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The proteinase/proteinase-activated receptor-2/transient receptor potential vanilloid-1 cascade impacts pancreatic pain in mice

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ABSTRACT

Aims: Proteinase-activated receptor-2 (PAR2) and transient receptor potential vanilloid-1 (TRPV1) are co-localized in the primary afferents, and the trans-activation of TRPV1 by PAR2 activation is involved in processing of somatic pain. Given evidence for contribution of PAR2 to pancreatic pain, the present study aimed at clarifying the involvement of TRPV1 in processing of pancreatic pain by the proteinase/PAR2 pathway in mice.
Main methods: Acute pancreatitis was created by repeated administration of cerulein in conscious mice, and the referred allodynia/hyperalgesia was assessed using von Frey filaments. Injection of PAR2 agonists into the pancreatic duct was achieved in anesthetized mice, and expression of Fos in the spinal cord was determined by immunohistochemistry.
Key findings: The established referred allodynia/hyperalgesia following cerulein treatment was abolished by post-treatment with nafamostat mesilate, a proteinase inhibitor, and with capsazepine, a TRPV1 antagonist, in mice. Injection of trypsin, an endogenous PAR2 agonist, or SLIGRL-NH_2, a PAR2-activating peptide, into the pancreatic duct caused expression of Fos protein in the spinal superficial layers at T8-T10 levels in the mice. The spinal Fos expression caused by trypsin and by SLIGRL-NH_2 was partially blocked by capsazepine, the former effect abolished by nafamostat mesilate.
Significance: Our data thus suggest that the proteinase/PAR2/TRPV1 cascade might impact pancreatic pain, in addition to somatic pain, and play a role in the maintenance of pancreatitis-related pain in mice.

Introduction

In patients with acute pancreatitis, severe abdominal pain is the most frequent reasons for seeking medical attention. Acute pancreatitis is a unique situation in which the pancreas is injured by trypsin that is prematurely autoactivated within the pancreas, and causes severe systemic inflammation. Proteinase-activated receptor-2 (PAR2), a G-protein-coupled receptor activated by trypsin and some other proteinases including tryptase and kallikreins (Bunnett 2006; Oikonomopoulou et al. 2006), plays a dual role in acute pancreatitis, being protective (anti-inflammatory) and pro-inflammatory depending on the experimental conditions (Kawabata et al. 2006, 2008; Laukkarinen et al. 2008; Matej et al. 2006; Namkung et al. 2004; Singh et al. 2007). PAR2 is expressed in the primary afferents (Steinhoff et al. 2000), and participates in processing of not only somatic pain (Kawabata et al. 2001; Vergnolle et al. 2001) but also visceral pain including pancreatic pain (Coelho et al. 2002; Hoogerwerf et al. 2004, 2001; Kawao et al. 2004).

Transient receptor potential vanilloid-1 (TRPV1), a capsaicin receptor, functions as a sensor for thermal and acidic nociception, and plays critical roles in the processing of somatic and visceral inflammatory pain (Cervero and Laird 2004; Stucky et al. 2009). We have shown that a TRPV1 antagonist abolishes the thermal hyperalgesia induced by intraplantar administration of SLIGRL-NH_2, a PAR2-activating peptide (Kawao et al. 2002) and the neurally mediated gastric mucus secretion following PAR2 stimulation (Kawabata et al. 2002). A delayed sensitization of TRPV1 occurs after intracolonic administration of SLIGRL-NH_2 (Kawao et al. 2004). Interestingly, two independent studies have provided direct evidence that PAR2 stimulation causes trans-activation of TRPV1 via activation of protein kinase C (PKC), leading to facilitation of pain signaling (Amadesi et al. 2004; Dai et al. 2004). Nonetheless, involvement of the PAR2/TRPV1 pathway in processing of visceral nociception including pancreatic pain has yet to be investigated.

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In the present study, to clarify the impact of the proteinase/PAR2/TRPV1 cascade on processing of pancreatic pain, we examined 1) if a single administration of nafamostat mesilate, a highly potent proteinase inhibitor, or capsazepine, a TRPV1 antagonist, reduces the established referred hyperalgesia in mice with acute pancreatitis, and 2) if they block expression of Fos in the spinal dorsal horn after infusion of PAR2 agonists, trypsin or SLIGRL-NH_2, in mice.

Materials and methods

Experimental animals

Male ddY mice weighing 18–24 g were purchased from Japan SLC Inc. (Shizuoka, Japan). All experimental protocols were approved by the Committee for the Care and Use of Laboratory Animals at Kinki University and were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 80-23, revised 1996).

Creation of cerulein-induced acute pancreatitis and drug administration schedules in conscious mice

As described previously (Kawabata et al. 2006), cerulein at 50 μg/kg was administered i.p. to mice 6 times at 1-h intervals. Referred allodynia/hyperalgesia in the upper abdomen was evaluated 30 min after the final dose of cerulein, as mentioned below. The establishment of acute pancreatitis was confirmed by measuring plasma amylase activity and pancreatic weight in the mice. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined as parameters of hepatic tissue damage. In inhibition experiments, nafamostat mesilate at doses of 0.1, 1.0 and 10 mg/kg was administered i.p. 15 min after the last dose of cerulein (15 min before the nociceptive testing), and capsazepine at 15 mg/kg was administered s.c. 10 min after the last dose of cerulein (20 min before the nociceptive testing). In prevention experiments, capsazepine at 15 mg/kg was administered s.c. twice, 30 min before the 1st and 4th doses of cerulein.

Assessment of sensitivity to mechanical stimulation to the upper abdomen in mice

The abdominal sensitivity of each mouse to mechanical stimuli was determined using von Frey filaments with strengths of 0.02, 0.16 and 1.0 g, as described previously (Kawabata et al. 2006). The mouse was placed on a raised wire mesh floor under a clear plastic box (23.5×16.6×12.4 cm), and acclimated to the experimental environment for 30 min. The upper abdomen of each mouse was stimulated with three distinct filaments, in ascending order of force. The mechanical stimulation with each filament was applied 5 times at intervals of 5–10 s, and, after a 1-min resting period, another 5 times in the same manner, namely 10 times in total. Attention was paid not to stimulate the same point twice in succession, considering "wind-up" effects or desensitization. Scoring of nociceptive behavior was defined as follows: score 0 = no response; score 1 = immediate escape or licking/scratching of site applied with von Frey hairs; score 2 = strong

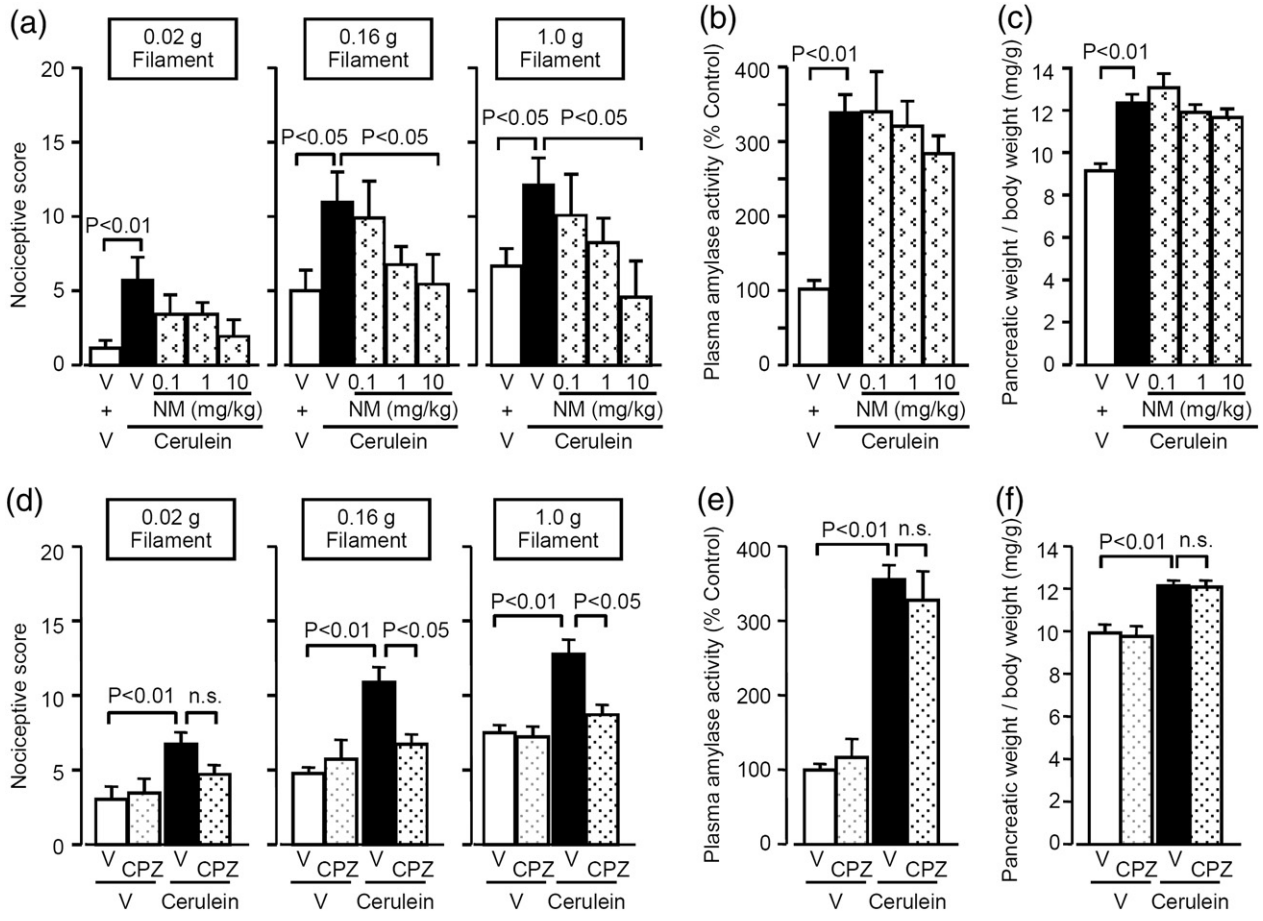

Fig. 1. Effect of post-treatment with nafamostat mesilate, a proteinase inhibitor, and capsazepine, a TRPV1 antagonist, on the established referred allodynia/hyperalgesia in mice with cerulein-induced pancreatitis. Cerulein at 50 μg/kg was administered i.p. to mice at 1-h intervals, 6 times in total. Nafamostat mesilate (NM) at 0.1, 1.0 and 10 mg/kg was administered i.p. to mice 15 min after the last dose of cerulein (a, b, c), and capsazepine (CPZ) at 15 mg/kg was administered s.c. to mice 10 min after the last dose of cerulein (d, e, f). The nociception test (a, d) was conducted 30 min after the last dose of cerulein, followed by determination of plasma amylase activity and pancreatic weight (b, c, e, f). Data show the mean with S.E.M. from 6–12 (a, b, c) or 4–8 (d, e, f) mice. V, vehicle. n.s., not significant.