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REGULAR ARTICLE

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mRNA for pancreatic uncoupling protein 2 increases in two models of acute experimental pancreatitis in rats and mice

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Abstract Uncoupling-protein 2 (UCP2) is a mitochondrial protein that appears to be involved in cellular oxidant defense and in the regulation of oncotic cell death, both of which are important features of acute pancreatitis. However, UCP2 expression in acute pancreatitis has not been previously reported. In the current experiments, pancreatic gene expression was studied by real-time reverse-transcription/polymerase chain reaction and Northern blots. Two models of acute experimental pancreatitis were investigated: cerulein-induced pancreatitis in mice at two different time points and taurocholate-induced pancreatitis in rats at two degrees of severity. After cerulein administration, acinar injury and leukocyte infiltration was significantly higher at 24 h compared with 12 h after the first injection of cerulein ($P<0.05$, $P<0.005$, respectively). UCP2 mRNA was unchanged at 12 h but was nearly 12-fold greater than control levels after 24 h ($P<0.001$). UCP2 gene expression correlated with acinar injury ($r=0.69$; $P<0.001$). By 72 h after taurocholate administration, the severe group had more necrosis than the mild group ($P<0.005$). Pancreatic UCP2 mRNA was increased fourfold in the severe group compared with controls ($P<0.01$). UCP2 expression correlated with parenchymal necrosis ($r=0.61$; $P<0.01$). Thus, pancreatic UCP2 mRNA increased in two models of acute pancreatitis. The increase in UCP2 gene expression was correlated with the severity of the disease. Up-regulation of UCP2 in the pancreas may be a protective response to oxidative stress, but this increase may also have a negative influence on cellular energy metabolism. Therefore, acinar UCP2 may be an important modifier of the severity of acute pancreatitis.

Keywords Acute pancreatitis · Oxidative stress · UCP2 · Uncoupling proteins · Mouse (female C57/B16) · Rat (male Zucker)

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Introduction

Acute pancreatitis is characterized by various degrees of acinar cell injury and by inflammation. Several experimental studies suggest that the extent of acinar cell injury is influenced by the inflammatory infiltration of activated leukocytes (Frossard et al. 1999; Poch et al. 1999). The ability of these cells to damage tissues in vivo depends on two events: the release of lytic enzymes and the formation of reactive oxygen species (ROS; Weiss 1989). The major source of excessive pancreatic ROS production in acute pancreatitis is infiltrating leukocytes (Telek et al. 1999; Yasar et al. 2002). Several lines of evidence suggest that ROS play an important role in the pathophysiology of acute pancreatitis. Accordingly, treatment with oxidant scavengers decreases pancreatic tissue injury in experimental pancreatitis (Demols et al. 2000; Poch et al. 1999; Schoenberg et al. 1994).

Uncoupling-protein 2 (UCP2) is a mitochondrial protein that is located on the inner mitochondrial membrane (Ricquier and Bouillaud 2000). UCP2 transcripts are expressed at various levels in a number of tissues, including

pancreas (Fleury et al. 1997; Gimeno et al. 1997). UCP2 increases re-entry of protons into the mitochondrial matrix (Krauss et al. 2002), thereby reducing the half-life of respiratory reactive intermediates capable of reducing O_2 to O_2^- (Skulachev 1998). Therefore, UCP2 has been proposed to help regulate the intracellular redox state and provides protection against ROS, a hypothesis that has been supported by studies in UCP2$^{-/-}$ mice (Arsenijevic et al. 2000; Negre-Salvayre et al. 1997). However, a consequence of the up-regulation of UCP2 is that heat is produced instead of ATP, cellular energy metabolites are depleted, and cell viability is compromised (Chavin et al. 1999). Indeed, Mills et al. 2002 have recently suggested that UCP2 is involved in the regulation of cellular oncosis, a form of cell death that is (1) often confused with necrosis, (2) induced by energy depletion, and (3) initially characterized by cellular swelling; they have found that increased expression of UCP2 promotes oncotic cell death and inhibits apoptosis (Mills et al. 2002). This could have implications during acute pancreatitis, because the severity of the disease may be a function of the relationship between apoptotic and oncotic cell death (Andersson and Wang 1998; Kaiser et al. 1995).

Although UCP2 may play a role in pancreatitis, UCP2 gene expression in acute pancreatitis has not been previously reported. The aim of the current study has been to investigate pancreatic UCP2 gene expression and its possible correlation with the severity of pancreatitis in two different well-established models of acute necrotizing pancreatitis: the cerulein hyper-stimulation model in mice and the taurocholate model in rats. In the mouse model, all animals receive the same supraphysiological dose of cerulein. The severity of pancreatitis has been shown to increase over time in this model and usually reaches its maximum by 24 h (Frossard et al. 2002; Halangk et al. 2000). We have measured UCP2 gene expression at 12 h and 24 h, time points by which significant tissue injury has been shown to occur. In the rat model, two different doses of taurocholate have been used to induce pancreatitis at two degrees of severity. UCP2 mRNA has been investigated at 72 h after treatment, a time point at which the necrotizing process has been shown to be most severe (Kruse et al. 1999). Untreated rats have been used as controls. We have also measured UCP2 mRNA in acini isolated from normal rat pancreas in order to determine whether UCP2 is expressed in that cell type.

Materials and methods

Animals and study design

All animals were kept at 21°C on a 12-h dark–light cycle with free access to water and standard chow for at least 1 week before the experiments. After the induction of pancreatitis, all animals received analgesia with buprenorphine (0.03 mg/kg s.c.) every 12 h as recommended by the Swedish National Board for Laboratory Animals. Animals were killed at the time points indicated below. The splenic part of pancreas was excised and immediately frozen in liquid nitrogen before being stored at −80°C for subsequent assays. The head of the pancreas was removed for histological examination. The research protocols (nos. S138-97, S29-03 and S29-04) were approved by the Local Animal Ethics Committee at the Karolinska Institutet, Stockholm, Sweden.

Experimental pancreatitis

Female C57/B16 mice (Scanbur-BK, Sollentuna, Sweden; mean weight: about 20 g) were injected i.p. hourly with either 50 μg/kg cerulein (n=22) or 0.9% saline (n=18) for 11 h; 13 cerulein-treated and 9 saline-injected mice were killed by cervical dislocation 12 h after the first injection. The remaining mice were killed 24 h after the first injection.

Necrotizing pancreatitis was induced in male Zucker rats (Charles River, Uppsala, Sweden; mean weight: 499± 23 g) by retrograde infusion of sodium taurocholate (Sigma, St Louis, Mo., USA) into the pancreatic duct as previously described (Segersvard et al. 2004). Rats in the mild acute necrotizing pancreatitis group (n=6) received 0.2 ml 3% taurocholate, whereas those in the severe acute necrotizing pancreatitis group (n=9) were given 0.4 ml 3.5% taurocholate. Control rats (n=9) were left untreated. At 72 h after the infusion, all animals were anesthetized and killed by exsanguination.

Histology

After removal, the pancreata were fixed in 4% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. An investigator who was blinded to the nature of the intervention assessed the pancreata. In the cerulein experiment, the amount of acinar cell necrosis and vacuolization was scored between 0 (normal) and 3 (severe) respectively, as previously described (Ethridge et al. 2002). The sum of these scores represented the degree of acinar injury. In the taurocholate rat experiment, inflammatory infiltration, fat necrosis, and parenchymal necrosis were scored according to Spormann et al. (1989).

Inflammatory infiltration assessment

Mouse pancreatic tissue samples (~70 mg) were ground and homogenized in ice-cold 20 mM phosphate-buffered saline (PBS) and then centrifuged. The resultant pellet was dissolved in 0.5% hexadecyltrimethylammonium bromide (HTAB) in 50 mM PBS, pH 6.0. The sample was sonicated and passed through 3 cycles of freezing and thawing before incubation for 2 h at 60°C. After centrifugation at 10,000g for 10 min, the supernatant was collected, and the protein concentration was measured. Samples of 30 μg protein from the supernatant were aliquoted in triplicate in a 96-well plate; subsequently, 100 μl 3,3′,5,5′-tetramethylbenzidine substrate (Vector laboratories, Burlingame, Calif., USA) was added, and the reaction was allowed to run for

exactly 3 min before being stopped with 100 µl 1 N sulphuric acid. Absorbance was read at 450 nm. Myeloperoxidase (MPO) activity was expressed as absorbance/min per milligram protein. In order to be able to compare our data with previous publications, we chose to use histology, the established way of assessing pancreatic inflammation in the rat model at the 72 h time point (Aho et al. 1980; Kruse et al. 1999).

Acinar isolation

Acini were isolated from normal rat pancreas by collagenase digestion as previously described (Adrian et al. 1990). Briefly, male Wistar rats (*n*=2) were anesthetized, and the pancreatic duct was cannulated. Then 3 ml ice-cold HEPES-buffered medium (pH 7.4) was injected slowly into the bilio-pancreatic duct. The medium contained 33 U/ml collagenase (CLSP grade, Worthington Biochemical, USA), 25 mM HEPES, 103 mM NaCl, 4.7 mM KCl, 0.6 mM $MgCl_2$, 1 mM NaH_2PO_4, 0.5 mM $CaCl_2$, 11 mM dextrose, 5.0 mM sodium pyruvate, 2.0 mM sodium glutamate, 5 mM sodium fumarate, minimal essential medium (2% v/v), bovine serum albumin (BSA; 0.2% w/v), and trypsin inhibitor (0.01% w/v). The gland was removed and placed in the same medium in a shaking incubator at 37°C for three 10-min periods under 100% oxygen. Gentle mechanical disruption was performed between incubations. The acinar suspension was then filtered through a 250-m nylon mesh and purified by being centrifuged three times (3 min each time) at 50 g through a 4% BSA-HEPES buffer gradient.

Quantitative real-time reverse-transcription/polymerase chain reaction

Tissue samples were ground in liquid nitrogen, and total RNA extracted and treated with DNAse (RNAqueous-4PCR; Ambion, Austin, Tex., USA). Total RNA was also extracted from samples from a normal rat lung and two normal rat pancreata and from isolated acini. The integrity of RNA was assessed by gel electrophoresis. Total RNA (1 µg) was reverse-transcribed by using a mixture of 0.5 µg oligodT and 400 pM 18S-specific primers. The resulting cDNA (20 ng) was subjected to multiplex real-time polymerase chain reaction (PCR) by using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, Calif.). Real-time amplification was run for 2 min at 50°C and 10 min at 95°C and then cycled for a total of 40 cycles (15 s at 95°C followed by 1 min at 60°C). The reaction mixture for the measurement of mouse UCP2 mRNA consisted of 2× TaqMan master mix, nuclease-free water, 200 nM 18S probe, 100 nM forward and reverse 18S primers, and 20× mouse UCP2 Assay-on-demand primer/probe mix. Total RNA from the rat pancreas and acinar cells was reverse-transcribed by using 200 nM UCP2 reverse primer and 400 pM 18S primers. The subsequent PCR employed 100 nM primers and 200 nM probe for both UCP2 mRNA and 18S.

Northern blots

Total RNA was extracted according to Chomczynski and Sacchi (1987). RNA (20 µg) was separated on a 1% agarose gel containing 2% formaldehyde and transferred to a nylon membrane (Zeta-Probe, Bio-Rad, Calif.). The UCP2 probe was labeled with [−32P] deoxycytidine triphosphate (Amersham, Pharmacia Biotech, UK) by using a nick translation kit (Roche Diagnostics, Mannheim, Germany). The 18S probe was end-labeled by using T4 polynucleotide kinase (Life Technologies, Täby, Sweden) and [−32P] ATP. Hybridization with the UCP2 probe was carried out overnight at 65°C in 250 mM sodium phosphate buffer (pH 6.8) containing 1 mM EDTA and 1% BSA. The filters were washed in 2× saline sodium citrate (SSC; 300 mM NaCl and 30 mM sodium citrate) for 10 min and then in 1× SSC for 25 min at the hybridization temperature. The blots were then stripped twice in 0.1× SSC and 0.5% SDS at 95°C for 30 min. Rehybridization with the 18S probe was carried out overnight at 37°C in the same buffer. The intensity of the bound probes was analyzed with a phosphoimager (Fujix, Bas 2000). Two of the mild pancreatitis samples and three of the severe pancreatitis samples were excluded because of degraded RNA.

Mitochondrial isolation and Western blot analysis

Mitochondrial isolations were performed as described previously (Pecqueur et al. 2001). Briefly, tissue samples were homogenized in TES buffer (10 mM TRIS-HCl, pH 7.5, 1 mM EDTA, 250 mM sucrose) supplemented with protease inhibitor cocktail (P8340, Sigma-Aldrich Sweden, Stockholm, Sweden). The homogenate was centrifuged at 750*g* for 10 min to remove unbroken cells and nuclei. The supernatant was subsequently centrifuged at 10,000*g* for 20 min to pellet the mitochondria. The pellet was resuspended in 1 ml TES buffer, and the centrifugal procedure was repeated. Finally, the mitochondrial pellet was suspended in protein lysis buffer (50 mM TRIS-HCl, 2% SDS, 10% glycerol), and the protein concentration was determined. Normal mouse spleen mitochondria were also isolated as described above for use as a positive control. Samples of 60 µg pancreatic and 30 µg spleen mitochondrial proteins were separated by SDS-polyacrylamide gel electrophoresis (12.5%) and then transferred onto an Immobilon-P polyvinyldienefluoride membrane (Millipore, Sundbyberg, Sweden). The membrane was blocked by drying at 37°C according to the manufacturer's instructions and then incubated overnight at 4°C with the C-terminal anti-UCP-2 goat polyclonal antibody (sc-6525; Santa Cruz Biotechnology, Santa Cruz, Calif., USA) diluted 1:500 (v/v) in 2% non-fat dry milk in TBS-T (0.1% Tween 20 in 10 mM TRIS-buffered saline, pH 7.4). The specificity of this antibody has been confirmed in UCP2

knockout mice (Horvath et al. 2003; Zhang et al. 2001). Duplicate samples were incubated with the primary antibody immuno-neutralized with 1:10 (v/v) blocking peptide (sc-6525P; Santa Cruz Biotechnology). After being washed, the membrane was incubated for 1 h at room temperature with horseradish-peroxidase-conjugated rabbit anti-goat antibody (Chemicon International, Temecula, Calif., USA) diluted 1:1000 (v/v). The membrane was washed, and immunoreactive bands were visualized with an enhanced chemiluminescence detection system (Santa Cruz Biotechnology).

Primers and probes

The UCP2 and 18S probes for Northern blot were as described previously (Rippe et al. 2000). All primers and probes and the 2× TaqMan master mix for real-time PCR were purchased from Applied Biosystems. Mouse UCP2 primers and probe were custom-made (assay ID: Mm00 495907_g1). The rat UCP2 primers and probe were intron-spanning (forward: 5′-AGC ACT GTC GAA GCC TAC AAG AC-3′; reverse: 5′-TGG CAT TTC GGG CAA CAT-3′; probe: FAM 5′-TTG CAC GAG AGG AAG GGA TCC GG-3′ TAMRA). The 18S primers and probe were part of TaqMan*reg* Ribosomal RNA Control Reagents. UCP2 was compared with 18S; 18S was used as the internal control because is unaffected by acute experimental pancreatitis, whereas both pancreatic β-actin and GAPDH gene expression increase during pancreatitis (Calvo et al. 1997; Iovanna et al. 1991; Yuan et al. 1999).

Statistics

Data are expressed as means ± SEM. Histological data were analyzed by Wilcoxon/Kruskal Wallis tests. Correlations were analyzed non-parametrically by Spearman's Rho test. UCP2 mRNA and MPO data were analyzed by one-way analysis of variance, followed by the Tukey-Kramer post-test for multiple comparisons. $P<0.05$ was considered statistically significant.

Results

Cerulein-induced pancreatitis

All cerulein-treated mice developed acute pancreatitis, with signs of inflammatory infiltration, edema, acinar cell necrosis, and vacuolization. No abnormal pancreatic morphology was seen in any control mouse. The acinar injury was significantly higher at 24 h compared with 12 h (3.8±0.4 vs. 2.8±0.2; $P<0.05$). As shown in Fig. 1, pancreatic MPO activity increased 8-fold at 12 h and 16-fold at 24 h in the cerulein-injected mice compared with the control mice at these time points ($P<0.005$, $P<0.001$, respectively). MPO activity in the cerulein-injected mice was significantly greater at 24 h than at 12 h ($P<0.005$). The levels of UCP2 mRNA in the cerulein-treated mice were not significantly different from those in the saline-injected mice 12 h after the first injection. However, at the 24-h time point, UCP2 mRNA values were nearly 11.5-fold greater than those in the control mice ($P<0.001$; Fig. 2). A significant correlation between UCP2 mRNA expression and acinar injury was observed ($r=0.69$; $P<0.001$).

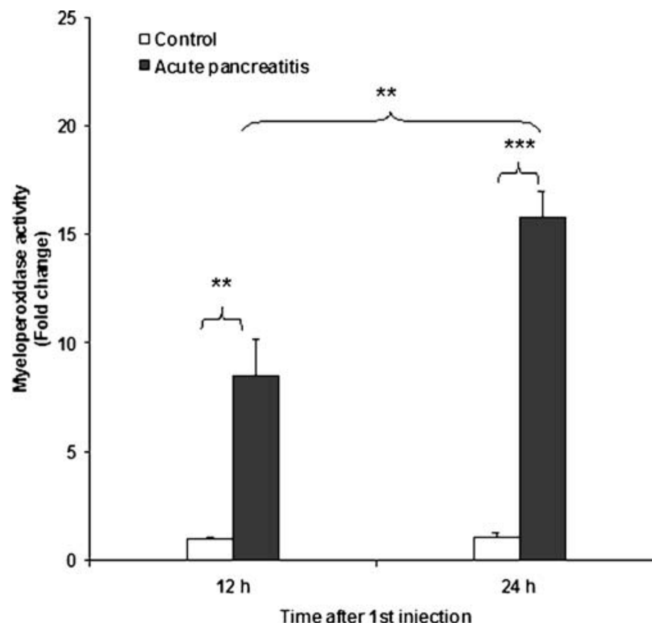

Fig. 1 Pancreatic myeloperoxidase (MPO) activity in mice 12 h and 24 h after the first injection of cerulein ($n_{12\ h}$=10; $n_{24\ h}$=6) or saline ($n_{12\ h}$=6; $n_{24\ h}$=6). Values are means ± SEM. $^{**}P<0.005$, $^{***}P<0.001$

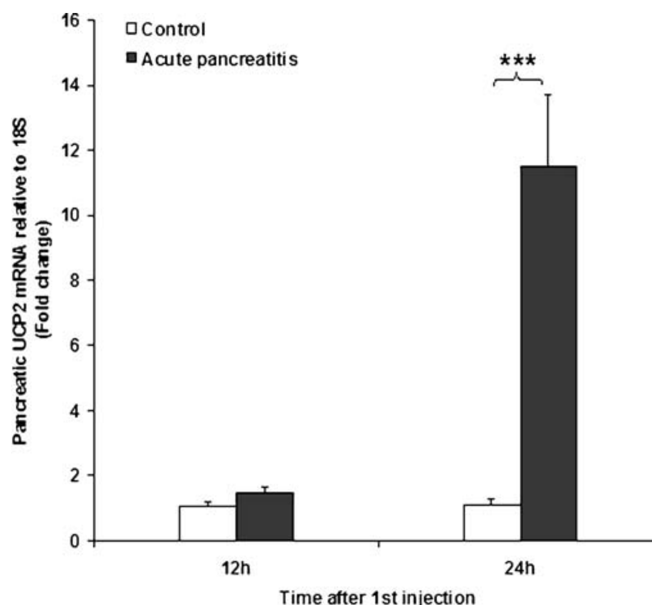

Fig. 2 Pancreatic UCP2 gene expression in mice with and without cerulein-induced acute pancreatitis. Fold change in relative UCP2 gene expression at 12 h and 24 h after the first injection of cerulein ($n_{12\ h}$=10; $n_{24\ h}$=6) or saline ($n_{12\ h}$=6; $n_{24\ h}$=6). Values are means ± SEM. $^{***}P<0.001$

Table 1 Pancreatic morphology in rats 72 h after taurocholate infusion. Data are means ± SEM

Histological abnormalities	Maximum possible score	Mild group (n=6)	Severe group (n=9)
Inflammatory infiltration	3	1.7±0.2	2.6±0.2
Parenchymal necrosis	7	3.7±0.4	6.3±0.3**
Fat necrosis	7	4.0±0.5	5.9±0.4*
Total score	17	9.3±1.1	14.8±0.8**

*P<0.05

**P<0.005 versus the mild group

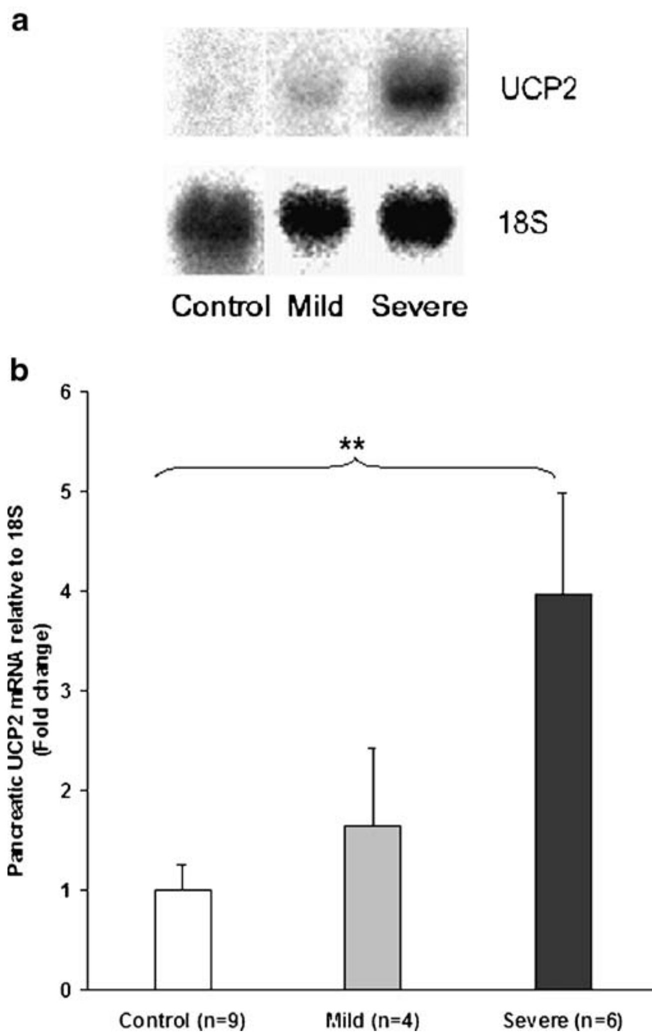

Fig. 3 Pancreatic UCP2 gene expression in rats with either mild (n=4) or severe (n=6) acute necrotizing pancreatitis. Normal rat pancreata were used as controls (n=9). **a** Representative Northern blot. **b** Fold change in relative UCP2 mRNA levels after sodium taurocholate infusion in mild and severe groups compared with control levels. Values are means ± SEM. **P<0.01

Taurocholate-induced pancreatitis

The severe pancreatitis group had more profound parenchymal necrosis and fat necrosis than the mild pancreatitis rats as shown in Table 1 (P<0.005, P<0.05, respectively vs. mild pancreatitis). No abnormal pancreatic morphology was seen in any control rat. Both the mild and severe pancreatitis groups had marked inflammatory infiltration (both P<0.001 vs. controls). A low basal level of pancreatic UCP2 mRNA expression was seen in the control animals (Fig. 3). At 72 h after taurocholate infusion, UCP2 mRNA levels in the mild pancreatitis animals were not significantly different from control values, but UCP2 mRNA in the severe pancreatitis group was 4-fold higher than in the control rats (P<0.01; Fig. 3). A significant correlation between UCP2 mRNA expression and parenchymal necrosis was observed (r=0.61; P<0.01). UCP2 transcripts were detected in the acini isolated from normal rat pancreas. The levels were about fourfold lower than in tissue from a normal whole pancreas and about 130-fold lower than those in the normal rat lung sample used as a positive control (data not shown).

Western blot

A strong 32-kDa UCP2 protein band was detected in mouse spleen mitochondria. In the pancreas, multiple bands of various molecular sizes were seen. The splenic band, but none of the pancreatic bands, was eliminated by preabsorption with blocking peptide (Fig. 4).

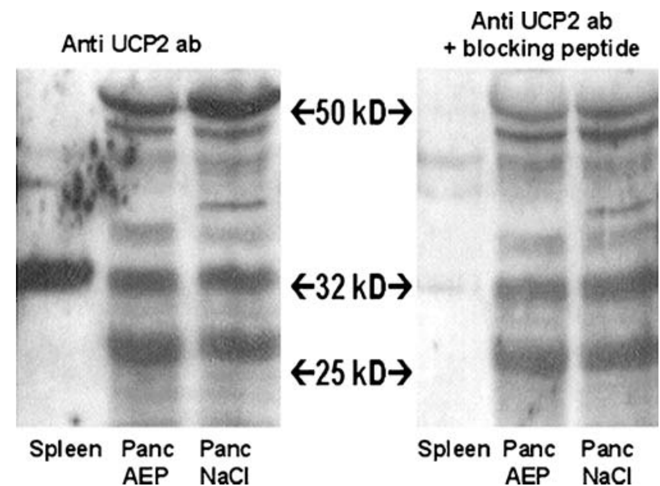

Fig. 4 Western blot of mitochondrial isolations from normal mouse spleen (*Spleen*; positive control) and pancreas with cerulein-induced acute experimental pancreatitis (*Panc AEP*) and saline injected controls (*Panc NaCl*). The strong 32-kDa UCP2 band detected in spleen mitochondria was abolished by preabsorption with blocking peptide, whereas the pancreatic bands were unaffected, indicating that these bands were not UCP2

Discussion

The current experiments have investigated pancreatic UCP2 gene expression in two etiologically different, well-established, animal models of acute experimental pancreatitis, thereby excluding any influence of species or model used. In both models, the observed parenchymal injury and leukocyte infiltration are in agreement with previous data showing a more severe pancreatitis at the late time point in mice and after the high dose of sodium-taurocholate in rats (Aho et al. 1980; Frossard et al. 2002; Halangk et al. 2000; Kruse et al. 1999). Increased UCP2 gene expression is seen in the pancreas at 24 h but not at 12 h in the cerulein pancreatitis model (Fig. 2). UCP2 mRNA increases in the rats with severe pancreatitis but not in those with mild pancreatitis (Fig. 3). Therefore, the increase of UCP2 mRNA is both time- and dose-dependent and is correlated with the amount of parenchymal injury in acute pancreatitis.

The present experiment shows, for the first time, that pancreatic acini isolated from normal pancreas also express UCP2 mRNA. Therefore, acinar cells may contribute to the observed increase in UCP2 mRNA expression in pancreatitis. UCP2 is highly expressed in immune cells (Arsenijevic et al. 2000), and the infiltration of activated immune cells is a well-known feature of acute pancreatitis. This might suggest that the observed increase in pancreatic UCP2 gene expression is attributable exclusively to mRNA produced in the infiltrating leukocytes. However, Alves-Guerra et al. (2003) have found that the contribution of immune cells to total UCP2 expression after endotoxin challenge in mice varies depending on the organ studied. In UPC2 knockout mice, they have demonstrated that immune cells are responsible for more than 90% of the UCP2 protein expression in the spleen, whereas immune cells produce almost no UCP2 in the intestine, an organ that resembles pancreas more closely with respect to its immune cell content (Alves-Guerra et al. 2003). No significant correlation between leukocyte infiltration and UCP2 was observed in the current rat experiment. These data suggest that cell types other than infiltrating immune cells contribute to the increased expression of UCP2 in the pancreas. In addition to acini, beta-cells of pancreatic islets express UCP2 (Zhang et al. 2001).

We have used pancreatic mitochondrial isolations to try to determine pancreatic UCP2 protein levels. In Western blot, preabsorption with blocking peptide eliminates the UCP2 band in the positive control (spleen) but does not affect the bands in the pancreas, indicating that the pancreatic bands are not UCP2 (Fig. 4). The multiple bands observed in the pancreas most likely arise by non-specific binding by the secondary antibody. Detection of UCP2 protein is difficult because the level of UCP2 protein is low, even in mitochondrial isolations (Alves-Guerra et al. 2003). To our knowledge, UCP2 protein has only been detected in mitochondria from tissues with relatively high levels of UCP2 transcripts, such as the spleen, the lung, the intestine, and the mouse reproductive tract (Pecqueur et al. 2001; Rousset et al. 2003). The presence of measurable UCP2 protein in spleen mitochondria has been confirmed in the current study. Detection of UCP2 protein in tissues with low levels of UCP2 mRNA, e.g., in muscle mitochondrial isolations, has been unsuccessful (Cadenas et al. 1999; Pecqueur et al. 2001). Because our real-time PCR data show that the amount of UCP2 transcripts in the pancreas is more than a hundred-fold lower than that in lung tissue, our inability to detect UCP2 protein in pancreatic mitochondrial isolations is not unexpected.

Regulation of protein expression can occur at several different levels, including the transcription of the gene, the stability of the mRNA, the control of translation, and the stability of the protein itself. UCP2 protein expression has been shown to be regulated at the transcriptional level, both in vivo and in vitro. Horvath et al. (2003) have shown that the up-regulation of UCP2 mRNA is followed by enhanced protein expression in transgenic mice. Increases of protein expression have also been seen in UCP2-transfected COS cells (Pecqueur et al. 2001). Evidence of the post-transcriptional regulation of UCP2 is provided by the study of Pecqueur et al. (2001) in which pulmonary mitochondrial UCP2 protein has been shown to increase significantly between 12 h and 18 h after one endotoxin injection and then to be eliminated within 24 h, without changes in mRNA levels. The matter is further complicated by the post-translational regulation of the uncoupling activity of UCP2 (Rial et al. 1999). Because of the complexity of the relationship between UCP2 mRNA, protein level, and activity, no definite conclusions can be drawn about UCP2 protein concentrations and function from our UCP2 gene expression data.

What could the role of acinar UCP2 be in acute pancreatitis? Several studies have reported an increase in UCP2 expression as a response to oxidative stress (Cortez-Pinto et al. 1999; Lee et al. 1999; Pecqueur et al. 2001), and UCP2 has, therefore, emerged as a potential regulator of the cellular redox state and as a protective factor against ROS (Arsenijevic et al. 2000; Negre-Salvayre et al. 1997). Because the current study has shown that acinar cells express UCP2, UCP2 may play an important role in the acinar defense system against ROS-mediated stress in acute pancreatitis.

On the other hand, progressive mitochondrial dysfunction with diminution of acinar ATP levels in cerulein-induced pancreatitis has been described (Halangk et al. 1998), an observation that may partly be explained by the findings of the current study. Up-regulation of UCP2 promotes oncotic cell death through decreased ATP synthesis and modulation of ROS-dependent signaling pathways (Mills et al. 2002). Regulation of the cell death pattern by UCP2 may therefore be one explanation of apoptotic cell death being promoted and disease severity decreasing when leukocyte action or oxidant production is inhibited (Fujimoto et al. 1997; Sandoval et al. 1996). We find this of great interest because the severity of acute pancreatitis seems to be a function of the ratio between apoptotic and oncotic cell death (Andersson and Wang 1998; Kaiser et al. 1995).

In conclusion, UCP2 mRNA increases in the pancreas in two models of acute pancreatitis that differ in the species used and the mechanism of induction of acute pancreatitis. The increase in UCP2 gene expression is correlated with the severity of the disease. We hypothesize that up-regulation of UCP2 in the pancreas may be a protective response to oxidative stress. However, this increase may also have a negative influence on cellular energy metabolism. Acinar UCP2 may, therefore, be an important modifier of the severity of acute pancreatitis. Further experiments generating targeted UCP2 mutations within acinar cells, such as an elastase-UCP2 transgene or knockouts, are needed to resolve this question, because global gene targeting would affect the function of all the different cell types involved in the pathophysiology of acute pancreatitis and make any experimental results difficult to interpret.

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