Local disruption of the celiac ganglion inhibits substance P release and ameliorates caerulein-induced pancreatitis in rats

Marc D. Noble, Joelle Romac, Yu Wang, Jay Hsu, John E. Humphrey, and Rodger A. Liddle

Department of Medicine, Duke University and Durham Veterans Affairs Medical Centers, Durham, North Carolina

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NEUROGENIC INFLAMMATION RESULTS from activation of specific afferent sensory nerves leading to the release of neurotransmitters from nerve terminals that ultimately cause vasodilation, plasma extravasation, and cellular infiltration (11, 14). These effects can lead to mast cell degranulation, neutrophil adhesion, and the production of reactive oxygen species (1, 32, 34). Neurogenic inflammation is a major factor in the inflammatory response to injury in a number of organs including the pancreas. Recent data suggest that activation of primary sensory neurons and release of inflammatory mediators are important in determining the severity of pancreatitis (25).

Substance P (SP) is a neurokinin located within primary afferent sensory neurons and has a substantial role in mediating neurogenic inflammation. SP release causes extravasation of plasma from postcapillary venules in the pancreas as well as other gastrointestinal organs (5). SP is the specific ligand for the neurokinin-1 receptor (NK-1R).

NK-1Rs are located on a variety of cell types including inflammatory cells, smooth muscle cells, vascular endothelial cells, surface epithelia, and pancreatic acinar cells (3, 24, 26, 31). Activation of this receptor has been shown to mediate the release of various proinflammatory agents (24). Further experiments using NK-1R knockout mice have shown that pancreatic inflammation can be significantly attenuated in caerulein-induced pancreatitis (7). Alternatively, local infusion of the NK-1R antagonist CP-96345 into the pancreatic duct has been shown to significantly reduce the severity of pressure-induced pancreatitis (8). These data demonstrate that activation of the NK-1R is an important step in initiating the inflammatory cascade in the pancreas leading to more severe forms of pancreatitis and that blocking this receptor can reduce the severity of pancreatitis. Activation of the vanilloid capsaicin receptor TRPV1 located on C and Aδ primary sensory neurons has been shown to mediate the release of SP (24). Pancreatic injury stimulates these neurons through the activation of TRPV1. Moreover, blocking TRPV1 significantly reduced SP release and pancreatitis severity (24).

Resiniferatoxin (RTX) is a specific TRPV1 agonist that, in high doses, selectively destroys C and Aδ fibers by dramatically increasing intracellular calcium concentrations and disrupting vital organelles (28). In the current study, we hypothesized that local denervation of the C and Aδ neurons innervating the pancreas via surgical gangliectomy or administration of RTX to the ganglion would reduce the severity of acute secretagogue-induced pancreatitis. The celiac ganglion has nerves coursing it that directly innervate the pancreas, thus making it an attractive target for surgical or pharmacological denervation. We demonstrate that surgical excision or application of RTX to the celiac ganglion inhibits SP release and reduces the severity of secretagogue-induced 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 Sprague-Dawley rats, weight-

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was removed. For those rats in the RTX group, 100 ng application of resiniferatoxin (RTX) to the celiac ganglion (***, *P* < 0.001). Optical sections (0.5 μm) of 512 × 512 pixels were obtained and processed using Adobe PhotoDeluxe. Quantification of NK-1R endocytosis was performed by analyzing 10 NK-1R immunoreactive acinar cells per mouse (*n* = 5) and determining the number of these cells containing >15 NK-1R-immunoreactive endosomes. Cytoplasmic endosomes were distinguished from plasma membrane-associated NK-1R immunoreactivity by ensuring that the nucleus of the acinar cells was in the same optical section as the NK-1R-immunoreactive endosomes.

**Statistical analysis.** Results are means ± SE. Statistical comparisons among groups were examined by one-way ANOVA with the Tukey posttest, using GraphPad Prism version 4.02. Statistical significance was set at *P* < 0.05.

**RESULTS**

Repeated administration of supramaximal-stimulating doses of caerulein caused pancreatitis as evidenced by elevations in pancreatic edema, MPO activity, and serum amylase. Caerulein-treated, sham-operated rats had a 3.25-fold increase in pancreas-to-body weight ratio (0.004 vs. 0.013, *P* < 0.001, *n* = 6) (Fig. 1). As shown in Fig. 2, caerulein substantially increased the size of the gross pancreas. Caerulein-treated, sham-operated rats also demonstrated a 2.8-fold increase in MPO levels (27.5 vs. 76.1 U/mg pancreatic protein, *P* < 0.01) (Fig. 3) and a 5.5-fold increase in amylase levels (1.2 vs. 6.6 mg/ml, *P* < 0.001) (Fig. 4) compared with vehicle-treated, sham-operated rats.

We hypothesized that primary sensory neuronal function, particularly SP release, was important in the pancreatic response to caerulein-induced injury. To investigate whether SP release occurred in the pancreas following caerulein administration, NK-1R internalization on pancreatic acinar cells was quantified. Caerulein treatment significantly increased NK-1R internalization with only 6% of cells demonstrating ≥15 vesicles per cell under basal conditions compared with 92% of cells following caerulein treatment (*P* < 0.0001) as shown in Figs. 5 and 6.

The axons of primary sensory nerves innervating the pancreas course through the celiac ganglion. To determine whether interruption of these nerves might affect SP release and the inflammatory response following caerulein administration, sur-
gical ganglionectomy was performed. Celiac ganglionectomy caused a 46% reduction in pancreas-to-body weight ratio (0.013 vs. 0.007, \( P < 0.001 \)) (Fig. 1), indicating a significant reduction in caerulein-induced pancreatic edema. Similarly, NK-1R internalization was reduced by 51% in caerulein-treated rats postceliac ganglionectomy. Although MPO activity was lower in rats that underwent ganglionectomy, this result was not statistically significant. Amylase activity was not statistically different between any of the observed groups, which is consistent with caerulein having a direct effect on the pancreas (24, 25).

To determine whether selective destruction of primary sensory neurons in the celiac ganglion may alter caerulein-induced pancreatitis, RTX was applied to the celiac ganglion. RTX is a selective agonist of TRPV1 that, in high concentrations, selectively destroys C and A\(\delta\) fibers. Importantly, RTX administration to the celiac ganglion reduced caerulein-induced pancreatic edema by 46% (0.013 vs. 0.007 g, \( P < 0.001 \)) (Fig. 1),
MPO activity by 47% (76.1 vs. 40.2 U/mg protein, \(P/<H_110210.05\)) (Fig. 3), and NK-1R internalization by 80% (Figs. 5 and 6). To confirm that RTX was acting locally at the ganglion and did not have a systemic effect, an eye-wipe test was performed. The eye-wipe responses to corneal application of 0.1% capsaicin were identical in both RTX- and sham-treated rats, indicating that RTX did not cause systemic destruction of primary sensory neurons (results not shown).

Ganglionectomy and RTX treatments alone had no effect on any pancreas measurements and were not different from rats that had sham surgery (results not shown).

The gross appearances of the pancreas from vehicle- and caerulein-treated rats with and without ganglionectomy or RTX pretreatment are shown in Fig. 2. Both celiac ganglionectomy and RTX substantially reduced the marked edematous enlargement of the pancreas stimulated by caerulein. Figure 7 shows histological sections of pancreas from the same groups. Caerulein treatment caused edema, cellular disruption, acinar cell vacuolization, and necrosis. These parameters were significantly reduced in pancreata from animals treated with celiac ganglionectomy or RTX.

**DISCUSSION**

It has previously been shown that SP mediates many of the changes that are characteristic of neurogenic inflammation (5). Through interaction with the NK-1R, SP induces plasma extravasation from postcapillary venules and neutrophil infiltration (22). Mice with genetic deletion of NK-1R are protected against secretagogue-induced pancreatitis (2) and choline-deficient, ethionine-supplemented diet-induced hemorrhagic pancreatitis (18), suggesting that blocking the effects of SP mitigates neurogenic inflammation, which appears to be important in the pathogenesis of acute pancreatitis. Conversely, it has been shown that preventing the degradation of SP by inhibition of the membrane protease, neutral endopeptidase, causes a relative increase in SP and can increase plasma extravasation (17). These studies suggest that SP through its interaction with...
the NK-1R is instrumental in the pathogenesis of neurogenic inflammation.

Primary sensory neurons of the C and Aδ subtype synthesize SP, which is released from nerve endings upon stimulation (9, 10, 29). It has been demonstrated in secretagogue and obstructive models of pancreatitis that primary sensory denervation reduces the severity of pancreatic inflammation (25). This finding supported a role for these neurons being a common pathway for the development of neurogenic inflammation.

TRPV1 is a nonselective cation channel that resides on C and Aδ fibers, which when activated causes the release of SP. Systemic administration of the TRPV1 inhibitor capsazepine has been shown to reduce the severity of secretagogue-induced pancreatitis (24). However, local denervation of primary sensory neurons of the pancreas as a means to reduce neurogenic inflammation has not been reported previously. We hypothesized that selective disruption of the nerves that directly innervate the pancreas could decrease the severity of secretagogue-

Fig. 7. Effects of caerulein, GN, and RTX treatment on pancreatic histology. A: representative histology sections of pancreas from rats treated with vehicle alone (A), caerulein alone (B), caerulein + GN (C), and caerulein + RTX (D). B: histological severity of pancreatitis was evaluated using a scale of 0–4 (see MATERIALS AND METHODS). ***P < 0.001.
induced pancreatitis. Innervation of the pancreas by primary sensory neurons originates from dorsal root ganglion cells (35). The axons of these nerve fibers course through the celiac ganglion making it a possible target for pharmacological or surgical manipulation.

In this study, we sought to compare surgical vs. pharmacological denervation of pancreatic primary sensory neurons. RTX is a member of the vanilloid family but is more potent than capsaicin for activating the TRPV1 receptor (30). When RTX is applied to TRPV1-expressing cells, a significant and prolonged increase in free intracellular calcium occurs (33). The resultant calcium toxicity destroys only the TRPV1-expressing cells (28). In the present study, we applied RTX to the celiac ganglion to selectively eliminate TRPV1-expressing primary sensory neurons. To confirm that the effect of RTX administration to the celiac ganglion was local rather than systemic, we evaluated chemogenic pain perception by forepaw eye-wiping movements in response to application of 0.1% capsaicin (16). Previous studies have shown that systemic denervation of primary sensory nerves attenuates the eye-wipe response (12). Our finding that the eye-wipe response was similar in RTX and sham groups indicates that animals did not have systemic denervation.

Both in vitro and in vivo studies have demonstrated that after binding SP, the NK-1R is rapidly internalized by endocytosis and eventually recycled to the plasma membrane after degradation of bound SP (3, 6, 19, 20, 21). In the current study, we used quantification of NK-1R-immunoreactive endosomes in pancreatic acinar cells to provide an estimate of endogenous SP release. This is a measure of dynamic SP release (rather than total SP content in the pancreas, which may not reflect SP release) and has been used in a number of other studies for evaluating SP release (3, 6, 19, 20, 21). In the current study, we demonstrated that caerulein-induced pancreatitis was associated with an increase in NK-1R internalization, which was reduced by both surgical celiac ganglionectomy and RTX treatment to the celiac ganglion. We believed that both of these maneuvers interrupt primary sensory nerve signaling, thereby reducing SP release in the pancreas. Both RTX administration and ganglionectomy reduced NK-1R internalization. The magnitude of this reduction was similar to the decline in other parameters of inflammation such as decreases in pancreatic MPO levels and edema. It is not surprising that RTX application to the celiac ganglion was not identical to celiac ganglionectomy. Treatment with RTX selectively destroys TRPV1-bearing neurons, which, in turn, prevents the subsequent release of SP and other transmitters of C and Aδ fibers (e.g., CGRP). Therefore, RTX treatment would be expected to have some actions similar to administration of an SP antagonist. Ganglionectomy, on the other hand, destroys the nerves and axons of the celiac ganglion. Because of this, ganglionectomy affects multiple types of neurons, some of which may even play a protective role against pancreatitis.

Although the application of RTX to the celiac ganglion and celiac ganglionectomy both appear to decrease the severity of pancreatitis as demonstrated by reductions in MPO activity, edema, and histological scoring, they do not completely prevent pancreatitis. These findings suggest that these interventions are affecting only one pathway that contributes to the development of pancreatitis. Extensive cellular injury can produce inflammation that involves other mediators that may not have a neurogenic component. Although it is unknown whether inhibition of other neural pathways could further reduce the severity of pancreatitis, we expect that neurogenic influences represent only one of several important processes in the pathogenesis of the disease.

Similar to other studies, reduction of pancreatitis severity was not accompanied by a decrease in serum amylase levels, which is consistent with direct hyperstimulation of the pancreas by caerulein (25). Even in experimental pancreatitis, hyperamylasemia is not synonymous with pancreatitis, and we believe that the hyperamylasemia observed in the current study is consistent with caerulein-induced pancreatic injury that does not necessarily lead to pancreatitis. Previous studies have also found that the levels of amylase in the disease course of pancreatitis can be variable and do not correlate accurately with the severity of pancreatitis (25, 27).

In conclusion, caerulein-induced pancreatitis is associated with an increase in NK-1R internalization, consistent with local release of SP; marked pancreatic edema and neutrophil infiltration; and changes in pancreatic histology, indicative of acute pancreatitis. Surgical disruption or local application of RTX to the celiac ganglion reduced NK-1R internalization and reduced the severity of acute secretagogue-induced pancreatitis. These findings indicate that activation of primary sensory nerves is involved in the pancreatic response to injury. It is possible that selectively disrupting TRPV1-bearing neurons could be used to reduce pancreatitis severity.

REFERENCES


