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The anti-inflammatory effect of methylprednisolone occurs down-stream of nuclear factor-κB DNA binding in acute pancreatitis

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Abstract

Glucocorticoids are potent anti-inflammatory drugs. The molecular mechanisms underlying these effects have not yet been fully revealed. The aim of the present study was to establish whether methylprednisolone pretreatment is beneficial and if it can block the pancreatic DNA binding of the transcription factor nuclear factor-κB (NF-κB) and proinflammatory cytokine synthesis during cholecystokinin-octapeptide (CCK)-induced acute pancreatitis in rats. Additionally, we set out to investigate the potential effects of methylprednisolone and CCK on pancreatic heat shock protein (HSP) synthesis. The dose–response (5–40 mg/kg) and time–course (6–72 h) curves of methylprednisolone on pancreatic HSP60 and HSP72 synthesis were evaluated following methylprednisolone treatment. We demonstrated that methylprednisolone specifically and dose-dependently induced HSP72 in the pancreas of rats, while it did not have a significant effect on HSP60 expression. The pancreatitis was induced near the peak level of HSP72 synthesis (2 × 30 mg/kg body weight [b.w.] methylprednisolone i.m. at an interval of 12 h, followed by a 12-h recovery period after the second injection of methylprednisolone) by administering 2 × 100 μg/kg CCK subcutaneously at an interval of 1 h. The injections of CCK in the vehicle-pretreated group significantly elevated the levels of pancreatic HSP60 and HSP72 2–4 h after the second CCK injection. Methylprednisolone pretreatment ameliorated many of the examined laboratory (the pancreatic weight/body weight [p.w./b.w.] ratio, the serum amylase activity, the plasma trypsinogen activation peptide concentration, the pancreatic levels of tumor necrosis factor-α and interleukin-6, the degree of lipid peroxidation, protein oxidation, nonprotein sulfhydryl group content and the pancreatic myeloperoxidase activity) and morphological parameters of the disease. Methylprednisolone pretreatment did not influence pancreatic NF-κB DNA binding, but decreased proinflammatory cytokine synthesis in this acute pancreatitis model. The findings suggest that the anti-inflammatory effect of large doses of methylprednisolone in secretagogue-induced pancreatitis occurs downstream of NF-κB DNA binding, and that increased pancreatic HSP72 synthesis may play a role in the protective effect of the drug.

Keywords: Heat shock protein; HSP72; NF-κB (nuclear factor-κB); Methylprednisolone; Cholecystokinin-octapeptide; Pancreatitis

1. Introduction

Nuclear factor-κB (NF-κB) is a pluripotent transcription factor that is involved in the regulation of many proinflammatory genes (Barnes, 1998; Barnes and Karin, 1997). These include genes encoding interleukins, chemokines, adhesion molecules, receptors and enzymes whose products contribute to the pathogenesis of the inflammatory disease (Barnes, 1998; Barnes and Karin, 1997). In most cells, NF-κB is normally sequestered in the cytoplasm in an inactive form associated with a class of inhibitory proteins called IκBs (Barnes, 1998). Upon stimulation, the IκBs are hyperphosphorylated, ubiquinated and degraded by the proteasomes. This process liberates NF-κB, allowing it to translocate to the nucleus (Barnes, 1998). In the nucleus, NF-κB binds to specific sequences in the promoter regions and transactivates the downstream genes (Barnes, 1998).

NF-κB is known to be rapidly activated after the induction of acute pancreatitis by cerulein, subsequently resulting

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in the increased transcription of various proinflammatory genes (Gukovsky et al., 1998; Steinle et al., 1999). Glucocorticoids, e.g. methylprednisolone, are widely used for the suppression of inflammatory responses (Barnes, 1998). Some of the anti-inflammatory effects of glucocorticoids have been shown to be mediated through the inhibition of NF-κB (Barnes, 1998; De Bosscher et al., 1997; Reichardt et al., 2001; Scheinman et al., 1995; Wissink et al., 1997).

Cells respond to stress (e.g. hyperthermia, hypoxia or heavy metals) in a highly conserved, stereotypical fashion (Lindquist, 1986; Welch, 1992). A characteristic feature of the stress or heat shock response is the rapid synthesis of a multigene family of proteins called heat shock proteins (HSPs) (Lindquist, 1986; Welch, 1992). The HSPs are well known to protect organisms against various stresses such as elevated temperatures, ischemia, infection by pathogens, toxins, etc. (Lindquist, 1986; Welch, 1992). HSPs not only help the cells to survive the stress conditions by repairing damaged proteins, but are also involved in gene regulation and the synthesis, degradation, folding, transport and translocation of proteins (Lindquist, 1986; Welch, 1992). HSPs range in size from 10 to 110 kDa, and the major HSPs have been classified into six families according to their molecular mass (e.g. HSP60 and HSP72).

It has been widely demonstrated that the preinduction of HSP expression (particularly HSP60 and HSP72) by hyper- or hypothermia may exert a protective effect against cerulein-induced acute pancreatitis (Frossard et al., 2001; Lee et al., 2000; Otaka et al., 1994, 1997; Rakonczay et al., 2001; Takács et al., 2002; Wagner et al., 1996). Recent reports from Frossard et al. (2002) indicated that both thermal and nonthermal stress protect against secretagogue-induced pancreatitis. We have also shown that the nontoxic HSP coinducer {(+)-/*R*/-*N*-[2-hydroxy-3-(1-piperidinyl)-propoxy]-pyridine-1-oxide-3-carboximidoil-chloride (*Z*)-maleate (1:1)} (BRX-220) has a protective effect against CCK-induced acute pancreatitis (Rakonczay et al., 2002a). Bhagat et al. (2002) found that HSP70 has an essential role in thermal stress-induced protection. This is an important observation since changes in the body temperature of animals have diverse effects on the organism in addition to the induction of HSPs (Roine et al., 1992; Sramek et al., 2000). However, Kruger et al. (2001) established that, although hyperthermia can directly abolish the earliest initiating event involved in the onset of pancreatitis (the premature and intracellular activation of digestive zymogens), this is independent of the increased pancreatic HSP synthesis. Similarly, we have found that the simple upregulation of HSP72 by a nonthermal method (sodium arsenite) does not seem sufficient for protection against cholecystokinin-octapeptide (CCK)-induced acute pancreatitis (Rakonczay et al., 2002b). Nevertheless, it is possible that the protective effect of heating or cooling is due not merely to increased HSP synthesis, but also to nonspecific effects such as the inhibition of NF-κB-binding activity (Frossard et al., 2001).

Large doses of glucocorticoids are known to induce HSP72 in the rat heart by a nonthermal method (Valen et al., 2000). The aim of the present study was to investigate the potential effects of methylprednisolone on the severity of CCK-induced edematous pancreatitis. Moreover, we wished to evaluate whether methylprednisolone can block the pancreatic NF-κB DNA binding and proinflammatory cytokine synthesis during CCK-induced acute pancreatitis. Additionally, we set out to investigate the potential effects of methylprednisolone and CCK on pancreatic HSP60 and HSP72 synthesis.

2. Methods

2.1. Experimental protocol

2.1.1. Animals

Male Wistar rats weighing 250–300 g were used. The animals were kept at a constant room temperature of 25 °C with a 12-h light–dark cycle, and were allowed free access to water and standard laboratory chow (Biofarm, Zagyvaszántó, Hungary). The rats were fasted for 16 h before the induction of acute pancreatitis. In each experimental group 4–6 rats were used. The experiments performed in this study were approved by the Animal Care Committee of the University.

2.1.2. HSP time–course and dose–response after methylprednisolone injection

Rats were injected intramuscularly (i.m.) with progressive doses of methylprednisolone (5, 10, 20, 30, or 40 mg/kg body weight [b.w.]) (Solumedrol, Pharmacia and Upjohn, Puurs, Belgium) twice at an interval of 12 h to investigate the dose response on HSP60 and HSP72 synthesis. The animals were killed by exsanguination through the abdominal aorta 12 h after the second methylprednisolone injection. The pancreas was quickly isolated and frozen at −70 °C until Western blot analysis was performed. In order to evaluate the time–course of HSP60 and HSP72 expression, a group of animals received 1 or 2 × 30 mg/kg b.w. of methylprednisolone (depending on the time of sacrifice) i.m. at an interval of 12 h and were killed at different time-points after the first methylprednisolone injection (6, 12, 18, 24, 30, 36, 48, 60 or 72 h). The control animals were untreated or received the drug vehicle i.m. and were killed 12 h after the second injection. The pancreas was processed for HSP determinations.

2.1.3. CCK-induced pancreatitis

Acute pancreatitis was induced near the peak level of HSP72 synthesis (2 × 30 mg/kg b.w. methylprednisolone i.m. at an interval of 12 h, followed by a 12-h recovery period after the second injection of methylprednisolone) by injecting 100 μg/kg b.w. CCK subcutaneously (s.c.) twice at an interval of 1 h (Fig. 1). Other rodents received vehicle

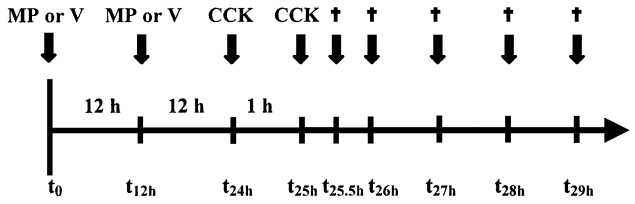

Fig. 1. Experimental protocol. Acute pancreatitis was induced near the peak level of HSP72 synthesis (2 × 30 mg/kg b.w. methylprednisolone i.m. at an interval of 12 h, followed by a 12-h recovery period after the second injection of methylprednisolone) by injecting 100 μg/kg b.w. CCK subcutaneously (s.c.) twice at an interval of 1 h. Other rats received vehicle (V) injections instead of methylprednisolone, but CCK was administered as mentioned above. The animals were sacrificed (†) by exsanguination through the abdominal aorta 30 min, 1 h, 2 h, 3 h or 4 h after the last CCK injection.

injections instead of the methylprednisolone, but CCK was administered as mentioned above. The animals were killed by exsanguination through the abdominal aorta 30 min, 1 h, 2 h, 3 h or 4 h after the last CCK injection. The control rats received methylprednisolone or the vehicle of the drug and physiological saline injections instead of CCK. The pancreas was quickly removed, cleaned from fat and lymph nodes, weighed, and frozen at −70 °C until use.

2.2. Western blotting

Samples of the pancreas were homogenized in a fourfold excess (w/v) of ice-cold buffer containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% sodium dodecylsulfate, 1% Triton X-100, 4 mM benzamidine, 5 mM iodoacetamide, 1.5 mM phenylmethylsulfhonyl fluoride and 100 IU/ml aprotinin, using an Ultra-Turrax homogenizer for 2 min. The homogenates were centrifuged at 20,000 × *g* for 20 min. The supernatants were collected and the protein concentrations were measured by the microbiuret method of Goa (1953). Fifty micrograms of protein was loaded per lane. Samples were electrophoresed on an 8–10% sodium dodecylsulfate-polyacrylamide gel according to the method of Laemmli (1970). The gels were either stained with Coomassie Brilliant Blue (to demonstrate equal loading of proteins for Western blot analysis) or transferred to a nitrocellulose membrane for 2.5 h at 30 V. Membranes were blocked in 5% nonfat dry milk for 1 h, and incubated with rabbit anti-HSP60 [produced by ourselves (Rakonczay et al., 2001), 1:50,000 dilution], anti-HSP72 (1:2,500 dilution) (a generous gift from István Kurucz, Biorex Laboratories, Veszprém, Hungary, which has been characterized previously, 1999), or anti-IκB-α (1:1,000 dilution) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibody for an additional 1–3 h at room temperature. The immunoreactive protein was visualized by enhanced chemiluminescence, using horseradish peroxidase-coupled anti-rabbit immunoglobulin at 1:10,000 dilution (Dako, Denmark). The intensities of the Western blot bands were quantified by using the Scanpack Image Analysis Program (Biometra, Göttingen, Germany).

2.3. Preparation of nuclear protein extracts, electrophoretic mobility shift assay (EMSA)

2.3.1. Nuclear protein extract

Nuclear protein extracts were prepared essentially as described by Dignam et al. (1983). A 250–300-mg pancreatic tissue sample was lysed on ice in hypotonic *buffer*

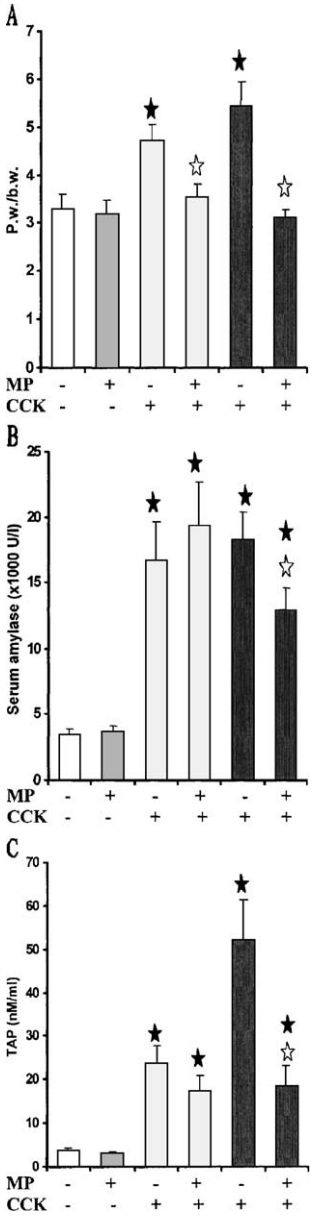

Fig. 2. Effects of methylprednisolone pretreatment on the (A) pancreatic weight/body weight ratio (p.w./b.w.), (B) serum amylase, and (C) plasma TAP levels during CCK-induced acute pancreatitis. Rats were given 2 × 30 mg/kg methylprednisolone (+) or the vehicle (−) of methylprednisolone i.m. were injected with 2 × 100 μg/kg CCK s.c. at an interval of 1 h, and were killed at 3 h (represented by black bars) or 5 h (represented by striped bars) after the first CCK injection. The control rats (represented by gray or white bars) were injected with methylprednisolone or the vehicle i.m. and physiological saline s.c. Means ± S.E.M. for six animals are shown. ☆ Significant difference ($P < 0.05$) vs. the respective vehicle-treated group or the ★ control groups.

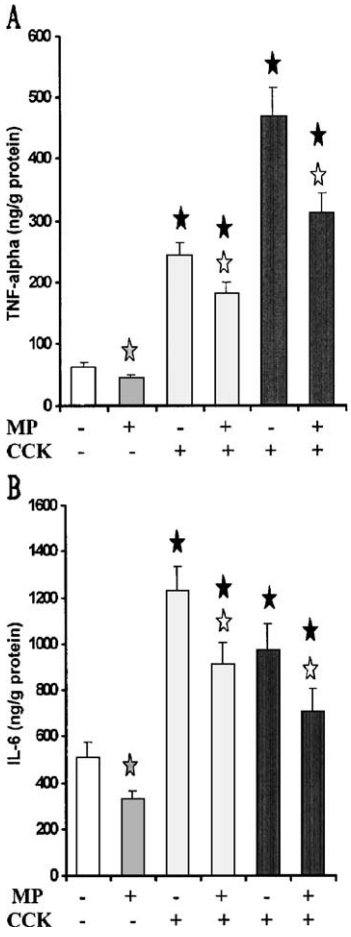

Fig. 3. Effects of methylprednisolone on pancreatic (A) tumor necrosis factor-α and (B) interleukin-6 levels during CCK-induced acute pancreatitis. Rats were treated as indicated in Fig. 2. Means ± S.E.M. for six animals are shown. ☆ Significant difference ($P<0.05$) vs. the physiological saline-treated group, the ☆ respective vehicle-treated group or the ★ control groups.

A by 20 strokes in a glass Dounce homogenizer. The hypotonic buffer was supplemented with 1 mM phenylmethylsulfhonyl fluoride, 4 mM benzamidine, 100 IU/ml aprotinin, and 1 mM dithiothreitol. The homogenate was left on ice for 20 min, and Nonidet P-40 was then added to a final concentration of 0.3–0.4% (v/v). The samples were briefly vortexed and incubated on ice for an additional 2 min. The nuclear pellet was collected by centrifugation of the lysed tissue for 20 s at 13,000 × *g* in a microfuge. The supernatant (cytosolic fraction) was saved for Western blot analysis of IκB-α. The nuclear pellet was resuspended in *buffer C* supplemented with 1 mM dithiothreitol, 1.5 mM phenylmethylsulfhonyl fluoride, 4 mM benzamidine, and 100 IU ml aprotinin. After rotation at 4 °C for 30–45 min, the nuclear membranes were pelleted by microcentrifugation for 10 min and the supernatant (nuclear extract) was aliquoted and stored at − 70 °C. The protein concentration of the nuclear extract was determined by the method of Goa (1953).

2.3.2. EMSA of NF-κB

A 21-basepair oligonucleotide 5′-GGCAGAGGGGACTTTCCGAGA-3′ containing the NF-κB consensus sequence (underlined) was annealed with its complementary oligonucleotide (with 5′ G overhangs at both ends) to generate a double-stranded probe and was end-labeled with [γ-^{32}P] by T_4 polynucleotide kinase. Labeled oligonucleotides were separated from the unincorporated isotope by PAGE and

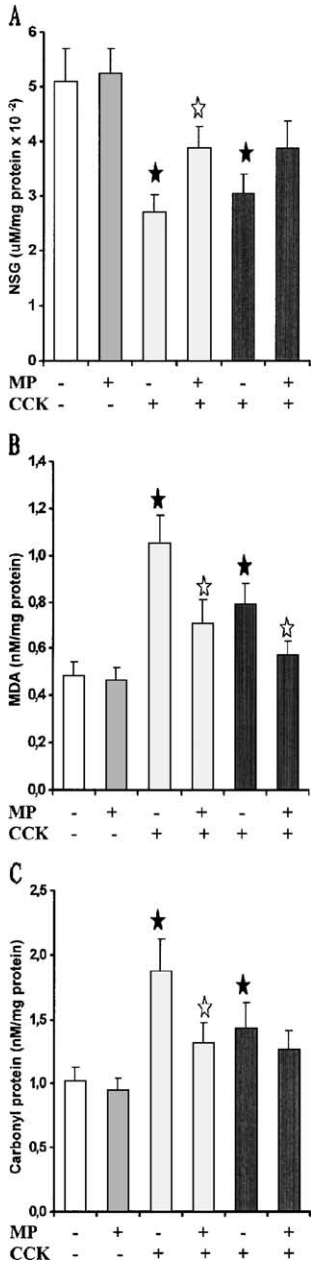

Fig. 4. Effects of methylprednisolone on pancreatic (A) nonprotein sulfhydryl group (NSG) content, (B) lipid peroxide (MDA) and (C) protein carbonyl levels during CCK-induced acute pancreatitis. Rats were treated as indicated in Fig. 2. Means ± S.E.M. for six animals are shown. ☆ Significant difference ($P<0.05$) vs. the respective vehicle-treated group or the ★ control groups.

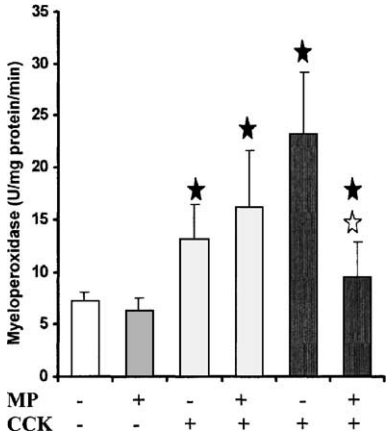

Fig. 5. Effects of methylprednisolone on pancreatic myeloperoxidase activities during CCK-induced acute pancreatitis. Rats were treated as indicated in Fig. 2. Data are means ± S.E.M. for six animals. ☆ Significant difference ($P<0.05$) vs. the respective vehicle-treated group or the ★ control groups.

were isolated from a 16% polyacrylamide gel. To determine the NF-κB binding activity, aliquots of nuclear protein (12 μg) were mixed with a buffer containing 10 mM HEPES (pH = 7.9), 50 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol, and 4.5 μg poly(d*I*/d*C*). The binding reaction was started by adding 3–5000 cpm of the radiolabeled double-stranded probe and was allowed to proceed for 30–40 min on ice. For cold competition, a 20× or 100× molar excess of specific unlabeled double-stranded wild-type or mutated oligonucleotide was added to the reaction mixture together with the labeled probe. In mutated oligonuleotide, the κB motif was changed to GGccACTaaC. DNA protein complexes were resolved by PAGE at 4 °C on a nondenaturing 4.5% gel in a buffer containing 6.7 mM Tris base, 3.3 mM sodium acetate, and 1 mM EDTA (pH = 7.5). Gels were vacuum-dried and exposed to Fuji RX films (Fuji Tokyo, Japan) with intensifying screens at −70 °C. The intensities of the bands were quantified by using the Scanpack Image Analysis Program (Biometra, Goettingen, Germany).

2.4. Assays

2.4.1. The pancreatic weight/body weight ratio (p.w./b.w.)

This ratio was utilized to evaluate the degree of pancreatic edema.

2.4.2. Serum amylase activity and plasma trypsinogen activation peptide concentration

All blood samples were centrifuged at 2500 × *g* for 20 min. The serum levels of amylase were determined by a colorimetric kinetic method (Dialab, Vienna, Austria). Plasma trypsinogen activation peptide (TAP) concentrations were determined with an ELISA kit (Biotrin, Dublin, Ireland) according to the manufacturer's instructions.

2.4.3. Pancreatic tumor necrosis factor-α and interleukin-6 levels

Tumor necrosis factor-α and interleukin-6 concentrations were measured in the pancreatic cytosolic fractions with ELISA kits (Bender Medsystems, Vienna, Austria) according to the manufacturers' instructions.

2.4.4. Pancreatic nonprotein sulfhydryl group content

Part of the pancreas was homogenized in fourfold excess (w/v) of ice-cold buffer containing 100 mM K_2HPO_4, 150 mM KCl and 100 mM EDTA (pH = 7.4), using an Ultra-Turrax homogeniser for 2 min. The homogenates were centrifuged at 10,000 × *g* for 20 min, and the supernatants' protein concentrations were measured by the microbiuret method of Goa (1953). Aliquots of 0.25 ml of the homogenates were mixed with 1.0 ml of 5% trichloroacetic acid, and were centrifuged at 3500 × *g* for 20 min. The nonprotein sulfhydryl group content was determined spectrophotometrically with Ellman's reagent (Sedlak and Lindsay, 1968) from the supernatant, and was corrected for the protein content of the tissue.

2.4.5. Pancreatic lipid peroxide levels

Lipid peroxides can undergo metal- or enzyme-catalyzed decomposition to form multiple products, including malon-

Table 1
Effects of MP pretreatment on the histologic parameters in cholecystokinin-octapeptide-induced acute pancreatitis

	V + CCK-27	30 mg/kg MP + CCK-27	2 × 30 mg/kg MP + CCK-27	V + CCK-29	30 mg/kg MP + CCK-29	2 × 30 mg/kg MP + CCK-29
Interstitial edema	1.44 ± 0.23	0.73 ± 0.12[a]	1.04 ± 0.12[a]	1.50 ± 0.13	0.96 ± 0.09[a]	0.71 ± 0.12[a]
Leukocyte infiltration	0.35 ± 0.07	0.15 ± 0.07[a]	0.23 ± 0.07	1.38 ± 0.39	0.68 ± 0.38[a]	0.37 ± 0.20[a]
Hyperemia	0.44 ± 0.10	0.27 ± 0.06	0.13 ± 0.04[a]	0.63 ± 0.25	0.70 ± 0.16	0.60 ± 0.13
Vacuolization	0.38 ± 0.05	0.21 ± 0.03[a]	0.23 ± 0.07	0.46 ± 0.10	0.27 ± 0.07[a]	0.40 ± 0.11
Necrosis (0–4)	0.56 ± 0.09	0.21 ± 0.08[a]	0.21 ± 0.07[a]	0.48 ± 0.10	0.27 ± 0.07	0.23 ± 0.08[a]
Apoptosis	0.42 ± 0.07	0.15 ± 0.02[a]	0.29 ± 0.16	0.67 ± 0.09	0.23 ± 0.06[a]	0.21 ± 0.07[a]
Basophilic lamellation	0.35 ± 0.08	0.33 ± 0.12	0.21 ± 0.06	0.54 ± 0.09	0.17 ± 0.05[a]	0.25 ± 0.05[a]
Total damage	3.94 ± 0.47	2.04 ± 0.26[a]	2.48 ± 0.43[a]	5.65 ± 0.79	2.38 ± 0.25[a]	2.50 ± 0.31[a]

Groups were treated as indicated in Fig. 1. Data are means ± S.E.M. for six animals.
[a] Significant difference ($P<0.05$) vs. the respective vehicle-treated group.

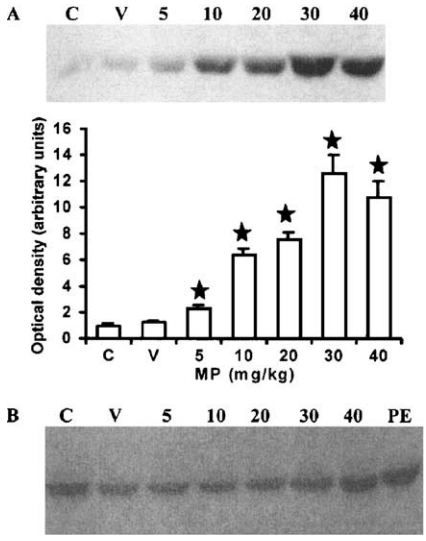

Fig. 6. Effect of pancreatic HSP72 and HSP60 expression to progressive doses of methylprednisolone injections. The figure depicts representative Western immunoblot analyses of protein lysates (50 μg/lane) from the pancreata of rats, showing the expression of (A) HSP72 and (B) HSP60 12 h after the injection of progressive doses of methylprednisolone or the drug vehicle. The levels of HSP72 were similar in the untreated (C) and vehicle-treated (V) control samples. Methylprednisolone administration (2 × 5, 10, 20, 30, or 40 mg/kg body weight) resulted in a dose-dependent increase of pancreatic HSP72 synthesis. The maximal amount of HSP72 was detected after 2 × 30 mg/kg b.w. methylprednisolone treatment. The bar chart shows the optical densities of the HSP72 Western blot bands for each group as means ± S.E.M., $n = 4$ animals/group. Methylprednisolone did not have a significant effect on HSP60 expression. PE indicates the protein extract of bacteria overexpressing human HSP60. ★ Significant difference ($P < 0.05$) vs. the vehicle-treated group.

dialdehyde. The malondialdehyde level was measured after the reaction with thiobarbituric acid, according to the method of Placer et al. (1966), and was also corrected for the protein content of the tissue.

2.4.6. Pancreatic protein carbonyl concentration

The concentration of protein carbonyls was determined by the 2,4-dinitrophenylhydrazine reaction according to the method of Levine et al. (1990) from the Western blot homogenates. Carbonyl protein content was calculated by using the absorption coefficient of 22,000 M/cm at 370 nm for aliphatic hydrazones and expressed as nmol carbonyl/mg protein.

2.4.7. Pancreatic myeloperoxidase activity

Pancreatic myeloperoxidase activity, as a marker of tissue leukocyte infiltration, was assessed by the method of Kuebler et al. (1996).

2.5. Histological examination

A 2–3 mm^3 portion of the pancreas was fixed in an 8% neutral formaldehyde solution and subsequently embedded in paraffin. Sections were cut at 4 μm thickness and stained with hematoxylin and eosin. The slides were coded and read for the traditional histological markers of pancreatic tissue injury by two independent observers who were blind to the experimental protocol. Semiquantitative grading of interstitial edema, leukocyte infiltration, hyperemia, and vacuolization, necrosis and apoptosis of acinar cells, was performed on 8–10 consecutive high-power fields (× 400) on a scale of 0–3 or 0–4. Additionally, basophilic lamellation of the cytoplasm of acinar cells was also graded since a pilot study revealed that, besides the traditional markers, the areas of basophilic lamellation were more extensive in the more severely damaged pancreata. The score for each graded parameter was averaged and the total pancreatic damage was calculated by adding all the averages together. The grading system and basophilic lamellation are described in more detail in one of our previous manuscripts (Rakonczay et al., 2001).

2.6. Statistical analysis

Results are expressed as means ± S.E.M. Differences between the experimental groups were evaluated by using the analysis of variance. Values of $P < 0.05$ were accepted as significant.

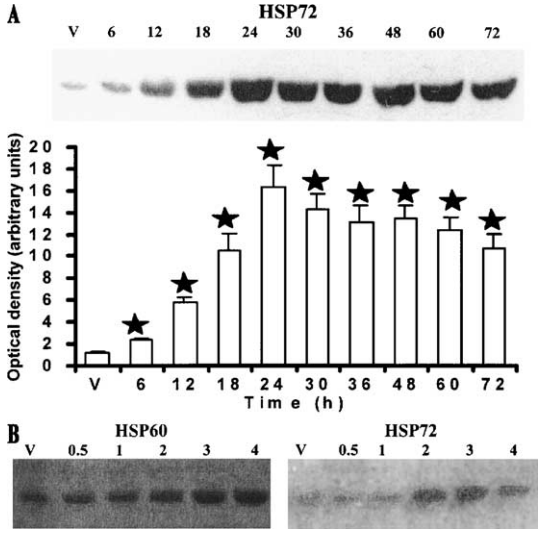

Fig. 7. Effects of methylprednisolone or CCK injections on the synthesis of HSP60 and HSP72 in the pancreas as a function of time. Representative Western immunoblot analysis of protein lysates (50 μg/lane) from the pancreata of rats, showing the expression of HSPs as a function of time after (A) methylprednisolone or (B) CCK injections. The control animals received the drug vehicle [V] i.m. instead of MP and were killed after 12 h. The level of HSP72 was already significantly increased at 6 h, and peaked at 24 h after the first methylprednisolone injection and remained elevated until 72 h. The bar chart shows the optical densities of the HSP72 bands for each group as means ± S.E.M., $n = 4$ animals/group. Methylprednisolone did not have a significant effect on HSP60 expression. The injections of CCK significantly elevated the levels of pancreatic HSP60 and HSP72 2–4 h after the second CCK injection. ★ Significant difference ($P < 0.05$) vs. the vehicle-treated group.

3. Results

3.1. The p.w./b.w. ratio, serum amylase activity and plasma TAP level

The administration of 2 × 100 μg/kg b.w. CCK increased the serum amylase activity, p.w./b.w. and plasma TAP level vs. the control (Fig. 2A,B). Methylprednisolone injections significantly decreased p.w./b.w. (3 and 5 h after the first CCK injection) and the serum amylase activity (5 h after the first CCK injection). The plasma levels of TAP were decreased dose-dependently by methylprednisolone 5 h after the first CCK injection (Fig. 2C).

3.2. Pancreatic tumor necrosis factor-α and interleukin-6 levels

Injections of CCK increased the pancreatic tumor necrosis factor-α and interleukin-6 levels over time (Fig. 3). Methylprednisolone in itself reduced the basal proinflammatory cytokine expressions vs. the physiological saline-treated group. Methylprednisolone pretreatment significantly decreased the levels of these pancreatic proinflammatory cytokines during CCK-induced acute pancreatitis vs. the vehicle-treated groups.

3.3. Pancreatic nonprotein sulfhydryl group content, lipid peroxide and protein carbonyl levels

CCK injections decreased the pancreatic nonprotein sulfhydryl group content and increased the malondialdehyde and protein carbonyl levels (Fig. 4). The administration of methylprednisolone significantly decreased the pancreatic nonprotein sulfhydryl group content 2 h after the last CCK injection (Fig. 4A). Methylprednisolone pretreatment decreased MDA levels 2 or 4 h after the last CCK injection (Fig. 4B). The protein carbonyl level was significantly ameliorated by the administration of methylprednisolone 2 h after the last CCK injection (Fig. 4C).

3.4. Pancreatic myeloperoxidase activity

The pancreatic myeloperoxidase activity was significantly elevated in CCK-induced pancreatitis. The administration of methylprednisolone decreased this parameter 4 h after the last CCK injection (Fig. 5).

3.5. Histological examination

The pancreatic morphological damage was significantly decreased in all of the groups treated with methylprednisolone as compared to the respective vehicle-treated group.

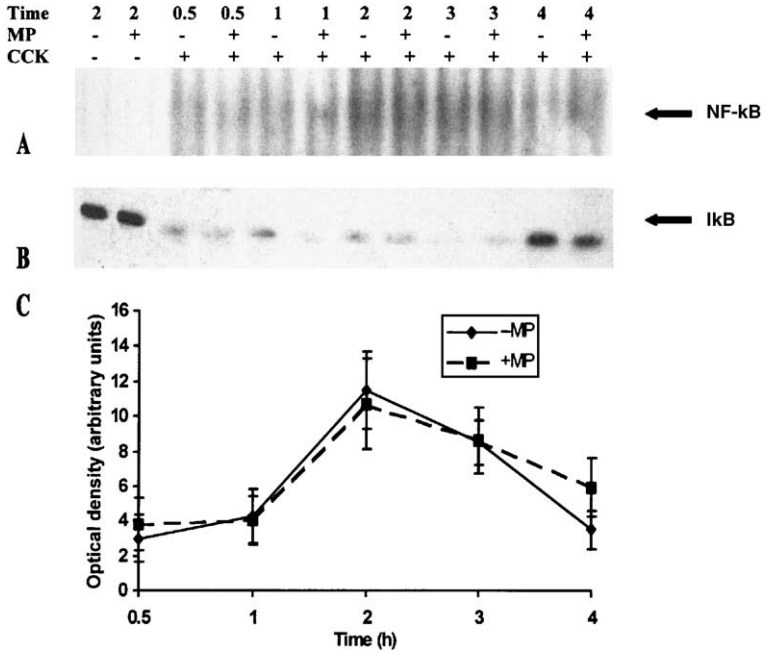

Fig. 8. The effect of 2 × 30 mg/kg b.w. methylprednisolone pretreatment on pancreatic nuclear factor-κB (NF-κB) binding activity and IκB-α levels during CCK-induced acute pancreatitis. (A) Nuclear protein extracts were prepared from the pancreas and subjected to electrophoretic mobility shift assay (EMSA) for NF-κB as described in Section 2. The figure shows a representative EMSA for pancreatic NF-κB DNA binding activity. NF-κB binding activity could hardly be detected in the control group. CCK injections increased the NF-κB binding activity over time. Maximal activation of the inducible DNA-binding activity was observed at 2 h. Methylprednisolone pretreatment did not affect the binding activity of NF-κB. (B) Cytosolic protein extracts were analyzed by Western blot, using a specific IκB-α antibody. The level of IκB-α was significantly decreased at the earliest time point studied (30 min), but it had recovered by 4 h after the second CCK injection vs. the control. (C) The intensities of NF-κB bands were quantified and are shown for each group as means ± S.E.M., $n = 3–6$ animals/group.

The point values for each of the scored parameters are shown in Table 1.

3.6. Effect of pancreatic HSP expression to methylprednisolone or CCK injections

The levels of HSP72 were similar in the untreated and vehicle-treated control samples. methylprednisolone administration resulted in a dose-dependent increase in pancreatic HSP72 synthesis (Fig. 6A). The maximal amount of HSP72 was detected after 2×30 mg/kg b.w. methylprednisolone treatment. Methylprednisolone did not have a significant effect on HSP60 expression (Fig. 6B). The time–course of HSP72 expression after methylprednisolone treatment was obtained by using the 30 mg/kg b.w. methylprednisolone dose. The level of HSP72 was already significantly increased at 6 h, peaked at 24 h after the first methylprednisolone injection and remained elevated until 72 h (Fig. 7A). The injections of CCK significantly elevated the levels of pancreatic HSP60 and HSP72 2–4 h after the second CCK injection (Fig. 7B).

3.7. The effect of methylprednisolone on NF-κB binding activity during CCK-induced acute pancreatitis

NF-κB binding activity could hardly be detected in the control groups. CCK injections increased the NF-κB binding activity over time (Fig. 8). Maximal activation of the inducible DNA-binding activity was observed at 2 h. A 2×30 mg/kg b.w. methylprednisolone pretreatment did not affect the binding activity of NF-κB. The specificity of NF-κB binding induced by CCK was confirmed in competition experiments. Incubation with increasing doses of the cold unlabeled oligonucleotide led to the inhibition of binding activity (Fig. 9). In contrast, incubation with an increased nonspecific DNA [poly(dI/dC)] concentration did not affect the NF-κB binding (results not shown).

3.8. Kinetics of IκB-α degradation in CCK-induced pancreatitis in the presence or absence of methylprednisolone treatment (Fig. 8)

The nuclear translocation of NF-κB is regulated by IκBs. The level of IκB-α was significantly decreased at the earliest time point studied (30 min), but it had recovered by 4 h after the second CCK injection vs. the control. Large doses of methylprednisolone pretreatment did not affect IκB-α degradation.

4. Discussion

Glucocorticiods are potent anti-inflammatory drugs (Barnes, 1998). The effects of glucocorticoids on the pancreas and acute pancreatitis have not yet been fully investigated. Our study was designed to examine the in vivo dynamics of pancreatic HSP induction (HSP60 and HSP72) in response to large doses of CCK or methylprednisolone, the effects of methylprednisolone on pancreatic NF-κB DNA binding and proinflammatory cytokine synthesis during CCK-induced pancreatitis, and the potential effects of methylprednisolone on this edematous pancreatitis model in the light of our previous findings. We demonstrated that methylprednisolone or CCK specifically and dose-dependently induces HSP72 in the pancreas of rats, while they did not have a significant effect on HSP60 expression. Acute pancreatitis was induced near the peak level of HSP72 synthesis after methylprednisolone treatment by administering high doses of CCK s.c. The injection of supramaximal doses of CCK resulted in the typical laboratory (e.g. hyperamylasemia) and morphological changes (interstitial edema, leukocyte infiltration and acinar cell injury) of acute pancreatitis 2 or 4 h after the second CCK injection (Lampel and Kern, 1977). Methylprednisolone pretreatment ameliorated many of the examined laboratory and morphological parameters of the disease. In accordance with the findings of Gukovsky et al. (1998) and Steinle et al. (1999), we demonstrated that NF-κB is rapidly and strongly activated while IκB-α is degraded in rat CCK-induced pancreatitis. Correspondingly, NF-κB-regulated genes (tumor necrosis factor-α and interleukin-6) were induced in CCK-pancreatitis. Methylprednisolone pretreatment did not affect the activation of NF-κB DNA binding (and IκB-α degradation) during acute pancreatitis. However, the anti-inflammatory drug decreased the levels of these proinflammatory cytokines.

The beneficial effects of exogenous corticosteroids in acute pancreatitis are a matter of dispute. The injection of

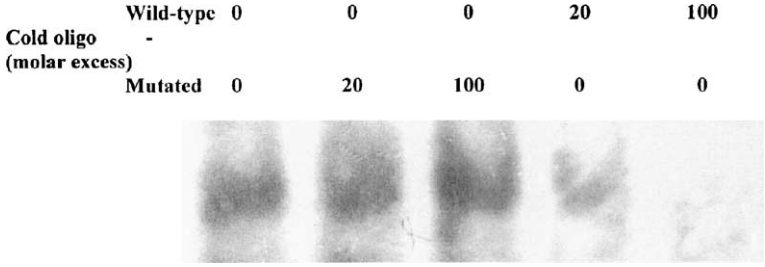

Fig. 9. Cold competition experiments confirming the specificity of NF-κB binding. Incubation with a 20 × or 100 × molar excess of the unlabeled wild-type double-stranded oligonucleotide led to the inhibition of binding activity while the mutated oligonucleotide had no such effect.

hydrocortisone during the development of the disease was observed to be harmful (De Dios et al., 1990; Pescador et al., 1995). In contrast, the protective effects of hydrocortisone in severe acute necrotizing pancreatitis were reported by several research groups (Gloor et al., 2001; Lázár et al., 1997; Osman et al., 1999). Glucocorticoids were found to attenuate pancreatic damage by protecting acinar cells during cerulein-induced acute pancreatitis (Kimura et al., 1998). The opposite was reported by Manso et al. (1995) in choline-deficient ethionine-supplemented diet-induced pancreatitis. In the former study, rats were injected with 10 mg/kg of hydrocortisone over 7 days before the induction of the pancreatitis. Gloor et al. (2001) administered 10 mg/kg hydrocortisone 10 min following the induction of cerulein-induced pancreatitis and found no protective effect, which is in contrast with our data. These controversial results might by explained by the different timing (pretreatment vs. treatment during the course of the disease) and doses of glucocorticoid treatment. We used a much higher dose of steroid than others (30 mg of methylprednisolone is equivalent to about 150 mg of hydrocortisone). This high dose was chosen to induce the synthesis of pancreatic HSPs.

The HSPs are involved in the synthesis, degradation, folding, transport and translocation of proteins (Lindquist, 1986). Cerulein pancreatitis has been reported to increase (Bhagat et al., 2002; Ethridge et al., 2000; Weber et al., 1995) or decrease (Rakonczay et al., 2002a; Strowski et al., 1997; Weber et al., 2000) the synthesis of pancreatic HSP60 and HSP72. The induction of the heat shock response helps the cells survive a subsequent, more serious stress event (Welch, 1992). HSP preinduction by thermal stress is known to protect the pancreas against cerulein-induced pancreatitis (Frossard et al., 2001; Lee et al., 2000; Otaka et al., 1994, 1997; Rakonczay et al., 2001; Takács et al., 2002; Wagner et al. 1996). Frossard et al. (2002) demonstrated that the nonthermal induction of HSP72 also protects against secretagogue-induced pancreatitis. We have also shown that the nontoxic HSP coinducer BRX-220 has a protective effect against CCK-induced acute pancreatitis (Rakonczay et al., 2002a). Bhagat et al. (2002) found that HSP70 has an essential role in thermal stress-induced protection. In contrast, Otaka et al. (1997) described that the specific preinduction of HSP72 by hot-water immersion did not have a preventive effect against cerulein-induced pancreatitis, whereas the preinduction of HSP60 did. Kruger et al. (2001) found that, although hyperthermia can directly abolish the premature and intracellular activation of digestive zymogens in cerulein-induced pancreatitis, this is independent of the synthesis of pancreatic HSPs. Similarly, we have found that the simple upregulation of HSP72 by a nonthermal method (sodium arsenite) is not sufficient for protection against CCK-induced acute pancreatitis (Rakonczay et al., 2002b). In the present experiment, we established that HSP72 can indeed have a protective effect against pancreatic damage. Therefore, the protective effect of methylprednisolone is (at least in part) likely to be mediated by increased pancreatic HSP72 synthesis. Absolutely decisive proof of the protective effects of HSP72 in this disease would require the blockade of the expression or function of these proteins.

Glucocorticoids are known to inhibit the expression of multiple inflammatory genes (Barnes, 1998). This effect is likely to be due to a direct inhibitory interaction between activated glucocorticoid receptors and transcription factors such as NF-κB and activator protein-1, which regulate the inflammatory gene expression (Barnes, 1998; De Bosscher et al., 1997; Reichardt et al., 2001; Scheinman et al., 1995; Wissink et al., 1997). The present experiments support these findings. Although methylprednisolone failed to inhibit pancreatic NF-κB DNA binding, it decreased the syntheses of tumor necrosis factor-α and interleukin-6. Previous descriptions of the effects of blocking NF-κB DNA binding in cerulein-induced acute pancreatitis are somewhat conflicting. Steinle et al. (1999) showed that following blocking of the cerulein-mediated NF-κB response with pyrrolidinedithiocarbamate, the severity of pancreatitis was more pronounced. In contrast, Gukovsky et al. (1998) observed that *N*-acetylcysteine blocked NF-κB DNA binding and significantly improved the parameters of pancreatitis. Ethridge et al. (2002) also demonstrated that selective inhibition of NF-κB by a novel peptide attenuates the severity of cerulein-induced acute pancreatitis.

Although methylprednisolone had no effect on NF-κB DNA binding in our hands, it did exert a protective effect against CCK-induced acute pancreatitis. The anti-inflammatory effect of methylprednisolone can also be independent of NF-κB (Bourke and Moynagh, 1999) and could be also explained by for example, the immunosuppressive, hemodynamic effects of the drug and/or its effects on the cell function (Barnes, 1998; Studley and Schenk, 1982). In our studies, the intrapancreatic leukocyte infiltration was reduced in the morphological specimens, which was partly supported by the decreased myeloperoxidase activities.

In conclusion, we have revealed that large doses of methylprednisolone pretreatment ameliorated the severity of CCK-induced pancreatitis in rat. In accordance with others we have demonstrated that NF-κB is rapidly and strongly activated during secretagogue-induced pancreatitis. Methylprednisolone pretreatment did not influence pancreatic NF-κB DNA binding, but it decreased proinflammatory cytokine synthesis in this acute pancreatitis model. Methylprednisolone or CCK specifically and dose-dependently induced the synthesis of HSP72 in the pancreas of rats. The findings suggest that the anti-inflammatory effect of large doses of methylprednisolone in CCK-induced pancreatitis occurs downstream of NF-κB DNA binding, and that increased pancreatic HSP72 synthesis may play a role in the protective effect of the drug. The beneficial nature of methylprednisolone in this mild acute pancreatitis model warrants further investigation.

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References

Barnes, P.J., 1998. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin. Sci. (Lond.) 94, 557–572.

Barnes, P.J., Karin, M., 1997. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N. Engl. J. Med. 336, 1066–1071.

Bhagat, L., Singh, V.P., Song, A.M., van Acker, G.J., Agrawal, S., Steer, M.L., Saluja, A.K., 2002. Thermal stress-induced HSP70 mediates protection against intrapancreatic trypsinogen activation and acute pancreatitis in rats. Gastroenterology 122, 156–165.

Bourke, E., Moynagh, P.N., 1999. Antiinflammatory effects of glucocorticoids in brain cells, independent of NF-kappa B. J. Immunol. 163, 2113–2119.

De Bosscher, K., Schmitz, M.L., Vanden Berghe, W., Plaisance, S., Fiers, W., Haegeman, G., 1997. Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. Proc. Natl. Acad. Sci. U. S. A. 94, 13504–13509.

De Dios, I., San Roman, J.I., Manso, M., Calvoo, J.J., Lopez, M.A., 1990. Glucocorticoids effects on exocrine pancreatic secretion in cerulein-induced acute pancreatitis in the rat. Arch. Int. Physiol. Biochim. 98, 361–369.

Dignam, J.D., Lebovitz, R.M., Roeder, R.G., 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. 11, 1475–1489.

Ethridge, R.T., Ehlers, R.A., Hellmich, M.R., Rajaraman, S., Evers, B.M., 2000. Acute pancreatitis results in induction of heat shock proteins 70 and 27 and heat shock factor-1. Pancreas 21, 248–256.

Ethridge, R.T., Hashimoto, K., Chung, D.H., Ehlers, R.A., Rajaraman, S., Evers, B.M., 2002. Selective inhibition of NF-kappaB attenuates the severity of cerulein-induced acute pancreatitis. J. Am. Coll. Surg. 195, 497–505.

Frossard, J.L., Pastor, C.M., Hadengue, A., 2001. Effect of hyperthermia on NF-kappaB binding activity in cerulein-induced acute pancreatitis. Am. J. Physiol. 280, G1157–G1162.

Frossard, J.L., Bhagat, L., Lee, H.S., Hietaranta, A.J., Singh, V.P., Song, A.M., Steer, M.L., Saluja, A.K., 2002. Both thermal and non-thermal stress protect against caerulein induced pancreatitis and prevent trypsinogen activation in the pancreas. Gut 50, 78–83.

Gloor, B., Uhl, W., Tcholakov, O., Roggo, A., Muller, C.A., Worni, M., Buchler, M.W., 2001. Hydrocortisone treatment of early SIRS in acute experimental pancreatitis. Dig. Dis. Sci. 46, 2154–2161.

Goa, J., 1953. Microbiuret method for protein determination of total protein in cerebrospinal fluid. Scand. J. Clin. Lab. Invest. 5, 218–222.

Gukovsky, I., Gukovskaya, A.S., Blinman, T.A., Zaninovic, V., Pandol, S.J., 1998. Early NF-kappaB activation is associated with hormone-induced pancreatitis. Am. J. Physiol. 275, G1402–G1414.

Kimura, K., Shimosegawa, T., Sasano, H., Abe, R., Satoh, A., Masamune, A., Koizumi, M., Nagura, H., Toyota, T., 1998. Endogenous glucocorticoids decrease the acinar cell sensitivity to apoptosis during cerulein pancreatitis in rats. Gastroenterology 114, 372–381.

Kruger, B., Weber, I.A., Albrecht, E., Mooren, F.C., Lerch, M.M., 2001. Effect of hyperthermia on premature intracellular trypsinogen activation in the exocrine pancreas. Biochem. Biophys. Res. Commun. 282, 159–165.

Kuebler, W.M., Abels, C., Schuerer, L., Goetz, A.E., 1996. Measurement of neutrophil content in brain and lung tissue by a modified myeloperoxidase assay. Int. J. Microcirc. Clin. Exp. 16, 89–97.

Kurucz, I., Tombor, B., Prechl, J., Erdő, F., Hegedűs, E., Nagy, Z., Vitai, M., Korányi, L., Laszló, L., 1999. Ultrastructural localization of HSP-72 examined with a new polyclonal antibody raised against the truncated variable domain of the heat shock protein. Cell Stress Chaperones 4, 139–152.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lampel, M., Kern, H.F., 1977. Acute interstitial pancreatitis in rats induced by excessive doses of a pancreatic secretagogue. Virchows Arch. 373, 97–113.

Lázár Jr., G., Varga, J., Lázár, G., Duda, E., Takács, T., Balogh, Á., Lonovics, J., 1997. The effects of glucocorticoids and a glucocorticoid antagonist (RU 38486) on experimental acute pancreatitis in rat. Acta Chir. Hung. 36, 190–191.

Lee, H.S., Bhagat, L., Frossard, J.L., Hietaranta, A., Singh, V.P., Steer, M.L., Saluja, A.K., 2000. Water immersion stress induces heat shock protein 60 expression and protects against pancreatitis in rats. Gastroenterology 119, 220–229.

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., Stadtman, E.R., 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 186, 464–478.

Lindquist, S., 1986. The heat-shock response. Ann. Rev. Biochem. 55, 1151–1191.

Manso, M.A., Reebello, A., Pescador, R., de Dios, I., 1995. Action of CCK on CDE diet-induced acute pancreatitis in rats treated with hydrocortisone. Comp. Biochem. Physiol., C Pharmacol. Toxicol. Endocrinol. 111, 257–263.

Osman, M.O., Jacobsen, N.O., Kristensen, J.U., Larsen, C.G., Jensen, S.L., 1999. Beneficial effects of hydrocortizone in a model of experimental acute pancreatitis. Dig. Surg. 16, 214–221.

Otaka, M., Itoh, H., Kuwabara, T., Zeniya, A., Fujimori, S., Otani, S., Tashima, Y., Masamune, O., 1994. Induction of heat shock protein and prevention of cerulein-induced pancreatitis by water-immersion stress in rats. Int. J. Biochem. 26, 805–811.

Otaka, M., Okuyama, A., Otani, S., Jin, M., Okayama, A., Itoh, S., Iwabuchi, A., Sasahara, H., Itoh, H., Tashima, Y., Komatsu, M., Masamune, O., 1997. Differential induction of HSP60 and HSP72 by different stress situations in rats. Dig. Dis. Sci. 42, 1473–1479.

Pescador, R., Manso, M.A., Rebollo, A.J., de Dios, I., 1995. Effect of chronic administration of hydrocortisone on the induction and evolution of acute pancreatitis induced by cerulein. Pancreas 11, 165–172.

Placer, Z.A., Cushman, L., Johnson, B.C., 1966. Estimation of product of lipid peroxidation (malonyl dialdehydes) in biochemical systems. Anal. Biochem. 16, 359–364.

Rakonczay Jr., Z., Takács, T., Mándi, Y., Iványi, B., Varga, I., Pápai, G., Boros, I., Lonovics, J., 2001. Water immersion pretreatment decreases pro-inflammatory cytokine production in cholecystokinin-octapeptide-induced acute pancreatitis in rats: possible role of HSP72. Int. J. Hyperther. 17, 520–535.

Rakonczay Jr., Z., Iványi, B., Varga, I.S., Boros, I., Jednákovits, A., Németh, I., Lonovics, J., Takács, T., 2002a. Non-toxic heat-shock protein co-inducer BRX-220 protects against acute pancreatitis in rats. Free Radic. Biol. Med. 32, 1283–1292.

Rakonczay Jr., Z., Mándi, Y., Kaszaki, J., Iványi, J., Boros, I., Lonovics, J., Takács, T., 2002b. Induction of HSP72 by sodium arsenite fails to protect against cholecystokinin-octapeptide-induced acute pancreatitis in rats. Dig. Dis. Sci. 47, 1594–1603.

Reichardt, H.M., Tuckermann, J.P., Gottlicher, M., Vujic, M., Weih, F., Angel, P., Herrlich, P., Schutz, G., 2001. Repression of inflammatory responses in the absence of DNA binding by the glucocorticoid receptor. EMBO J. 20, 7168–7173.

Roine, R., Luurila, O.J., Soukas, A., Heikkonen, E., Koskinen, P., Ylikahri, R., Toivonen, L., Harkonen, M., Salaspuro, M., 1992. Alcohol

and sauna bathing: effects on cardiac rhythm, blood pressure, and serum electrolyte and cortisol concentrations. J. Intern. Med. 231, 333–338.

Scheinman, R.I., Gualberto, A., Jewell, C.M., Cidlowski, J.A., Baldwin Jr., A.S., 1995. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. Mol. Cell. Biol. 15, 943–953.

Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound and nonprotein sulfhydryl group in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205.

Sramek, P., Simeckova, M., Jansky, L., Savlikova, J., Vybiral, S., 2000. Human physiological responses to immersion into water of different temperatures. Eur. J. Appl. Physiol. 81, 436–442.

Steinle, A.U., Weidenbach, H., Wagner, M., Adler, G., Schmid, R.M., 1999. NF-kappaB/Rel activation in cerulein pancreatitis. Gastroenterology 116, 420–430.

Strowski, M.Z., Sparmann, G., Weber, H., Fiedler, F., Printz, H., Jonas, L., Göke, B., Wagner, A.C., 1997. Caerulein pancreatitis increases mRNA but reduces protein levels of rat pancreatic heat shock proteins. Am. J. Physiol. 273, G937–G945.

Studley, J.G., Schenk Jr., W.G., 1982. Pathophysiology of acute pancreatitis. Evaluation of the effect and mode of action of steroids in experimental pancreatitis in dogs. Am. J. Surg. 143, 761–764.

Takács, T., Rakonczay Jr., Z., Varga, I.S., Iványi, B., Mándi, Y., Boros, I., Lonovics, J., 2002. The comparative effects of water immersion pretreatment on three different acute pancreatitis models in rats. Biochem. Cell Biol. 80, 241–251.

Valen, G., Kawakami, T., Tahepold, P., Dumitrescu, A., Lowbeer, C., Vaage, J., 2000. Glucocorticoid pretreatment protects cardiac function and induces cardiac heat shock protein 72. Am. J. Physiol., Heart Circ. Physiol. 279, H836–H843.

Wagner, A.C., Weber, H., Jonas, L., Nizze, H., Strowski, M., Fiedler, F., Printz, H., Steffen, H., Göke, B., 1996. Hyperthermia induces heat shock protein expression and protection against cerulein-induced pancreatitis in rats. Gastroenterology 111, 1333–1342.

Weber, C.K., Gress, T., Muller-Pillasch, F., Lerch, M.M., Weidenbach, H., Adler, G., 1995. Supramaximal secretagogue stimulation enhances heat shock protein expression in the rat pancreas. Pancreas 10, 360–367.

Weber, H., Wagner, A.C., Jonas, L., Merkord, J., Hofken, T., Nizze, H., Leitzmann, P., Göke, B., Schuff-Werner, P., 2000. Heat shock response is associated with protection against acute interstitial pancreatitis in rats. Dig. Dis. Sci. 45, 2252–2264.

Welch, W.J., 1992. Mammalian stress response: cell physiology, structure, function of stress proteins, and implications for medicine and disease. Physiol. Rev. 72, 1063–1081.

Wissink, S., van Heerde, E.C., Schmitz, M.L., Kalkhoven, E., van der Burg, B., Baeuerle, P.A., van der Saag, P.T., 1997. Distinct domains of the RelA NF-kappaB subunit are required for negative cross-talk and direct interaction with the glucocorticoid receptor. J. Biol. Chem. 272, 22278–222784.