Primary sensory neurons: a common final pathway for inflammation in experimental pancreatitis in rats

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Primary sensory neurons: a common final pathway for inflammation in experimental pancreatitis in rats. Am J Physiol Gastrointest Liver Physiol 283: G938–G946, 2002. First published June 5, 2002; 10.1152/ajpgi.00105.2002.—We hypothesized that neurogenic inflammation is a common final pathway for parenchymal inflammation in pancreatitis and evaluated the role of primary sensory neurons in secretagogue-induced and obstructive pancreatitis. Neonatal rats received either the primary sensory neuron-denervating agent capsaicin (50 mg/kg sc) or vehicle. At 8 wk of age, pancreatitis was produced by six hourly injections of caerulein (50 μg/kg ip) or by common pancreaticobiliary duct ligation (CPBDL). The severity of pancreatitis was assessed by serum amylase, pancreatic myeloperoxidase (MPO) activity, histological grading, pancreatic plasma extravasation, and wet-to-dry weight ratio. Caerulein significantly increased MPO activity and wet-to-dry weight ratio, produced histological evidence of edematous pancreatitis, induced plasma extravasation, and caused hyperamylasemia. CPBDL increased MPO activity and produced histological evidence of pancreatitis. Neonatal capsaicin administration significantly reduced tissue MPO levels, histological severity scores, and wet-to-dry weight ratio and abolished plasma extravasation. These results demonstrate that primary sensory neurons play a significant role in the inflammatory cascade in experimental pancreatitis and appear to constitute a common final pathway for pancreatic parenchymal inflammation.

caeulen; capsaicin; common pancreaticobiliary duct ligation; neurogenic inflammation; substance P

NEUROGENIC INFLAMMATION REFERS to the local arteriolar vasodilatation, increased vascular permeability, edema, and neutrophil infiltration that result following stimulation of nociceptive primary sensory neurons (13, 21). This inflammatory cascade is mediated by peripheral release of chemical transmitters including the neuropeptide substance P (SP) from nerve terminals of depolarized primary sensory neurons. Subsequent binding of SP to its receptor, the neurokinin-1 receptor (NK-1R), on target cells, such as immune cells and vascular endothelial cells (3, 10, 29), and involvement of other neurotransmitters lead to manifestations of inflammation, including tissue edema and neutrophil infiltration.

Several recent studies have suggested a role for neurogenic factors in the inflammation of pancreatitis. Figini et al. (6) demonstrated that administration of SP to mice stimulates plasma extravasation from postcapillary venules in the pancreas, and this effect is blocked by the administration of antagonists to the NK-1R. In 1998, Bhatia et al. (2) reported that genetic deletion of the NK-1R in mice markedly reduced the severity of secretagogue-induced pancreatitis and pancreatitis-associated lung injury. NK-1R knockout mice have also been shown to have a reduced severity of diet-induced hemorrhagic pancreatitis and a markedly improved survival in this model (26). In a more recent study, the same group reported that NK-1R antagonists blocked plasma extravasation in a model of secretagogue-induced pancreatitis in rats (9). Although it is clear that activation of the NK-1R plays a central role in the neurogenic inflammatory process, additional evidence is required to demonstrate the involvement of primary sensory neurons in this cascade and to determine whether neurogenic inflammation is a common final pathway in the development of pancreatitis.

Although primary sensory neurons are well known to contain SP, studies have also localized SP to intrinsic enteric nerve plexuses. With the use of colchicine treatment to block axonal transport of neuropeptide granules, Su et al. (38) demonstrated the presence of local SP-immunoreactive neuronal cell bodies within the pancreas of rats. In addition, upper abdominal sympathectomy was shown to reduce SP-containing nerve fibers in the pancreas, further supporting the notion that there are dual intrinsic and extrinsic origins of SP-immunoreactive neurons in rat pancreas. Thus the relative significance of each of these pathways to neurogenic pancreatic inflammation in experimental pancreatitis also requires elucidation.

The specificity of primary sensory neurons for the actions of capsaicin, a sensory neuron excitotoxin, provides a useful model for the evaluation of these ques-

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tions. Neonatal capsaicin administration has been demonstrated to cause permanent loss of capsaicin-sensitive primary sensory neurons as well as an irreversible reduction in SP in regions normally innervated by these neurons (17, 19, 32). Selective destruction of primary sensory neurons by capsaicin attenuates the inflammatory response induced by peripheral nerve stimulation or by injection of noxious substances (7, 8).

The technique of neonatal capsaicin denervation has been used to demonstrate that primary sensory neurons modulate the severity of joint injury in a rat model of adjuvant-induced arthritis (24). In addition, with the use of this method, other investigators have shown that primary sensory neurons mediate toxin A-induced pancreatitis (28), dermal inflammation (29), and irritant-induced tracheal edema (18).

In the current study, we tested the hypotheses that primary sensory neurons play a role in pancreatic inflammation in experimental pancreatitis and that primary sensory neurons are a common final pathway for neurogenic inflammation in pancreatitis. More specifically, we examined whether primary sensory denervation diminishes inflammation in rat models of secretagogue-induced pancreatitis and obstructive pancreatitis. We demonstrate that neonatal capsaicin administration causes primary sensory denervation and reduces the severity of pancreatic inflammation in both models of pancreatitis, suggesting that primary sensory neurons are a common final pathway for inflammation in experimental pancreatitis.

MATERIALS AND METHODS

Animal protocol and experimental design. All animal experiments were performed with approval of the Duke University Institutional Animal Care and Use Committee. Male newborn Sprague-Dawley rats were purchased from Charles River (Wilmington, MA) and housed in climate-controlled rooms with a 12:12-h light-dark cycle. On day (of life) 2, animals received either the primary sensory denervating agent capsaicin (50 mg/kg sc) (17) or vehicle [absolute ethanol/Tween 80/isotonic saline (10:10:80, vol/vol/vol)]. Animals were weaned at ~4 wk of age and then fed standard laboratory chow and water ad libitum throughout the experiment. Rats were permitted water ad libitum throughout the experiment. When rats were 8 wk of age, primary sensory denervation was confirmed by testing the eye-wipe response to assess chemogenic pain perception (25). The number of protective forepaw eye wipes was counted in the first minute after application of 0.1% capsaicin to the cornea of the right eye. As additional confirmation of primary sensory denervation, we observed that rats that received capsaicin neonatally lacked the normal withdrawal response to tail pinch and had a reduced eye-wipe response to corneal application of dilute acid (0.1 M HCl). It also should be noted that the pancrea of rats that received capsaicin neonatally were grossly and histologically indistinguishable from the pancrea of rats that received vehicle neonatally. Rats were assigned to the following experiments: caerulein-induced pancreatitis or obstructive pancreatitis.

Caeerulein-induced pancreatitis. The cholecystokinin analog caerulein was purchased from Bachem California (Torrance, CA) and dissolved in 0.1 M NaHCO₃ followed by dilution in isotonic saline. Caerulein was prepared the morning of the experiment and stored on ice. Caerulein was administered as six hourly intraperitoneal injections at a supramaximal stimulating dose of 50 μg/kg per injection (22).

Control rats received six hourly intraperitoneal injections of isotonic saline. In one group, 1 h after the last caerulein or vehicle injection, animals were euthanized and mixed arteriovenous blood was collected by decapitation for measurement of serum amylase concentration. The pancreas was then quickly removed and divided for histological grading and for measurement of tissue myeloperoxidase (MPO) activity. In a second group, Evans blue was used to quantify pancreatic plasma extravasation (35).

Obstructive pancreatitis. Rats were anesthetized with xylazine (25 mg/kg im) and ketamine (50 mg/kg im), and the abdomen was entered via midline laparotomy. The common pancreaticobiliary duct was doubly ligated adjacent to the duodenal wall (33). Control rats underwent laparotomy with double ligation of the bile duct at the hilum of the liver. Postoperatively, animals were permitted laboratory chow and water ad libitum. On postoperative day 3, animals were euthanized and mixed arteriovenous blood was collected by decapitation for measurement of serum amylase concentration. The pancreas was then quickly removed and divided for histological grading and for measurement of tissue MPO activity.

Serum amylase concentration. Mixed arteriovenous blood was centrifuged for 10 min at 1,500 g. The serum amylase concentration was measured by using the precin yellow starch assay previously described (20). A standard curve was prepared by using crude type VI-B α-amylase (Sigma, St. Louis, MO), and serum amylase levels were expressed as milligrams per milliliter.

MPO activity. Portions of the harvested pancreata were immediately frozen at ~80°C. We measured the tissue activity of MPO, an enzyme produced by neutrophils and used as a marker of inflammation associated with neutrophil infiltration, as previously described using the substrate tetramethylbenzidine (39). Pancreatic tissue MPO activity was expressed as units per milligram total protein.

Histological grading. Portions of the pancreata were fixed overnight at room temperature in a pH-neutral, phosphate-buffered, 10% formalin solution. The tissue was then embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and coded for examination by a blinded pathologist unaware of the experimental design. The pathologist graded the severity of pancreatitis using modified scoring criteria previously described (37). The results were expressed as a score of 0–3 for the histological parameters of edema and neutrophil infiltration. Tissue necrosis was graded on a scale of 0–14 and consisted of the sum of parenchymal necrosis (graded from 0 to 7) and fat necrosis (graded from 0 to 7). Total histological score is the combined scores of edema, neutrophil infiltration, and necrosis.

Plasma extravasation. The Evans blue technique (35) was used to quantify pancreatic plasma extravasation in caerulein-induced pancreatitis. Evans blue is known to bind quantitatively to albumin and therefore remains intravascular until gaps form in the endothelial cell layer allowing leakage into tissues. One hour after the last caerulein or vehicle injection, rats were anesthetized with xylazine (25 mg/kg im) and ketamine (50 mg/kg im). A right femoral vein was cuffed down, and Evans blue (30 mg/kg of a 3% solution in isotonic saline) was injected intravenously. After 7 min, rats were transcardially perfused with 70 ml PBS containing 100 U/ml heparin sodium, followed by 200 ml 4% paraformaldehyde in PBS. The pancreas was removed,
RESULTS

Eye-wipe response. To confirm primary sensory denervation, we assessed chemogenic pain perception when rats were 8 wk old by evaluating the protective eye-wipe response to application of capsaicin to the cornea. In vehicle-treated rats, the mean number of ipsilateral forepaw eye wipes following application of 0.1% capsaicin to the right cornea was ~40 (Fig. 1). In rats that received capsaicin as neonates, this response was significantly reduced to only nine eye wipes per minute (P < 0.0001; n = 28), confirming primary sensory denervation.

Caerulein-induced pancreatitis. Administration of supramaximal stimulating doses of caerulein (6 hourly injections of 50 μg·kg⁻¹·dose⁻¹) to noncapsaicinized adult rats produced evidence of pancreatitis, as determined by serum amylase levels, tissue MPO activity, and histological grading. The serum amylase concentration in rats that received caerulein increased 20-fold compared with vehicle-treated animals (P < 0.001; n = 6; Fig. 2). In addition, caerulein treatment caused a significant increase in the level of pancreatic MPO activity, from 0.015 ± 0.002 to 0.654 ± 0.228 U/mg (P < 0.01; n = 6; Fig. 3). Histologically, caerulein treatment produced moderately severe pancreatitis characterized by pancreatic edema, neutrophil infiltration, and parenchymal necrosis as well as an injury pattern of acinar cell vacuolization (Fig. 4). All histological severity parameters were significantly elevated in noncapsaicinized rats receiving caerulein (Table 1), with an increase in total histological score from 0.25 ± 0.11 to 0.68 ± 0.54 (P < 0.001; n = 6; Fig. 5).

Neonatal capsaicin administration resulted in a reduction in the caerulein-induced elevation in pancreatic MPO activity. Compared with noncapsaicinized animals, capsaicinized rats receiving caerulein exhibited an 82% reduction in MPO activity (p < 0.05; Fig. 3) to a level indistinguishable from vehicle-treated rats. In contrast to noncapsaicinized rats, the capsaicin-treated animals receiving caerulein developed markedly less pancreatic edema, neutrophil infiltration, and parenchymal necrosis as illustrated histologically (Fig. 4). As shown in Table 1, neonatal capsaicin administration significantly reduced the scores of edema by 36% (P < 0.01), neutrophil infiltration by 41% (P < 0.01), and necrosis by 59% (P < 0.05). The total histological severity score was diminished by 47% in rats that were treated with capsaicin neonatally (P < 0.001; Fig. 5). Although the serum amylase concentration
tended to be lower in capsaicinized rats, there was no statistically significant difference compared with noncapsaicinized animals.

In a second group of rats, we used the Evans blue technique to quantify plasma extravasation into the pancreatic interstitium in caerulein-induced pancreatitis. Caerulein treatment of noncapsaicinized rats increased Evans blue extravasation by greater than fivefold, from $7.2 \pm 3.3$ to $37.8 \pm 4.8$ ng/mg dry weight ($P < 0.001$; $n = 4$; Fig. 6). In addition, caerulein treatment caused a significant increase in the pancreas wet-to-dry weight ratio, from $2.6 \pm 0.3$ to $7.2 \pm 1.4$ ($P < 0.05$; $n = 4$; Fig. 7). Compared with noncapsaicinized animals, capsaicinized rats receiving caerulein had an 85% reduction in Evans blue extravasation ($P < 0.001$; Fig. 6) to a level indistinguishable from vehicle-treated rats. Similarly, neonatal capsaicin administration diminished the caerulein-induced elevation in pancreas wet-to-dry weight ratio ($P < 0.05$; Fig. 7) to the level of vehicle-treated rats.

### Table 1. Effects of caerulein and neonatal capsaicin administration on pancreatic histology

<table>
<thead>
<tr>
<th>Edema</th>
<th>Neutrophil Infiltration</th>
<th>Necrosis</th>
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<tbody>
<tr>
<td>Vehicle + vehicle</td>
<td>$0.00 \pm 0.00$</td>
<td>$0.25 \pm 0.11$</td>
</tr>
<tr>
<td>Capsaicin + vehicle</td>
<td>$0.08 \pm 0.08$</td>
<td>$0.33 \pm 0.11$</td>
</tr>
<tr>
<td>Vehicle + caerulein</td>
<td>$1.83 \pm 0.11^*$</td>
<td>$1.83 \pm 0.11^*$</td>
</tr>
<tr>
<td>Capsaicin + caerulein</td>
<td>$1.17 \pm 0.17^+$</td>
<td>$1.08 \pm 0.20^+$</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE ($n = 6$ rats). Histological parameters were scored by a pathologist blinded to the experimental design. *$P < 0.001$ vs. vehicle + vehicle; †$P < 0.01$ vs. vehicle + caerulein; ‡$P < 0.05$ vs. vehicle + caerulein.

Obstructive pancreatitis. Common pancreaticobiliary duct ligation (CPBDL) in noncapsaicinized adult rats resulted in biochemical and microscopic evidence of acute pancreatitis. Ligation of the common pancreaticobiliary duct caused a significant increase in the level of pancreatic MPO activity, from $3.18 \pm 1.04$ to $10.59 \pm 0.71$ U/mg ($P < 0.01$; $n = 4$; Fig. 8). Histologically, CPBDL produced severe pancreatitis characterized by edema formation, neutrophil infiltration, acinar cell vacuolization, and parenchymal and fat necrosis (Fig. 9). Both the edema score and the necrosis score were significantly elevated in noncapsaicinized rats that underwent ligation of the pancreaticobiliary duct (Table 2). Compared with rats undergoing biliary duct liga-

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**Fig. 4.** The effects of caerulein and neonatal capsaicin administration on pancreatic histointerarchitecture. Representative histological sections of rat pancreas fixed in 10% neutral-buffered formalin, paraffin embedded, and stained with hematoxylin and eosin from control (A), caerulein alone (B), and caerulein + neonatal capsaicin (C)-treated rats. Caerulein administration caused pancreatic edema, neutrophil infiltration, and parenchymal injury and necrosis. Neonatal capsaicin administration inhibited the effects of caerulein on pancreatic histoarchitecture. Magnification: ×250.

**Fig. 5.** The effects of caerulein and neonatal capsaicin administration on total histological score of pancreatitis. Caerulein administration increased the total histological severity score of pancreatitis, and this effect was significantly inhibited by neonatal capsaicin administration. Results are expressed as means ± SE ($n = 6$). *$P < 0.001$ vs. vehicle + vehicle; †$P < 0.001$ vs. vehicle + caerulein.
tion, the total histological score in the CPBDL group increased from 1.38 ± 0.43 to 13.88 ± 1.20 (P < 0.001; n = 4; Fig. 10). The serum amylase concentration in noncapsaicinized rats that underwent CPBDL did not statistically differ from the control group (Fig. 11).

Neonatal capsaicin administration resulted in a reduction in the CPBDL-induced elevation in pancreatic MPO activity. Capsaicinized rats undergoing CPBDL had a 59% reduction in MPO activity (P < 0.01; Fig. 8) compared with noncapsaicinized animals. In contrast to noncapsaicinized rats, the capsaicin-treated animals that underwent CPBDL demonstrated less pancreatic edema, neutrophil infiltration, and parenchymal and fat necrosis histologically (Fig. 9), although only the necrosis score was significantly reduced, from 9.50 ± 1.23 to 4.88 ± 0.55 (P < 0.05; Table 2). The total histological severity score was diminished by 43% in rats that were treated with capsaicin neonatally (P < 0.05; Fig. 10). Serum amylase concentrations were not significantly different in capsaicinized animals.

Recent studies have suggested that neurogenic factors play a significant role in the inflammatory cascade in experimental pancreatitis. SP-induced pancreatic plasma extravasation in mice has been demonstrated to be blocked by NK-1R antagonists (6). NK-1R knockout mice have been reported to have a markedly reduced severity of secretagogue-induced pancreatitis (2) and diet-induced hemorrhagic pancreatitis (26). In addition, plasma extravasation in a rat model of secretagogue-induced pancreatitis is blocked by administration of antagonists to the NK-1R (9). These studies have suggested an important role for SP stimulation of the NK-1R in the pathogenesis of experimental pancreatitis. However, the specific involvement of SP-containing primary sensory neurons has not yet been reported.

It is well established that a subpopulation of cell bodies of primary sensory neurons synthesize the undecapeptide neurotransmitter SP, which is released from nerve endings following nerve stimulation and depolarization (11, 12, 34). Furthermore, Won et al. (43) demonstrated by retrograde tracing and immunohistochemistry that the rat pancreas is innervated by SP-immunoreactive nerve fibers originating from dorsal root ganglion cells. However, other studies (38) reported the presence of SP-immunoreactive nerve fibers in the pancreas after colchicine treatment as well as partial abolition of SP-immunoreactive nerve fibers by surgical denervation. These findings suggest that SP nerve fibers in the pancreas originate from both intrinsic (i.e., enteric) and extrinsic (i.e., primary sensory) cells. The potential contribution of each of these sources of SP to neurogenic pancreatic inflammation in pancreatitis is unknown. In this study, we hypothesized that primary sensory neurons play a role in pancreatic inflammation in experimental pancreatitis and that primary sensory neurons are a common final pathway for neurogenic inflammation in pancreatitis. More specifically, we examined whether primary sensory denervation would ameliorate pancreatic inflam-
nation in rat models of secretagogue-induced and obstructive pancreatitis.

We used the neonatal capsaicin administration model to produce primary sensory denervation. Capsaicin, a chemical derivative of vanillyl amide and the pungent agent in red pepper, has been used extensively as a probe for primary sensory neuron mechanisms, and its value as a pharmacological tool is dependent on the specificity of its neurotoxic actions for primary sensory neurons. Systemic administration of a single dose of 50 mg/kg capsaicin in neonatal rats causes permanent degeneration of 90–95% of unmyelinated primary sensory neurons, with no significant change in myelinated afferent fibers, and irreversible depletion of sensory neuron SP (4, 23, 31, 32). Of particular importance is the finding in the gastrointestinal tract that neonatal capsaicin administration ablates primary sensory neurons while leaving intrinsic SP-containing neurons intact (16). Investigators have used this denervation model to demonstrate the involvement of primary sensory neurons in various inflammatory conditions, including toxin A-induced intestinal inflammation (28), irritant-induced tracheal edema (18), and adjuvant-induced experimental arthritis (24).

To confirm capsaicin-induced primary sensory denervation, we evaluated chemogenic pain perception by counting the number of protective forepaw eye-wiping movements in response to instillation of 0.1% capsaicin into the eye (25). The eye-wipe response is a well-accepted method of confirming primary sensory denervation, because it provides gross behavioral evidence of impairment of chemonociceptive primary sen-

Table 2. Effects of CPBDL and neonatal capsaicin administration on pancreatic histology

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<th>Edema</th>
<th>Neutrophil Infiltration</th>
<th>Necrosis</th>
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<td>Vehicle + BDL</td>
<td>0.63 ± 0.24</td>
<td>0.75 ± 0.25</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Capsaicin + BDL</td>
<td>0.38 ± 0.24</td>
<td>0.88 ± 0.55</td>
<td>1.13 ± 1.13</td>
</tr>
<tr>
<td>Vehicle + CPBDL</td>
<td>2.75 ± 0.25*</td>
<td>1.63 ± 0.13</td>
<td>9.50 ± 1.23†</td>
</tr>
<tr>
<td>Capsaicin + CPBDL</td>
<td>1.50 ± 0.46</td>
<td>1.50 ± 0.35</td>
<td>4.88 ± 0.55‡</td>
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Results are expressed as mean ± SE (n = 4). Histological parameters were scored by a pathologist blinded to the experimental design. *P < 0.01 vs. vehicle + biliary duct ligation (BDL); †P < 0.001 vs. vehicle + BDL; ‡P < 0.05 vs. vehicle + common pancreatic BDL (CPBDL).

Fig. 9. The effects of duct ligation and neonatal capsaicin administration on pancreatic histoarchitecture. Representative histological sections of pancreas fixed in 10% neutral-buffered formalin, paraffin embedded, and stained with hematoxylin and eosin from rats that underwent BDL (A), CPBDL (B), and CPBDL + neonatal capsaicin administration (C). CPBDL caused pancreatic edema, neutrophil infiltration, and parenchymal injury and necrosis. Neonatal capsaicin administration inhibited the effects of CPBDL on pancreatic histoarchitecture. Magnification: ×250.

Fig. 10. The effects of duct ligation and neonatal capsaicin administration on total histological score of pancreatitis. CPBDL increased the total histological severity score of pancreatitis, and this effect was significantly inhibited by neonatal capsaicin administration. Results are expressed as means ± SE (n = 4). *P < 0.001 vs. vehicle + BDL; †P < 0.05 vs. vehicle + CPBDL.
Sensory innervation. Furthermore, suppression of chemonociceptive pain responses by neonatal capsaicin administration has been demonstrated to correlate with the prevention of neurogenic inflammatory responses (17). In our study, the eye-wipe response was reduced by 78% in rats that received capsaicin as neonates, and there was an increased latency of ~5 s in the initiation of protective eye wipes after the instillation of capsaicin into the eye (data not shown). Although these data suggest that primary sensory denervation was not complete, which is in keeping with prior studies demonstrating degeneration of up to 95% of unmyelinated sensory afferent neurons, it is clear that neonatal capsaicin administration resulted in permanent loss of the majority of primary sensory neurons.

The current study demonstrates that repeated caerulein administration in rats causes biochemical and histological evidence of acute pancreatitis. Moreover, neonatal capsaicin administration causes primary sensory denervation and reduces the severity of secretagogue-induced pancreatitis. To determine whether neurogenic inflammation is a common final pathway for parenchymal inflammation in pancreatitis, we evaluated the role of primary sensory neurons in a surgical model of obstructive pancreatitis. We report that ligation of the common pancreaticobiliary duct in rats causes biochemical and histological evidence of acute pancreatitis and that primary sensory denervation induced by neonatal capsaicin treatment reduces the severity of obstructive pancreatitis. These findings suggest that primary sensory neurons play a critical role in the tissue inflammatory response to injury in pancreatitis and that neurogenic factors constitute a common final pathway for parenchymal inflammation in pancreatitis. Furthermore, we propose that initial parenchymal injury, by caerulein hyperstimulation or by common pancreaticobiliary duct obstruction, generates a signal that activates primary sensory neurons resulting in release of peptide transmitters, including SP, with subsequent amplification and propagation of the inflammatory cascade. Whether primary sensory neurons play a role in other models of experimental pancreatitis, such as diet-induced hemorrhagic pancreatitis and trypsin/taurocholate-induced pancreatitis, remains to be determined.

It is worth noting that there were differences in the extent of effects of capsaicin in the two models of pancreatitis. Specifically, the percentage reductions in necrosis scores were 59% in caerulein-induced pancreatitis and 49% in obstructive pancreatitis; in total histological score, 47% and 43%, respectively; and in tissue MPO activity, 82% and 59%, respectively. It also should be noted that the overall severity of pancreatitis differed between the two models, with obstructive pancreatitis being more severe. However, a difference in severity was not unexpected, because the pathogenesis of caerulein-induced pancreatitis differs from that of obstructive pancreatitis. Because of the disparity in severity between the two models, it is also not surprising that intervention (i.e., neonatal capsaicin administration) had a slightly different effect in the two models. It is possible that the effects of capsaicin were less impressive in obstructive pancreatitis, because the severity of pancreatitis in this model was greater than in caerulein-induced pancreatitis.

Plasma extravasation is an early and major component of neurogenic inflammation in the trachea (1), urinary bladder (30), and skin (36). In this study, using the Evans blue technique, we have demonstrated that caerulein treatment in rats stimulates pancreatic microvascular permeability and plasma extravasation with edema formation. In contrast to the partial reductions in biochemical and histological parameters of pancreatitis, primary sensory denervation with neonatal capsaicin administration completely abolished caerulein-induced pancreatic plasma extravasation and edema. This finding is in agreement with earlier studies that reported that SP-induced pancreatic plasma extravasation in mice (6) and caerulein-induced pancreatic plasma extravasation in rats (9) are abolished by antagonists to the NK-1R. This suggests that secretagogue-induced plasma extravasation in rat pancreas is mediated entirely by activation of primary sensory neurons, whereas other components of neurogenic inflammation, such as neutrophil infiltration and parenchymal necrosis, are predominantly, but not entirely, mediated by sensory afferent neurons.

Neonatal capsaicin administration reduced the severity of tissue damage induced by caerulein by significantly diminishing pancreatic edema, neutrophil infiltration, parenchymal necrosis, and plasma extravasation. Although serum amylase levels tended to be lower in the rats that were treated with capsaicin neonatally, these data were not significantly different. This finding suggests that caerulein-stimulated amylase release is not mediated by primary sensory neurons and does not correlate with the severity of experimental pancreatitis. In our model of obstructive pancreatitis, serum amylase concentrations on postoperative day 3 after CPBDL did not statistically differ from amylase levels in control animals. These data are in agreement with Ohshio et al. (33), who reported that the elevated serum amylase concentrations resulting within 3 h of CPBDL in rats fell to the level of control.
Sensory Neurons in Pancreatitis

G945

Rats by 6 h postoperatively. It is clear from our results, as well as from reports of our colleagues, that serum amylase levels are variable throughout the course of the disease and are not predictive of severity of pancreatic inflammation.

Studies from another laboratory (40–42) using an adult model of capsaicin administration to cause ablation of sensory neurons have suggested a protective role of primary sensory neurons and, specifically, calcitonin gene-related peptide in caerulein-induced pancreatitis. This technique to cause sensory denervation differs substantially from administration of capsaicin in the neonatal period. Although adult capsaicin administration can ablate sensory neurons, the effect of capsaicin in adult rats is not permanent, and sensory deficits and neuropeptide depletion are often reversible (4). In addition, it is known that the neurotoxic effects of capsaicin in adult rats are not as widespread as they are in neonates, and thus the deleterious effect on sensory neuropeptides is not as extensive (4, 15). Because of the confounding factors associated with adult capsaicin administration, we suggest that the differences in sensory nerve ablation technique preclude an adequate comparison between our results and the results of these prior studies.

In conclusion, the results of this study demonstrate that caerulein-induced and obstructive pancreatitis in rats are mediated, in part, by primary sensory neurons. Because neonatal capsaicin administration specifically destroys small, unmyelinated primary sensory neurons, the integrity of these afferent fibers is critical to the development of pancreatic inflammation. It is important to note that primary sensory neurons are known to contain several neuropeptides, including SP, calcitonin gene-related peptide, and neurokinin A (5, 14, 27). Although SP is by far the most potent inflammatory neuropeptide derived from primary sensory neurons, calcitonin gene-related peptide has been demonstrated to induce vasodilatation, and neurokinin A is a mediator of plasma extravasation (14). Thus additional studies are required to elucidate the role of each of these neuropeptides as it relates to the reduction in pancreatic inflammation following primary sensory denervation. Nevertheless, the current study demonstrates the specific involvement of primary sensory neurons in the inflammatory cascade of experimental pancreatitis and the role of primary sensory neurons as a common final pathway in pancreatic inflammation.

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