Localized pancreatic NF-κB activation and inflammatory response in taurocholate-induced pancreatitis

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Anna S. Gukovskaya, and Stephen J. Pandol. Localized pancreatic NF-κB activation and inflammatory response in taurocholate-induced pancreatitis. Am J Physiol Gastrointest Liver Physiol 280: G1197–G1208, 2001.—Transcription factor nuclear factor-κB (NF-κB) is activated in cerulein pancreatitis and mediates cytokine expression. The role of transcription factor activation in other models of pancreatitis has not been established. Here we report upregulation of NF-κB and inflammatory molecules, and their correlation with local pancreatic injury, in a model of severe pancreatitis. Rats received intraductal infusion of taurocholate or saline, and the pancreatic head and tail were analyzed separately. NF-κB and activator protein-1 (AP-1) activation were assessed by gel shift assay, and mRNA expression of interleukin-6, tumor necrosis factor-α, KC, monocyte chemotactic protein-1, and inducible nitric oxide synthase was assessed by semiquantitative RT-PCR. Morphological damage and trypsin activation were much greater in the pancreatic head than tail, in parallel with a stronger activation of NF-κB and cytokine mRNA. Saline infusion mildly affected these parameters. AP-1 was strongly activated in both pancreatic segments after either taurocholate or saline infusion. NF-κB inhibition with N-acetylcysteine ameliorated the local inflammatory response. Correlation between localized NF-κB activation, cytokine upregulation, and tissue damage suggests a key role for NF-κB in the development of the inflammatory response of acute pancreatitis.

Acute pancreatitis; nuclear factor-κB; activator protein-1; cytokines; chemokines; inducible nitric oxide synthase

Although the mechanism of acute pancreatitis has not been established, there is emerging evidence that upregulation of inflammatory mediators, such as cytokines, chemokines, adhesion molecules, and inducible nitric oxide synthase (iNOS), is central to the process (for recent reviews, see Refs. 6, 36, and 43). Upregulation of these molecules has been shown in human (5, 20, 24, 28) and experimental pancreatitis (11, 12, 16, 17, 38, 39, 41, 48, 52), and their role in the disease has been demonstrated by using pharmacological inhibitors or antagonists (17, 21, 35, 37, 41, 47), neutralizing antibodies (16, 38, 48, 52), and genetic approaches (7, 8, 12).

The cellular mechanism of regulation of these inflammatory molecules involves activation of transcription factors such as nuclear factor (NF)-κB (3, 14, 51) and activator protein (AP)-1 (50). Activation of NF-κB in the pancreas has recently been demonstrated in rat cerulein pancreatitis (17, 44). Inhibition of NF-κB activation resulted in a decrease in the expression of cytokines KC [rat analog of interleukin (IL)-8/Gro-α] and IL-6 (17).

NF-κB and AP-1 represent two early response transcriptional complexes essential for the gene expression of inflammatory molecules (3, 14, 15, 50). NF-κB exists as a complex of homo- or heterodimers composed of members of the Rel family of proteins (3, 14). In most resting cells, NF-κB is sequestered within the cytoplasm in an inactive form. After activation, NF-κB complexes translocate into the nucleus and activate transcription from target genes. AP-1 represents a homo- or heterodimer complex composed of Jun, Fos, or activating transcription factor subunits (22, 49). These components normally reside in the nucleus and after stimulation bind to phorbol ester- or cAMP-responsive sites on DNA, inducing gene expression.

The involvement of the inflammatory molecules and their regulation has mostly been investigated in rat cerulein-induced pancreatitis, a model with mild severity (1, 26). To establish a universal role for these mechanisms in the development of pancreatitis, we designed the present set of experiments in a model of pancreatitis induced in rats by the taurocholate biliary-pancreatic duct infusion. This model is associated with severe pancreatic necrosis and a high mortality (2, 27). We analyzed early molecular events, namely activation of inflammatory molecules [IL-6, tumor necrosis factor (TNF)-α, KC, monocyte chemoattractant protein (MCP)-1, and iNOS] and the transcription factors involved in their regulation (NF-κB and AP-1).

In the taurocholate model, pancreatic damage is mainly localized to the head of the pancreas, whereas in cerulein-induced pancreatitis and other models the
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Damage is evenly distributed throughout the organ. Taking advantage of this characteristic of taurocholate-induced pancreatitis, we studied the early molecular events separately in the pancreatic head and tail and correlated them with the local morphological and biochemical changes (e.g., neutrophil infiltration and trypsin activation). Furthermore, because several factors (chemical and mechanical effect, oxidative stress, ischemia-reperfusion; see Refs. 2, 25, 31, 45) are involved in the mechanism of this model, we investigated the pancreatic response to saline intraductal infusion, in which the trigger is restricted to increased hydrostatic pressure. Characterization of the injury induced by saline infusion may have a direct clinical implication for understanding the acute pancreatitis that some patients develop as a complication of the endoscopic retrograde cholangiopancreatography (18, 29).

We found localized activation of NF-κB (but not AP-1) and localized upregulation of inflammatory molecules in the pancreatic head. These molecular changes correlated with the severity of morphological changes, including inflammatory cell infiltration, and with trypsin activation. Moreover, pharmacological blockade of NF-κB activation resulted in attenuation of the local inflammatory response. Saline infusion caused, although to a lesser degree, characteristic morphological changes of pancreatitis and NF-κB and AP-1 activation and upregulation of inflammatory molecules. The results indicate that taurocholate infusion causes a localized activation of NF-κB that, in turn, upregulates the expression of inflammatory molecules. These signals may be important for the infiltration of inflammatory cells and localized parenchymal damage in pancreatitis.

MATERIALS AND METHODS

Materials

Ketamine hydrochloride was from Fort Dodge Laboratories (Fort Dodge, IA), and xylazine was from Miles (Shawnee Mission, KS); Boc-Gln-Ala-Arg-7-amido-4-methylcoumarin (AMC) was from Bachem (Torrance, CA); PE-50 tubing was from Becton-Dickinson (Sparks, MD); trypsin was from Worthington (Freehold, NJ); MES was from ICN Biomedicals (Aurora, OH); Tissue-Tek optimum cutting temperature (OCT) compound was from Sakura Finetek (Torrance, CA); polyclonal rabbit anti-rat neutrophil antibody was from Santa Cruz Biotechnology (San- ta Cruz, CA); TRIzol reagent and the SuperScript II Pream- ridase staining kit were from Santa Cruz Biotechnology (San- ta Cruz, CA); poly(dI-dC) was from Boehringer-Mannheim (NY); Exo(−)Klenow DNA polymerase I was from Stratagene (La Jolla, CA); poly(dL-dC) was from Boehringer-Mannheim (Indianapolis, IN); Bio-Rad protein assay was from Bio-Rad Laboratories (Hercules, CA). Other reagents were from Sigma Chemical (St. Louis, MO).

Experimental Procedure

Care and handling of the animals were approved by the Animal Research Committee of the Veterans Affairs Greater Los Angeles Healthcare System, in accordance with National Institutes of Health guidelines. Male Sprague-Dawley rats (Harlan, Madison, WI), weighing 300–340 g, were random-
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Slides were then washed with PBS three times for 10 min and incubated for 2 h at room temperature with biotin-conjugated secondary antibody diluted in the working buffer (1:200). Sections were washed with PBS/glycine buffer and incubated with ABC peroxidase reagent for 30 min, then washed with PBS/glycine buffer three times for 10 min, incubated with diaminobenzidine substrate and H₂O₂, washed in PBS/glycine buffer three times for 10 min, and examined by light microscopy. The specificity of p65 staining was confirmed by the use of secondary reagents, omitting the primary antibody.

Values were obtained by counting the number of neutrophils (or the number of acinar cell nuclei immunoreactive for p65) per 100 acinar cells. An average of 100 fields was counted for neutrophils, and an average of 20 fields was counted for p65 immunolocalization from four to six rats for each condition.

Assays

Measurement of active trypsin. Trypsin activity in pancreatic tissue homogenates was measured by a fluorimetric assay according to the method of Kawabata et al. (23). Briefly, the tissue was homogenized on ice in 3 vol of a buffer containing 5 mM MES (pH 6.5), 1 mM MgSO₄, and 250 mM sucrose using a glass-Teflon homogenizer. A 25-μl aliquot of the homogenate was added to 2 ml of the assay buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1 mM CaCl₂, and 0.1 mg/ml BSA in a stirred cuvette at 37°C. The reaction was started by adding the Boc-Glu-Ala-Arg-AMC substrate and was followed for 5 min. The increase in fluorescence (excitation 380 nm, emission 440 nm) was linear during the observation. Trypsin activity in the homogenate was calculated using a standard curve for purified trypsin obtained by the same procedure.

Preparation of nuclear extracts. Nuclear protein extracts from pancreatic tissue were prepared for gel shift assay as described earlier (17, 39). Briefly, 100–200 mg of frozen tissue were powdered in a mortar in liquid nitrogen and lysed in 1 ml of a low-salt buffer (17) with 20 strokes in a glass Dounce homogenizer. Before use, the lysis buffer was supplemented with phenylmethylsulfonyl fluoride (PMSF) and di-thiothreitol (DTT) to a final concentration of 1 mM each and with the protease-inhibitor cocktail containing 5 μg/ml each of pepstatin, leupeptin, chymostatin, antipain, and aprotinin. After 20 min, 10% Igepal CA-630 was added to a final concentration of 0.3% (vol/vol), and then nuclei were collected by 30 s microcentrifugation. Nuclear protein was extracted from pelleted nuclei in a high-salt buffer (17) supplemented with 1 mM PMSF, 1 mM DTT, and the protease inhibitor cocktail described previously for up to 1 h at 4°C. Nuclear membranes were pelleted by microcentrifugation for 10 min, and the clear supernatant (nuclear extract) was separated into aliquots and stored at −80°C. The protein content of the nuclear extract was determined using the Bio-Rad protein assay.

Electrophoretic mobility shift assay. Electrophoretic mobility shift assay (EMSA) experiments were performed using double-stranded oligonucleotides comprising the consensus sequences (underlined) for NF-κB (5′-GGCAGAGGGACTT-TCCGAGA) and AP-1 (5′-GGCTTTAGATGTCAGCCGGA). The binding site for AP-1 comprised the phorbol ester-responsive element. Oligonucleotides were end labeled with [³²P]dCTP using Klenow DNA polymerase I. In the mutated NF-κB oligonucleotide, the κB motif was changed to GGGCCAT-TaaCC. Nuclear proteins (7–10 μg) were incubated with the [³²P]-labeled oligonucleotide probe under binding conditions (10 mM HEPES (pH 7.8), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 10% (vol/vol) glycerol, and 3 μg poly(dI-dC)) for 20 min at room temperature in a final volume of 20 μl. For cold competition, a 100× molar excess of unlabeled wild-type or mutated oligonucleotides was added with the probe. For supershift experiments, 2–4 μg of specific antibodies against NF-κB proteins p65 (Rel A), p50, p52, and c-Rel or against AP-1 proteins c-Fos, c-Jun, Jun-B, Jun-D, and Fra-2 were incubated with the binding reaction mixture for 40 min at room temperature before addition of the probe. After binding, protein-DNA complexes were electrophoresed on a native 4.5% polyacrylamide gel at 200 volts using 0.5× TBE buffer (1× TBE: 89 mM Tris base, 89 mM boric acid, and 2 mM EDTA). After being dried, the gels were quantified in the PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Semiquantitative RT-PCR analysis of mRNA expression. The procedure for semiquantitative RT-PCR was essentially as described previously (17, 39). Total RNA was extracted from pancreatic tissue using the TRzol reagent. RNA quality was verified by ethidium bromide staining of ribosomal RNA bands on denaturing agarose gel. Total RNA (5 μg) was reverse-transcribed using oligo(dT) as a primer (SuperScript II Preamplification System). cDNA obtained from 0.5 μg total RNA was subjected to PCR using rat gene-specific primers for IL-6, TNF-α, KC, MCP-1, and iNOS. The primer sequences were described previously (17, 39). Amplification was for a different number of cycles (between 22 and 32) to yield visible products within the linear amplification range. For a given target sequence, the same cycle number was applied for cDNAs from all animals studied. Negative controls were performed by omitting the RT step or the cDNA template from PCR amplification. The identity of the RT-PCR products was confirmed by direct sequencing. They were separated on an agarose gel containing ethidium bromide, photographed, and quantified using an AMBI image analysis system (Scanalytics, San Diego, CA). Background correction in the densitometry was performed using an area immediately adjacent to the RT-PCR product band. mRNA levels were normalized to the density of the RT-PCR product for acidic ribosomal phosphoprotein P0 (ARP), used as a housekeeping gene, in the same sample, as we described previously (17, 39).

Statistical Analysis

Data are presented as means ± SE of the values. Statistical differences between values from two groups were determined by the unpaired Student’s t-test. For normally distributed parameters, differences between more than two groups were determined by one-way ANOVA, and multiple comparisons were performed by using Newman-Keuls post hoc analysis. For parameters without normal distribution, differences between more than two groups were determined by the Kruskal-Wallis test and Dunn’s post hoc analysis. A P value <0.05 was considered statistically significant.

RESULTS

Macroscopic and Histological Examination of the Pancreas

Rats that received intraductal infusion of saline showed slight pancreatic edema, and light microscopy revealed a mild degree of interstitial edema. By contrast, after 1 or 6 h of taurocholate infusion, all of the animals had severe necrohemorrhagic pancreatitis, observed grossly and by light microscopy, that mostly affected the head segment of the pancreas. The pancreatic tail had normal histology in most of the animals.
Parameters of Pancreatitis: Serum Amylase and Lipase, Pancreatic Neutrophil Infiltration, and Trypsin Activation

Figure 1 shows the levels of serum amylase and lipase and the infiltration of neutrophils in pancreatic tissue of untreated rats and in those at 1 or 6 h after receiving intraductal infusion of either saline or taurocholate. Animals infused with taurocholate showed a time-dependent increase of serum amylase and lipase that was already significant after 1 h of the infusion. Intraductal saline infusion induced a smaller increase in serum amylase and lipase that was significantly higher compared with the control group. The number of infiltrating neutrophils in the pancreatic head of taurocholate-infused rats showed a similar time-dependent pattern as the amylase and lipase levels. The number of neutrophils in the pancreatic head increased also in the 6-h saline group, but to a lesser extent. In control pancreata, no inflammatory infiltration was seen (Fig. 1C).

Trypsin activation was also evaluated separately in the pancreatic head and tail (Fig. 2). In the head of the pancreas, trypsin activity increased at 1 h and then further at 6 h after taurocholate infusion. The saline treatment induced a smaller increase that was statistically significant in the 6-h saline group compared with control and 1-h saline groups. Trypsin activity in the pancreatic tail after taurocholate infusion showed a much smaller increase than in the head \((P < 0.05)\) and was not statistically different from the control group.

Activation of Pancreatic NF-κB in Taurocholate-Induced Pancreatitis

In the pancreas of normal rats, DNA binding activity of NF-κB was virtually undetectable. As shown in Fig. 3, taurocholate infusion caused a time-dependent NF-κB activation in the pancreatic head. The course of NF-κB activation paralleled that of serum amylase and lipase levels and neutrophil infiltration, with the highest increase (5-fold over the control group) detected 6 h after the taurocholate infusion (Fig. 3B). Saline infusion caused minor NF-κB activation. The specificity of NF-κB binding activity was demonstrated by cold competition experiments using wild-type and mutated B oligonucleotides (Fig. 4A). Only wild-type, and not mutated, κB oligonucleotide competed with the probe for the NF-κB DNA binding.

Composition of NF-κB Complexes

NF-κB exists as homo- or heterodimer composed of members of the NF-κB/Rel protein family. To characterize NF-κB complexes activated in taurocholate pancreatitis, nuclear extracts from the pancreatic head of 6-h taurocholate-infused animals were analyzed in supershift experiments using antibodies against p65 (Rel A), p50, p52, or c-Rel. As shown in Fig. 4B, antibody against p65 decreased the intensity of the NF-κB-specific band, and the antibody against p50 produced a

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Fig. 1. Serum amylase (A) and lipase (B) and neutrophil infiltration (C) in the pancreatic head in taurocholate-induced pancreatitis. Animals were either killed without any treatment (control) or at 1 or 6 h after receiving intraductal infusion of either saline or taurocholate (TC). Values are means ± SE for at least 4 animals in each group. *Values are significantly greater \((P < 0.05)\) than for all other conditions. †Values are significantly greater \((P < 0.05)\) than for control when taurocholate and control groups are compared. ‡Values are significantly greater \((P < 0.05)\) than for control when saline and control groups are compared.

Fig. 2. Trypsin activity in the pancreatic head and tail from control rats and from rats 1 or 6 h after intraductal infusion of either saline or taurocholate. Values are means ± SE for at least 5 animals in each group. *Values are significantly greater \((P < 0.05)\) than those for all other conditions. †Values are significantly greater \((P < 0.05)\) than for control when saline and control groups are compared. **Values are significantly different \((P < 0.05)\) between pancreatic head and the corresponding tail.
clear supershift. The presence of p52 or of c-Rel was not detected by the supershift assay. These data indicate that p65 and p50 proteins are present in the NF-κB complexes activated in taurocholate pancreatitis.

To further analyze cellular localization of the activated NF-κB, we performed immunostaining in pancreatic tissue with a specific antibody to p65. NF-κB is kept in inactive form in the cytoplasm and translocates to the nucleus upon activation. Thus immunostained nuclei indicate the presence of the activated NF-κB. In accordance with EMSA data, p65 immunoreactivity in the cell nuclei was not observed in control pancreas (Fig. 5A). In contrast, pancreatic tissue from the 6-h taurocholate group, in which a strong NF-κB activation was detected by EMSA, displayed immunostained nuclei, confirming the activation of NF-κB (Fig. 5B). In both control and pancreatitis tissue, no staining was observed without primary antibody (data not shown). Most of immunoreactivity was confined to the acinar cell population, and ~10% of the acinar cell nuclei stained for p65 (Fig. 5B). A small portion of infiltrated neutrophils also showed nuclear p65 localization (Fig. 5C).

**Activation of Pancreatic AP-1 Complexes in Taurocholate-Induced Pancreatitis**

In the pancreas of normal rats, there was essentially no AP-1 DNA binding activity. As shown in Fig. 6, AP-1 was strongly activated in pancreata from rats at 1 and 6 h after infusion of either saline or taurocholate. Thus, compared with NF-κB, pancreatic AP-1 activation developed faster. Also, the induction of AP-1 binding activity in both saline- and taurocholate-infused animals was stronger than that for NF-κB (Fig. 6B).

The specificity of AP-1 binding was confirmed in cold competition experiments (Fig. 7A) using unlabeled wild-type or unrelated (Oct-1) oligonucleotide.
AP-1 is a dimeric transcription factor that consists of homodimers of the Jun family proteins (c-Jun, Jun-B, and Jun-D) or Jun/Fos heterodimers. Members of the Fos family (c-Fos, Fos-B, Fra-1, and Fra-2) can associate with different Jun proteins to form the heterodimeric AP-1 complexes. The composition of AP-1 complexes in pancreata of taurocholate-infused animals was determined by supershift analysis using antibodies against Jun or Fos proteins (Fig. 7B). A supershifted band was detected with c-Fos, Jun-B, and Fra-2 antibodies. The addition of Jun-B antibody also caused a significant decrease of the specific AP-1 band (Fig. 7B). c-Jun, Jun-D, and Fos-B did not demonstrate a clear supershift or intensity decrease of the AP-1 band. Thus the results obtained indicate the presence of c-Fos, Jun-B, and Fra-2 in AP-1 complex(es) activated in the pancreas of taurocholate-infused rats.

Cytokine, Chemokine, and iNOS mRNA Expression in Pancreatic Tissue

To determine whether there is upregulation of gene expression for inflammatory molecules in the pancreas, mRNA levels of the cytokines IL-6 and TNF-α, chemo-
Kines KC and MCP-1, and the inflammatory enzyme iNOS were evaluated by semiquantitative RT-PCR (Fig. 8). The values were expressed relative to the levels in saline-infused rats. As shown in Fig. 8, IL-6, KC, and MCP-1 expression were not or were barely detectable within the normal pancreas, whereas low constitutive levels of mRNA were observed for TNF-α and iNOS. After the infusion of taurocholate, IL-6, KC, and MCP-1 were upregulated, showing increases at both 1 and 6 h. In comparison, an increase of iNOS mRNA expression was delayed, showing upregulation at 6 h. A particularly dramatic increase (>100-fold) was observed for KC mRNA at 6 h after taurocholate infusion. In contrast, we did not detect significant changes in TNF-α expression after taurocholate infusion. Saline infusion, although to a lesser degree than taurocholate infusion, also increased mRNA expression of IL-6, KC, and MCP-1. At 1 h, the saline-induced upregulation of IL-6 and MCP-1 was comparable to that induced by taurocholate infusion.

Regional Activation of NF-κB, AP-1, and Inflammatory Molecule Gene Expression in the Pancreas

Morphological observations (this study and Refs. 31 and 46) show that retrograde intraductal infusion of taurocholate causes severe tissue damage in the head of the pancreas with preservation of the pancreatic tail. This characteristic of the model led us to investigate whether the early molecular responses displayed a similar localized profile. Representative specimens of each segment (head or tail) from the same pancreas were collected and analyzed separately by EMSA and RT-PCR. As shown in Fig. 9A, NF-κB activation was much stronger in the head segment than in the corresponding tail at both 1 and 6 h after taurocholate infusion. This localized activation paralleled the more intense morphological damage, neutrophil infiltration, and trypsin activation detected in the same pancreatic segment (Fig. 2). By contrast, AP-1 showed a similar increase in DNA binding activity in the head and in the tail after taurocholate infusion (Fig. 9B).

Similarly, the mRNA expression for IL-6, MCP-1, iNOS (Fig. 9C), and KC (data not shown) also displayed a localized response, with strong upregulation in the head of the pancreas contrasting the absent or weak expression in the corresponding tail segment.

Abrogation of the Local Inflammatory Response in Taurocholate-Induced Pancreatitis by NF-κB Inhibition

To determine the role of NF-κB in the inflammatory response of taurocholate pancreatitis, rats were treated with an NF-κB inhibitor, the antioxidant NAC (15, 17, 51). NF-κB activation and mRNA expression of inflammatory molecules were measured in pancreatic head specimens collected 6 h after taurocholate infusion in rats treated with NAC or vehicle. As shown in Fig. 10A, NAC clearly suppressed pancreatic NF-κB activation caused by taurocholate infusion. NAC inhibited NF-κB activation by >50% (Fig. 10B). Blockade of NF-κB activation markedly ameliorated the following parameters of the local inflammatory response: neutrophil infiltration (Fig. 10C) and mRNA expression of IL-6, KC, and iNOS (Fig. 10D). However, NAC treatment did not change serum amylase and lipase levels at 6 h after taurocholate infusion (data not shown).

DISCUSSION

There is emerging consensus that inflammatory mediators (cytokines, in particular) play a central role in the development of experimental acute pancreatitis (6, 36, 43). Recently, NF-κB has been identified as an important regulator that controls the expression of many of those inflammatory mediators in the pancreas (9, 17, 19, 39, 44, 52). These molecular events have been studied in more detail in the rat cerulein model of pancreatitis. Administration of the CCK analog cerulein in rats causes a very reproducible but mild form of pancreatitis limited to changes in the pancreas, without major systemic complications or lethality (1, 26). By contrast, taurocholate infusion into the rat biliopancreatic duct is associated with severe local pan-
creatic damage (edema, necrosis, hemorrhage, and abundant leukocyte infiltration), major systemic complications, and high mortality (2). This model closely reproduces the necrohemorrhagic pancreatitis in humans (27). In the present study, we chose the taurocholate-induced pancreatitis model to investigate whether the above-described molecular events are common between different experimental models and, hence, represent a common pathway in the early phase of the disease. In addition to NF-κB, we also studied AP-1, another important transcription factor in the early inflammatory response (15, 50). Because in the taurocholate model the damage is mostly localized to the pancreatic head, we asked whether activation of transcription factors and inflammatory molecules correlated with parameters of pancreatitis. Finally, we analyzed responses to the mechanical effect of saline intraductal infusion.

Morphologically, taurocholate infusion caused severe pancreatic damage in the head of the pancreas, evident at both 1 and 6 h after the infusion. This was accompanied by a progressive increase in serum amylase and lipase, pancreatic neutrophil infiltration, and trypsin activation in the head segment of the gland. After a parallel time course, NF-κB was rapidly activated in the pancreatic head, with increasing activation at 6 h after taurocholate infusion. These observations extend previous findings of early pancreatic NF-κB activation in the rat cerulein model (17, 44) and in pancreatitis induced by occlusion of the pancreatic duct (9). In those studies, NF-κB activation was detected within the first 15 min of cerulein induction (17, 44) and 1 h after pancreatic duct occlusion (9). NF-κB activation has also been reported in peritoneal and alveolar macrophages from rats with taurocholate-induced pancreatitis (42).

With the supershift assay, we showed that p65 (Rel A) and p50 subunits are present in the activated NF-κB complexes in taurocholate pancreatitis. We immunolocalized the translocated p65 subunit to the acinar cell nuclei. p65 translocation into acinar cell nuclei has been also observed in the cerulein model (44). Although neutrophil infiltration into the pancreas was abundant 6 h after taurocholate infusion, the immunostaining revealed that the main source of nuclear p65 was the acinar cells. This suggests that NF-κB activation in the acinar cells represents a primary event in the early phase of acute pancreatitis. The finding of pancreatic NF-κB activation in several experimental models of pancreatitis indicates that NF-κB activation may be an important common mechanism in the development of acute pancreatitis.

We observed a strong AP-1 response already at 1 h after taurocholate infusion. However, in contrast to NF-κB, AP-1 binding activity in the pancreas did not
further increase at 6 h after taurocholate infusion. Different homo- and heterodimeric AP-1 complexes can differently regulate transcription of target genes (50). Our supershift assay demonstrated the presence of c-Fos, Jun-B, and Fra-2 in the AP-1 complexes activated in response to taurocholate infusion. AP-1 activation in the pancreas has not been described previously. So far, two studies reported pancreatic induction of the protooncogenes c-fos and c-jun during cerulein-induced pancreatitis in mice (13) and rats (10). The role of the different AP-1 subunits we found activated in taurocholate pancreatitis in the expression of genes mediating the inflammatory response remains to be determined.

We measured the pancreatic mRNA expression of proinflammatory cytokines (IL-6 and TNF-α), chemokines (KC and MCP-1), and iNOS in saline- and taurocholate-infused rats. Promoters of all of these genes contain binding sites for NF-κB and AP-1 (4, 33, 34, 40). Our results show that taurocholate pancreatitis is

![Fig. 9](http://ajpgi.physiology.org/)

**Fig. 9.** Regional activation of NF-κB and AP-1 and cytokine mRNA expression in pancreatic head and tail of rats from taurocholate group. *A* and *B*: representative EMSA showing much greater NF-κB activation in the pancreatic head than in the corresponding tail (*A*) and similar AP-1 activation in both pancreatic segments (*B*). *C*: representative RT-PCR showing localized mRNA upregulation for cytokines and iNOS in the pancreatic head compared with the corresponding tail.

![Fig. 10](http://ajpgi.physiology.org/)

**Fig. 10.** Effect of N-acetylcysteine (NAC) on NF-κB activation (*A* and *B*), neutrophil infiltration (*C*), and mRNA expression of inflammatory molecules (*D*) in 6-h taurocholate pancreatitis. In addition to taurocholate treatment, rats received NAC or saline iv infusion as described in MATERIALS AND METHODS. *A*: representative EMSA showing inhibition of NF-κB DNA binding activity in pancreata of rats treated with NAC. *B*: intensities of the NF-κB band were quantified in the PhosphorImager and are shown as means ± SE for the taurocholate + NAC group (open bar) relative to the taurocholate + saline group (solid bar) (*n* = 4). *C*: pancreatic neutrophil infiltration. *P* < 0.05 compared with the taurocholate + saline group. *D*: representative RT-PCR for pancreatic expression of the housekeeping gene ARP, cytokine IL-6, chemokine KC, and iNOS. Values are means ± SE of 4 animals in each group.
associated with an early upregulation in the head of the pancreas of all the mediators studied except TNF-α. Because TNF-α induction has been reported for other models of pancreatitis (9, 13, 16, 38), this finding may be dependent on the specific characteristics of taurocholate-induced pancreatitis. It also may be that TNF-α activation in this model is delayed beyond the 6-h observation period. Of particular interest is the strong upregulation we observed for the chemokine KC, a potent neutrophil chemoattractant (30).

In contrast to other experimental models of acute pancreatitis, in which the pancreas is entirely affected, the intraductal infusion of taurocholate renders a regional affection mostly limited to the pancreatic head. Such localized damage is an inherent characteristic of this model. This and previous studies (25, 31, 45, 46) show more pronounced morphological injury, microcirculatory changes, trypsin activation, and neutrophil infiltration in the pancreatic head than in the tail. In this study, we found a localized activation of NF-κB and expression of inflammatory molecules in the pancreatic head. The correlation between regional activation of NF-κB (and the cytokines regulated by NF-κB) and the parameters that reflect pancreatic damage adds new evidence to the key role of this transcription factor in tissue injury. In contrast to NF-κB, there was not much difference in AP-1 activation between the pancreatic head and tail. Also, the increase in AP-1-binding activity was similar in saline- and taurocholate-infused rats. This suggests that, although AP-1 may be necessary for the full activation of specific inflammatory molecules, it is not sufficient by itself to do so.

To establish whether the changes observed in taurocholate-induced pancreatitis were dependent on NF-κB activity, we used the NF-κB inhibitor NAC. This antioxidant is well known to inhibit NF-κB activation in vivo (15, 17, 51). In agreement with what we previously reported for cerulein pancreatitis (17), NAC markedly inhibited NF-κB activation in the 6-h of taurocholate pancreatitis, with significant amelioration of the local inflammatory response (neutrophil infiltration and expression of inflammatory molecules). This finding supports the idea that NF-κB activation is a key event in triggering the local cascade of inflammatory mediators associated with acute pancreatitis. NAC did not affect serum amyrase and lipase levels at 6 h after taurocholate infusion. This finding may be explained by the multifactorial nature of pancreatic injury associated with the taurocholate model, one of the factors being “mechanical” injury. Once the integrity of the pancreatic tissue is disrupted by the direct action of the taurocholic bile salt, pancreatic enzymes may reach the circulation independently of any mechanism regulating the inflammatory process. The absence of such mechanical factors in cerulein pancreatitis may account for the observation of a greater effect of NAC on serum amyrase and lipase in the cerulein model (17).

It is of note that the beneficial effects of NF-κB and cytokine inhibition may also be pronounced at later stages of taurocholate-induced pancreatitis. Inhibition of NF-κB with another antioxidant, pyrrolidinedithiocarbamate, has been reported to improve the survival of rats with taurocholate pancreatitis (42). Our study focused on the early events in this model.

Finally, our data provide characterization of some molecular events in the pancreatic response to saline intraductal infusion. Saline infusion into the pancreatic duct is not a harmless procedure (2, 31, 45). The hydrostatic pressure increase is one of several factors that can trigger the inflammatory reaction in the pancreas (32). In this regard, intraductal saline infusion may be extrapolated to the endoscopic retrograde cholangiopancreatography used in humans that is associated with a risk of pancreatitis (18, 29). In the present study, although saline infusion did not cause the necrohemorrhagic changes as observed after taurocholate infusion, it did induce significant hyperamylasemia and hyperlipasemia, inflammatory cell infiltration, and trypsin activation in the head of the pancreas. Furthermore, we observed saline-induced activation of NF-κB and some cytokines/chemokines (e.g., IL-6, KC, and MCP-1) in response to saline infusion. A particularly strong activation was observed for AP-1, which was comparable to that induced by taurocholate. These results indicate that an increase in intrapancreatic hydrostatic pressure in itself can trigger some pathways initiating an inflammatory response.

In summary, our study demonstrated that taurocholate infusion results in activation of the transcription factors NF-κB and AP-1 and the expression of proinflammatory cytokines/chemokines and iNOS in the pancreas. NF-κB (but not AP-1) activation and upregulation of proinflammatory molecules were more pronounced in the head than in the tail segment of the pancreas. The localized responses correlated with the severity of the lesion, including morphological changes, neutrophil infiltration, and pancreatic trypsin activation. Inhibition of NF-κB activation resulted in amelioration of the local inflammatory response. Intraductal saline infusion also caused morphological and molecular changes that reflect pancreatic injury, although much milder compared with taurocholate infusion. The results indicate that activation of transcription factors and inflammatory molecule expression is a common mechanism in the development of acute pancreatitis in different models of pancreatitis.

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