CEP-1347 inhibits caerulein-induced rat pancreatic JNK activation and ameliorates caerulein pancreatitis

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Wagner, Andreas C. C., Luca Mazzucchelli, Matthew Miller, Anna Marie Camoratto, and Burkhard Göke. CEP-1347 inhibits caerulein-induced rat pancreatic JNK activation and ameliorates caerulein pancreatitis. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G165–G172, 2000.—Pancreatic caerulein-induced activation of c-j un NH2-terminal kinase (JNK) has been reported, and JNK has been proposed as a mediator during induction of hyperstimulated pancreatitis. CEP-1347 has recently been described as a specific JNK inhibitor. We tested whether CEP-1347 inhibits caerulein-induced pancreatic JNK activation in isolated acini and in vivo. CEP-1347 dose dependently inhibited acinar caerulein-induced JNK activation with nearly complete inhibition at 2 µM but had no effect on digestive enzyme release. For in vivo studies, rats were pretreated with CEP-1347 before caerulein hyperstimulation. For assessment of JNK activation and histological alterations, animals were killed 30 min or 2 and 4 h after caerulein hyperstimulation, respectively. Pancreatic wet weight, serum enzyme levels, and pancreatic activity of p38 and extracellular signal-regulated kinase (ERK) were also determined. Caerulein hyperstimulation strongly activated JNK, JNK, p38, and ERK. CEP-1347 pretreatment dose dependently reduced caerulein-induced pancreatic JNK activation without p38 or ERK inhibition. JNK inhibition also reduced pancreatic edema formation and reduced histological severity of pancreatitis. Thus we show that CEP-1347 inhibits JNK activation in vivo and ameliorates caerulein-induced pancreatitis.

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ACUTE PANCREATITIS is a common gastrointestinal disorder but remains enigmatic in its unpredictable clinical course, which ranges from mild to very severe and life threatening. It is now widely accepted that intracellular, preliminary activation of zymogens such as trypsinogen to active forms such as trypsin plays a major role in the development of pancreatitis (11). It is also clear that the regular secretion of digestive enzymes into the pancreatic duct is disturbed during pancreatitis (30). Instead, digestive enzymes now appear in the serum, and increased serum amylase and lipase levels are the single most important laboratory value for the diagnosis of acute pancreatitis. In addition, in a second phase of the disease, an inflammatory reaction ensues, which finally determines the course and severity of acute pancreatitis (30). The sequelae of the inflammatory reaction generally include round cell infiltration of pancreatic tissues as well as an increase in vascular permeability leading to pancreatic edema. The inflammatory reaction may ultimately progress to a very severe systemic inflammatory response syndrome leading to multiple organ failure. Due to our still limited understanding of its underlying pathophysiology, treatment of acute pancreatitis is confined to general supportive measures with no causal approach. To obtain a better understanding of the pathophysiology of pancreatitis, we are interested in characterizing the molecular mechanisms that mediate this disorder.

Within the mitogen-activated protein kinase (MAPK) family, some members are generally believed to be part of the cellular stress response machinery. Thus, c-j un NH2-terminal kinase (JNK) was originally described as a kinase activated by several kinds of stress such as ultraviolet irradiation, cytokines, hyperosmolarity, or tumor necrosis factor (18, 28). The yeast HOG-kinase homologue, p38 MAPK, is also activated in response to stress (13, 26).

In the pancreas, it has been shown that hyperstimulation with the CCK receptor agonist caerulein, which induces acute pancreatitis in rats, can activate JNK in the rat pancreas (3, 9). We and others have recently found that p38 is also expressed in the pancreas and rapidly activated by caerulein (22, 29, 32). Due to the pattern and time course of activation in response to a variety of different secretagogues, JNK has been proposed as an important mediator early during caerulein-induced pancreatitis (9, 32).

Recently, an inhibitor of JNK, CEP-1347 (KT-7515), has been described and characterized (7, 15, 21). CEP-1347 is a semisynthetic derivative of a Nocardioopsis sp. fermentation product, the indolocarbazole K-252a (16). CEP-1347 has been shown to inhibit JNK activation in cell culture systems in vitro and to protect neurons against apoptotic cell death in animal experiments in vivo (7, 21). However, direct inhibition of JNK activation in vivo so far has not been demonstrated.

Thus we have now investigated whether CEP-1347 inhibits JNK activation in our system in vitro, using acutely isolated rat pancreatic acini, as well as in vivo, using caerulein hyperstimulation to induce pancreatitis, and have used this compound as a tool to...
examine whether JNK inhibition influences caerulein pancreatitis.

MATERIALS AND METHODS

Chemicals and antibodies. SDS, polyacrylamide, and molecular weight and isoelectric focusing standards were from Bio-Rad (Hercules, CA); minimal essential amino acids were from GIBCO (Grand Island, NY); and nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). Acetonitrile, l-ascorbic acid, methanol, ethyl acetate, and methylene chloride were purchased from Fisher Scientific (Pittsburgh, PA). All reagents were of HPLC grade. HPLC grade water was obtained using a Millipore Milli-Q plus system. All other chemicals were from Sigma (St. Louis, MO). The amino acid (AA)-(1—79) glutathione S-transferase (GST) c-Jun construct cloned into pGEX used for JNK activity assessment was a generous gift of J. Dixon (Ann Arbor, MI). CEP-1347 was kindly provided by Cephalon (West Chester, PA).

Preparation and treatment of pancreatic acini. Acini were prepared as previously described (35). Briefly, pancreata from male white Sprague-Dawley rats were digested with purified collagenase and dispersed by pipetting through polypropylene pipettes of decreasing orifice, followed by filtration through a 150-µm Nytex screen. Acini were purified by centrifugation through 4% BSA (wt/vol) and then preincubated for 30 min at 37°C in HEPES-buffered Ringer solution, pH 7.4, supplemented with 11.1 mM glucose, MEM amino acids, 5 mg/ml BSA, and 0.1 mg/ml soybean trypsin inhibitor. Buffers were gassed with 100% O2. Acini were then kept resting at room temperature for 2 h with and without the indicated concentrations of CEP-1347. Incubation at 37°C was then continued, and acini were stimulated with the indicated concentrations of caerulein. After a 30-min stimulation, acini were pelleted and homogenized for assessment of MAPK, JNK, and p38 kinase activity in kinase/immunoprecipitation (IP) buffer containing 50 mM glycerophosphate, 2 mM Na3VO4, 1 mM each of NaF, EGTA, EDTA, DTT, phenylmethylsulfonyl fluoride (PMSF), 5% glycerol in PBS (pH 7.4, 150 mM NaCl, 16 mM Na2HPO4 ·2H2O, and 4 mM NaH2PO4 ·2H2O). Supernatants were used for measurement of amylase release, expressed as percent total amylase content.

In vivo experiments. Rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg). After insertion of a tail vein catheter, rats were then treated with a hyperstimulatory dose of caerulein (10 µg/kg iv). Controls received isotonic saline only.

To assess CEP-1347-induced changes in caerulein-induced pancreatic stress kinase activation, rats were treated with 3 or 30 mg/kg CEP-1347, dissolved in Solutol (Boehringer Mannheim), by subcutaneous injection 4 h before caerulein injection. In additional experiments, animals received a total of 6 or 60 mg/kg CEP-1347 divided into two doses, with 3 or 30 mg/kg given 4 and 2 h before caerulein treatment. Controls received Solutol alone.

Rats were then killed 30 min after caerulein injection, and pancreata were homogenized in stress kinase/P buffer. This time point was chosen because it is known that pancreatic extracellular signal-regulated kinase (ERK), JNK, and p38 are all strongly activated 30 min after caerulein hyperstimulation in vivo (3, 9, 22, 29, 32). Pancreatic wet weight and serum lipase and amylase levels were also determined.

To maximize JNK inhibition in vivo, animals used to assess histology all received 6 or 60 mg/kg sc CEP-1347, which was divided into two injections of 3 or 30 mg/kg, one at 4 h and one at 2 h before caerulein treatment. Pancreata were then removed, and tissue sections were stained for further analysis. Serum lipase and amylase levels as well as pancreatic wet weight were also determined. At these later time points, caerulein-induced pancreatic stress kinase activity was no longer elevated as reported previously (22, 32); these data are not shown.

Preparation of GST fusion proteins and measurement of ERK and JNK by in-gel kinase assay. For measurement of JNK activation, AA-(1—79) c-Jun substrate was bacterially expressed as GST fusion protein and purified according to previously published protocols (22, 32). In-gel kinase assays for ERK and JNK, using myelin basic protein and AA-(1—79) GST c-Jun as substrates, respectively, were also performed according to previously published protocols (3, 4, 22, 32).

To assess effects of caerulein and CEP-1347 on the p38 MAPK pathway, activation of MAPK activated protein kinase 2 was assessed following immunoprecipitation in an in vitro assay using recombinant heat shock protein 27 as substrate as described previously (12).

SDS-PAGE. One-dimensional gel electrophoresis was performed according to the method of Laemmli (19) as previously described (24). Five micrograms of protein were loaded per lane.

Quantitative analysis of kinase activation. SDS gels were dried, and incorporation of 32P into kinase substrates was visualized and quantified using a phosphorimager system (STORM860, Molecular Dynamics, Sunnyvale, CA).

Light microscopy and histological evaluation. For histological evaluation, freshly removed pancreata were formalin (4%) fixed, ethanol dehydrated, and embedded in paraffin. Six-micrometer slices were then stained with hematoxylin and eosin and subjected to conventional light microscopy. Sections for quantification were prepared directly from frozen pancreata in the Department of Pathology (University of Bern) without awareness of the underlying treatment. Severity of pancreatitis was assessed by a pathologist (L. Mazzucchelli), who was blinded to the experimental protocol. Assessment of histological alterations was performed as previously described (33). Pancreatitis severity was quantified using seven parameters, including acidophilia and vesiculation and vacuolization of acinar cells as well as hyperemia, edema, and granulocyte and round cell infiltration of the interstitial space. According to severity, a score of 0–3 could be given for each parameter. The maximum score was 21.Pancreata from normal, untreated controls on average were given a score of 3.

Serum amylase and lipase measurements. Measurement of serum amylase and lipase activity was performed using commercially available kits (Boehringer Mannheim) following the manufacturer's instructions.

CEP-1347 serum level measurements. Serum samples were removed from the tail vein and stored at -20°C until analyses were performed. To maximize the stability of CEP-1347 in this biological matrix, l-ascorbic acid (1.2%) was added before drug extraction. Also, because CEP-1347 is a light-sensitive compound, samples were protected from exposure to light at all times. Drug was extracted in ethyl acetate-methylene chloride (4:1, vol/vol). Serum levels of CEP-1347 were measured by reverse-phase chromatography on a Zorbax Rx C18 column (Hewlett-Packard, Rockville, MD; 5 µm, 4.6 mm ID × 12.5 mm). The isocratic mobile phase consisted of 58.3% acetonitrile, 33.3% HPLC grade water, and 8.3% methanol (vol/vol/vol) and was pumped at a flow rate of 1.0 ml/min. The eluant was monitored by a fluorescent detector (gamma excitation at 303 nm; gamma emission was total emission with a 360-nm cut-off filter). The quantifiable range of the assay was from 4.00 to 100 ng/ml.
RESULTS

CEP-1347 inhibits caerulein-induced JNK activation but not amylase release in acutely isolated rat pancreatic acini. To assess whether CEP-1347 inhibits JNK activation in our system, acutely isolated acini were used. Although a high dose of caerulein (10 nM) led to strong activation of p54 and p46 isoforms of JNK (Fig. 1, lanes 3 and 4), pretreatment with CEP-1347 dose dependently reduced caerulein-induced JNK activation (Fig. 1, lanes 5–12). CEP-1347 at 20 nM had little effect, whereas 2 µM nearly and 20 µM CEP-1347 completely abolished caerulein-induced JNK activation. CEP-1347 at 200 nM inhibited JNK by ~50%.

Acini not exposed to hyperstimulation stress showed very little JNK activation (Fig. 1, lanes 1 and 2). Although our data show a clear dose-response relationship for inhibition of caerulein-induced acinar JNK activation, the concentrations needed to achieve significant or complete inhibition were between 5- and 10-fold higher than those previously reported for inhibition of JNK activity in neuronal cell lines (IC50 = 50 nM, Refs. 7, 21). There was no apparent inhibition of caerulein-induced ERK or p38 activation in acini in vitro (not shown, see Fig. 3 for lack of effects on ERK and p38 in vivo). Caerulein-induced amylase release was completely unaffected by pretreatment with 20 µM CEP-1347 (Fig. 2).

CEP-1347 dose dependently inhibits pancreatic JNK activation in vivo. To assess effects of CEP-1347 on caerulein-induced JNK activation in the pancreas in vivo, rats were treated with 3, 6, 30, or 60 mg/kg CEP-1347 before caerulein hyperstimulation (10 µg/kg iv). According to the time course of caerulein-induced activation of pancreatic stress kinases (3, 4, 22, 29, 32), animals were then killed 30 min after caerulein treatment. Similar to the in vitro situation, very little to no JNK activity was apparent in pancreata of control rats, whereas caerulein hyperstimulation induced strong JNK activation (Fig. 3A, top, lanes 1 and 2). CEP-1347 at 3 mg/kg sc 4 h before caerulein treatment only weakly inhibited caerulein-induced JNK activation, whereas 30 mg/kg CEP-1347 led to 50% inhibition (Fig. 3A, top, Fig. 4). Even greater inhibition down to 25% of caerulein-induced JNK activation seen without CEP-1347 pretreatment could be achieved by giving two subcutaneous injections of 30 mg/kg CEP-1347, one at 4 h and one at 2 h before caerulein treatment, amounting to a total dose 60 mg/kg CEP-1347 (Fig. 3A, top, lanes 7 and 8, and Fig. 4). Effects of amounts of CEP-1347 even greater than 60 mg/kg were not assessed due to concerns about specificity. However, similar to the in vitro situation, even at the highest dose of CEP-1347 at 60 mg/kg, caerulein-induced pancreatic ERK and also p38 activation were not appreciably reduced by CEP-1347 pretreatment (Fig. 3A, middle and bottom). Subcutaneous injection of the solvent Solutol alone had no effect on pancreatic stress kinase activation (not shown).

To further assess the relation between CEP-1347 concentration and reduction of caerulein-induced JNK activation, we also measured serum levels of CEP-1347 at the time of death. Thus, 4.5 h after 3 mg/kg sc CEP-1347, serum levels ranged between 20 and 40 ng/ml. After administration of 6 mg/kg, serum levels were ~60 ng/ml. The corresponding serum levels after 30 and 60 mg/kg CEP-1347 ranged between 200 and 800 ng/ml. As can be seen in Fig. 5, there was a very good correlation between actual CEP-1347 serum levels and degree of inhibition of caerulein-induced pancreatic JNK activation.

CEP-1347 treatment reduces pancreatic edema and histological severity of caerulein pancreatitis. To further assess CEP-1347-mediated effects on biological parameters of hyperstimulation stress pancreatitis, pancreatic wet weight as well as serum amylase and lipase levels were also assessed in the same animals used for determination of CEP-1347-mediated pancreatic JNK inhibition. Interestingly, parallel to the ob-

Statistical analysis. Analysis was done using standard software (Sigma Plot, Systat). Data were compared by Student's t-test; P < 0.05 was considered significant.
served inhibition of caerulein hyperstimulation-induced JNK activation 30 min after caerulein treatment. Animals were treated with the indicated amounts of CEP-1347 (subcutaneously) before caerulein hyperstimulation. A: dose response of CEP-1347 treatment on caerulein-induced activation of JNK (top), extracellular signal-regulated kinase (ERK; middle), and p38 (bottom). CEP-1347 treatment reduces caerulein-induced JNK activation dose dependently (lanes 3-8) without apparent effects on ERK or p38 (middle and bottom). HSP27, heat shock protein 27. B: effects of maximal CEP-1347 pretreatment on JNK (top), ERK (middle), and p38 (bottom) with and without caerulein. CEP-1347 has no apparent effect on basal pancreatic JNK, ERK, and p38 activity. Samples from 2 representative independent experiments out of up to 10 are shown.

Fig. 4. CEP-1347 treatment reduces both hyperstimulation-induced JNK activation and hyperstimulation-induced pancreatic wet weight increase but does not reduce hyperstimulation-induced increase of serum lipase levels 30 min after caerulein treatment. To assess edema formation, pancreata from animals used for measurement of caerulein-induced JNK activation were weighed before homogenization. Reduction of pancreatic JNK activation through CEP-1347 treatment was quantitatively determined with a phosphorimager. Serum samples for determination of lipase levels were also collected. Data are expressed as percent reduction of caerulein-induced effects, since CEP-1347 pretreatment decreased caerulein-induced pancreatic wet weight (open bars) and JNK activation (solid bars). In contrast, CEP-1347 pretreatment did not significantly inhibit caerulein-induced increase of serum lipase levels (gray bars).

Fig. 5. Correlation between CEP-1347 serum levels and reduction of caerulein-induced pancreatic JNK activation 30 min after caerulein treatment. Pancreatic JNK activation was quantified with a phosphorimager and plotted against CEP-1347 serum levels at the time of death. Correlation was determined using standard software (Sigma Plot).
Thus effects of CEP-1347 pretreatment on caerulein-induced pancreatitis appeared to divergently affect two typical endpoints of pancreatitis. The secretory disturbance, expressed as the increase in serum digestive enzyme levels, on one hand was unaffected by CEP-1347 pretreatment. On the other hand, the accumulation of interstitial fluid and edema, expressed as increased wet weight, was strongly reduced. Therefore, histological assessment of caerulein-induced pancreatitis in animals killed 2 and 4 h after caerulein hyperstimulation with and without CEP-1347 pretreatment was also performed. Pancreatic wet weight and serum amylase and lipase levels were again determined. In this set of experiments, all animals received two CEP-1347 injections, one at 4 h and one at 2 h before caerulein treatment. Similar to the effects of CEP-1347 pretreatment on biological parameters of pancreatitis observed at 30 min after caerulein hyperstimulation, a high dose of CEP-1347 (60 mg/kg) reduced the increase in pancreatic wet weight both at 2 and 4 h after caerulein treatment, whereas low-dose CEP-1347 (6 mg/kg) pretreatment had little effect on caerulein-induced pancreatic wet weight after hyperstimulation. After pretreatment with 60 mg/kg CEP-1347, pancreatic wet weight after caerulein hyperstimulation now ranged between 0.61 and 0.99 g. pancreatic edema formation. However, serum lipase levels were not significantly reduced by CEP-1347 treatment. Data from four independent experiments.

Fig. 6. CEP-1347 pretreatment reduces caerulein-induced increase of pancreatic wet weight but not serum lipase levels 2 and 4 h after caerulein-induced hyperstimulation. Animals were pretreated with 6 or 60 mg/kg CEP-1347 and killed at 2 and 4 h after caerulein hyperstimulation for assessment of histological changes (Fig. 7) as well as wet weight and serum lipase levels. Data are depicted as in Fig. 4 for better comparison. Low-dose CEP-1347 (6 mg/kg) pretreatment had little effect on caerulein-induced pancreatic wet weight increase, whereas high-dose CEP-1347 (60 mg/kg) reduced pancreatic edema formation. However, serum lipase levels were not significantly reduced by CEP-1347 treatment. Data are from four independent experiments.

DISCUSSION

In our study, we have investigated effects of the novel JNK inhibitor CEP-1347 on caerulein-induced pancreatic JNK activation in vitro and in vivo as well as on biological parameters of hyperstimulation pancreatitis. Similar to previously published data on CEP-1347-mediated JNK inhibition in neural cell lines, CEP-1347 also inhibited caerulein-induced JNK activation in acutely isolated acini. However, the amounts of CEP-1347 needed to achieve inhibition in isolated acini were between 5- and 10-fold higher compared with cell culture systems. The reasons for this difference are unclear but might involve less efficient penetration of CEP-1347 through acinar cell membranes as well as possibly higher amounts of JNK present in acini. In addition, CEP-1347 does not inhibit JNK directly but interferes with the JNK cascade upstream of JNK itself (21). Because there are several upstream kinases such as MAPK kinase kinase (MKK)4, MKK7, or MKK1, which can all activate JNK, it is also possible that the different active doses of CEP-1347 needed to inhibit JNK activation may reflect different mechanisms of activation in pancreatic acinar cells. CEP-1347-induced inhibition of acinar JNK activation was, however, clearly dose dependent and despite the relatively high amounts needed for inhibition also specific with respect to other dually phosphorylated kinases such as ERK and p38.

As reported previously (3, 9, 22, 32), in our study, pancreatic JNK was only activated following secretory hyperstimulation in vitro and in vivo. This is interesting because it is believed that, in acini, the CCK receptor has different affinity states, mediating different responses to low-dose and high-dose treatment with CCK receptor agonists (6). Thus lower, secretory amounts of CCK or caerulein mainly act through the high-affinity state to induce secretion of digestive enzymes. In contrast, supramaximal, hyperstimulatory...
doses, acting through the low-affinity state of the CCK receptor, produce a blockade of digestive enzyme release and lead to the second, descending phase of the secretory-hormone dose response curve (Fig. 2). It is further thought that the appearance of digestive enzymes in the blood during pancreatitis is another expression of the low-affinity CCK receptor-mediated secretory disturbance observed during pancreatitis (6), and the low-affinity state of the CCK receptor has been implicated in mediating signals leading to hyperstimulation pancreatitis in vivo (27).

In contrast to p38 and ERK, which both show basal activity in the pancreas and are activated by submaximal amounts of secretagogues (22, 32), pancreatic JNK is exclusively regulated in response to hyperstimulation. This raised the possibility that JNK activation, triggered through the low-affinity CCK receptor, might be important for signaling of the hyperstimulation-induced secretory disturbance. However, inhibition of caerulein-induced JNK activation did not measurably influence the ascending or the descending phase of the caerulein dose-response curve for acinar amylase release (Fig. 2). Thus, in accordance with recent data on pancreatic p38 (22, 29, 32), pancreatically phosphorylated kinases are clearly not involved in hormone-regulated stimulus-secretion coupling in pancreatic acinar cells.

This is also evident from the observation that JNK inhibition does not influence the appearance of pancreatic digestive enzymes in the blood following caerulein hyperstimulation in vivo. However, although JNK inhibition had no effect on the hyperstimulation-induced secretory disturbance, other sequelae typically observed during caerulein pancreatitis were clearly reduced. In particular, CEP-1347 treatment reduced accumulation of interstitial fluid and pancreatic edema formation. Clearly, even after CEP-1347 treatment, histological appearance and edema during caerulein pancreatitis were only ameliorated but not abolished. This might indicate only a modulatory role of the JNK cascade for the determination of severity of hyperstimulation-induced pancreatitis. However, in contrast to our in vitro experiments in isolated acini, only partial inhibition (75%) of pancreatic JNK activation during pancreatitis induction could be achieved in vivo. Thus it is possible that complete JNK inhibition might have resulted in even greater effects on histological changes and edema formation.

In view of the unaffected increase in serum digestive enzyme levels after caerulein hyperstimulation together with the reduction of pancreatic edema formation and histological severity, we therefore speculate that inhibition of JNK activation through CEP-1347 might dissociate the secretory disturbance within acinar cells.

Fig. 7. CEP-1347 pretreatment reduces caerulein hyperstimulation-induced interstitial fluid accumulation. Animals were treated as in Fig. 6 with and without 60 mg/kg CEP-1347. Pancreata were then removed 2 or 4 h after caerulein hyperstimulation, and tissue sections were stained with hematoxylin and eosin for light microscopy. Original magnification = ×50. Although caerulein hyperstimulation induced typical changes such as vacuolization and interstitial edema (20) in animals killed 2 and 4 h after treatment (A, left), edema formation was particularly reduced following CEP-1347 pretreatment (A, right). B: for comparison, a tissue section from an untreated rat is also shown. Tissue sections are representative of 4 independent experiments. C: tissue sections from pancreata were evaluated by a blinded pathologist and caerulein-induced histological alterations were quantified as described in the text. Pretreatment with 60 mg/kg CEP-1347 reduced the histology score by 50% (P < 0.05 2 and 4 h after caerulein), whereas 6 mg/kg had no significant effect on histological scoring. Data are from 4 independent experiments.
nar cells from other, inflammatory sequela typically observed during pancreatitis. This is interesting because it is now believed that development of pancreatitis involves several steps (30). After the causative agent such as hyperstimulation has induced pancreatic damage, the second step involves an inflammatory reaction with increased vascular permeability, production of cytokines, and migration of macrophages and lymphocytes in the pancreas (23, 30). It is this secondary reaction that finally determines the actual severity of pancreatitis, which may range from a mild, localized edematous disease to a systemic inflammatory response syndrome with multiorgan failure. Although the determinants of the severity of the inflammatory response during acute pancreatitis are unclear, recent data indicate that the pancreas itself may actively participate in triggering an inflammatory reaction through production of chemokines (10).

In conclusion, we show for the first time that CEP-1347 treatment reduces stress-induced JNK activation not only in vitro but also in vivo. JNK activation appears to play no role in acinar stimulus-secretion coupling or the secretory blockade observed during pancreatitis. On the other hand, we provide evidence that JNK activation during hyperstimulation stress might represent an important signal for the occurrence of the inflammatory response, observed during pancreatitis.

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REFERENCES


