BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **227,** 1–7 (1996)
ARTICLE NO. 1458

Stress-Activated Protein Kinase Activation Is the Earliest Direct Correlate to the Induction of Secretagogue-Induced Pancreatitis in Rats

Terrence Grady, Andrzej Dabrowski, John A. Williams, and Craig D. Logsdon

Department of Physiology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0622

Received August 22, 1996

We compared the cellular events induced by hyperstimulation of rats with caerulein which induces acute pancreatitis, to bombesin, which does not induce pancreatitis. Both secretogogues induced the intracellular activation of trypsinogen and the colocalization of lysosomal hydrolases and zymogen granules within 10–15 minutes. These data indicate that these parameters, previously thought to be crucial initiating events of pancreatitis, are not definitive cellular markers of the disease. We then compared the abilities of the two secretagogues to activate stress-activated protein kinase (SAPK). Significant effects of caerulein hyperstimulation on SAPK activity were observed within 5 minutes, the maximum (57-fold) activation was evident after 15 minutes, and levels remained above control for at least 3 hours. In comparison, hyperstimulation with bombesin induced a maximal 5-fold increase of SAPK activity which returned to basal within one hour. These data indicate that SAPK activity is the earliest and best correlated cellular marker associated with secretagogue-induced pancreatitis.

The earliest factors involved in the development of acute pancreatitis remain a mystery. Many experimental *in vivo* models of pancreatitis are available for the study of this disease process, and the secretagogue-hyperstimulation model offers the greatest control over the earliest initiating events. When CCK, or its stable analogue caerulein, is administered in a supraphysiologic dose, a non-lethal edematous pancreatitis quickly develops. This model reproduces many features of the human disease with its sequelae of elevated pancreatic enzyme concentrations in the serum, increased pancreatic edema, and histological features of inflammation including leukocyte infiltration, cell death, minor hemorrhage and extensive cytoplasmic vacuolization (1). Previous studies show that acetylcholine can also induce pancreatitis when administered in high doses (2). Both CCK and acetylcholine exhibit biphasic dose response curves for enzyme secretion, whereas bombesin or the CCK analog JMV-180 have monophasic dose-response curves for secretion (3). It has previously been noted in mice that there is a relationship between the abilities of secretagogues to induce high-dose inhibition and to cause pancreatitis (4). These potential differences in the abilities of secretagogues to induce pancreatitis was the starting point for our investigations into the earliest mechanisms involved in the initiation of pancreatitis.

Initially we confirmed the predicted differences in the abilities of caerulein and bombesin to induce pancreatitis in rats. Bombesin did not induce any of the common parameters of the disease. It is widely assumed that a contributing factor in the pathophysiology of acute pancreatitis is the intracellular activation of pancreatic zymogens to their active forms. Therefore, we compared the abilities of these two secretagogues to stimulate intrapancreatic zymogen activation and lysosomal enzyme redistribution. Surprisingly, we found that both of these cellular parameters were induced nearly equally by supraphysiological doses of either caerulein or bombesin. Thus, we next tested the possibility that high concentrations of caerulein might activate signaling pathways distinct from those activated by bombesin. The pathways typically thought to be involved in the secretory effects of these hormones, such as activation of phospholipase C and protein kinase C, do not provide insight into pancreatitis, as both hormones

0006-291X/96 $18.00

appear equally able to activate these pathways (5). Therefore, we focused on the abilities of the two secretagogues to activate pathways involved in mediating a stress response.

A major cellular pathway stimulated by cell stress involves the stress activated protein kinase (SAPK) signal cascade. SAPKs, also called c-Jun amino-terminal kinases, are related to the MAP kinases and are the dominant c-Jun amino-terminal protein kinases activated in response to stressful stimuli including bacterial endotoxins or ultraviolet irradiation (6). SAPKs can also be activated by inhibitors of protein synthesis, such as anisomycin, inflammatory cytokines such as TNF-α, heat shock, or changes in osmolarity (7). We have recently shown that CCK can activate this pathway in pancreatic acinar cells (8). In the current study we found that while SAPK activity was increased by both secretagogues, the effects of supraphysiological concentrations of caerulein were much greater (57-fold versus 5-fold) and more prolonged ($>$3 hours versus 1 hour) than those of bombesin. These data indicate that hyper-activation of SAPK within the pancreatic tissue is the earliest observable event known to correlate with the development of pancreatitis. Thus, SAPK activity may act as an intracellular marker of pancreatitis and may provide a link between CCK receptor activation and the other events that occur in pancreatitis.

METHODS

Materials. Male Wistar rats (100-125g) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The trypsin substrate (Boc-Gln-Ala-Arg-MCA) and chymotrypsin substrate (Suc-Ala-Ala-Pro-Phe-MCA) were purchased from Peptides International, Inc. (Louisville, KY). The cathepsin B substrate Z-Arg-Arg-βNA salt was purchased from Bachem Bioscience, Inc. (King of Prussia, PA). Guanidine hydrochloride was from GIBCO-BRL; leupeptin and aprotinin were from Boehringer Mannheim; [γ-^{32}P] ATP (3000 Ci/mmol) was from New England Nuclear (Wilmington, DE); caerulein and CCK-JMV-180 were from Research Plus (Bayonne, NJ). All other reagents of the highest purity were obtained from Sigma (St. Louis, MO). The SAPK lysis buffer was prepared fresh just before use and kept ice-cold [50 mM β-glycerophosphate, 1.5 mM ethyleneglycol-bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), 1 mM phenylmethylsulfonyl fluoride(PMSF), 1 mM Na_3VO_4, 1 mM dithiotreitol (DTT), 10 μg/ml leupeptin, and 10 μg/ml aprotinin, pH 7.4]. The fractionation buffer contained 250 mM sucrose, 1mM $MgSO_4$, and 5mM 3-(N-morpholino)-propanesulfonic acid (MOPS) pH 6.5 at 4°C. Protein was assayed by the Bio-Rad protein assay reagent.

In vivo stimulation with secretagogues. Under sterile conditions, rats were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg). A PE-50 polyethylene cannula (Biolab Products, Lake Havasu City, AZ) was introduced through the femoral vein into the inferior vena cava. The cannula was plugged, tunneled subcutaneously and brought out behind the head in the cervical region to allow complete freedom of movement. Cannula patency was maintained by a heparin lock (1000 U/ml), and the animal was allowed to recover overnight, fasted with water *ad libitum.* The animals were housed individually in shoe box cages and remained conscious and unrestrained throughout the experimental period. The following morning, the cannula patency was confirmed by the entry of blood into the cannula which was then flushed with 0.9% NaCl. For measurement of serum enzymes, pancreatic edema and histological examination, all well accepted indicators of pancreatitis, rats with patent cannulas were infused with either 0.9% NaCl (controls), a supramaximal dose of caerulein equivalent to 10 μg/kg/h, or a supramaximal dose of bombesin equivalent to 500 μg/kg/h for 5, 10, 15, 30, 60, or 180 min. The secretagogues used throughout this study were dissolved in 0.9% NaCl saline and sterilized by filtration through a 0.22μm filter prior to each infusion.

Collection of blood and determination of serum amylase and lipase. Blood was collected by cardiac puncture while the rats were anesthetized with pentobarbital. The whole blood was allowed to clot, centrifuged at 10,000×g for 5 min, then stored at −20°C until assayed for amylase activity by the method of Ceska *et al.* (9). Lipase activity was determined by the method of Tietz *et al.* (10).

Determination of pancreatic edema. Pancreatic edema was quantified by weighing the freshly harvested tissue (wet weight) and comparing that to the same sample after desiccation to a constant weight at 90°C for 36 hours (dry weight). The results were calculated and expressed as a percentage (wet weight-dry weight / wet weight ×100).

Measurement of active zymogens in pancreatic homogenates. Pancreatic tissue samples were homogenized in ice-cold fractionation buffer with the use of a Potter-Elvehjem homogenizer. The lysates were subjected to differential subcellular fractionation according to the modified method of Tartakoff and Jamieson (11,12). Active trypsin and chymotrypsin were measured in pancreatic homogenates prior to the differential centrifugation. Trypsin and chymotrypsin activity were assayed fluorimetrically using Boc-Gln-Ala-Arg-MCA and Suc-Ala-Ala-Pro-Phe-MCA as substrates, respectively. The total homogenates were kept in ice-cold fractionation buffer until used in the fluorescent assay. For both hydrolases, a sample of the homogenate was mixed with the assay buffer (50mM TRIS, 150mM NaCl, 1mM $CaCl_2$, and 0.1% BSA, pH 8.0) and incubated to 37°C. The respective peptidyl-MCA substrate was then added, and

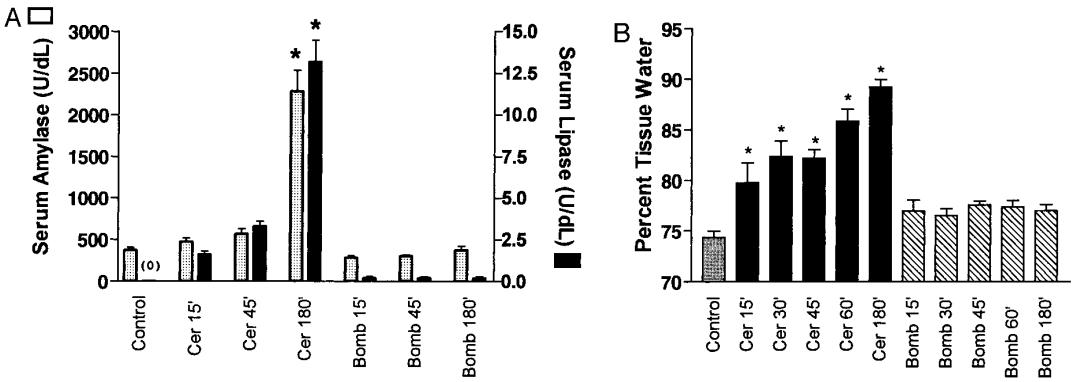

FIG. 1. Effects of caerulein and bombesin on parameters of pancreatitis in the rat. Rats were infused for the indicated times with saline (Control), caerulein (Cer) (10 μg/kg/h), or bombesin (Bomb) (500 μg/kg/h). Panel A shows serum amylase (dotted bars) and lipase (solid bars) values after the infusion while panel B shows the pancreatic edema determination as described in the text. Data shown are means ± standard error of the mean for at least four animals in each group (*$p<0.05$ relative to Control values).

the fluorescence emitted at 460nm was immediately recorded using a SPEX DM 3000 fluorometer following excitation at 380nm. The enzyme activity in the homogenates was calculated using standard curves produced using purified enzymes in an identical manner, and the activity was normalized to the protein content of the homogenates.

Measurement of subcellular cathepsin B distribution. The cathepsin B activity in each enriched fraction was determined by the method of McDonald and Ellis and is expressed as a percent of the activity in the total homogenates, *i.e.* that homogenate before the differential centrifugation process (13). The results are presented as a ratio of the cathepsin B activity in the zymogen granule fraction to that activity in the lysosomal fraction. A larger value is indicative of a redistribution to the more dense zymogen granule fraction.

Measurement of SAPK activity. Rats were infused with either 0.9% NaCl (controls), a bolus secretory dose of caerulein equivalent to 0.1 μg/kg/h, a supramaximal dose of caerulein equivalent to 10 μg/kg/h, or a supramaximal dose of bombesin equivalent to 500 μg/kg/h for several different time points. To confirm the maximal effects on SAPK activity by another secretagogue which does not cause pancreatitis, CCK-JMV-180 was infused at a supermaximal dose equivalent to 5 mg/kg/h for 15 min. The animals were sacrificed by exsanguination and pancreatic tissue samples were homogenized in ice-cold lysis buffer. The lysates were then centrifuged in a Beckman GPR swinging bucket centrifuge (2000×g at 4°C for 15 min) and the supernatant was assayed for SAPK activity.

Kinase assays in SDS-polyacrylamide gels were performed using our modified method of Kameshita and Fujisawa (8,14). Briefly, the pancreatic extracts were boiled in sample buffer for 5 min, and equal amounts (40 μg protein) were subjected to SDS-PAGE in polyacrylamide gels (10%) containing 0.3 mg/ml of glutathione *S*-transferase (GST)-c-$Jun_{1\text{-}79}$ fusion protein (15). After washing, the renatured gel was incubated in the kinase buffer (40mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) pH 8.0, 10mM $MgCl_2$, 2mM DTT, and 0.1mM EGTA) which contained 20μM ATP and 90μCi [γ-^{32}P]ATP. The activity of the SAPK kinases were then visualized and quantified in the dried gels by the use of a GS-250 Molecular Imager System (Bio-Rad Laboratories, Inc.).

Statistical analysis. The results are presented as the mean ± standard error of the mean (SEM) for at least four separate animals in each group. Differences between the treated groups and the control groups were considered to be significant when $p<0.05$ as determined by analysis of variance (ANOVA). Dunnet's multiple comparison post-hoc test was utilized to assess the difference between individual groups.

RESULTS

In vivo stimulation with secretagogues. The infusion of a supraphysiologic dose of caerulein and bombesin had distinct effects on the exocrine pancreas of rats. Caerulein hyperstimulation resulted in the formation of an edematous pancreatitis, as defined by the increase in tissue water content and increased digestive enzyme levels in the serum (FIG 1). In contrast, bombesin hyperstimulation induced none of the parameters indicative of acute pancreatitis. There was no difference in serum enzyme levels or tissue edema in animals administered supraphysiologic concentrations of bombesin when compared to control animals that received saline alone (FIG 1). In addition, histologic examination of caeruelin-hyperstimulated rats revealed a gross

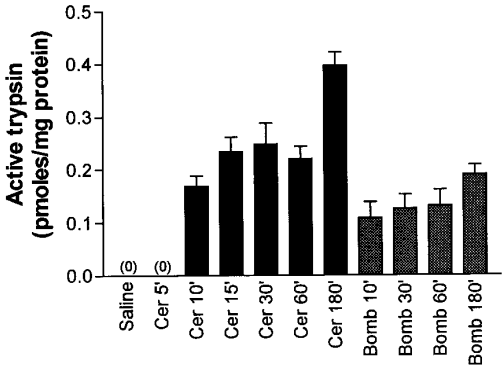

FIG. 2. Effects of caerulein and bombesin on trypsinogen activation. Male Wistar rats were infused intravenously with saline, caerulein equivalent to 10 μg/kg/h (Cer), or bombesin equivalent to 500 μg/kg/h (Bomb) for the times indicated. Data shown are means $\pm$ standard error of the mean for at least four animals in each group (*$p<0.05$ relative to saline values). Intracellular activation of trypsinogen was measured using a sensitive fluorometric assay of trypsin as described in the text.

interstitial edema, acinar cell vacuolization, and an inflammatory cell infiltrate, whereas bombesin hyperstimulation revealed none of these histological changes (data not shown).

Measurement of active zymogens in pancreatic homogenates. To evaluate the usefulness of active intracellular zymogens as markers of pancreatitis we compared the abilities of caerulein and bombesin to induce zymogen activation at early time points. Both caerulein and bombesin induced the presence of activated zymogens in cellular homogenates (FIG 2). Active trypsin was first detected ten minutes after the initiation of the caerulein and bombesin hyperstimulation and the levels continued to rise for at least three hours. The cellular content of active trypsin was greater after caerulein stimulation, but the difference was not large. No active trypsin was detectable in the control animals or in those which received either secretagogue for 5 minutes. The measurement of active chymotrypsin in the same samples revealed identical findings to the active trypsin data (data not shown).

Measurement of subcellular cathepsin B distribution. The redistribution of the lysosomal hydrolase cathepsin B to the zymogen granule fraction has been postulated as a potential cause of intravacuolar digestive enzyme activation, for it occurs in other models of pancreatitis with diverse etiologies. We observed that both caerulein and bombesin caused redistribution (FIG 3). The ratio of the cathepsin B in the zymogen fraction to that in the lysosomal fraction was identical in the bombesin treated animals to that seen in the caerulein-induced pancreatitis animals after three hours hyperstimulation. The lysosomal enzyme cathepsin B was detected in the zymogen granule enriched fraction in a time dependent manner. Detectable levels observed after 15 minutes of caerulein hyperstimulation and levels continued to rise over the three hours examined.

Measurement of tissue SAPK activity. The SAPK activity detected in the pancreatic homogenates following secretagogue stimulation is presented in Figure 4. The histogram is a quantification of the respective p55 SAPK bands located at 55kD. Both caerulein and bombesin treatments significantly increased SAPK activity in rat pancreas. However, infusion of a supraphysiologic dose of caerulein resulted in a much greater increase in SAPK activity (57-fold) than did either a secretory dose of caerulein (5-fold) or a supraphysiologic dose of bombesin (5-fold) or JMV-180 (4-fold) (FIG 4). Furthermore, the effect of hyperstimulation with caerulein were more prolonged than those of bombesin. In both cases maximal effects on SAPK were observed after 15 minutes stimulation. SAPK activity returned to basal within 1 hour

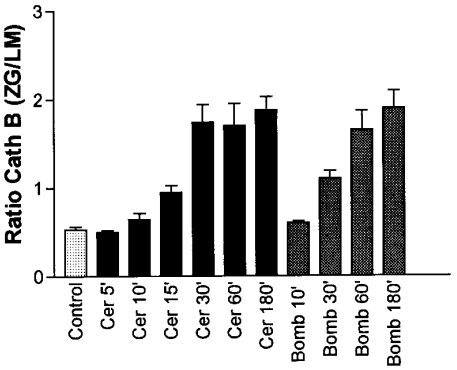

FIG. 3. Effects of caerulein and bombesin on lysosomal enzyme redistribution. Male Wistar rats were infused intravenously with saline (Control), caerulein equivalent to 10 μg/kg/h (Cer), or bombesin equivalent to 500 μg/kg/h which is a supraphysiologic dose (Bomb) for the times indicated. Data shown are means $\pm$ standard error of the mean for at least four animals in each group (*$p<0.05$ relative to Control values). The subcellular redistribution of the lysosomal hydrolase cathepsin B to the more dense zymogen granule fraction was determined by subcellular fractionation.

after treatment a supraphysiologic concentration of bombesin. In contrast, SAPK activity remained elevated for at least 3 hours following the infusion of a supraphysiologic dose of caerulein (FIG 4).

DISCUSSION

Pancreatitis induced by caerulein-hyperstimulation is a widely accepted and well characterized model for examining the early events in the development of pancreatitis (1,16,17). Our investigation utilized secretagogues with different abilities to induce pancreatitis to determine

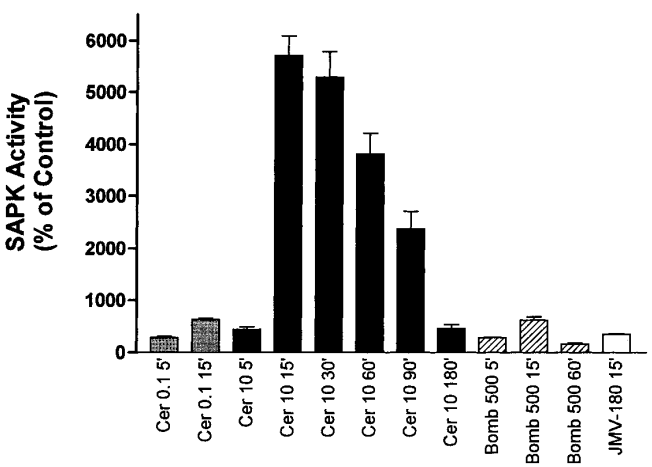

FIG. 4. Effects of pancreatic secretagogues on tissue SAPK activity. Male Wistar rats were infused intravenously with saline, caerulein equivalent to 0.1 μg/kg/h which is a maximal secretory dose (Cer 0.1), caerulein equivalent to 10 μg/kg/h which is a supraphysiologic dose (Cer 10), bombesin equivalent to 500 μg/kg/h which is a supraphysiologic dose (Bomb), or JMV-180 equivalent to 5 mg/kg/h which is a supraphysiologic dose for the times indicated. Data shown are means $\pm$ standard error of the mean for at least four animals in each group. SAPK activity was determined using an in-gel kinase assay as described in text.

which intracellular changes best correlate with the initiation of this disease. We found that none of the previously identified cellular parameters accurately correlated with the ability of a secretagogue to induce pancreatitis. In contrast, the abilities of the secretagogues to stimulate large and prolonged increases in SAPK activity correlated well with their abilities to induce pancreatitis. Thus, SAPK activity is the earliest detectable event directly linked to the initiation of acute pancreatitis.

While SAPK activity was mildly elevated over the basal state in rats stimulated with a secretory dose of caerulein or a supramaximal dose of bombesin or JMV-180, these effects did not match the tremendous increase in SAPK activity seen with a supraphysiologic dose of caerulein. These observations confirm and extend our previous studies on the detection of activated SAPK in dispersed acinar cells (8). It may be that the mild elevation observed with physiologic concentrations of caerulein indicate that there is crosstalk between the SAPK and MAPK pathways, the latter of which we have reported to be regulated by CCK both *in vivo* and *in vitro* (8). A recent report described Ras as an upstream mediator of both SAPK and MAPK (18). The additional upstream regulators that determine which of these pathways is activated to a greater extent by secretagogues are yet to be identified. Furthermore, we have reported that CCK and bombesin stimulate c-*jun* expression in rat pancreatic acini, and this activation was magnified with increasing concentrations of CCK (19). Numerous studies show that SAPK is the major regulator of the transcription factor c-*jun* (20-23). Thus, the mild activation of the SAPK pathway may represent a physiological event under secretagogue control, and high concentrations of CCK may overstimulate SAPK activation in a pathophysiologic response. In the present study we have demonstrated for the first time that CCK activates SAPK in a manner which correlates strongly with the initiation of pancreatitis. A similar response to cellular stress was recently observed by others in a rat model of kidney ischemia-reperfusion injury (22). These authors suggested that strong activation of SAPK very early after reperfusion of an ischemic kidney may transduce an important signal to the nucleus and trigger the genetic response to ischemia. The complex cellular responses to the events preceding the development of acute pancreatitis may be regulated in a similar manner.

It has long been postulated that the inappropriate activation of digestive enzymes within the pancreas was the sole initiating event. However, this study provides evidence suggesting otherwise. Infusion of supramaximally stimulating dose of caerulein for 3 hours resulted in an edematous pancreatitis, whereas bombesin hyperstimulation had no deleterious effects on the pancreas. Yet, active trypsin was detected in whole tissue homogenates following both caerulein *and* bombesin hyperstimulation. The bombesin treated rats revealed an active trypsin level that was 50% of that seen in the homogenates of caerulein-hyperstimulated rats. This correlates with earlier work done *in vitro* where active zymogens were identified with high doses of caerulein and bombesin, and where the active enzymes in the bombesin treated acini were found outside the cells in the incubation media while the caerulein stimulated cells retained their active enzymes (24). The mechanisms underlying the activation of zymogens within the acinar cell and interstitial spaces are unclear, but the time-course studies presented here indicate SAPK activation precedes the detection of active zymogens.

Cell fractionation studies have shown that in several models of acute pancreatitis lysosomal hydrolases are redistributed into a fraction that contains zymogen granules along with the appearance of large vacuoles within the apical regions of acinar cells. These changes occur within 30 minutes which precedes the appearance of cell injury and other parameters of pancreatitis (16,25). Thus, one theory of zymogen activation involves the activation of trypsinogen by cathepsin B, a lysosomal hydrolase shown to activate trypsinogen *in vitro,* which would then initiate the zymogen activation cascade. However, this remains controversial, as infusion of inhibitors that nearly eliminated lysosomal activity did not prevent the development of pancreatitis (26,27), and a recent study investigating models of biliary pancreatitis revealed

no apparent correlation to this redistribution and the onset of pancreatitis (28). In the current study hyperstimulation with bombesin led to a similar lysosomal redistribution as seen with caerulein while showing none of the symptoms of pancreatitis. It is apparent that, like zymogen activation, the colocalization of lysosomal hydrolases and secretory enzymes occur even under conditions in which pancreatitis does not develop, and the two may represent parallel physiological events. Thus, it does not appear that lysosomal redistribution is important to the initiation of pancreatitis, and it may be a cellular housekeeping process in response to activated zymogens.

By whatever mechanism the edema and digestive enzymes within the serum arise, it appears that the activation of SAPK within the pancreatic tissue precedes the cellular changes leading to those pathophysiological events. Moreover, this pathway is activated within 5 minutes of caerulein hyperstimulation, making it the earliest known event which directly correlates with initiation of pancreatitis. Because SAPK activation can be measured in isolated acinar cells, *in vitro* studies will provide the opportunity to manipulate the SAPK pathway. This opens the possibility of developing an *in vitro* model of pancreatitis which would allow for more detailed cellular manipulation into factors initiating this disease process and exploration of possible therapeutic interventions.

ACKNOWLEDGMENTS

This work was supported by NIH Grants DK 41122, DK 41225, and DK 35912, a National Research Service Award (DK 09273) to T.G., and by the Michigan Gastrointestinal Peptide Center (DK 34933).

REFERENCES

1. Lampel, M., and Kern, H. F. (1977) *Virchows Arch. A. Pathol. Pathol. Anat.* **373,** 97.
2. Adler, G., Gerhards, G., Schick, J., Rohr, G., and Kern, H. F. (1983) *Am. J. Physiol.* **244,** G623–G629.
3. Williams, J. A., and Yule, D. I. (1993) The Pancreas: Biology, Pathobiology, and Disease (Go, V. L. W., *et al.,* Eds.), p. 167, Raven Press, New York.
4. Powers, R. E., Grady, T., Orchard, J. L., and Gilrane, T. B. (1993) *Pancreas* **8,** 58.
5. Matozaki, T., Zhu, W. Y., Tsunoda, Y., Goke, B., and Williams, J. A. (1991) *Am. J. Physiol.* **260,** G858.
6. Kyriakis, J. M., *et al.* (1994) *Nature* **369,** 156.
7. Derijard, B., *et al.* (1994) *Cell* **76,** 1025.
8. Dabrowski, A., Grady, T., Logsdon, C. D., and Williams, J. A. (1996) *J. Biol. Chem.* **271,** 5686.
9. Ceska, M., Birath, K., and Brown, B. (1969) *Clin. Chim. Acta* **26,** 437.
10. Tietz, N. W., and Fiereck, E. A. (1966) *Clin. Chim. Acta* **13,** 352.
11. Tartakoff, A. M., and Jamieson, J. D. (1974) *Methods Enzymol.* **31,** 41.
12. Grady, T., Saluja, A., Kaiser, A. M., and Steer, M. (1996) *Am. J. Physiol.* **271,** G20.
13. McDonald, J. K., and Ellis, S. (1975) *Life Sci.* **17,** 1269.
14. Kameshita, I., and Fujisawa, H. (1989) *Anal. Biochem.* **183,** 139.
15. Chruscinski, A. J., Link, R. E., Daunt, D. A., Barsh, G. S., and Kobilka, B. K. (1992) *Biochem. Biophys. Res. Commun.* **186,** 1280.
16. Watanabe, O., Baccino, F. M., Steer, M. L., and Meldolesi, J. (1984) *Am. J. Physiol.* **246,** G457.
17. Kloppel, G., Dreyer, T., Willemer, S., Kern, H. F., and Adler, G. (1986) *Virchows Arch. A. Pathol. Anat. Histopathol.* **409,** 791.
18. Lin, A., *et al.* (1995) *Science* **268,** 286.
19. Lu, L., and Logsdon, C. D. (1992) *Am. J. Physiol.* **263,** G327.
20. Sanchez, I., *et al.* (1994) *Nature* **372,** 794.
21. Woodgett, J. R., *et al.* (1993) *Adv. Second Messenger Phosphoprotein Res.* **28,** 261.
22. Pombo, C. M., *et al.* (1994) *J. Biol. Chem.* **269,** 26546.
23. van Dam, H., *et al.* (1995) *EMBO J.* **14,** 1798.
24. Grady, T., Gorelick, F. S., and Powers, R. E. (1992) *Pancreas* **7,** 738.
25. Koike, H., Steer, M. L., and Meldolesi, J. (1982) *Am. J. Physiol.* **242,** G297.
26. Lerch, M. M., Saluja, A. K., Dawra, R., Saluja, M., and Steer, M. L. (1993) *Gastroenterology* **104,** 1768.
27. Saluja, A. K., *et al.* (1991) *Pancreas* **6,** 718.
28. Luthen, R., Niederau, C., Niederau, M., Ferrell, L., and Grendell, J. H. (1995) *Gastroenterology* **109,** 573.