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Relationship between NF-κB and Trypsinogen Activation in Rat Pancreas after Supramaximal Caerulein Stimulation

Antti J. Hietaranta, Ashok K. Saluja, Lakshmi Bhagat,
Vijay P. Singh, Albert M. Song, and Michael L. Steer[1]
Department of Surgery, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, Massachusetts 02215

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Intra-acinar cell nuclear factor-κB (NF-κB) and trypsinogen activation are early events in secretagogue-induced acute pancreatitis. We have studied the relationship between NF-κB and trypsinogen activation in rat pancreas. CCK analogue caerulein induces early (within 15 min) parallel activation of both NF-κB and trypsinogen in pancreas *in vivo* as well as in pancreatic acini *in vitro*. However, NF-κB activation can be induced without trypsinogen activation by lipopolysaccharide in pancreas *in vivo* and by phorbol ester in pancreatic acini *in vitro*. Stimulation of acini with caerulein after 6 h of culture results in NF-κB but not trypsinogen activation. Protease inhibitors (AEBSF, TLCK, and E64d) inhibit both intracellular trypsin activity and NF-κB activation in caerulein stimulated acini. A chymotrypsin inhibitor (TPCK) inhibits NF-κB activation but not trypsin activity. The proteasome inhibitor MG-132 prevents caerulein-induced NF-κB activation but does not prevent trypsinogen activation. These findings indicate that although caerulein-induced NF-κB and trypsinogen activation are temporally closely related, they are independent events in pancreatic acinar cells. NF-κB activation per se is not required for the development of early acinar cell injury by supramaximal secretagogue stimulation.

Key Words: **acinar cell injury; caerulein; digestive enzymes; nuclear factor-κB; pancreas; transcription factor.**

Abbreviations used: AEBSF, 4-(2-aminoethyl)-benzenesulfonyl fluoride; ARDS, adult respiratory distress syndrome; EMSA, electrophoretic mobility shift assay; E64d, (2S,3S)-trans-epoxysuccinyl-L-leucyamido-3-methylbutane ethyl ester; IκB, inhibitory-kappaB; LPS, lipopolysaccharide; NF-κB, nuclear factor-kappaB; SDS-PAGE, sodium dodecylsulfate-polyacrylamide electrophoresis; TPA, Phorbol 12-myristate 13-acetate; TLCK, Nα-p-tosyl-L-lysine chloromethyl ketone; TPCK, N-tosyl-L-phenylalanine chloromethyl ketone.

[1] To whom correspondence should be addressed. Department of Surgery, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215. Fax: 617-667-8679. E-mail: msteer@bidmc.harvard.edu.

Rats given a supramaximally stimulating dose of the CCK analogue caerulein develop acute pancreatitis (1). Incubation of rat pancreatic tissue, *in vitro,* with a supramaximally stimulating concentration of caerulein also results in pancreatic acinar cell injury (2). Under both the *in vivo* and *in vitro* conditions, intra-acinar cell activation of the digestive enzyme zymogen trypsinogen occurs shortly after exposure to caerulein and the potentially damaging effects of intracellularly activated zymogens is believed to play an important role in the cell injury which follows (2, 4). Activation of the transcription factor NF-κB is another event which has been noted to occur shortly after exposure of the pancreas to a supramaximally stimulating concentration of caerulein or CCK under either *in vivo* (5, 6) or *in vitro* (7, 8) conditions. NF-κB is a family of transcription factors sharing a conserved Rel homology domain which plays a key role in inflammatory processes by regulating the transcription of genes coding for a variety of inflammatory mediators (9).

We have found that, under both *in vivo* and *in vitro* conditions, caerulein-induced NF-κB and trypsinogen activation occur with a similar time dependence (10). This, combined with our previously reported findings which indicate that caerulein-induced activation of trypsinogen is a Ca^{2+}-dependent event (2) and the observation, reported by others (8), that caerulein-induced NF-κB activation in the pancreas is also a Ca^{2+}-dependent process, suggested that the two activation responses to supramaximal secretagogue stimulation might be interrelated. The current manuscript reports the results of studies which were designed to evaluate the relationship between NF-κB and trypsinogen activation in pancreatitis.

MATERIALS AND METHODS

Materials. Male Wistar rats weighing 100–200 g (Charles River Laboratories, Wilmington, MA) were used as experimental animals. The animals were housed in temperature controlled (23 ± 2°C) rooms with 12 h light/dark cycle, fed standard laboratory chow and allowed to drink ad libitum. Caerulein was from Research Plus

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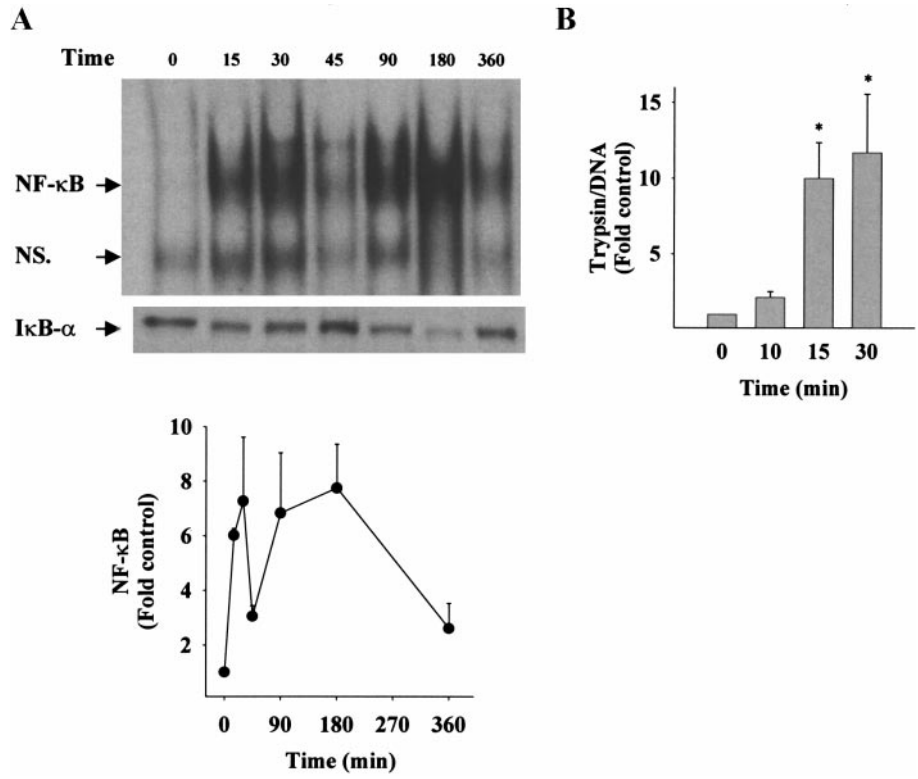

FIG. 1. Caerulein infusion induces NF-κB and trypsinogen activation in rat pancreas. (A) NF-κB DNA binding activity and IκB-α protein levels. Nuclear- and cytoplasmic extracts were prepared from pancreatic tissue samples collected at various times of supramaximal caerulein stimulation. The upper panel represents NF-κB electrophoretic mobility shift assay (EMSA), middle panel IκB-α Western blot, and lower panel densitometric quantitation of NF-κB binding activity. Values represent fold increase over nonstimulated controls. (B) Trypsin activity in rat pancreas in response to caerulein hyperstimulation. The values represent fold increase over nonstimulated controls. *p value <0.05 as compared to controls.

(Bayonne, NJ), Lipopolysaccharide (LPS, *E. coli* serotype 026:B6) from Sigma Chemical (St. Louis, MO), collagenase from Worthington Biochemical (Freehold, NJ), trypsin substrate (Boc-Glu-Ala-Arg-MCA) from Peptides International (Louisville, KY), propidium iodide from Molecular Probes (Eugene, Oregon), Z-Leu-Leu-Leu-CHO (MG-132) from Calbiochem (La Jolla, CA), 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) from Europa Bioproducts Ltd. (Cambridge, U.K.). Penicillin-streptomycin was from Gibco Labs (Grand Island, NY). Phorbol 12-myristate 13-acetate (TPA, PMA), (2S,3S)-trans-epoxysuccinyl-L-leucyamido-3-methylbutane ethyl ester (E64d), Nα-p-tosyl-L-lysine chloromethyl ketone (TLCK) and N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) as well as all other reagents and chemicals were purchased from Sigma Chemical (St. Louis, MO). All experimental protocols were approved by the institutional Animal Use Committee of the Beth Israel Deaconess Medical Center, Boston, MA.

In vivo experiments. The time dependent effect of caerulein on NF-κB and trypsinogen activation *in vivo* was studied in previously cannulated rats. The animals were anesthetized by intraperitoneal pentobarbital (Veterinary Laboratories, Lenexa, KY). A PE-50 cannula (Biolab Products, Lake Havasu City, AZ) was introduced into right jugular vein and tunneled subcutaneously to exit behind the neck. The cannula was filled with heparin (200 U/ml) to prevent blood clotting. Water was given *ad libitum* during the 12 h recovery period until the experiments. Caerulein was administered at a dose of 5 μg/kg/h following administration of an initial 5 μg/kg bolus dose. In the LPS studies, LPS was administered as a single intraperitoneal dose (200 μg). For comparison in these studies, control animals received either no injection or a single intra-peritoneal injection of caerulein (50 μg/kg) and animals were sacrificed 1.5 h later in a CO_2 chamber and pancreatic tissue samples collected.

In vitro experiments. Dispersed rat pancreatic acini were prepared by collagenase digestion and studied as previously described (4, 11, 12). The acini were either stimulated with caerulein or incubated in the buffer alone after a short equilibration period (5 min). In another set of experiments, the acini were preincubated in the presence of various agents (AEBSF; 2.5 mM in H_2O, E64d; 0.1 mM in DMSO, TLCK; 0.2 mM in methanol, TPCK; 25 μM in methanol, MG-132; 25 μM in DMSO) or in buffer alone for 30 min before adding caerulein. The concentration of caerulein used in all experiments was 0.1 μM. The acini were collected at various time points, washed in ice cold HEPES-Ringer buffer, and processed for trypsin assay and for nuclear- and cytolasmic protein extracts. For experiments using cultured acini, freshly prepared acini were suspended in RPMI-1640 medium supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 U/ml) for 6 h at 37°C in an atmosphere of 5% CO_2. They were then washed and resuspended in HEPES-Ringer buffer for study. Viability of acini after culture and washing, as assessed by trypan blue exclusion, was >90%.

Assays. Trypsin activity was quantitated as previously described using the fluorimetric method of Kawabata *et al.* (13). A standard curve was generated using purified trypsin and data expressed as trypsin activity per μg DNA. To allow for pooling of results, activity was calculated as a fold increase over normal control animals for *in vivo* experiments or unstimulated acini in *in vitro* experiments. DNA was quantitated fluorimetrically using propidium iodide (excitation at 536 nm, emission at 617 nm). For evaluation of NF-κB activation

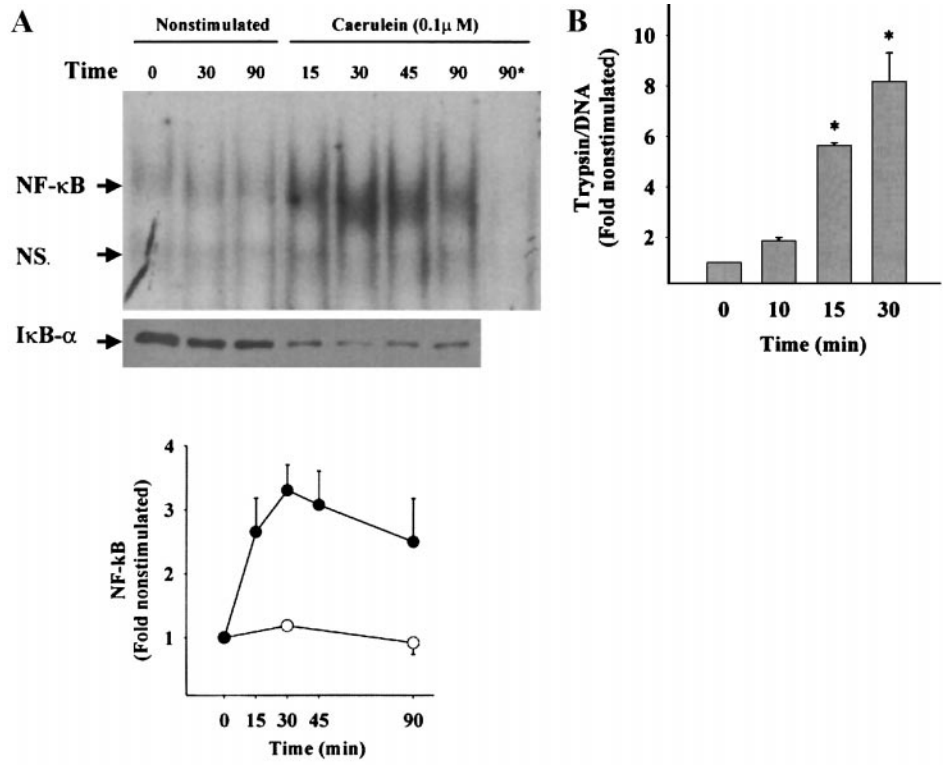

FIG. 2. Supramaximal caerulein stimulation (0.1 μM) induces NF-κB and trypsinogen activation in pancreatic acini. (A) NF-κB DNA binding activity and IκB-α protein levels. Nuclear- and cytoplasmic extracts were prepared from freshly prepared acini after various times of caerulein stimulation. The upper panel represents NF-κB electrophoretic mobility shift assay (EMSA, *competition assay with 1.75 pmol of unlabelled NF-κB oligonucleotide), middle panel IκB-α Western blot, and lower panel densitometric quantitation of NF-κB binding activity after various times of supramaximal caerulein stimulation. Values represent fold increase over nonstimulated acinar cells at 0-time. (B) Trypsin activity in pancreatic acini in response to supramaximal caerulein stimulation. The values represent fold increase over nonstimulated acini at 0-time. *p value <0.05 as compared to nonstimulated acini.

and IκB degradation, nuclear- and cytoplasmic extracts were prepared as described by Dyer and Herzog (14). Protein concentrations were determined by the method of Bradford (15).

Electrophoretic mobility shift assay (EMSA). Aliquots of 7.5–10 μg of nuclear protein were mixed in 25 μl reactions containing 5 mM Tris pH 7.5, 100 mM NaCl, 1 mM DDT, 1 mM EDTA, 4% (vol/vol) glycerol, 0.08 mg/ml salmon sperm DNA and H_2O. The oligonucleotide probe (5′-AGT TGA GGG GAC TTT CCC AGG C-3′, Promega, Madison, WI) containing κB binding motif was end labeled with [γ-^{32}P]ATP using T_4 polynucleotide kinase and purified over two successive 1 ml G-50 columns (Amersham Pharmacia Biotech Inc., Piscataway, NJ). 1×10^6 cpm of the probe was added to the mixture and the binding reaction allowed to proceed for 20 min at room temperature. Unlabeled oligonucleotide was used for specific competition assay. DNA-protein complexes were resolved in a 6% nondenaturating polyacrylamide gel in a TBE buffer at 140 V for 2–2.5 h. Gels were dried and exposed to Kodak Bio Max MR films at −70°C. NF-κB bands from films were quantitated by using HP Scanjet 4100C scanner and Scion Image analysis program.

Western blot analysis. Equal amounts of cytoplasmic protein extracts (5–10 μg) were diluted in Laemmli sample buffer with 5% mercaptoethanol. The samples were then boiled for 5 min and resolved in 10% polyacrylamide gels in Tris-Glycine-SDS buffer. The gels were then transferred into nitrocellulose membranes and blocked for 1 h in 5% non-fat dry milk in phosphate buffered saline (PBS), pH 7.5, containing 0.1% (vol/vol) Tween-20 (PBST-milk). Blots were the incubated with polyclonal rabbit anti-IκB-α antibody (sc-371) (Santa Cruz Biotechnology Inc., Santa Cruz, CA), at 1:1000 (vol/vol) dilution in PBST-milk at 4°C overnight. The membranes were washed for 1 h in PBST and incubated with horseradish peroxidase conjugated anti-rabbit IgG at 1:5000 (vol/vol) dilution in PBST-milk for 1 h at room temperature. After washing the membranes for 1 h in PBST, IκB-α protein bands were visualized by enhanced chemiluminiscense (NEN Life Science Products, Boston, MA).

Cell injury. LDH leakage from acini was used to quantitate cell injury (16) during *in vitro* exposure of pancreatic acini to caerulein as previously described (2). LDH activity was measured as described by Amador *et al.* (17) and expressed as a percent of total cellular LDH activity.

Analysis of data. The results reported in this communication represent mean ± standard error (SEM) of the mean values obtained from 3 or more separate experiments. In all figures, vertical bars denote SEM values. Statistical evaluation of data was accomplished by analysis of variance (ANOVA) and p values of less than 0.05 were considered significant.

RESULTS

Time Dependence of Trypsinogen and NF-κB Activation

Under *in vivo* conditions, supramaximal caerulein stimulation results in rapid activation of NF-κB which is apparent within 15 min of the start of caerulein

TABLE 1

The Effect of Protease Inhibitors on Caerulein-Induced Trypsinogen- and NF-κB Activation in Pancreatic Acini

Treatment	Trypsin/DNA	NF-κB
None	11.05 ± 0.30	3.79 ± 0.23
AEBSF (2.5 mM)	0.03 ± 0.02	0.37 ± 0.08
E64d (0.1 mM)	1.64 ± 0.59	0.42 ± 0.09
TLCK (0.2 mM)	1.28 ± 0.05	0.21 ± 0.02
TPCK (25 μM)	11.89 ± 3.26	0.31 ± 0.08

Note. The acini were preincubated for 30 min with inhibitors or in buffer alone before stimulation with caerulein (0.1 μM) for 30 min. Trypsinogen activation is expressed as fold of nontreated and nonstimulated acini. NF-κB activation was determined by electrophoretic mobility shift assay (EMSA), calculated by densitometry and is expressed as a fold of nontreated and nonstimulated acini.

administration, peaks at 30 min, declines towards resting levels at 45 min, and then rises again over the subsequent 2.5 h (Fig. 1A). IκB-α levels are inversely effected with the same time dependence (Fig. 1A). A rapid *in vivo* activation of trypsinogen is also observed (Fig. 1B) which is first apparent 15 min after the start of caerulein administration. Similar observations are also noted after *in vitro* exposure of freshly prepared acini to a supramaximally stimulating concentration of caerulein. NF-κB activation and IκB-α degradation can be detected within 15 min of caerulein addition (Fig. 2A). Trypsinogen activation can also be observed at that time (Fig. 2B).

Effects of Enzyme Inhibitors on NF-κB and Trypsinogen Activation

The effects of various enzyme inhibitors on caerulein-induced *in vitro* NF-κB and trypsinogen activation were evaluated and the results are shown in Table 1. In each case, freshly prepared acini were preincubated in the presence or absence of the inhibitor for 30 min and then caerulein (0.1 μM) was added. NF-κB activation and trypsin activity were evaluated 30 min after caerulein addition. The trypsin inhibitors AEBSF and TLCK as well as the cathepsin B inhibitor E64d prevented both NF-κB activation and trypsin activity. On the other hand, the chymotrypsin inhibitor TPCK prevented NF-κB activation but did not reduce intracellular trypsin activity.

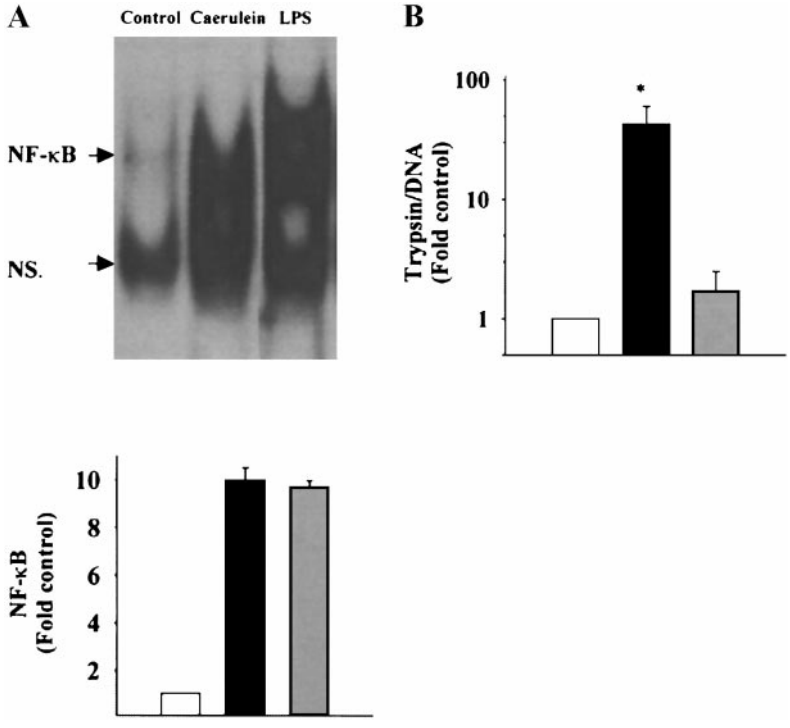

FIG. 3. Effect of LPS on NF-κB and trypsinogen activation, *in vivo.* (A) NF-κB DNA binding activity. The upper panel represents electrophoretic mobility shift assay (EMSA), and lower panel corresponding densitometric quantitation of the NF-κB DNA binding activity. Nuclear extracts were prepared from pancreatic tissue samples collected at 1.5 h after a single injection of either a supramaximally stimulating dose of caerulein (50 μg/kg, i.p.) (black bar) or lipopolysaccharide (LPS) (200 μg/animal, i.p.) (grey bar). Values represent fold increase over nonstimulated controls (white bar). (B) Trypsin activity in pancreas 1.5 h after injection of either caerulein (black bar) or LPS (grey bar). The values in logarithmic scale represent fold increase over nonstimulated controls (white bar). *p value <0.05 as compared to controls.

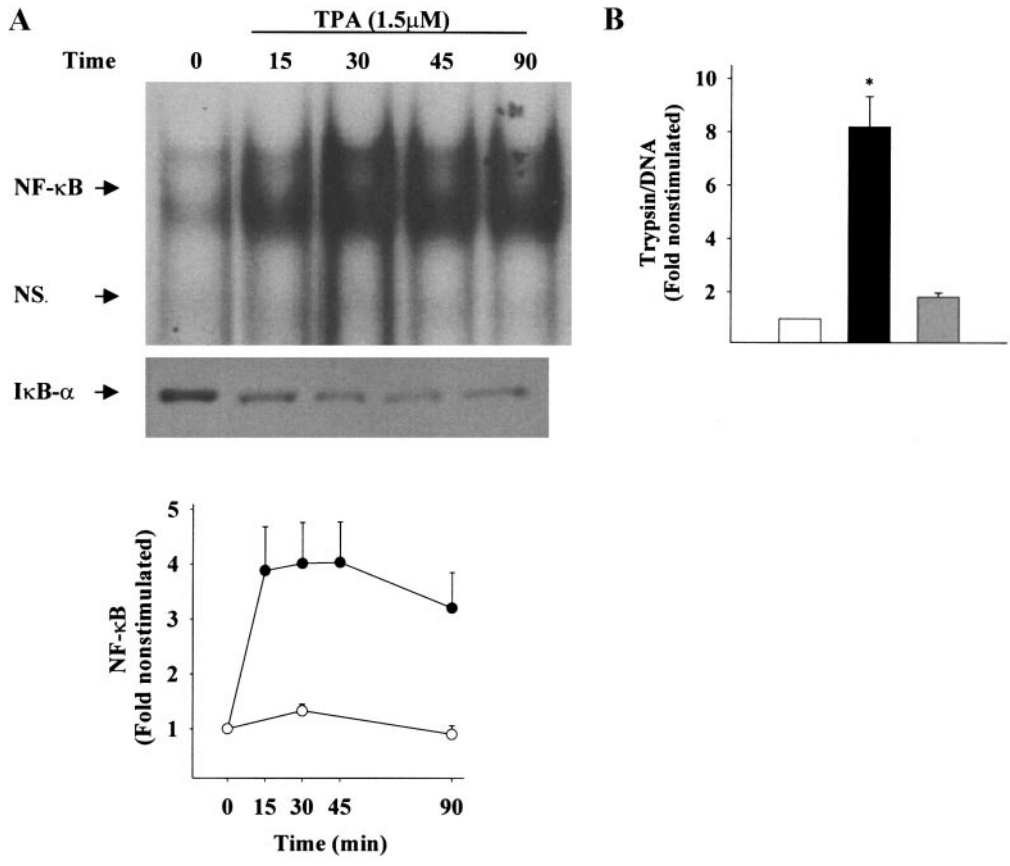

FIG. 4. Effect of TPA on NF-κB and trypsinogen activation in isolated acini. (A) NF-κB DNA binding activity and IκB-α protein levels. Nuclear- and cytoplasmic extracts were obtained from freshly prepared acini after various periods of TPA (1.5 μM) stimulation. The upper panel represents NF-κB electrophoretic mobility shift assay (EMSA) and middle panel IκB-α Western blot. The lower panel represents densitometric quantitation of NF-κB binding activity in nonstimulated (open circles) and TPA stimulated (black circles) acini. Values represent fold increase over nonstimulated acini at 0-time. (B) Trypsin activity in freshly prepared pancreatic acini 30 min after stimulation with caerulein (0.1 μM) (black bar) or TPA (1.5 μM) (grey bar). The values represent fold increase over nonstimulated acini (white bar) at 30 min of incubation. *p value <0.05 as compared to nonstimulated acini.

Effects of LPS on in Vivo Pancreatic NF-κB and Trypsinogen Activation

Rats were given either LPS or, for comparison, caerulein by intraperitoneal injection and pancreatic NF-κB as well as trypsinogen activation were evaluated 1.5 h later. As shown in Fig. 3A, both caerulein and LPS cause NF-κB activation but only caerulein, and not LPS, causes trypsinogen activation (Fig. 3B).

Effect of TPA on in Vitro Pancreatic NF-κB and Trypsinogen Activation

Freshly prepared acini were incubated with TPA for varying times and NF-κB activation, IκB-α degradation, and trypsinogen activation were studied. As shown in Fig. 4A, TPA causes rapid NF-κB activation and IκB-α degradation which is apparent within 15 min of TPA addition. On the other hand, addition of TPA does not result in a rise in pancreatic trypsin activity (Fig. 4B).

Effect of Short-Term Culture on NF-κB and Trypsinogen Activation

We have previously noted that supramaximal secretagogue stimulation of pancreatic acini after short-term (i.e., 6 h) culture does not lead to intra-acinar cell activation of trypsinogen or to subsequent cell injury as manifest by LDH leakage after caerulein addition (18). To evaluate the effects of short-term culture on caerulein-induced NF-κB activation, acini were incubated under culture conditions for 6 h and both NF-κB and trypsinogen activation were evaluated 30 min after caerulein addition. As shown in Fig. 5, addition of caerulein to acini after 6 h of culture does not result in trypsinogen activation (Fig. 5B) but it does lead to NF-κB activation (Fig. 5A).

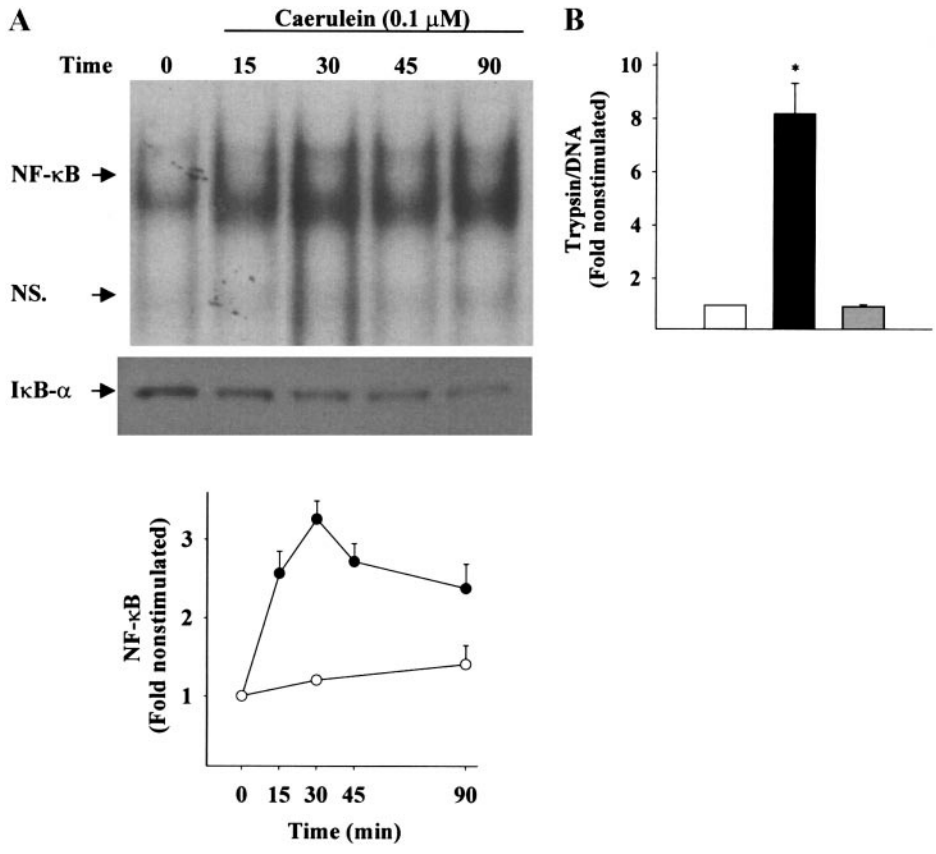

FIG. 5. Effect of supramaximal caerulein stimulation (0.1 μM) in cultured acini on NF-κB and trypsinogen activation. Pancreatic acini were cultured for 6 h in RPMI-1640 medium. The acini were then washed and resuspended in HEPES-Ringer buffer and stimulated with caerulein. (A) NF-κB DNA binding activity and IκB-α protein levels. Nuclear- and cytoplasmic extracts were prepared from cultured acini after various times of caerulein stimulation. The upper panel represent NF-κB electrophoretic mobility shift assay (EMSA) and middle panel IκB-α Western blot. The lower panel represents densitometric quantitation of NF-κB binding activity in nonstimulated (open circles) and caerulein stimulated (black circles) cultured acini. Values represent fold increase over nonstimulated cultured acini at 0-time. (B) Trypsin activity in pancreatic acini after 30 min of caerulein stimulation. Black bar represents fresh acini and grey bar cultured acini. The values represent fold increase over corresponding fresh or cultured nonstimulated acini (white bar) at 30 min of incubation. *p value <0.05 as compared to nonstimulated acini.

Effect of the Proteasome Inhibitor MG-132 on Caerulein-Induced NF-κB Activation, Trypsinogen Activation, and Cell Injury

In previously reported studies (2), we have shown that *in vitro* exposure of freshly prepared acini to a supramaximally stimulating concentration of caerulein results in rapid activation of trypsinogen and to subsequent (90 min later) cell injury as manifested by LDH leakage from acinar cells. Under these conditions, NF-κB activation (Fig. 6A) and IκB-α degradation (not shown) are also observed. The proteasome inhibitor MG-132 prevents IκB-α degradation thus aborting the process of NF-κB activation (Fig. 6A) yet, as shown in Fig. 6B, MG-132 does not interfere with caerulein-induced intra-acinar cell activation of trypsinogen and it also does not prevent caerulein-induced cell injury (Fig. 6C).

DISCUSSION

Acute pancreatitis is an inflammatory disease of the pancreas which results in pancreatic acinar cell injury as well as generation of various chemokines, cytokines, adhesion molecules and other inflammatory mediators. Recent reports emanating from several groups, including our own, have indicated that intrapancreatic activation of trypsinogen and other digestive enzyme zymogens plays a critical role in the evolution of pancreatitis (3, 4). More recently, the importance of inflammatory mediator generation has been recognized and the role of NF-κB in regulating this process has become the focus of current interest.

NF-κB is a family of homo- and heterodimeric proteins which, under normal conditions, are coupled to an inhibitor (IκB) in the cytoplasmic space (19). In response to stress, a cascade of phosphorylation events

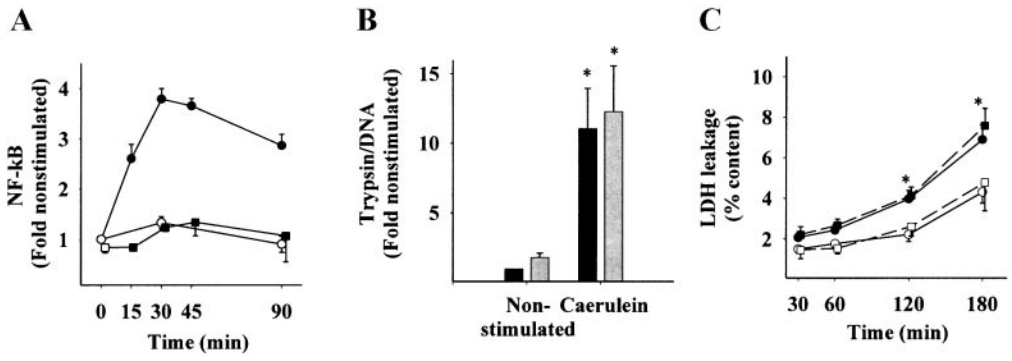

FIG. 6. Effect of proteasome inhibitor MG-132 on caerulein induced NF-κB activation, trypsinogen activation and cell injury in pancreatic acini. Freshly prepared acini were preincubated with or without MG-132 (25 μM) for 30 min and then stimulated with caerulein (0.1 μM) for various times. (A) Densitometric quantitation of NF-κB DNA binding activity in caerulein stimulated pancreatic acinar cells with (black squares) or without (black circles) MG-132 treatment. Values represent fold increase over nonstimulated acinar cells (open circles) at 0-time. (B) Trypsin activity in acini after 30 min of caerulein stimulation. Acini were preincubated for 30 min with either buffer alone (black bars) or in buffer containing MG-132 (25 μM) (grey bars) before caerulein stimulation. The values represent fold increase over nontreated and nonstimulated acini at 30 min of incubation. (C) LDH leakage from acini into the incubation medium at various times of caerulein stimulation (black symbols) or without stimulation (open symbols). Acini were preincubated for 30 min with either buffer alone (circles) or in buffer containing MG-132 (25 μM) (squares) before caerulein stimulation. Values represent percentage of the total cellular LDH activity, measured after lysing the acinar cells, that was released into the medium prior to lysis. *p value <0.05, between caerulein stimulated and nonstimulated acini within respective pretreatment groups (i.e., buffer alone or MG-132).

results in IκB phosphorylation, IκB/NF-κB dissociation, proteasomal degradation of IκB, and nuclear translocation of NF-κB with subsequent upregulation of the expression of genes coding for a variety of inflammatory factors/mediators including cytokines, chemokines, and adhesion molecules (9, 19, 20). Recent studies have shown that NF-κB activation occurs at a very early time during the evolution of secretagogue (i.e., caerulein) induced pancreatitis but whether NF-κB activation has a pro or anti-inflammatory effect in this model of pancreatitis is the subject of some controversy (5, 6).

The currently reported studies were designed to explore the relationship between intrapancreatic trypsinogen activation and pancreatic NF-κB activation during pancreatitis. We were intrigued by the similar time dependence of both events under *in vivo* as well as *in vitro* conditions (Figs. 1 and 2) as well as recent reports which indicate that, in response to supramaximal caerulein stimulation, both events are Ca^{2+} dependent—i.e., both can be prevented, under *in vitro* conditions, by either removal of Ca^{2+} from the extracellular medium or chelation of intracellular Ca^{2+} with BAPTA (2, 8). These observations, as well as the finding that both caerulein-induced NF-κB and trypsinogen activation could be inhibited by the trypsin inhibitors AEBSF and TLCK as well as the cathepsin B inhibitor E64d (Table 1) suggested, to us, that the two events might be interdependent. On the other hand, our finding that the chymotrypsin inhibitor TPCK inhibits NF-κB but not trypsinogen activation indicated that the situation might, in actuality, be more complex.

We considered two models by which the relationship between NF-κB and trypsinogen activation might be explained (Fig. 7). In both, the initial stress (i.e., caerulein supramaximal stimulation) is assumed to raise intracellular Ca^{2+} levels resulting in a series of events that culminates in both NF-κB and trypsinogen activation. In one model, the two activation events would occur independent of each other while, in the other, the activation of one (either NF-κB or trypsinogen) would be dependent upon activation of the other. To discriminate between these two alternative models, a series of experiments was performed using LPS and TPA to activate NF-κB, MG-132 to inhibit NF-κB activation, and culture-stress to prevent caerulein-induced trypsinogen activation.

In vivo administration of LPS results in NF-κB activation in the absence of trypsinogen activation (Fig. 3). Similarly, exposure of acini, *in vitro,* to TPA results in NF-κB (Fig. 4) but not trypsinogen activation. Conversely, the proteasomal inhibitor MG-132 prevents *in vitro* caerulein-induced NF-κB activation but it does

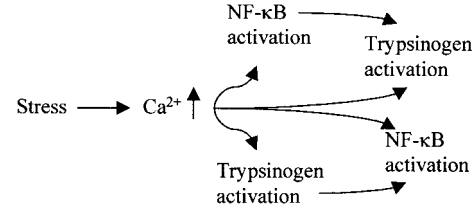

FIG. 7. The possible relationship between NF-κB and trypsinogen activation in pancreatic acinar cells. Intracellular Ca^{2+} rise (induced by stress, i.e. caerulein stimulation) results in both NF-κB and trypsinogen activation. The activation of both of these might be independent of each other or, alternatively, the activation of one might be required for the activation of the other.

not alter either caerulein-induced trypsinogen activation or cell injury (Fig. 6). Thus, these studies indicate that NF-κB activation does not, by itself, lead to trypsinogen activation and that blockade of NF-κB activation does not prevent caerulein-induced cell injury.

We have recently noted that short-term culture of pancreas fragments and acini protects them from caerulein-induced *in vitro* intra-acinar cell trypsinogen activation (18). We have employed the short-term culture system in the currently reported studies to examine caerulein-induced NF-κB activation under conditions in which caerulein-induced trypsinogen activation does not occur. We have found that, under these conditions, caerulein-induced NF-κB activation is not altered. Thus, we can conclude that trypsinogen activation is not a pre-requisite for NF-κB activation.

Taken together, the observations reported in this communication lead us to the following conclusions:

1. Trypsinogen- and NF-κB activation are early events in secretagogue-induced pancreatitis which occur within 15 min of supramaximal stimulation and which preceed evidence of cell injury.
2. Caerulein-induced NF-κB activation is not the result of trypsinogen activation and it can occur independent of trypsinogen activation.
3. Caerulein-induced trypsinogen activation is not the result of NF-κB activation and it can occur independent of NF-κB activation.
4. Caerulein-induced cell injury can occur by mechanisms which do not involve NF-κB activation.

These studies suggest that NF-κB activation plays little or no role in acinar cell events which are critical to the earliest stages of pancreatitis—those that involve digestive enzyme activation and culminate in acinar cell injury. We suspect that NF-κB activation, and subsequent generation of inflammatory mediators, plays a more important role in the later pancreatitis-associated events that involve generation of an inflammatory response, determination of the extent of pancreatic injury, and coupling of pancreatic to nonpancreatic events such as ARDS.

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