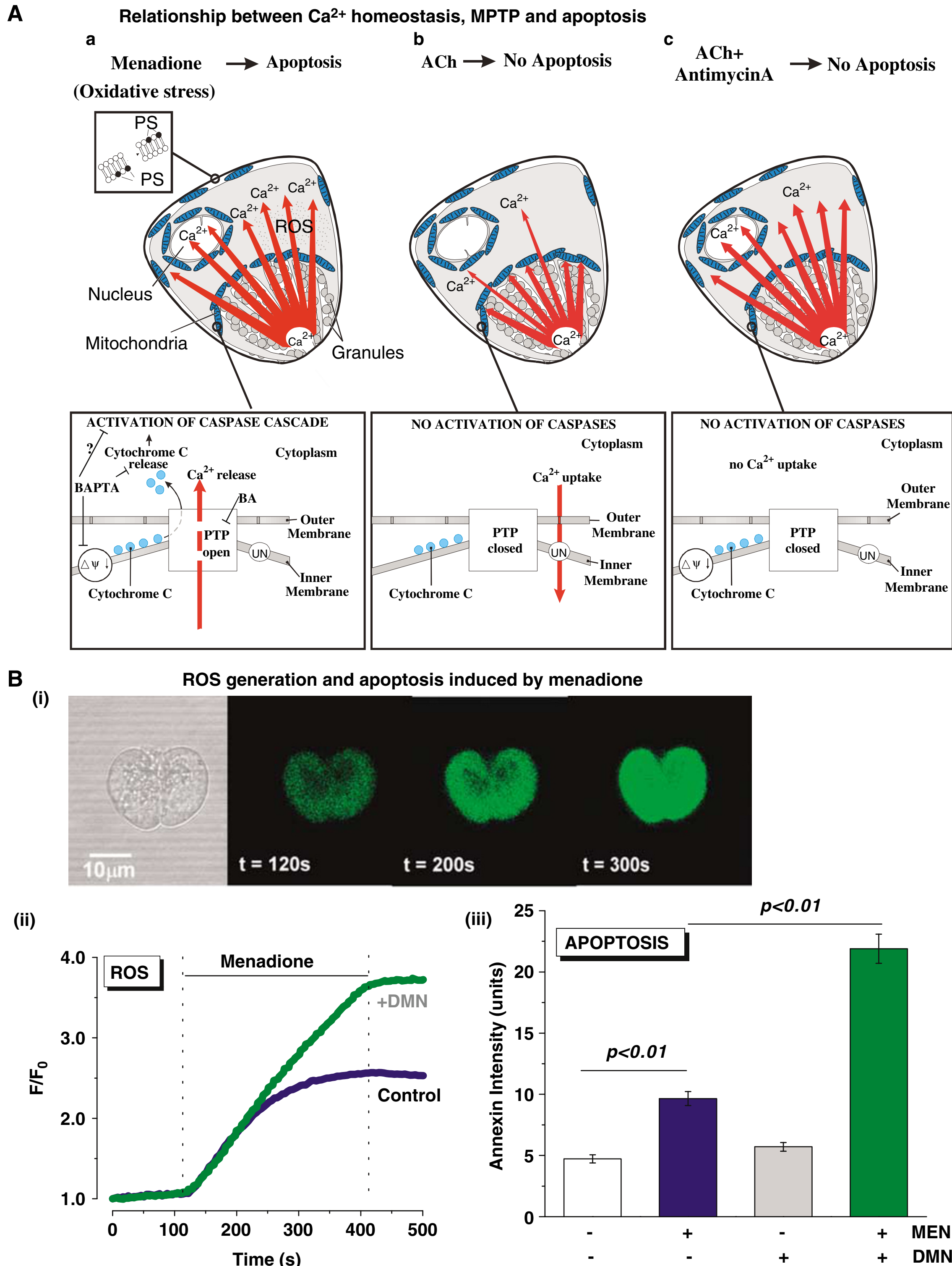

Figure 2 (**A**) Schematic model illustrating the differences in Ca^{2+} homeostasis and induction of the PTP after stimulation with (a) menadione, (b) ACh or (c) stimulation with ACh plus antimycin A. Generation of ROS by menadione may facilitate opening of the mitochondrial PTP and spread of Ca^{2+} waves from the apical to basolateral regions of the cell (BA, bongkrekic acid; PS, phosphatidylserine; ROS, reactive oxygen species; UN, Ca^{2+} uniporter).[10] (**B**) (i) Transmitted light and CM-H_2DCFDA fluorescence images of a doublet of acinar cells, showing ROS generation induced by menadione (30 μM) leads to (iii) apoptosis (measured with annexin V FITC) in pancreatic acinar cells. This effect of menadione on ROS and cell fate was significantly greater when NQO1 was inhibited by DMN, whereas DMN alone did not generate ROS or cause apoptosis *per se*[36]

We have recently demonstrated that generation of ROS is essential for oxidant-induced apoptosis of pancreatic acinar cells.[36] Quinones, such as menadione, enter fast redox cycles within the cell, which consume NAD(P)H and produce ROS. Inhibition of the enzyme NQO1, to prevent menadione detoxification by two-electron reduction, potentiated both ROS generation and consequent apoptosis, while the novel NQO1 inhibitor 4-dimethoxy-2-methylnaphthalene (DMN), a menadione analogue designed not to undergo redox cycling, neither produced ROS nor affected cell fate *per se* (Figures 2b and 3). NQO1 is thought to be an important cellular defence mechanism to counteract electrophile and oxidant damage,[68] possibly by maintaining co-enzyme Q in a reduced, anti-oxidant state.[69] It is overexpressed in acute pancreatitis and many cancers including pancreatic adenocarcinoma,[70] and may be an important early biomarker of disease. A recent study has shown that increased expression of NQO1 reduced ROS generation induced by tert-butyl hydroperoxide and also suppressed tumour necrosis factorα- and interferonγ-induced NO production via iNOS,[71] while menadione-induced toxicity was augmented in NQO1-deficient mice.[72] Since earlier observations have indicated that transient Ca^{2+} signals alone are insufficient to induce opening of the MPTP and apoptosis *per se*,[10] our recent data strongly suggest generation of ROS may constitute an important additional component that promotes acinar cell death. This is in accord with a previous study in hepatocytes, demonstrating MPTP opening via menadione-induced oxidative stress[73] and consistent with a model in which oxidation of MPTP components sensitises Ca^{2+}-dependent pore opening.[74,75]

Cell fate and energetics: the importance of mitochondrial ATP production. Under physiological conditions mitochondria respond to local increases of cytosolic Ca^{2+} by generating ATP via stimulus-metabolism coupling.[13,14,16] However, Ca^{2+} overload can drastically reduce ATP production and this may constitute a vital switch between apoptosis and necrosis that ultimately determines cell fate.[76] It has been suggested that promotion of necrosis through ATP depletion might in part be mediated via an inability of the apoptosome to activate the initiator caspase 9.[18] Recently, an important mechanism whereby alcohol may induce Ca^{2+}-dependent necrotic acinar cell death has been identified.[12] Fatty acid ethyl esters (FAEEs), non-oxidative metabolites of ethanol, are generated at higher concentrations within the pancreas than any other organ,[77–79] and unlike ethanol *per se*, are able to induce experimental pancreatitis *in vivo*.[80] Non-oxidative ethanol metabolites induce persistent, global, cytosolic Ca^{2+} signals in a concentration-dependent manner,[11] initiated via IP_3 receptor-mediated Ca^{2+} release and sustained by depolarisation of mitochondria,[12] the organelle at which FAEE accumulation and hydrolysis to fatty acids (FA) is thought to occur.[81] The consequent mitochondrial impairment leads to a depletion of intracellular ATP, causing run down of the SERCA and plasma membrane Ca^{2+}-dependent pumps and consequent inadequate clearance of raised cytosolic Ca^{2+} (Figure 4).

Interestingly, FAEE-induced mitochondrial impairment in pancreatic acinar cells occurs as a result of the formation of FAs from FAEE hydrolysis, since FAEE esterase inhibition prevents FAEE-induced mitochondrial impairment, allowing ATP to be generated and thus protecting the cell from cytosolic Ca^{2+} overload.[12] This mechanism explains not only how ethanol may induce severe acute pancreatitis through mitochondrial inhibition but also provides a basis for acinar cell

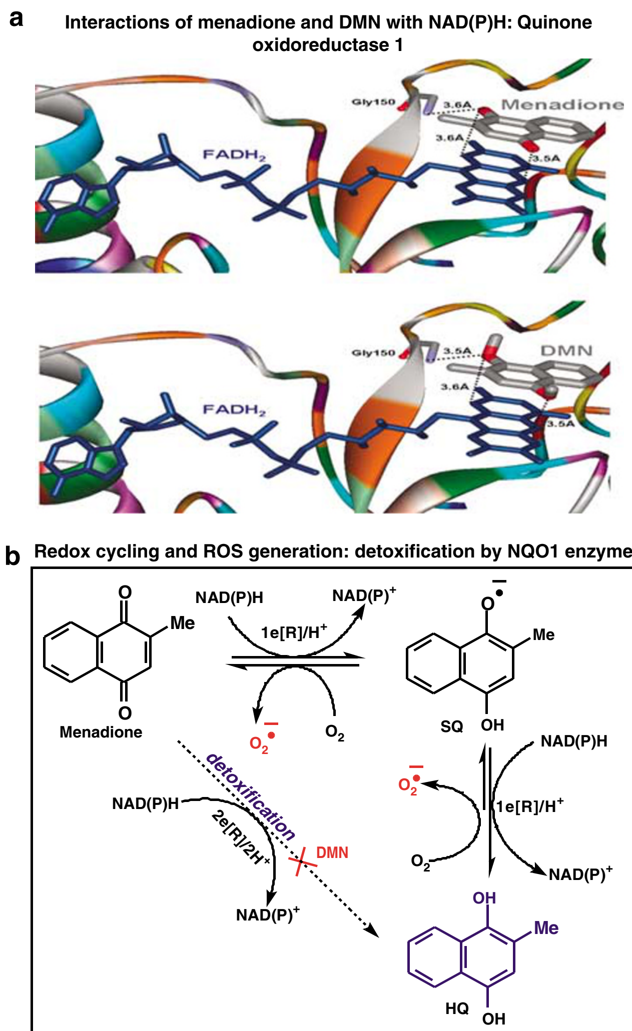

Figure 3 Structural modification of the menadione molecule to prevent redox cycling and inhibit NAD(P)H:Quinone oxidoreductase (NQO1). (**a**) Molecular modelling of putative interactions between NQO1 enzyme and menadione (upper) and DMN (lower) suggests that menadione and DMN are flexibly docked into the active site of NQO1 (in close proximity to the bound $FADH_2$ – blue), utilising the N^1 and N^5 of the $FADH_2$ and the N of Gly-150 for increased interaction. In the case of menadione, the O^1 and O^4 positions of menadione are in close proximity to N^1-$FADH_2$ and N^5-$FADH_2$ (3.6 and 3.5 Å, respectively) enabling electron transfer to occur. However, no electron transfer is feasible for DMN, although it is stabilised by $FADH_2$ (MeO^1-N^1FADH_2, 3.6 Å; MeO^4-N^5FADH_2, 3.5 Å) and Gly-150 (MeO^1-*N*-Gly, 3.5 Å) interactions, and consequently DMN inhibits the effects of menadione at this site.[36] (**b**) Proposed model for the mechanism of action of DMN. Metabolism of menadione by one-electron ($1e/H^+$) reducing enzymes generates an unstable semiquinone radical, with further reduction to the stable hydroquinone; back oxidation generates ROS (O_2^-) when oxygen is present. Menadione may also be metabolised by one-step, two-electron ($2e/2H^+$) reduction via NQO1 directly to the hydroquinone, with no ROS production. Inhibition of NQO1 by DMN causes preferential metabolism of menadione by one-electron reductive processes leading to enhanced ROS generation[36]

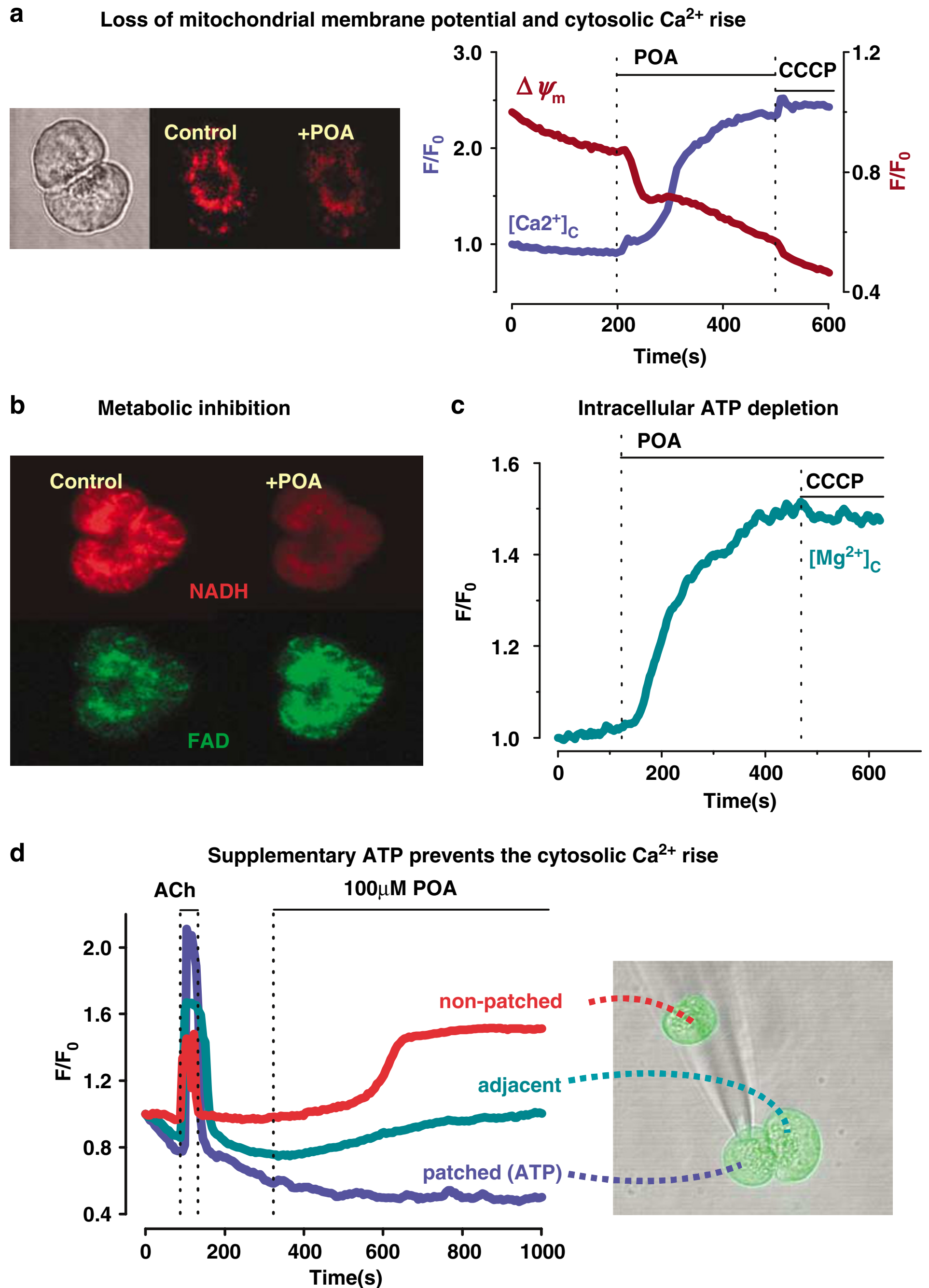

Figure 4 Excess fatty acids, which are formed from the breakdown of non-oxidative ethanol metabolites (FAEEs) in mitochondria,[12,81] induce sustained increases of cytosolic Ca^{2+} and inhibit mitochondrial function. Palmitoleic acid (POA; 50–100 μM) (**a**) depolarised mitochondrial membrane potential (see light-transmitted and TMRM fluorescence images (left), and graph red trace) and elevated cytosolic [Ca^{2+}] (Fluo4 fluorescence blue trace) measured simultaneously in dual-loaded pancreatic acinar cells, (**b**) concomitantly decreased NADH (red) and increased FAD autofluorescence (green) in the perigranular mitochondrial region, (**c**) depleted cellular ATP, seen as an increase in Mg Green fluorescence; subsequent addition of the protonophore CCCP, which depolarises the inner mitochondrial membrane, caused no further change. (**d**) Provision of supplementary ATP to the interior of the cell, via patch-pipette, prevented the rise of cytosolic Ca^{2+} induced by POA in the patched cell (blue), whereas a typical sustained Ca^{2+} response was obtained in a nearby non-patched cell (red) that did not receive ATP[12]

injury in pancreatitis induced by hyperlipidaemia, a recognised risk factor for the disease. In accord, it has previously been demonstrated that infusion of oleic acid, to induce acute pancreatitis *in vivo*, caused dramatic decreases of intracellular ATP,[62] a feature also common to cerulein hyperstimulation.[82] In the cerulein model of pancreatitis, isolated mitochondria exhibit damage, including swelling and disruption of cristae.[83] The importance of a decline of the ATP : ADP ratio in pancreatic acinar cells has also been shown recently from experiments in which energy-dependent

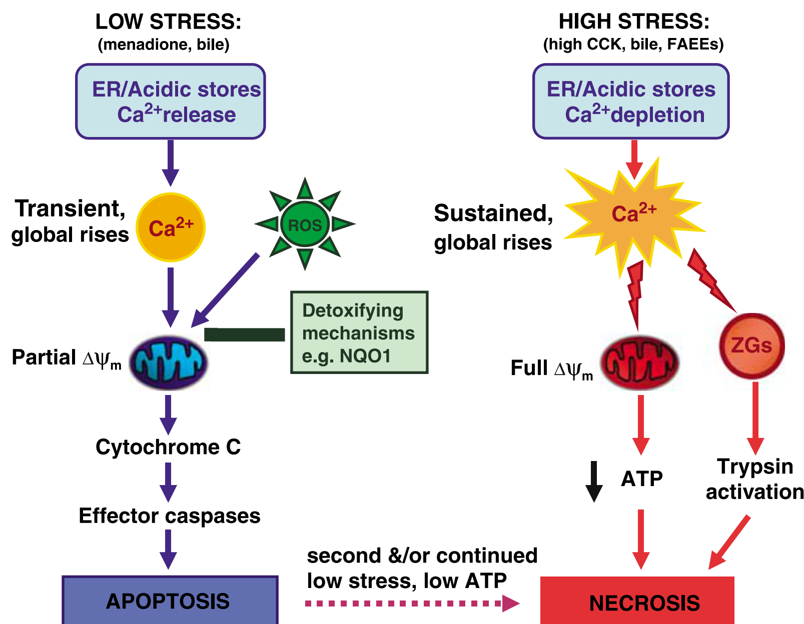

Figure 5 A simplified schematic model illustrating how cytosolic Ca^{2+} signals might influence cell fate in the pancreatic acinar cell. Oscillatory global rises of cytosolic Ca^{2+}, triggered by release of Ca^{2+} from endoplasmic reticulum (ER) and acidic Ca^{2+} stores by moderate stress to the cell, for example, menadione, cause transient mitochondrial depolarisation and promote apoptosis, when additional factors such as ROS generation are present; endogenous detoxifying protective mechanisms such as NQO1 may influence the outcome. More severe insults to the cell, however, cause depletion of the ER/acidic stores, sustained pathological elevations of cytosolic $[Ca^{2+}]$ dependent on extracellular Ca^{2+} entry, which lead to irreversible inhibition of mitochondrial function, cellular ATP depletion and paralysis of energy-dependent Ca^{2+} pumps, and to premature activation of pancreatic digestive enzymes. The net effect of such changes, induced by known precipitants of acute pancreatitis, is necrotic death of the pancreatic acinar cell. It should be noted that potentially contradictory data regarding the effects of bile acids are apparent in the literature. Bile acids induce cytosolic Ca^{2+} signals that are inherently variable[32] and may be associated with apoptosis (especially under conditions of NQO1 inhibition[36]) or apparent necrosis,[30] differences which may relate to the type and combination of bile acids used and/or level of stimulation

necrosis is promoted by endotoxin following chronic alcohol exposure in rats.[84] Furthermore, bile salts induce prolonged, global, cytosolic Ca^{2+} signals[31] that are associated with mitochondrial depolarisation,[32] although this effect on mitochondria appears less pronounced than with non-oxidative alcohol metabolites and may indicate important differences between the toxins. Such mitochondrial inhibition appears to provoke compensatory protective measures in the cell, including an upregulation of mitochondrial ATP synthase, observed after both cerulein hyperstimulation and chronic alcohol exposure.[85] The importance of ATP depletion for pancreatic acinar cell fate is further underscored by experiments in which addition of ATP to the cell interior, administered via a patch pipette, was able to reverse the detrimental Ca^{2+} signals induced by alcohol metabolites. For example, FA-induced sustained cytosolic Ca^{2+} rises, via the release from ER Ca^{2+} stores and subsequent Ca^{2+} entry, were completely abolished in cells receiving supplementary ATP, whereas control cells produced large, sustained elevations of cytosolic Ca^{2+} (Figure 4)[12] that cause cellular necrosis.[11]

Conclusions

It is clear that Ca^{2+} signalling is tightly regulated within subcellular microdomains in the pancreatic acinar cell for normal physiological processes,[24] and evidence suggests that different patterns of cytosolic Ca^{2+} rises influence both apoptotic and necrotic cell death pathways. The balance between these two principal types of cell death might influence the severity of acute pancreatitis; however, whether induction of apoptosis would be beneficial in a clinical setting remains unproven. The current data in pancreatic acinar cells indicate that transient release of Ca^{2+} from the ER and acidic stores, induced by mild stimuli, such as oxidant stress, promotes apoptosis via the intrinsic pathway, when an additional factor, for example, the generation of ROS is present. This action may depend on a partial mitochondrial depolarisation and transient opening of the MPTP, which does not adversely influence ATP production.[41] More severe insults, on the other hand, cause depletion of Ca^{2+} stores with the induction of sustained global Ca^{2+} elevations that inhibit mitochondrial function with a consequent drastic fall of ATP production, paralysing energy-dependent processes such as the plasmalemmal and ER Ca^{2+} pumps, and also prematurely activate digestive enzymes (Figure 5). Interventions that address either inhibition of sustained Ca^{2+} rises or protection of mitochondrial function may prove beneficial in the treatment of acute pancreatitis.

Acknowledgements. We thank Professor A.V. Tepikin for critical reading of this article.

1. Sutton R, Criddle D, Raraty MG, Tepikin A, Neoptolemos JP, Petersen OH. Signal transduction, calcium and acute pancreatitis. *Pancreatology* 2003; **3**: 497–505.

2. Raraty M, Ward J, Erdemli G, Vaillant C, Neoptolemos JP, Sutton R *et al.* Calcium-dependent enzyme activation and vacuole formation in the apical granular region of pancreatic acinar cells. *Proc Natl Acad Sci USA* 2000; **97**: 13126–13131.
3. Kruger B, Albrecht E, Lerch MM. The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis. *Am J Pathol* 2000; **157**: 43–50.
4. Swaroop VS, Chari ST, Clain JE. Severe acute pancreatitis. *JAMA* 2004; **291**: 2865–2868.
5. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P *et al.* Classification of cell death: recommendations of the nomenclature Committee on cell death. *Cell Death Differ* 2005; **12** (Suppl 2): 1463–1467.
6. Melino G, Knight RA, Nicotera P. How many ways to die? How many different models of cell death? *Cell Death Differ* 2005; **12** (Suppl 2): 1457–1462.
7. Orrenius S, Gogvadze V, Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. *Annu Rev Pharmacol Toxicol* 2007; **47**: 143–183.
8. Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. *Trends Biochem Sci* 2007; **32**: 37–43.
9. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol* 2003; **4**: 552–565.
10. 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* 2002; **115**: 485–497.
11. 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 USA* 2004; **101**: 10738–10743.
12. Criddle DN, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP *et al.* Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 2006; **130**: 781–793.
13. Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. Decoding of cytosolic calcium oscillations in the mitochondria. *Cell* 1995; **82**: 415–424.
14. Rizzuto R, Brini M, Murgia M, Pozzan T. Microdomains with high Ca2+ close to IP3-sensitive channels that are sensed by neighboring mitochondria. *Science* 1993; **262**: 744–747.
15. Voronina S, Sukhomlin T, Johnson PR, Erdemli G, Petersen OH, Tepikin A. Correlation of NADH and Ca^{2+} signals in mouse pancreatic acinar cells. *J Physiol* 2002; **539**: 41–52.
16. Petersen OH. Ca^{2+} signalling and Ca^{2+}-activated ion channels in exocrine acinar cells. *Cell Calcium* 2005; **38**: 171–200.
17. Kloppel G, Maillet B. Pathology of acute and chronic pancreatitis. *Pancreas* 1993; **8**: 659–670.
18. Gukovskaya AS, Pandol SJ. Cell death pathways in pancreatitis and pancreatic cancer. *Pancreatology* 2004; **4**: 567–586.
19. Bhatia M. Apoptosis of pancreatic acinar cells in acute pancreatitis: is it good or bad? *J Cell Mol Med* 2004; **8**: 402–409.
20. Bhatia M, Wallig MA, Hofbauer B, Lee HS, Frossard JL, Steer ML *et al.* Induction of apoptosis in pancreatic acinar cells reduces the severity of acute pancreatitis. *Biochem Biophys Res Commun* 1998; **246**: 476–483.
21. Mareninova OA, Sung KF, Hong P, Lugea A, Pandol SJ, Gukovsky I *et al.* Cell death in pancreatitis: caspases protect from necrotizing pancreatitis. *J Biol Chem* 2006; **281**: 3370–3381.
22. Gukovskaya AS, Vaquero E, Zaninovic V, Gorelick FS, Lusis AJ, Brennan ML *et al.* Neutrophils and NADPH oxidase mediate intrapancreatic trypsin activation in murine experimental acute pancreatitis. *Gastroenterology* 2002; **122**: 974–984.
23. Ward JB, Petersen OH, Jenkins SA, Sutton R. Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? *Lancet* 1995; **346**: 1016–1019.
24. Petersen OH, Sutton R, Criddle DN. Failure of calcium microdomain generation and pathological consequences. *Cell Calcium* 2006; **40**: 593–600.
25. Tinel H, Cancela JM, Mogami H, Gerasimenko JV, Gerasimenko OV, Tepikin AV *et al.* Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca(2+) signals. *EMBO J* 1999; **18**: 4999–5008.
26. Dolman NJ, Gerasimenko JV, Gerasimenko OV, Voronina SG, Petersen OH, Tepikin AV. Stable golgi-mitochondria complexes and formation of golgi Ca(2+) gradients in pancreatic acinar cells. *J Biol Chem* 2005; **280**: 15794–15799.
27. Ward JB, Sutton R, Jenkins SA, Petersen OH. Progressive disruption of acinar cell calcium signaling is an early feature of cerulein-induced pancreatitis in mice. *Gastroenterology* 1996; **111**: 481–491.
28. Waterford SD, Kolodecik TR, Thrower EC, Gorelick FS. Vacuolar ATPase regulates zymogen activation in pancreatic acini. *J Biol Chem* 2005; **280**: 5430–5434.
29. Mooren FC, Hlouschek V, Finkes T, Turi S, Weber IA, Singh J *et al.* Early changes in pancreatic acinar cell calcium signaling after pancreatic duct obstruction. *J Biol Chem* 2003; **278**: 9361–9369.
30. Kim JY, Kim KH, Lee JA, Namkung W, Sun AQ, Ananthanarayanan M *et al.* Transporter-mediated bile acid uptake causes Ca^{2+}-dependent cell death in rat pancreatic acinar cells. *Gastroenterology* 2002; **122**: 1941–1953.
31. 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* 2002; **540**: 49–55.
32. 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* 2004; **279**: 27327–27338.
33. Fischer L, Gukovskaya AS, Young SH, Gukovsky I, Lugea A, Buechler P *et al.* Phosphatidylinositol 3-kinase regulates Ca2+ signaling in pancreatic acinar cells through inhibition of sarco(endo)plasmic reticulum Ca2+-ATPase. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G1200–G1212.
34. Singh VP, Saluja AK, Bhagat L, Van Acker GJ, Song AM, Soltoff SP *et al.* Phosphatidylinositol 3-kinase-dependent activation of trypsinogen modulates the severity of acute pancreatitis. *J Clin Invest* 2001; **108**: 1387–1395.
35. Gukovsky I, Cheng JH, Nam KJ, Lee OT, Lugea A, Fischer L *et al.* Phosphatidylinositide 3-kinase gamma regulates key pathologic responses to cholecystokinin in pancreatic acinar cells. *Gastroenterology* 2004; **126**: 554–566.
36. Criddle DN, Gillies S, Baumgartner-Wilson HK, Jaffar M, Chinje EC, Passmore S *et al.* Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. *J Biol Chem* 2006; **281**: 40485–40492.
37. Nicholson DW. Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ* 1999; **6**: 1028–1042.
38. Kagedal K, Zhao M, Svensson I, Brunk UT. Sphingosine-induced apoptosis is dependent on lysosomal proteases. *Biochem J* 2001; **359**: 335–343.
39. Wyllie AH, Morris RG, Smith AL, Dunlop D. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. *J Pathol* 1984; **142**: 67–77.
40. Jiang S, Chow SC, Nicotera P, Orrenius S. Intracellular Ca^{2+} signals activate apoptosis in thymocytes: studies using the Ca(2+)-ATPase inhibitor thapsigargin. *Exp Cell Res* 1994; **212**: 84–92.
41. Halestrap AP. Calcium, mitochondria and reperfusion injury: a pore way to die. *Biochem Soc Trans* 2006; **34**: 232–237.
42. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM *et al.* Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 1999; **397**: 441–446.
43. Schafer ZT, Kornbluth S. The apoptosome: physiological, developmental, and pathological modes of regulation. *Dev Cell* 2006; **10**: 549–561.
44. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP *et al.* The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 2004; **427**: 461–465.
45. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H *et al.* Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 2005; **434**: 652–658.
46. Rizzuto R, Pinton P, Ferrari D, Chami M, Szabadkai G, Magalhaes PJ *et al.* Calcium and apoptosis: facts and hypotheses. *Oncogene* 2003; **22**: 8619–8627.
47. Joseph SK, Hajnoczky G. IP(3) receptors in cell survival and apoptosis: Ca(2+) release and beyond. *Apoptosis* 2007, Feb 6 (E-pub ahead of print).
48. Pinton P, Rizzuto R. Bcl-2 and Ca2+ homeostasis in the endoplasmic reticulum. *Cell Death Differ* 2006; **13**: 1409–1418.
49. Opie EL. The relation of cholelithiasis to disease of the pancreas and to fat necrosis. *Bull Johns Hopkins Hosp* 1901; **12**: 119–121.
50. Senninger N. Bile-induced pancreatitis. *Eur Surg Res* 1992; **24** (Suppl 1): 68–73.
51. Niederau C, Niederau M, Luthen R, Strohmeyer G, Ferrell LD, Grendell JH. Pancreatic exocrine secretion in acute experimental pancreatitis. *Gastroenterology* 1990; **99**: 1120–1127.
52. Gerasimenko JV, Sherwood M, Tepikin AV, Petersen OH, Gerasimenko OV. NAADP, cADPR and IP3 all release Ca2+ from the endoplasmic reticulum and an acidic store in the secretory granule area. *J Cell Sci* 2006; **119**: 226–238.
53. Menteyne A, Burdakov A, Charpentier G, Petersen OH, Cancela JM. Generation of specific Ca(2+) signals from Ca(2+) stores and endocytosis by differential coupling to messengers. *Curr Biol* 2006; **16**: 1931–1937.
54. Gerasimenko JV, Maruyama Y, Yano K, Dolman NJ, Tepikin AV, Petersen OH *et al.* NAADP mobilizes Ca^{2+} from a thapsigargin-sensitive store in the nuclear envelope by activating ryanodine receptors. *J Cell Biol* 2003; **163**: 271–282.
55. 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* 1996; **84**: 473–480.
56. Gerasimenko JV, Flowerdew SE, Voronina SG, Sukhomlin TK, Tepikin AV, Petersen OH *et al.* 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* 2006; **281**: 40154–40163.
57. Petersen OH, Sutton R. Ca(2+) signalling and pancreatitis: effects of alcohol, bile and coffee. *Trends Pharmacol Sci* 2006; **27**: 113–120.
58. Quesada I, Chin WC, Verdugo P. ATP-independent luminal oscillations and release of Ca^{2+} and H^{+} from mast cell secretory granules: implications for signal transduction. *Biophys J* 2003; **85**: 963–970.
59. Nguyen T, Chin WC, Verdugo P. Role of Ca^{2+}/K^{+} ion exchange in intracellular storage and release of Ca^{2+}. *Nature* 1998; **395**: 908–912.
60. Sanfey H, Sarr MG, Bulkley GB, Cameron JL. Oxygen-derived free radicals and acute pancreatitis: a review. *Acta Physiol Scand Suppl* 1986; **548**: 109–118.

61. Reinehr R, Becker S, Keitel V, Eberle A, Grether-Beck S, Haussinger D. Bile salt-induced apoptosis involves NADPH oxidase isoform activation. *Gastroenterology* 2005; **129**: 2009–2031.
62. Nordback IH, Clemens JA, Chacko VP, Olson JL, Cameron JL. Changes in high-energy phosphate metabolism and cell morphology in four models of acute experimental pancreatitis. *Ann Surg* 1991; **213**: 341–349.
63. Altomare E, Grattagliano I, Vendemiale G, Palmieri V, Palasciano G. Acute ethanol administration induces oxidative changes in rat pancreatic tissue. *Gut* 1996; **38**: 742–746.
64. Urunuela A, Sevillano S, de la Mano AM, Manso MA, Orfao A, De DI. Time-course of oxygen free radical production in acinar cells during acute pancreatitis induced by pancreatic duct obstruction. *Biochim Biophys Acta* 2002; **1588**: 159–164.
65. Tsai K, Wang SS, Chen TS, Kong CW, Chang FY, Lee SD *et al.* Oxidative stress: an important phenomenon with pathogenetic significance in the progression of acute pancreatitis. *Gut* 1998; **42**: 850–855.
66. 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* 2001; **20**: 1863–1874.
67. Crompton M. The mitochondrial permeability transition pore and its role in cell death. *Biochem J* 1999; **341** (Part 2): 233–249.
68. Dinkova-Kostova AT, Talalay P. Persuasive evidence that quinone reductase type 1 (DT diaphorase) protects cells against the toxicity of electrophiles and reactive forms of oxygen. *Free Radic Biol Med* 2000; **29**: 231–240.
69. Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D. NAD(P)H:quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. *Chem Biol Interact* 2000; **129**: 77–97.
70. Lyn-Cook BD, Yan-Sanders Y, Moore S, Taylor S, Word B, Hammons GJ. Increased levels of NAD(P)H: quinone oxidoreductase 1 (NQO1) in pancreatic tissues from smokers and pancreatic adenocarcinomas: a potential biomarker of early damage in the pancreas. *Cell Biol Toxicol* 2006; **22**: 73–80.
71. Dinkova-Kostova AT, Liby KT, Stephenson KK, Holtzclaw WD, Gao X, Suh N *et al.* Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc Natl Acad Sci USA* 2005; **102**: 4584–4589.
72. Radjendirane V, Joseph P, Lee YH, Kimura S, Klein-Szanto AJ, Gonzalez FJ *et al.* Disruption of the DT diaphorase (NQO1) gene in mice leads to increased menadione toxicity. *J Biol Chem* 1998; **273**: 7382–7389.
73. Petronilli V, Miotto G, Canton M, Brini M, Colonna R, Bernardi P *et al.* Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcein fluorescence. *Biophysical J* 1999; **76**: 725–734.
74. Chernyak BV, Bernardi P. The mitochondrial permeability transition pore is modulated by oxidative agents through both pyridine nucleotides and glutathione at two separate sites. *Eur J Biochem* 1996; **238**: 623–630.
75. Szalai G, Krishnamurthy R, Hajnoczky G. Apoptosis driven by IP3-linked mitochondrial calcium signals. *EMBO J* 1999; **18**: 6349–6361.
76. Nicotera P, Leist M, Ferrando-May E. Intracellular ATP, a switch in the decision between apoptosis and necrosis. *Toxicol Lett* 1998; **102-103**: 139–142.
77. Laposata EA, Lange LG. Presence of nonoxidative ethanol metabolism in human organs commonly damaged by ethanol abuse. *Science* 1986; **231**: 497–499.
78. Haber PS, Apte MV, Moran C, Applegate TL, Pirola RC, Korsten MA *et al.* Non-oxidative metabolism of ethanol by rat pancreatic acini. *Pancreatology* 2004; **4**: 82–89.
79. Hamamoto T, Yamada S, Hirayama C. Nonoxidative metabolism of ethanol in the pancreas; implication in alcoholic pancreatic damage. *Biochem Pharmacol* 1990; **39**: 241–245.
80. Werner J, Saghir M, Warshaw AL, Lewandrowski KB, Laposata M, Iozzo RV *et al.* Alcoholic pancreatitis in rats: injury from nonoxidative metabolites of ethanol. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G65–G73.
81. Lange LG, Sobel BE. Mitochondrial dysfunction induced by fatty acid ethyl esters, myocardial metabolites of ethanol. *J Clin Invest* 1983; **72**: 724–731.
82. Halangk W, Matthias R, Schild L, Meyer F, Schulz HU, Lippert H. Effect of supramaximal cerulein stimulation on mitochondrial energy metabolism in rat pancreas. *Pancreas* 1998; **16**: 88–95.
83. Schild L, Matthias R, Stanarius A, Wolf G, Augustin W, Halangk W. Induction of permeability transition in pancreatic mitochondria by cerulein in rats. *Mol Cell Biochem* 1999; **195**: 191–197.
84. Fortunato F, Deng X, Gates LK, McClain CJ, Bimmler D, Graf R *et al.* Pancreatic response to endotoxin after chronic alcohol exposure: switch from apoptosis to necrosis? *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G232–G241.
85. Li HS, Zhang JY, Thompson BS, Deng XY, Ford ME, Wood PG *et al.* Rat mitochondrial ATP synthase ATP5G3: cloning and upregulation in pancreas after chronic ethanol feeding. *Physiol Genomics* 2001; **6**: 91–98.