Free radicals and the pancreatic acinar cells: role in physiology and pathology

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Reactive oxygen and nitrogen species (ROS and RNS) play an important role in signal transduction and cell injury processes. Nitric oxide synthase (NOS)—the key enzyme producing nitric oxide (NO)—is found in neuronal structures, vascular endothelium and, possibly, in acinar and ductal epithelial cells in the pancreas. NO is known to regulate cell homeostasis, and its effects on the acinar cells are reviewed here. ROS are implicated in the early events within the acinar cells, leading to the development of acute pancreatitis. The available data on ROS/RNS involvement in the apoptotic and necrotic death of pancreatic acinar cells will be discussed.

Keywords: nitric oxide; reactive oxygen species; reactive nitrogen species; pancreatic acinar cells; pancreatitis

1. INTRODUCTION

Reactive oxygen and nitrogen species (ROS and RNS) are groups of related molecules with individually distinct chemical and biological properties. ROS refers to all oxidation and excitation states of molecular oxygen that arise in physiological conditions, including superoxide, singlet oxygen, hydroxyl radical, hydrogen peroxide, other peroxides and hypohalites. RNS refers to nitric oxide (NO) and its intermediate oxidation/reduction products that arise in physiological settings including nitroxyl, nitrous oxide, nitrosonium, nitric dioxide, and some unstable adducts of NO with biomolecules such as *S*-nitrosothiols and dinitrosyl iron complexes. ROS/RNS are integral parts of intracellular signalling, but become damaging at high levels of production.

Acute pancreatitis (AP) is an often-fatal human disease in which the pancreas digests itself. The disease process is triggered by activation of proteases inside the dominant acinar cells and there is a substantial body of evidence demonstrating that ROS/RNS contribute to the acinar cell damage during the early phases of pancreatitis (Sanfey *et al.* 1984; Tsai *et al.* 1998; Schulz *et al.* 1999). However, if the generation of NO and ROS in physiological and pathological conditions is firmly established at the level of the whole pancreas as well as in many non-acinar cell types, the data from the acinar cells are more ambiguous. Here we try to review the existing information on RNS/ROS production inside pancreatic acinar cells and the downstream effects of these species on the cells.

2. LOCALIZATION OF NITRIC OXIDE SYNTHASE IN PANCREAS

Nitric oxide (NO) is a messenger molecule that is involved in many signalling processes, via the cellular

cyclic guanosine-3',5'-monophosphate (cGMP) pathway and through the nitrosylation of regulatory thiols (Blaise *et al.* 2005). It is produced *de novo* by a family of enzymes named nitric oxide synthase (NOS), of which neuronal and endothelial isoforms (nNOS and eNOS) are expressed constitutively, regulated by calmodulin and requiring a $[Ca^{2+}]_i$ elevation above the basal level. These constitutive NOS isoforms are responsible for regulated low-level NO generation. eNOS can also be activated via phosphorylation by various protein kinases (Fleming & Busse 2003). An inducible isoform (iNOS) is expressed in response to immunological stimuli. iNOS is Ca-insensitive and can produce large amounts of NO as long as it has enough substrate (L-arginine). Mitochondria may contain their own constitutive NOS (Ghafourifar & Richter 1997; Giulivi *et al.* 1998; Lacza *et al.* 2003), although it is not entirely clear whether this isoform is of neuronal, endothelial or a new type. In addition to *de novo* generation from L-arginine by NOS, NO can be recycled back from nitrite, a stable end-product of NO metabolism, by enzymes such as nitrite reductase (Kozlov *et al.* 1999) and xanthine oxidase (Millar *et al.* 1998). Superoxide dismutase and copper-containing enzymes can also produce NO by processing hydroxyurea (Sato *et al.* 1997).

The main sources of constitutive NO generation in the pancreas are neurons in intrapancreatic ganglia, intra- and extrapancreatic nerve endings (Kirchgessner *et al.* 1994) and vascular endothelium (Konturek *et al.* 1993), expressing high levels of neuronal and endothelial type of NOS, respectively. The potential targets of these nitrergic axons are acini, ducts and blood vessels (Kirchgessner *et al.* 1994; Umehara *et al.* 1997). Lower levels of constitutive NOS were found in the islets of Langerhans (Burrell *et al.* 1996; Salehi *et al.* 1996; Umehara *et al.* 1997; Nakada *et al.* 2003). The data on the presence of NOS in the pancreatic acinar and ductal epithelia are ambiguous and species specific. Some investigations show absence of constitutive NOS in pancreatic acini (Worl *et al.* 1994; Umehara *et al.*

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1997; Al Mufti *et al.* 1998; Ember *et al.* 2000), while others report detectable levels of NOS mRNA (Jaworek *et al.* 2000) and protein (Xu *et al.* 1997; Nam *et al.* 1998; DiMagno *et al.* 2004b). The work of DiMagno and collaborators (2004b) shows that 30% of the eNOS in the mouse pancreas is of acinar origin, but this proportion is less (9%) for nNOS (which the authors attributed to possible contamination from other cell types). In contrast, work of Xu *et al.* (1997) points to predominant nNOS expression in rat acinar cells. In the above-mentioned studies, intrapancreatic NOS was detected by means of Western blotting, immunocytochemistry (using nNOS and eNOS selective antibodies) and histochemistry (NADPH diaphorase activity). Apart from this, nuclei in the exocrine pancreas, including acinar cells, showed immunoreactivity against a new eNOS-interacting protein (Konig *et al.* 2002).

NOS presence in the pancreatic acinar cells was also confirmed in functional studies. NOS activity in isolated cell/acini in response to secretagogues was detected using arginine to citrulline conversion (Gukovskaya & Pandol 1994; Wrenn *et al.* 1994; Ahn *et al.* 1998), by evaluating nitrite concentration (Wrenn *et al.* 1994; Ahn *et al.* 1998; Jaworek *et al.* 2000), or cGMP production (Haymovits & Scheele 1976; Gardner & Rottman 1980; Pandol & Schoeffield-Payne 1990; Wrenn *et al.* 1994; Gilon *et al.* 1995; Gukovskaya & Pandol 1995; Xu *et al.* 1997; Yoshida *et al.* 1997; Ahn *et al.* 1998). Leptin receptor (Jaworek *et al.* 2002a) and protease-activated receptor-2 stimulation (Kawabata *et al.* 2002) was also able to generate NO in acinar cells. Additional circumstantial evidence for the ability of pancreatic acinar cells to produce NO during stimulation with agonists came from the observation that NOS blockade can modulate cell-to-cell coupling through gap junctions in isolated acini (Chanson *et al.* 1999).

Thus, there seems to be a substantial body of evidence that pancreatic acinar cells are capable of producing NO by constitutive NOS, though in much lower quantities than many neighbouring cells.

NO lifetime in physiological solutions allows for free diffusion up to several cell size distances from the production site (Beckman & Tsai 1994). Thus, the specificity of NO as a messenger depends on the spatial NO concentration profile determined by NO sources and sinks. In this respect, pancreatic acinar cells appear to be weak NO releasers, while they can be effective free radical sinks/buffers (Niedermaier *et al.* 1996). It is interesting to note that pancreatic beta cells are both NO releasers and NO targets. Beta cells from different islets may synchronize their Ca^{2+} oscillations in response to the ambient glucose concentration, via diffusion of NO (Grapengiesser *et al.* 2001) and carbon monoxide (CO; Lundquist *et al.* 2003) and subsequent activation of soluble guanylate cyclase. In this situation, acinar cells should probably serve as a buffering medium, limiting NO/CO communication between the islets. It is, however, conceivable that these diffusible messengers could also coordinate the activity of beta cells and surrounding acinar cells.

3. NO AND PANCREATIC ACINAR CELL FUNCTION

The major function of the acinar cells in the pancreas is to synthesize and secrete digestive enzymes. The reported effects of NO on the regulation of pancreatic exocrine secretion are controversial. Studies *in vivo* suggest a positive influence of NO upon pancreatic secretion. Inhibition of endogenous NO synthesis decreased stimulated secretion of pancreatic enzymes in rats (Konturek *et al.* 1994; Molero *et al.* 1995; Vaquero *et al.* 1998; Jyotheeswaran *et al.* 2000; Zoucas *et al.* 2001), mice (DiMagno *et al.* 2004a), pigs (Holst *et al.* 1994), humans (Konturek *et al.* 1997), cats (Patel *et al.* 1995) and dogs (Konturek *et al.* 1993). NOS blockade was also shown to suppress basal zymogen secretion in fasting dogs (Maczka *et al.* 1994) and rats (Vaquero *et al.* 1998), although no effect was observed in mice (DiMagno *et al.* 2004a) and fed dogs (Konturek *et al.* 1993). The effects of NOS inhibition on *in vitro* secretion are variable. NOS inhibition was reported to reduce CCK-8-stimulated amylase secretion from isolated rat pancreatic acinar cells (Ahn *et al.* 1998), carbachol-stimulated amylase secretion from isolated rat pancreatic acini (Wrenn *et al.* 1994) and carbachol and CCK-stimulated secretion in guinea-pig acini (Gardner & Rottman 1980), while cGMP analogues caused amylase secretion in guinea-pig pancreatic lobules (Haymovits & Scheele 1976). In contrast, another study reported reduction of electric field-stimulated amylase secretion by the NO donor SNP and 8-Br-cGMP (Yago *et al.* 2002). Other studies showed no effect of NOS inhibition on CCK-8 or carbachol-stimulated or basal secretion in isolated rat pancreatic acini (Konturek *et al.* 1994; Molero *et al.* 1995; Yoshida *et al.* 1997), lobules (Kirchgessner *et al.* 1994) and isolated guinea-pig (Gunther & Jamieson 1979) and canine pancreatic acini (Konturek *et al.* 1993). Given such discrepancy between *in vivo* and *in vitro* data, NO effects on zymogen secretion in the acinar cells should be indirect and mediated (at least partly) by NO production and action at non-acinar cell types. This may include vasculature, nervous, endocrine cells and other NO producers/targets. The effects of NO were shown to be influenced by the site of NO generation, the amount of NO produced, and depended on the specific NOS isoforms involved in NO synthesis (Vaquero *et al.* 1998; Zoucas *et al.* 2001; DiMagno *et al.* 2004a).

The digestive enzyme secretion by acinar cells is controlled by intracellular Ca^{2+} signalling. The relationship between NO and calcium signalling has been studied at the level of isolated acini and cells. The reported effects of NO on $[\text{Ca}^{2+}]_i$ regulation are contradictory: studies from two groups showed that NO has a stimulatory effect on calcium oscillations and calcium influx (Pandol & Schoeffield-Payne 1990; Bahnson *et al.* 1993; Gukovskaya & Pandol 1994; Xu *et al.* 1994, 1997; Chanson *et al.* 1999), while other investigations described little or no effects on Ca^{2+} signals (Gilon *et al.* 1995; Yoshida *et al.* 1997; Yago *et al.* 2002), concluding that the NO/cGMP system is not significantly involved in the mediation of Ca^{2+} signalling. The NO-stimulated Ca^{2+} influx in acinar cells observed by the laboratories of Muallem & Pandol

was dependent on cGMP. This is different from the Ca^{2+} influx regulation by direct S-nitrosylation (independent of cGMP) of the influx channel expressed in HEK-293 cells reported by Gill's group (van Rossum *et al.* 2000). The latter may be a possible NO regulating mechanism of Ca^{2+} entry in parotid acinar cells, which is also cGMP independent (Watson *et al.* 1999). The reports of NO influence on Ca^{2+} homeostasis in pancreatic acinar cells contrast with data from their structural and morphological analogues—acinar cells from salivary glands, which display more complex patterns of NO— Ca^{2+} interplay. In acinar cells isolated from salivary glands, NO was shown to influence Ca^{2+} release from intracellular stores via cGMP by increasing IP3 production and simultaneously affecting ryanodine-sensitive Ca^{2+} stores (Looms *et al.* 2002).

Overall the data on the direct role of NO in fast pancreatic acinar cell reactions such as Ca^{2+} signalling and enzyme secretion are controversial. It was proposed that the homeostatic role of ROS and RNS 'may be to link the behavioural and differentiative commitments of a cell to its metabolic budget' (Nathan 2003). In this respect, the main effects of endogenous NO may be regulation of metabolism and maintenance of pancreatic tissue.

4. NO LINKS TO PANCREATIC ACINAR CELL HOMEOSTASIS AND PATHOLOGY

Endogenous NO may be implicated in regulation of pancreatic acinar cell homeostasis in terms of control of gene expression, protein synthesis, cytoskeletal organization and energy metabolism, growth and apoptosis. NO was shown to exert a tonic inhibition of apoptosis in the pancreas during both basal conditions and growth stimulated by CCK-8, thus regulating the balance between proliferation and apoptosis (Trulsson *et al.* 2002). In rat pancreatic acini, exogenous NO was found to increase tyrosine phosphorylation of the focal adhesion non-receptor tyrosine kinase p125^{FAK} and the cytoskeleton-associated protein paxillin (Garcia-Benito *et al.* 2000), which may both be implicated in cell growth, migration and mitogenesis. In this cell type, p125^{FAK} and paxillin may be important mediators of EGF action (Tapia *et al.* 1999). Another regulatory role of NO comes from its ability, as well as that of some ROS, to stimulate TALK-1 and TALK-2 K^+ channels, which are highly and specifically expressed in pancreatic acinar cells and important in setting the membrane potential (Duprat *et al.* 2005).

Non-selective blockade of NOS generally leads to a decrease in the pancreatic blood flow, increase of pancreatic weight, pancreatic enzyme elevation and pancreatic acinar cell degeneration *in vivo* (Konturek *et al.* 1994; Kolaja *et al.* 2004). However, these pancreatic changes appear to be only transient, suggesting a progressive adaptation to NOS inhibition (Kolaja *et al.* 2004). Administration of high doses of L-arginine causes oxidative and nitrosative stress in the pancreas (Czako *et al.* 1998) and leads to pancreatic tissue damage (Mizunuma *et al.* 1984; Hegyi *et al.* 2004).

Evidence suggests that NO is implicated in certain pathological processes in the pancreas. It was shown that patients with AP have reduced levels of serum L-arginine and L-citrulline (Sandstrom *et al.* 2003). This indicates the potential significance of arginine metabolism and NO in the pathogenesis of this disease. The role of NO produced by either constitutive or inducible NOS in experimental models of AP is controversial. Generally, NO generated by iNOS, which during AP is induced in infiltrating neutrophils and macrophages (Satoh *et al.* 1998) and also in the other cells of pancreatic tissue (Al Mufti *et al.* 1998; Rau *et al.* 2001; Vaquero *et al.* 2001; Qader *et al.* 2003; Um *et al.* 2003), has injurious effects (Lomis *et al.* 1995; Ayub *et al.* 2001; Simsek *et al.* 2001; Cuzzocrea *et al.* 2002; Ozturk *et al.* 2003; Um *et al.* 2003; Chen *et al.* 2004; Isik *et al.* 2004; Sandstrom *et al.* 2005). The inducible form of cyclooxygenase, producing CO in a manner similar to NO production by iNOS, also exacerbates the severity of pancreatitis (Song *et al.* 2002). A beneficial effect of iNOS on the course of pancreatitis was, however, shown in the model of AP caused by coxsackievirus B4 infection (Zaragoza *et al.* 1999; Flodstrom *et al.* 2001) and in one iNOS knockout mice study (Qui *et al.* 2001). The effect of constitutive NOS during AP is complex. First, eNOS, but not nNOS, was shown to be protective in the initiation of caerulein-induced AP in rats (DiMagno *et al.* 2004b). Meanwhile, another study on the same model of AP implied that neuronal secretion of NO may cause significant protection (Dembinski *et al.* 1996). It is also worth noting that, unlike iNOS, constitutive NOS activity becomes progressively depleted during AP (Takacs *et al.* 2002; Qader *et al.* 2003). This may explain adaptation to non-selective NOS inhibition over time (Kolaja *et al.* 2004).

The pancreas is highly susceptible to ischaemic damage. There is increasing evidence that pancreatic ischaemia plays an important role in initiating and aggravating AP. NO appears to reduce pancreatic ischaemia/reperfusion injury (Tanaka *et al.* 1996; Benz *et al.* 2002; Obermaier *et al.* 2004a,b; Yuan *et al.* 2004). However, while the overall protective effect of NO supplementation was firmly established, the effect of NO synthesis inhibition is not always equally clear (Hotter *et al.* 1995; Tanaka *et al.* 1996; Benz *et al.* 2002; Yuan *et al.* 2004). One study suggests that NOS can be induced following ischaemia and reperfusion of the pancreas (Viola *et al.* 2000), contributing to local and systemic damage.

The protective action of NO on pancreatic acinar tissue occurs mainly at the extra-acinar level, as seen from comparison of studies *in vitro* and *in vivo* (Werner *et al.* 1997, 1998; DiMagno *et al.* 2004b), and includes pancreatic blood flow enhancement (Konturek *et al.* 1993; DiMagno *et al.* 2004b; Obermaier *et al.* 2004a) and leukocyte adherence inhibition (Dembinski *et al.* 1996; Obermaier *et al.* 2004b). NO also contributes to prostanoid biosynthesis in the earlier stages of AP (Closa *et al.* 1994b), which may play a role in cellular defence mechanisms (Closa *et al.* 1994c). However, the amelioration of pancreatitis by leptin (Jaworek *et al.* 2002b; Konturek *et al.* 2002; Warzecha *et al.* 2002) and PAR-2 activation (Namkung *et al.* 2004; Sharma *et al.*

Figure 1. (a) Sources of NO in the pancreas and NO effects on the pancreatic acini. (b) Role of ROS/RNS in cell pathology during acute pancreatitis.

2005), which both involve NO (Jaworek *et al.* 2002a; Kawabata *et al.* 2002; Konturek *et al.* 2002), may at least partly result from the primary effects on the acinar cells. The beneficial effects of leptin appear to be dependent on the increase in pancreatic cell growth and the limitation of pro-inflammatory interleukin-1 β synthesis, whereas the PAR-2 effect is possibly mediated by inhibiting ERK1/2 translocation to the nucleus in the acinar cells. NO increases non-apoptotic cell death and can contribute to hypoplasia caused by repetitive injections of high concentrations of CCK-8 (Trulsson *et al.* 2001, 2004). On the other hand, NO suppresses apoptosis and facilitates pancreatic hyperplasia induced by slow infusion of CCK-8 (Andrzejewska *et al.* 2002; Trulsson *et al.* 2002). Some of these effects may be due to universal mechanisms of cell metabolism regulation by NO such as tonic modulation of cell respiration and inhibition of caspases. NO was also reported to inhibit cathepsin B (Stamler *et al.* 1992), which may mediate intrapancreatic trypsinogen activation (Greenbaum *et al.* 1959; Halangk & Lerch 2004) and lead to AP. The stimulatory effect of NO on exocytosis may also exert a beneficial action on the cells, since inhibition of secretion is associated with pancreatic acinar cell pathology (Lerch & Adler 1994; figure 1).

In contrast to the protective action of low levels of NO, excess of NO causes injurious effects by potentiating oxidative stress (Dabrowski & Gabrylewicz 1994; Al Mufti *et al.* 1998).

5. MECHANISMS OF ROS AND RNS INDUCED DAMAGE IN PANCREATIC ACINAR CELLS

There are numerous indications that ROS/RNS play a significant role in various forms of pancreatic pathology including chronic (Acheson *et al.* 1985; Schoenberg *et al.* 1995) and acute pancreatitis (Sanfey *et al.* 1984).

AP is a rapidly developing systemic disease. According to the current view, the primary injury of the acinar cells leads to intracellular trypsinogen activation and inhibition of acinar cell secretion. There are data suggesting that the acinar cell damage is followed within minutes by the production of ROS. The primary free radical production may exacerbate the cell damage, causing lesions of cells membranes and cytoskeleton, impairing functions of intracellular proteins, damaging DNA, evoking lipid peroxidation, decreasing the level of antioxidants and activating NF- κ B. The translocation of NF- κ B into the nucleus results in intra-acinar transcription of chemokines and cytokines. These molecules lead to invasion and activation of inflammatory cells, which produce more ROS/RNS and are responsible for acinar necrosis and amplification of the inflammation in the pancreas.

Oxidative stress was documented in pancreatic tissue by detecting generation of ROS with chemiluminescence (Gough *et al.* 1990) or fluorescence (Urunuela *et al.* 2002) and accumulation of products of ROS-mediated lipid peroxidation (Nonaka *et al.* 1989b, 1990; Guyan *et al.* 1990; Schoenberg *et al.*

1991, 1995; Tsai *et al.* 1998; Rau *et al.* 2000) and protein modification (Reinheckel *et al.* 1998), with concomitant depletion of low molecular weight antioxidants (Neuschwander-Tetri *et al.* 1994; Luthen *et al.* 1995; Tsai *et al.* 1998; Schulz *et al.* 1999; Kruse *et al.* 2001; Rau *et al.* 2001; Rahman *et al.* 2004). It was also confirmed by ESR spectroscopy using spin traps (Nonaka *et al.* 1989a). In one case of human AP, an *in situ* histological study using confocal microscopy showed ROS-derived cerium perhydroxide depositions in pancreatic acini and neighbouring capillaries, indicating strong local oxidative stress (Telek *et al.* 2001).

However, lipid peroxidation may perhaps be a consequence rather than the cause of pancreatic inflammation. Moreover, it was shown that artificial ROS production or intracellular glutathione depletion, leading to oxidative stress similar to that observed in models of AP, could not bring about equal acinar cell damage and necrosis (Fu *et al.* 1997; Rau *et al.* 2000). Thus, ROS/RNS may play a secondary role in the pathogenesis of AP. In addition, ROS-induced lipid peroxidation could not be proven in haemorrhagic necrotizing pancreatitis in humans (Schoenberg *et al.* 1995). Only limited clinical studies have indicated the therapeutic efficacy of antioxidants against pancreatic necrosis (Schulz *et al.* 1999). Nevertheless, evidence from clinical trials suggests the importance of free radicals in the genesis of oedema in AP (Schulz *et al.* 1999). Moreover, the level of ROS production during pancreatitis can be strongly dependent on the experimental type of AP model (Nordback & Cameron 1993).

Among four models of AP in dogs used by Nordback *et al.* (intra-arterial infusion of oleic acid that mimics hyperlipemic pancreatitis, partial obstruction of the pancreatic duct that mimics gallstone pancreatitis, a 2 h period of ischaemia before perfusion that mimics shock pancreatitis, and the infusion of caerulein at supramaximal stimulatory doses, which lacks an obvious clinical counterpart), all but caerulein pancreatitis exhibited the presence of toxic oxygen metabolites, highlighting the importance of ROS in the early pathogenesis of AP (Nordback & Cameron 1993). In that study, an antioxidant, dithiothreitol, ameliorated all except the caerulein form of pancreatitis. ROS scavenger therapy was also found effective in taurocholate- (Gough *et al.* 1990; Schoenberg *et al.* 1991, 1994), caerulein- (Guice *et al.* 1986; Wisner *et al.* 1988; Schoenberg *et al.* 1994) and pancreatic duct obstruction (Sevillano *et al.* 2003) induced AP in rats, as well as in caerulein- and choline-deficient/ethionine-enriched diet induced AP in mice (Nonaka *et al.* 1991, 1992; Cuzzocrea *et al.* 2004). However, in other studies of caerulein and choline-deficient/ethionine-enriched diet models of pancreatitis in rodents, administration of ROS scavengers and suppression of ROS production was unable to reduce the degree of the acinar cell injury (Steer *et al.* 1991; Schulz *et al.* 2001). Antioxidants also inhibited caerulein-induced NF- κ B activation in isolated rat pancreatic acinar cells (Yu *et al.* 2002). Interestingly, the synthetic capacity of the pancreas for the endogenous intracellular antioxidant glutathione is substantially less than that of the kidney or liver (Neuschwander-

Tetri *et al.* 1997), which may explain the susceptibility of pancreatic acinar cells and the pancreas as a whole to oxidative damage.

At the molecular level, the main cellular sources of ROS include the mitochondrial electron transport chain, NADPH oxidase, xanthine oxidase and cytochrome 450 isoenzymes. NADH oxidase is strongly expressed in inflammatory cells and is capable of producing vast amounts of superoxide. Neutrophil NADPH oxidase was found to potentiate caerulein-induced trypsin activation (Gukovskaya *et al.* 2002). This enzyme seems to be the main source of ROS after development of pancreatic inflammation during AP. However, so far, none of its isoforms have been found in pancreatic acinar cells (Gukovskaya *et al.* 2002). Xanthine oxidoreductase enzyme usually (under physiological conditions) functions as xanthine dehydrogenase and uses NAD^+ as electron acceptor. However, under certain circumstances it may reversibly or irreversibly convert to xanthine oxidase, which transfers electrons to molecular oxygen, giving rise to superoxide and hydrogen peroxide. Limited proteolytic cleavage switches the enzyme to oxidase irreversibly, and such conversion in experimental AP models has been reported (Closa *et al.* 1994a). In contrast, reversible conversion of the enzyme via sulphydryl group oxidation was suggested in another study (Nordback & Cameron 1993). Xanthine oxidase was proven to be a source of ROS contributing to both local and systemic effects of AP, studied *in vivo* and *in situ* (Nonaka *et al.* 1989b; Cassone *et al.* 1991; Telek *et al.* 2001). Furthermore, xanthine oxidase has been shown to generate reactive oxygen metabolites in the caerulein-stimulated isolated acinar cells (Suzuki *et al.* 1993). While xanthine oxidase is the main suspected source of primary ROS during early stages of AP, cytochrome P-450 isoenzymes, i.e. phase I xenobiotic-metabolizing enzymes, are incriminated in ROS production in chronic pancreatitis (CP). Induction of cytochrome P-450 was reported during CP (Acheson *et al.* 1985; Foster *et al.* 1993; Wacke *et al.* 1998) as well as in other forms of exocrine pancreatic disease such as AP (Acheson *et al.* 1985) and pancreatic cancer (Acheson *et al.* 1985; Foster *et al.* 1993). The increased oxidant load from long-term cytochrome P-450 induction can eventually lead to depletion of the antioxidant defence in pancreatic acinar cells and to cell injury (Braganza 1998), and may be a significant factor in pathogenesis of CP. This central role of ROS production, by phase I metabolizing enzymes, and glutathione depletion, by ensuing ROS and phase II conjugating enzymes (glutathione S-transferase pi), in the evolution of CP, however, was questioned in several studies (Niedermaier *et al.* 1991; Wallig 1998).

In vitro, exposure of isolated pancreatic acinar cells to oxidative stress caused rapid cell damage and death. Oxidizing agents induce Ca^{2+} oscillations, which occur before other functional and morphological alterations (Thorn *et al.* 1992; Niedermaier *et al.* 1996; Klonowski-Stumpe *et al.* 1997; Gerasimenko *et al.* 2002). These oscillations were mediated by Ca^{2+} release from both thapsigargin sensitive and insensitive intracellular stores (Klonowski-Stumpe *et al.* 1997; Gonzalez *et al.* 2002). Interestingly, ROS-induced Ca^{2+} oscillations

produce no protein secretion (Klonowski-Stumpe *et al.* 1997; Sweiry *et al.* 1999). It is worth noting, however, that xanthine oxidase—used together with the substrate hypoxanthine as a standard ROS production system in many studies *in vitro*—gives rise to Ca^{2+} signals itself in a substrate independent way. The proteinase activity in commercial xanthine oxidase has been reported (Ager *et al.* 1984). Therefore, commercial preparations of xanthine oxidase may activate PAR-2 receptors and, consequently, bring about Ca^{2+} oscillations (Namkung *et al.* 2004), and contribute to some effects attributed to ROS.

Microsomes, lysosomes and mitochondria were found to be the organelles most susceptible to oxidative damage in caerulein-induced AP (Sanchez-Bernal *et al.* 2004). Another study showed that isolated zymogen granules were markedly more sensitive to oxidative stress than intact acinar cells (Niederau *et al.* 1996). Early changes induced by oxidative stress in the acinar cells also included vacuolization and structural alterations of mitochondria, endoplasmic reticulum and nucleus (Han *et al.* 2000). Treatment of the pancreatic AR4-2J cell line with hydrogen peroxide resulted in selective mitochondrial DNA damage with nuclear DNA unaffected (Ehlers *et al.* 1999). In mitochondria, ROS evoked a decrease in the dehydrogenase activity (Weber *et al.* 1998), inner membrane depolarization, opening of the permeability transition pore and cytochrome *c* release, which results in activation of programmed cell death (Ehlers *et al.* 1999; Gerasimenko *et al.* 2002). ROS was shown to affect actin filament polymerization in mouse pancreatic acinar cells, and this effect depended on Ca^{2+} mobilization, but not on protein kinase C. Interestingly, in this study H_2O_2 almost completely inhibited CCK-8-induced amylase release (Rosado *et al.* 2002). The action of ROS on actin filaments might be the mechanism of free radical-induced injury of pancreatic acinar cells (Jungermann *et al.* 1995). A link between ROS and MAPK, JNK/SAPK and p38 MAPK signalling cascades has been established in pancreatic acinar cells (Dabrowski *et al.* 2000). It was found that hydrogen peroxide and menadione strongly activated these pathways in the isolated cells. It has been hypothesized that this activation may be one of the mechanisms of ROS-induced dysregulation of the cytoskeleton (in addition to the more direct ROS-induced disruption of the actin network; Dabrowski *et al.* 2000; Dabrowski 2003).

NO overproduction by iNOS in the pancreas is implicated in the exacerbation of cell injury during AP (Lomis *et al.* 1995; Viola *et al.* 2000; Ayub *et al.* 2001; Simsek *et al.* 2001; Cuzzocrea *et al.* 2002; Ozturk *et al.* 2003; Um *et al.* 2003; Chen *et al.* 2004; Isik *et al.* 2004; Sandstrom *et al.* 2005). One possible mechanism of NO-mediated damage is potentiation of oxidative stress by NO. First, inhibition of cell respiration by NO may favour generation of superoxide (Moncada & Erusalimsky 2002). Second, peroxynitrite, the product of the reaction of NO with superoxide anion, is a more aggressive radical than superoxide or NO and can participate in some specific cell-damaging chemical reactions such as irreversible tyrosine nitration (Ischiropoulos 2003). Peroxynitrite may also be

produced inside the mitochondria as a result of superoxide generation by the mitochondrial electron transport chain and NO production by mitochondrial NOS activated by mitochondrial Ca^{2+} uptake (Bringold *et al.* 2000). Peroxynitrite formed inside mitochondria may cause cytochrome *c* release (Ghaffourifar *et al.* 1999) and lead to cell apoptosis.

6. CONCLUSIONS

Mounting evidence suggests that NO may participate in the regulation of pancreatic acinar cell homeostasis at the level of energy supply, Ca^{2+} signalling, enzyme secretion, cell growth and death. This regulation occurs partly at the intra-acinar level. Pancreatic acinar cells appear to possess some relatively weak NOS activity, but many other cell types of the pancreas were shown to be potent NO releasers. The acinar cells probably serve as both targets and scavengers of free radicals (both RNS and ROS). Free radicals may play an important role in the pathogenesis of AP and CP and may mediate cell injury following pancreatic ischaemia/reperfusion. The importance of ROS/RNS as mediators of cell damage strongly depends on the experimental model of pancreatitis. In several models of AP, ROS/RNS were shown to be involved in the amplification of local and systemic inflammation. The source of free radicals can be external to the acinar cells; nevertheless, some studies also suggest that acinar cells, when exposed to noxious stimuli, may produce ROS themselves. Further research in this field may yield important information about the general mechanisms of regulation and pancreatic pathology.

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