Protection against acute pancreatitis by activation of protease-activated receptor-2

Anupriya Sharma, Xiaohong Tao, Arun Gopal, Brooke Ligon, Patricia Andrade-Gordon, Michael L. Steer, and George Perides

1Department of Surgery, Tufts-New England Medical Center and Tufts University School of Medicine, Boston; 2Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts; and 3Drug Discovery, Johnson and Johnson Pharmaceutical Research and Development, Spring House, Pennsylvania

Submitted 30 July 2004; accepted in final form 23 September 2004

Protection against acute pancreatitis by activation of protease-activated receptor-2. Am J Physiol Gastrointest Liver Physiol 288: G388–G395, 2005. First published September 30, 2004; doi:10.1152/ajpgi.00341.2004.—Protease-activated receptor-2 (PAR-2) is one of the four members of the PAR family (8, 11, 18). It is proteolytically activated by trypsin and other trypsin-like serine proteases that cleave the NH2-terminal extracellular domain of the murine receptor at SKGR-SLIGRL, releasing a tethered ligand that contains the activating peptide SLIGRL. This proteolytic cleavage permits the activating peptide to bind, intramolecularly, to activate the receptor. In addition to proteolytic activation, PAR-2 responses can also be nonproteolytically elicited by exposing receptor-bearing cells to soluble preparations of the activating peptide SLIGRL. In contrast to the activating peptide, scrambled-sequence peptides and the reverse activating peptide LRGILS do not cause PAR-2 activation under these conditions (9). In addition to triggering a series of signal-transduction events and a cellular response that varies depending on the cell type, PAR-2 activation is followed by β-arrestin-mediated receptor phosphorylation, receptor internalization, and receptor degradation that together result in desensitization to further PAR-2 stimulation and downregulation of PAR-2 levels (9).

PAR-2 is widely expressed in the gastrointestinal tract, and PAR-2-mediated responses in the exocrine pancreas have been previously reported. In the exocrine pancreas, PAR-2 activation has been found to accelerate acinar cell secretion of digestive enzymes and to alter duct cell ion channel function, suggesting that both acinar and duct cells express PAR-2 (13, 17). On the basis of studies involving other tissues, it is also likely that PAR-2 is expressed by pancreatic endothelial cells, resident macrophages, and nociceptive nerves, but studies evaluating PAR-2 expression by these other elements of the exocrine pancreas have not been reported.

It is generally believed that PAR-2-mediated events are proinflammatory (24) and that interference with PAR-2-mediated events has an anti-inflammatory effect (4). Viewed from...

Address for reprint requests and other correspondence: G. Perides, Tupper 205, Dept. of Surgery #37, Tufts-New England Medical Center, 750 Washington St., Boston, MA 02111 (E-mail: gperides@tufts-nemc.org).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
that perspective, we presumed that genetic deletion of PAR-2 would protect mice from pancreatitis and that pharmacological activation of PAR-2 would worsen pancreatitis.

MATERIALS AND METHODS

Reagents. SLIGRL-NH2 and LRGILS-NH2 were synthesized by the Tufts University Core Facility (Boston, MA). Caerulein was obtained from Bachem Bioscience (King of Prussia, PA). The substrate for amylase, 2-chloro-4-nitrophenyl-a-D-malto-trioside, was obtained from Diagnostics Chemical (Oxford, CT). The fluorogenic substrate for trypsin, Boc-Gln-Ala-Arg-MCA, and amastatin were purchased from Peptides International (Louisville, KY). Anti-PAR-2 mouse monoclonal antibody SAM11, raised against amino acids 37–52 of PAR-2, was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-ERK1/2, anti-phospho ERK1/2, JNK, and anti-phospho-JNK were purchased from Cell Signaling (Beverly, MA). All other chemicals were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO).

Mice. All experiments were performed using wild-type male C57BL/6 mice (25–30 g) purchased from Charles River Laboratories (Wilmington, MA) or PAR-2−/− mice bred into the C57BL/6 background (6). The animals were housed in temperature-controlled (23 ± 2°C) rooms with a 12:12-h light/dark cycle, fed standard laboratory chow, fasted overnight before each experiment, and given water ad libitum. All experiments conformed to protocols approved by the Institutional Animal Care and Use Committee of the Tufts-New England Medical Center.

Induction of pancreatitis. Secretagogue-induced pancreatitis was elicited by giving mice 12 hourly intraperitoneal injections of caerulein (50 μg/kg per injection). Unless otherwise stated, animals were killed by CO2 asphyxiation 1 h after the final caerulein injection. For measurement of intrapancreatic trypsin activity, animals were killed 30 min after the initial caerulein injection. Mice receiving SLIGRL-NH2 were intravenously injected with 4 μmol/kg SLIGRL-NH2 along with 4 μmol/kg amastatin 1 h before the first caerulein injection and were subsequently given hourly (×12) intraperitoneal injections of SLIGRL-NH2/amastatin along with caerulein. Mice given caerulein and amastatin according to the same protocol, but not SLIGRL-NH2, served as the control group for these experiments.

Severity of pancreatitis. The severity of pancreatitis was evaluated as reported previously (12). Pancreas samples stained with hematoxylin/eosin were examined by an experienced pancreatic morphologist who was not aware of the sample identity, and the extent of acinar cell necrosis, as a percentage of acinar cell mass, was quantitated. Pancreatic edema was quantitated by measuring pancreatic water content and was expressed as a percentage of tissue wet weight. Serum amylase activity and pancreatic trypsin activity were quantitated spectrophotometrically as previously described (15, 23). Bromide-enhanced chemiluminescence was used to quantitate pancreas myeloperoxidase content as described previously (12).

Immunoblot analysis. Protein extracts were prepared by homogenizing pancreatic tissue with a Tissue Tearor (Biospec Products, Bartlesville, OK) in 10 mM Tris acetate pH 7.5 buffer containing protease inhibitors (Complete; Roche Molecular Biochemicals, Indianapolis, IN) and 1 mM PMSF. The extracted proteins were diluted in Laemmli sample buffer with 5% β-mercaptoethanol. After being boiled, 50 μg of protein were resolved in 10% polyacrylamide gels in Tris-glycine-SDS buffer. The proteins were transferred to PVDF membranes, blocked in 5% nonfat dry milk in Tris-buffered saline, pH 7.5, containing 0.05% (wt/vol) Tween 20 (TBST). Blots were incubated at 4°C overnight with the SAM11 anti-PAR-2 antibody or with anti-phospho-ERK1/2 or anti-phospho-JNK antibodies diluted in TBST (0.5 μg/ml). The membranes were washed in TBST and incubated with horseradish peroxidase-conjugated anti-rabbit IgG at 1:5,000 (vol/vol) dilution in TBST-milk for 1 h. After being washed, immunoreactive bands in the membranes were visualized by enhanced chemiluminescence (PerkinElmer Life Sciences, Boston, MA) and quantitated by densitometry. To determine the total ERK1/2 or JNK, membranes were stripped and reblotted with antibodies against ERK1/2 or JNK.

Analysis of data. Results are reported as means ± SE obtained from three or more independent experiments. In all figures, vertical bars denote SE values and the absence of vertical bars indicates that the SE values are too small to depict. Statistical evaluation of data was accomplished by analysis of variance, and P < 0.05 was considered to indicate significant differences.

RESULTS

Genetic deletion of PAR-2 worsens the severity of experimental pancreatitis. Wild-type and PAR-2−/− deficient mice were given 12 hourly injections of either saline (control) or a supramaximally stimulating dose of caerulein (50 μg/kg per injection) to induce pancreatitis, and they were killed 1 h after the final caerulein injection. Representative photomicrographs of pancreas sections taken from caerulein-treated animals are shown in Fig. 1. As has been repeatedly observed by us and others, supramaximal stimulation of wild-type mice with caerulein results in acinar cell vacuolization, pancreatic edema, and acinar cell necrosis along with an intrapancreatic inflammatory response (Fig. 1A). Samples obtained from PAR-2−/− mice that were identically treated reveal more acinar cell vacuolization, intralobular edema, and acinar cell necrosis and a greater inflammatory response (Fig. 1B). The magnitude of the changes that define pancreatitis severity were quantitated

---

Fig. 1. Genetic deletion of protease-activated receptor-2 (PAR-2) worsens the severity of secretagogue-induced pancreatitis. Wild-type (WT) and PAR-2−/− mice were given 12 hourly injections of caerulein and were killed 1 h after the last injection. Representative hematoxylin and eosin-stained samples of pancreas taken from WT (A) and PAR-2−/− mice (B) with caerulein-induced pancreatitis are shown. Note the increased acinar cell vacuolization, intralobular edema, and acinar cell necrosis in the sample taken from the PAR-2−/− mouse.
Fig. 2. Genetic deletion of PAR-2 worsens the severity of secretagogue-induced pancreatitis. WT and PAR-2−/− mice were given 12 hourly injections of caerulein (WT.C and PAR-2−/− C, respectively) and were killed 1 h later. Serum amylase activity (A), pancreatic edema (B), pancreatic myeloperoxidase activity (C), and acinar cell necrosis (D) were quantitated as described in the text. Values are means ± SE obtained from 10 mice in each group. *P < 0.01 for PAR-2-deficient mice given caerulein vs. WT mice receiving caerulein.

and, as shown in Fig. 2, pancreatic edema (i.e., pancreatic water content), pancreatic inflammation (i.e., myeloperoxidase activity in the pancreas), and acinar cell necrosis are all increased in samples obtained from PAR-2-deficient mice compared with the wild-type control animals. Serum amylase levels, which correlate poorly with the severity of pancreatitis, are increased to a similar extent in both animal groups (Fig. 2). The results indicate that genetic deletion of PAR-2 worsens the severity of secretagogue-induced pancreatitis.

PAR-2 is activated during secretagogue-induced pancreatitis. To monitor the fate of PAR-2 during caerulein-induced pancreatitis, we employed immunoblot. We first tested our ability to detect PAR-2 in the pancreas. Pancreata from wild-type and PAR-2−/− mice were removed, proteins were extracted in 20 mM Tris acetate (pH 7.4) containing protein inhibitors (Complete), and proteins were then subjected to SDS-PAGE. Proteins were examined by immunoblot analysis using the SAM11 monoclonal antibody raised against amino acids 37–50 of PAR-2. As shown in Fig. 3, PAR-2 immunoreactivity is noted in samples prepared from wild-type mice but not PAR-2−/− animals. The apparent sharp band has an electrophoretic mobility corresponding to ~55 kDa. This is consistent with some publications reporting a sharp 52-to-55-kDa band (2), whereas other publications identify PAR-2 as a diffuse 80- to 90-kDa band (3). It is possible that our band represents a nonglycosylated form or a degradation product of PAR-2.

Wild-type mice were given a single intraperitoneal injection of caerulein (50 µg/kg) and were killed at varying times over the subsequent 2 h. The pancreas was removed, and extracted proteins were subjected to immunoblot analysis with anti-PAR-2 monoclonal antibodies. As shown in Fig. 3, a time-dependent loss of PAR-2 immunoreactivity is observed, indicating that PAR-2 is degraded during the early stages of secretagogue-induced pancreatitis. Theoretically, loss of PAR-2 immunoreactivity could be the result of either PAR-2 activation (and subsequent internalization/degradation) or, alternatively, PAR-2 degradation caused by acinar cell injury in pancreatitis. We favor the former explanation (see DISCUSSION) and conclude that PAR-2 is activated during the early stages of caerulein-induced pancreatitis. After the initial decline in PAR-2 immunoreactivity that follows administration of a single supramaximally stimulating dose of caerulein, PAR-2 immunoreactivity gradually increases, most likely reflecting the appearance of new PAR-2 (Fig. 3).

Parenteral administration of SLIGRL-NH₂ causes loss of PAR-2 immunoreactivity. Wild-type mice were given SLIGRL-NH₂ (4 µmol/kg) along with amastatin (4 µmol/kg) by tail-vein injection and were killed at varying intervals. Control animals received only amastatin or amastatin with the reverse PAR-2 peptide LRGLS-NH₂. The pancreas was removed, and extracted proteins were subjected to immunoblot analysis with anti-PAR-2 antibodies. As shown in Fig. 4, administration of the PAR-2-activating peptide leads to time-dependent loss of PAR-2 immunoreactivity, indicating that PAR-2 has been pharmacologically activated, internalized, and

Fig. 3. Pancreatic PAR-2 levels decrease during secretagogue-induced pancreatitis. Pancreatic proteins from PAR-2−/− and WT mice were subjected to SDS-PAGE followed by immunoblot analysis. Note the lack of immunoreactivity in the PAR-2−/− samples. WT mice were given a single intraperitoneal injection of caerulein (50 µg/kg) and were killed at varying intervals. The pancreas was removed, and PAR-2 levels were measured by immunoblot as described in the text and were quantitated by densitometry. Representative immunoblot is shown; values are means ± SE from 3 independent experiments. *P < 0.05 vs. 0-time control.
degraded. No change in PAR-2 immunoreactivity is observed when the reverse PAR-2 peptide is administered. The activation and degradation of PAR-2 after administration of a single dose of SLIGRL-NH₂ is transient and, within 2 h of SLIGRL-NH₂ administration, PAR-2 levels are restored as newly synthesized receptors appear (not shown). With repeated hourly doses of SLIGRL-NH₂/amastatin, however, PAR-2 immunoreactivity remains markedly depressed for up to 4 h.

**Pharmacological activation of PAR-2 lessens the severity of pancreatitis.** On the basis of the above findings, we reasoned that parenteral administration of SLIGRL-NH₂ could induce PAR-2 activation and that this approach could be used to evaluate the effect of PAR-2 activation on the severity of secretagogue-induced pancreatitis. To achieve this goal, mice were given SLIGRL-NH₂ along with amastatin 1 h before caerulein administration, and repeated doses of SLIGRL-NH₂/amastatin were subsequently given, at hourly intervals, along with the caerulein. Control animals received only amastatin. To be certain that the responses to SLIGRL-NH₂ administration were mediated by PAR-2, a parallel series of identical experiments were performed using PAR-2-deficient mice. After 12 hourly injections of caerulein (with or without SLIGRL-NH₂/amastatin), all of the animals were killed and the severity of pancreatitis was evaluated.

As shown in Figs. 5 and 6, the severity of pancreatitis is markedly reduced by administration of the PAR-2-activating peptide to wild-type but not to PAR-2⁻/⁻ mice. Microscopic examination of samples show that acinar cell vacuolization, intralobular edema, acinar cell necrosis, and pancreatic edema are each diminished by administration of SLIGRL-NH₂/amastatin to wild-type but not to PAR-2⁻/⁻ mice (Fig. 5). Quantitation of the changes related to pancreatitis severity shows that pancreatic edema (i.e., pancreatic water content), inflammation (i.e., myeloperoxidase activity), and acinar cell necrosis are each decreased in caerulein-treated wild-type mice given SLIGRL-NH₂/amastatin compared with those given only amastatin but that no decrease in pancreatitis severity is observed when PAR-2⁻/⁻ mice are given the PAR-2-activating peptide (Fig. 6).

Intrapancreatic activation of trypsinogen is an early event in caerulein-induced pancreatitis and, in previously reported studies, we have shown that trypsinogen activation occurs within acinar cells (10). As shown in Fig. 6, intrapancreatic trypsinogen activation is not altered in either wild-type or PAR-2-deficient animals by administration of the PAR-2-activating peptide.

**MAPKs are activated during caerulein-induced pancreatitis, and the nuclear translocation of activated ERK1/2 is decreased by administration of the PAR-2-activating peptide.** Wild-type mice were injected with caerulein along with amastatin either with or without SLIGRL-NH₂. After 30 min, the pancreata were removed and the nuclear fraction was separated from the cytosolic fraction. Proteins were subjected to immunoblot analysis with anti-phospho-ERK1/2 antibody. As shown in Fig. 7, total ERK1/2 in whole cells is not altered at this time but the

---

**Fig. 4.** Administration of SLIGRL-NH₂ leads to loss of PAR-2 immunoreactivity. WT mice were given hourly injections of SLIGRL-NH₂ along with amastatin and were killed at 1, 2, and 4 h after the first injection. PAR-2 levels were evaluated by immunoblot analysis as described in the text. Representative immunoblot is shown; values are means ± SE from 3 independent experiments. 4LS indicates group given the reverse, nonactivating PAR-2 peptide. *P < 0.05 vs. 0-time control.

**Fig. 5.** Pharmacological activation of PAR-2 reduces the severity of secretagogue-induced pancreatitis. WT and PAR-2⁻/⁻ mice were given 12 hourly doses of caerulein (50 µg/kg per injection) and amastatin (4 µmol/kg per injection) with or without SLIGRL-NH₂ (4 µmol/kg per injection) as described in the text and were killed 1 h after the last caerulein injection. Photomicrographs show representative images from WT (A and C) and PAR-2⁻/⁻ (B and D) given caerulein with amastatin (A and B) or caerulein, amastatin, and SLIGRL-NH₂ (C and D).
level of phospho-ERK1/2 is markedly increased, indicating that it is activated during the initial 30 min of supramaximal secretagogue stimulation. Nuclear translocation of phospho-ERK1/2 as well as total ERK1/2 is observed, but the nuclear translocation of the total and activated forms is markedly reduced by administration of SLIGRL-NH₂. In other studies using anti-JNK and anti-phospho-JNK antibodies, activation (i.e., phosphorylation) and nuclear translocation of JNK following supramaximal stimulation with caerulein was also observed, but PAR-2 activation did not alter the nuclear translocation (not shown). Activation and nuclear translocation of the proinflammatory transcription factors activator protein-1

Fig. 6. Pharmacological activation of PAR-2 reduces the severity of secretagogue-induced pancreatitis. WT and PAR-2⁻/⁻ mice were given 12 hourly doses of caerulein (50 μg/kg per injection) and amastatin (4 μmol/kg per injection) with 4 μmol/kg of injection of SLIGRL-NH₂ (CS) or without SLIGRL-NH₂ (C) as described in the text and were killed 1 h after the 12th caerulein injection. Serum amylase activity (A), pancreatic edema (B), pancreatic myeloperoxidase activity (C), and acinar cell necrosis (D) were quantitated as described in the text. In a separate group of animals, pancreatic trypsin activity was measured 30 min after the first caerulein injection (E). Trypsin activity in control samples taken from non-caerulein-treated animals were routinely 15–20% of the value noted in samples taken from caerulein-treated animals not given SLIGRL-NH₂. Values are means ± SE from 5 animals per group; *P < 0.01 for WT mice receiving caerulein/amastatin plus SLIGRL-NH₂ (CS) vs. WT mice receiving only caerulein/amastatin (C).

Fig. 7. Activation of PAR-2 attenuates ERK1/2 translocation to the nucleus. WT mice were injected 50 μg/kg caerulein along with 4 μmol/kg amastatin either with 4 μmol/kg SLIGRL-NH₂ (CS) or without SLIGRL-NH₂ (C). After 30 min, the pancreata were removed and the nuclear fraction was separated from the nonnuclear fraction. Proteins were subjected to immunoblot analysis with the anti-phospho-ERK1/2 antibody. For normalization purposes, the blots were stripped and reprobed with antibodies raised against total ERK1/2. Representative immunoblots are shown; values are means ± SE of densitometric analysis of phospho-ERK1/2 from 3 independent experiments. *P < 0.01 for WT mice receiving caerulein/amastatin plus SLIGRL-NH₂ vs. WT mice receiving only caerulein/amastatin.
deletion of PAR-2 would reduce the severity of secretagogue-induced pancreatitis, but contrary to our expectations that genetic deletion of PAR-2 is ideal for such studies because it is an easily elicited and highly reproducible model of severe, necrotizing pancreatitis. Mice is ideal for such studies because it is an easily elicited and highly reproducible model of severe, necrotizing pancreatitis.

Pancreas-derived digestive enzyme zymogens are inappropriately activated within the pancreas during the early stages of clinical as well as experimental pancreatitis, and both the pancreatic injury and the inflammatory reaction that ultimately characterize pancreatitis are generally believed to be the result of the nonspecific digestive and injurious effects of those activatedzymogens. The currently reported studies were stimulated by a number of recent reports that have convincingly shown that, in addition to its digestive and injurious effects, the pancreas-derived protease trypsin can also act as a specific signaling ligand by proteolytically activating the tethered ligand receptor PAR-2 (18). PAR-2 is a seven-transmembrane G protein-coupled receptor that is expressed by many types of cells both within and outside of the pancreas. Within the exocrine pancreas, PAR-2 has been found to be functionally present on both acinar and duct cells. On the basis of these observations, we hypothesized that, in addition to its digestive and injurious effects, the trypsinogen that is activated within the pancreas during pancreatitis might also specifically interact with PAR-2 to initiate signal-transduction events that could modulate the severity of pancreatitis. Because activation of PAR-2 in most of the systems studied to date has been noted to exert a proinflammatory effect, we expected that the interaction of trypsin with PAR-2 would worsen the severity of pancreatitis.

We have employed an experimental model of pancreatitis that is induced by the repeated administration of supramaximally stimulating doses of the cholecystokinin analog caerulein to test our hypothesis. This secretagogue-induced model of pancreatitis has been extensively employed by us as well as many other groups to define the early events and severity determinants in pancreatitis. It is the most well-characterized of the various pancreatitis models and, although its mechanism of induction clearly differs from that which is likely to trigger clinical pancreatitis, its widespread application to studies of pancreatitis is based on the generally held beliefs that secretagogue-induced pancreatitis involves many of the same biochemical and cell biological events that are responsible for the clinical disease and that factors that regulate the severity of caerulein-induced pancreatitis are also likely to regulate the severity of clinical pancreatitis. Furthermore, since it is not possible to access the pancreas of patients during the early stages of clinical pancreatitis, studies dealing with the cell biology of acute pancreatitis and the factors that regulate pancreatitis severity must, for pragmatic reasons, be performed using experimental models of the disease. Of the various models that might be used, the secretagogue-induced model in mice is ideal for such studies because it is an easily elicited and highly reproducible model of severe, necrotizing pancreatitis.

To test our hypothesis, we have used mice with genetic deletion of PAR-2, but contrary to our expectations that genetic deletion of PAR-2 would reduce the severity of secretagogue-induced pancreatitis, we have found that genetic deletion of PAR-2 actually leads to a marked worsening of the disease. As shown in Figs. 1 and 2, the magnitude of pancreatic edema, acinar cell vacuolization, acinar cell injury/necrosis, and intrapancreatic inflammation are all increased in PAR-2-deficient mice when those animals are compared with the PAR-2-sufficient control group. The hyperamylasemia of pancreatitis is not altered by PAR-2 deletion, but as noted by many groups including our own, the magnitude of hyperamylasemia does not correlate well with the severity of pancreatitis.

We have employed an experimental model of pancreatitis that is induced by the repeated administration of supramaximally stimulating doses of the cholecystokinin analog caerulein to test our hypothesis. This secretagogue-induced model of pancreatitis has been extensively employed by us as well as many other groups to define the early events and severity determinants in pancreatitis. It is the most well-characterized of the various pancreatitis models and, although its mechanism of induction clearly differs from that which is likely to trigger clinical pancreatitis, its widespread application to studies of pancreatitis is based on the generally held beliefs that secretagogue-induced pancreatitis involves many of the same biochemical and cell biological events that are responsible for the clinical disease and that factors that regulate the severity of caerulein-induced pancreatitis are also likely to regulate the severity of clinical pancreatitis. Furthermore, since it is not possible to access the pancreas of patients during the early stages of clinical pancreatitis, studies dealing with the cell biology of acute pancreatitis and the factors that regulate pancreatitis severity must, for pragmatic reasons, be performed using experimental models of the disease. Of the various models that might be used, the secretagogue-induced model in mice is ideal for such studies because it is an easily elicited and highly reproducible model of severe, necrotizing pancreatitis.

To test our hypothesis, we have used mice with genetic deletion of PAR-2, but contrary to our expectations that genetic deletion of PAR-2 would reduce the severity of secretagogue-induced pancreatitis, we have found that genetic deletion of PAR-2 actually leads to a marked worsening of the disease. As shown in Figs. 1 and 2, the magnitude of pancreatic edema, acinar cell vacuolization, acinar cell injury/necrosis, and intrapancreatic inflammation are all increased in PAR-2-deficient mice when those animals are compared with the PAR-2-sufficient control group. The hyperamylasemia of pancreatitis is not altered by PAR-2 deletion, but as noted by many groups including our own, the magnitude of hyperamylasemia does not correlate well with the severity of pancreatitis.

Our finding that PAR-2 deletion worsens the severity of experimental pancreatitis strongly suggests that the presence of PAR-2 protects mice against pancreatitis and that PAR-2 exerts a protective effect by mediating events that reduce the extent of acinar cell injury/necrosis and pancreatic inflammation. If valid, this conclusion would predict that 1) in wild-type animals, PAR-2 is activated during pancreatitis, and 2) pharmacological activation of PAR-2 in wild-type animals will reduce the severity of pancreatitis. Our studies have confirmed both of those predictions.

As shown in Fig. 3, pancreatic PAR-2 immunoreactivity is rapidly lost following the administration of a single, supramaximally stimulating dose of caerulein. Theoretically, loss of PAR-2 immunoreactivity could reflect the nonspecific digestive changes of pancreatitis, but this is unlikely because PAR-2 immunoreactivity is lost very shortly after the administration of caerulein, before evidence of cell injury can be detected. On the other hand, it is much more likely that the loss of PAR-2 immunoreactivity that we have observed reflects trypsin-induced activation of the receptor, an event that is known to be followed by receptor phosphorylation, arrestin-mediated receptor internalization, and receptor degradation (7). Our observations, therefore, lead us to conclude that, in wild-type mice, PAR-2 is activated during the early stages of pancreatitis, and we are led to speculate that the worsening of pancreatitis, which is noted in PAR-2-deficient animals, results from the loss of protection against pancreatitis that is otherwise afforded to wild-type animals by PAR-2 activation.

We performed a series of experiments designed to define the conditions under which PAR-2 could be pharmacologically activated before and during the onset of secretagogue-induced pancreatitis. In addition to proteolytic activation of PAR-2, the tethered ligand receptor can be nonproteolytically activated by exposure to the activating peptide SLIGRL-NH2. As shown in Fig. 4, pancreatic PAR-2 immunoreactivity is rapidly lost following the parenteral administration of the activating peptide SLIGRL-NH2, which does not activate PAR-2, is administered.

To achieve long-lasting PAR-2 activation in our studies, we gave animals repeated doses of SLIGRL-NH2 along with the aminopeptidase inhibitor amastatin and, as shown in Figs. 5 and 6, the severity of secretagogue-induced pancreatitis is reduced by repeated administration of SLIGRL-NH2/amastatin. Pancreatic edema (i.e., pancreatic water content), pancreatic inflammation (i.e., myeloperoxidase activity within the pancreas), and pancreatic acinar cell necrosis are all markedly diminished by administration of the PAR-2-activating peptide to wild-type animals. To be certain that the effects of SLIGRL-NH2 administration reflect the effects of the PAR-2-activating peptide on PAR-2-bearing cells, we carried out a control series of identical experiments using PAR-2-deficient animals, and as

Downloaded from http://ajpgi.physiology.org/ by 10.220.33.1 on June 25, 2017
shown in Fig. 6, administration of SLIGRL-NH₂ to those animals did not alter the severity of pancreatitis. Together, our observations have led us to conclude that pharmacological activation of PAR-2, achieved by parenteral administration of the PAR-2-activating peptide, reduces the severity of secretagogue-induced pancreatitis in wild-type mice and that, in pancreatitis, PAR-2 activation is an anti-inflammatory event.

We have examined the following three mechanisms by which PAR-2 activation might reduce the severity of pancreatitis: 1) that PAR-2 activation might interfere with the intrapancreatic activation of digestive enzyme zymogens; 2) that PAR-2 activation might interfere with the activation and/or nuclear translocation of the proinflammatory transcription factors AP-1 or NF-κB; and 3) that PAR-2 activation might interfere with the activation and/or nuclear translocation of MAPKs. Intrapancreatic activation of trypsinogen and other digestive zymogens is a critical event in the evolution of secretagogue-induced and other models of pancreatitis, and in previous studies, we have found that interventions that prevent trypsin activation lead to a reduction in pancreatitis severity (20). However, as shown in Fig. 6, pharmacological activation of PAR-2 does not reduce the level of trypsin activity within the pancreas during secretagogue-induced pancreatitis, and thus it is unlikely that PAR-2 protects against pancreatitis by interfering with intrapancreatic zymogen activation. In other studies (not shown), we found that the intrapancreatic activation and nuclear translocation of both AP-1 and NF-κB, which occur during secretagogue-induced pancreatitis (20) are not altered by pharmacological activation of PAR-2, and based on these findings, we have concluded that PAR-2 activation protects against pancreatitis by mechanisms that do not involve either AP-1 or NF-κB.

Studies previously reported by others have indicated that pancreatic JNK and ERK1/2 are each activated during the early stages of secretagogue-induced pancreatitis (5, 19), and we have confirmed these findings (Fig. 7). In addition, we have found that ERK1/2 is translocated to the nucleus during pancreatitis, where, on the basis of studies in many other systems, it presumably acts to regulate a large number of cellular events (1, 14). In our studies, we have found that pharmacological activation of PAR-2 during pancreatitis does not alter the activation (i.e., phosphorylation) of either ERK1/2 or JNK or the nuclear translocation of activated JNK. On the other hand, we have found that PAR-2 activation does profoundly reduce the nuclear translocation of ERK1/2 and phospho-ERK1/2 (Fig. 7).

Recently, DeFea and co-workers (7) have shown that, in neutrophils, PAR-2 activation causes ERK1/2 to associate with the PAR-2/arrestin complex. As a result, ERK1/2 translocation to the nucleus is prevented by PAR-2 activation, and this causes the activated ERK1/2 to be redirected to the plasmalemma. A similar mechanism may explain the effects of PAR-2 activation on the intracellular trafficking of activated ERK1/2 within the pancreas during pancreatitis. Regardless of the mechanisms involved, however, our findings that PAR-2 activation reduces the severity of pancreatitis and interferes with nuclear translocation of activated MAPKs suggests that the two events are interrelated, i.e., that PAR-2 activation protects against pancreatitis by trapping ERK1/2 within the cytoplasmic compartment and that this alteration in MAPK trafficking downregulates proinflammatory events and/or upregulates anti-inflammatory events that are critical to pancreatitis.

It is perhaps important to point out that the studies reported in this communication were performed under in vivo conditions, that our knockout mice had global deletion of PAR-2, and that parenteral administration of the PAR-2-activating peptide was likely to cause PAR-2 activation in many, if not all, PAR-2-bearing cell types. Thus, although our studies clearly indicate that PAR-2 activation exerts a protective effect on pancreatitis, we are unable to identify the cell type responsible for that protective effect. The functional expression of PAR-2 on pancreatic acinar cells and the fact that trypsinogen is activated within pancreatic acinar cells during the early stages of pancreatitis certainly support the notion that it is activation of acinar cell PAR-2 that exerts the protective effect on pancreatitis, but studies employing cell type-specific silencing of PAR-2 and/or in vitro studies using isolated acinar cells will be needed to confirm this.

Finally, it may be of interest to consider the potential survival advantage that accompanies PAR-2 expression by pancreatic acinar cells. Studies by our group (10) and others have shown that, even in the absence of pancreatitis, small amounts of digestive zymogens, including trypsinogen, become activated within pancreatic acinar cells. It has been assumed that the gland is protected against injury from these inappropriately activated enzymes by the presence of potent trypsin inhibitors within the secretory compartment of acinar cells and by the fact that, before their secretion, the zymogens are sequestered from the cytoplasmic space by being enclosed within membrane-bound organelles. From a purely teleological standpoint, however, it is also tempting to speculate that a trypsin receptor-mediated anti-inflammatory response might convey a considerable survival advantage by dampening the potentially injurious effects of intrapancreatic trypsinogen activation.

In summary, we have shown that genetic deletion of PAR-2 in mice worsens the severity of experimental pancreatitis and that pharmacological activation of mouse PAR-2 has the opposite effect. As this manuscript was being submitted for publication, Namkung et al. (16) reported studies that indicated that pharmacological activation of rat PAR-2 could reduce the severity of secretagogue-induced pancreatitis in those animals, but their studies did not address the effects of PAR-2 deletion or the mechanisms responsible for the protective effects of PAR-2 activation. Our own studies have suggested that PAR-2 activation may reduce the severity of pancreatitis by interfering with the intracellular trafficking and action of the MAPK ERK1/2. Regardless of the mechanisms involved, however, these studies suggest that interventions that trigger PAR-2 activation may beneficially effect the severity of pancreatitis and that they may be of value in the treatment and/or prevention of the clinical disease.

ACKNOWLEDGMENTS

We wish to acknowledge the important contributions to the early stages of this project made by Drs. Vijay Singh (Mayo Clinic) and Ashok Saluja (University of Massachusetts Medical School, Worcester, MA).

GRANTS

This study was supported by Grants RO1-AM-31396–20 (to M. L. Steer) and P30-DK-34928–20 (to Tufts-NEMC GRASP Center) from the National Institutes of Health.
REFERENCES