Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Pancreas. Author manuscript; available in PMC 2017 November 16.

Published in final edited form as:

Pancreas. 2010 November ; 39(8): 1220–1225. doi:10.1097/MPA.0b013e3181df1c90.

Attenuation of Persistent Experimental Pancreatitis Pain by a Bradykinin B2 Receptor Antagonist

Qingmin Chen, PhD, **Louis P. Vera-Portocarrero, PhD**, **Michael H. Ossipov, PhD**, **Marina Vardanyan, MD, PhD**, **Josephine Lai, PhD**, and **Frank Porreca, PhD**

Department of Pharmacology, University of Arizona College of Medicine, Tucson, AZ

Abstract

Objective—The role of bradykinin (BK) receptors in activating and sensitizing peripheral nociceptors is well known. Recently, we showed that spinal dynorphin was pronociceptive through direct or indirect BK receptor activation. Here, we explored the potential role of BK receptors in pain associated with persistent pancreatitis in rats.

Methods—Experimental pancreatitis and abdominal hypersensitivity were induced by intravenous administrations of dibutyltin dichloride (DBTC). [*des*-Arg^9-Leu^8]BK (B1 antagonist) and HOE 140 (B2 antagonist) were given by intraperitoneal or intrathecal injection. Dynorphin antiserum was given intrathecally. Reverse transcription–polymerase chain reaction was used to detect spinal mRNA for BK receptors.

Results—Dibutyltin dichloride–induced pancreatitis upregulated B1 and B2 mRNA in the thoracic dorsal root ganglion and B2, but not B1, in the pancreas. No changes in spinal B1 or B2 mRNA were observed. Intraperitoneal or intrathecal administration of HOE 140 dose dependently abolished DBTC-induced abdominal hypersensitivity, whereas [*des*-Arg^9-Leu^8]BK was without effect by either route of administration. Antiserum to dynorphin (intrathecal) abolished DBTC-induced hypersensitivity.

Conclusions—These results suggest that blockade of peripheral or spinal BK B2 receptors may be an effective approach for diminishing pain associated with pancreatitis. Moreover, it is suggested that spinal dynorphin may maintain pancreatitis pain through direct or indirect activation of BK B2 receptors in the spinal cord.

Keywords

bradykinin receptor; bradykinin receptor antagonists; pancreatitis pain

Chronic pancreatitis is an ongoing inflammatory disorder characterized by irreversible destruction of the pancreas associated with disabling chronic pain and permanent loss of function. Pain is the most challenging and debilitating symptom associated with chronic pancreatitis present in most patients.[1] Chronic pancreatitis–induced pain is very difficult to manage clinically.[2] Because the mechanisms driving visceral pain remain relatively obscure,

Reprints: Frank Porreca, PhD, Department of Pharmacology, College of Medicine, University of Arizona Health Science Center, 1501 N Campbell Ave, Tucson, AZ 85724 (frankp@u.arizona.edu).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

treatment protocols aimed at relieving pancreatic pain tend to be empirical.[2,3] Initial treatment is generally conservative and includes nonopioid analgesics (non-steroidal anti-inflammatory drugs and acetaminophen), followed by opioids (morphine, meperidine, tramadol).[4] Previous studies have demonstrated that, during the course of chronic pancreatitis, pancreatic sensory nerves are exposed to inflammatory mediators causing neuroimmune interactions, potentially contributing to the persistence of pain.[5] Persistent pain associated with peripheral tissue injury and inflammation has been shown to result from long-term changes in nociceptive processing, including sensitization of nociceptors.[6,7] Known inflammatory mediators including bradykinin (BK), prostanoids, cytokines, nitric oxide, and protons are likely to play a role in sensitization of pancreatic nociceptors in patients with pancreatitis.[8]

In the periphery and viscera, the kinins BK and kallidin (KD) are generated during inflammatory processes and tissue injury and are responsible for direct activation and sensitization of nociceptors.[9–12] Bradykinin and KD exert their pronociceptive effects through activation of the inducible B1 receptors and the B2 receptors, which are constitutively expressed and widely distributed throughout central and peripheral tissues (for review, see Marceau and Bachvarov[13]). Clinical and experimental evidence showed an increased level of BK and KD, as well as the precursor kininogen, in inflammatory conditions.[14–17] Acute pancreatitis is associated with significant elevations in serum BK levels.[18] Pharmacological blockade of the B2 receptor with a selective antagonist alleviated hyperalgesia in acute and chronic inflammatory models,[19–23] further demonstrating a critical role played by BK in inflammatory pain. Moreover, treatment with BK receptor antagonists has been shown to be effective in reducing edema and severity of disease in experimental models of acute pancreatitis.[24–27] However, the contribution of BK B1 or B2 receptors to persistent visceral pain is not known. Therefore, the aims of this study were to investigate the role of BK receptors in pain associated with persistent pancreatitis and to explore the possibility that such pain is mediated through peripheral and/or central BK receptors.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, Ind) weighing 175 to 200 g were used for all experiments. Rats were maintained 3 to a cage in a climate-controlled room on a 12-hour light-dark cycle (lights on at 6:00 AM), and food and water were available ad libitum. All experimental protocols were approved by the Animal Care and Use Committee of the University of Arizona. This study was performed in accordance with the policies and recommendations of the International Association for the Study of Pain.

Induction of Pancreatitis

Experimental pancreatitis was induced according to a protocol reported by Sparmann and colleagues.[28] Dibutyltin dichloride (DBTC; Aldrich, Milwaukee, Wis) was dissolved in 2 parts of 100% ethanol and 3 parts of glycerol and was injected into the tail vein at a dose of 6 mg/kg under isoflurane anesthesia (2–3 L/min, 4.0% until anesthetized and then 2.5% throughout the procedure). Controls received the same volume of vehicle solution only

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(ethanol-glycerol, 2:3). The animals were allowed to recover for 7 days after DBTC injection, before any pharmacological manipulations were performed.

Intrathecal Catheter Implantation

Animals were anesthetized with a combination of ketamine (80 mg/kg) and xylazine (12 mg/kg). Rats were placed in a stereotaxic head-holder, and a midline incision was made at the back of the skull to expose the atlanto-occipital membrane. A PE-10 intrathecal catheter (polyethylene-10 tubing; 4.5–5.0 cm) was inserted into the subarachnoid space and advanced to the midthoracic level of the spinal cord. The catheter was then secured to the musculature at the site of incision, which was then closed. The rats received 4.4 mg/kg gentamicin intramuscularly as a prophylactic precaution after the surgery and were allowed to recover for 3 days before receiving the DBTC injection. Drugs were administered in a volume of 5 μL through a length of PE20 tubing connecting the catheter with the injection syringe. The catheter was cleared by flushing with 9 μL saline after drug administration.

Drugs and Injection

Rats were randomized to experimental groups receiving a B2 antagonist (HOE 140; American Peptide Company, Inc, Sunnyvale, Calif), B1 antagonist ([*des*-Arg^9-Leu^8]BK; Bachem Inc, Torrance, Calif), vehicle (distilled water), dynorphin antiserum (Peninsula Laboratories Inc, San Carlos, Calif), or the control serum. For systemic administration, HOE 140 (0.1, 0.3, 1, 3 μmol/kg) and [*des*-Arg^9-Leu^8]BK (1, 10, 100 μmol/kg) were given intraperitoneally.

Quantitative Real-Time PCR

Total RNA was isolated from the pancreas, the thoracic spinal cord, and the thoracic dorsal root ganglia (DRGs) (T8-T12) by TRIzol method (Invitrogen, Carlsbad, Calif). Two-step reverse transcription (RT) was performed using 1 μg total RNA and the Retro script kit (Ambion, Austin, Tex). Real-time polymerase chain reaction (PCR) analysis was performed on an iCycler MyiQ Single Color Real-Time PCR Detection System (Bio-Rad, Hercules, Calif). The sequences of the genes of interest were taken from GenBank accession numbers B1 receptor NM_030851, B2 receptor X69681, and prodynorphin NM_019374. Gene-specific primers for the amplification were obtained from the Midland Certified Reagent Company. Real-time PCR primers sequences for the amplification were as follows:

- B1 receptor/forward primer: 5′-GCATCTTCCTGGTGGTGG-3′ (nucleotides 540–557)
- B1 receptor/reverse primer: 5′-CAGAGCGTAGAAGGAATGTG-3′ (nucleotides 682–701)
- B2 receptor/forward primer: 5′-CTTTGTCCTCAGCGTGTTC-3′ (nucleotides 364–382)
- B2 receptor/reverse primer: 5′-CAGCACCTCTCCGAAC AG-3′ (nucleotides 506–523)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

- prodynorphin/forward primer: 5′-GCAAATACCCCAAGAGGAG-3′ (nucleotides 670–688)
- prodynorphin/reverse primer: 5′-CGCAGAQAAACCACCATAGC-3′’ (nucleotides 817–835)
- β-actin/forward primer: 5′-CACCATGTACCCAGGCATTG-3′ (nucleotides 990–1009)
- β-actin/reverse primer: 5′-CCACATCTGCTGGAAGGTG-3′ (nucleotides 1131–1149)

The real-time PCR reactions were carried out in a total reaction volume of 20 μL containing the final concentration of 1× SYBR Green Master Mix (Bio-Rad), 300 nM of forward and reverse primers, and 200 ng of cDNA from the RT step. All samples from different animals were run in triplicate using an annealing temperature of 60°C. The expression of target genes was normalized to that of β-actin. The differences of target mRNA expression between treatment and control were analyzed using the comparative CT method. The threshold cycle (CT) is defined as the cycle at which the fluorescence generated from a certain amount of amplified PCR product reaches a fixed threshold. For each sample, the CT value for the control gene (β-actin) was subtracted from the CT value for the gene of interest (B1 receptor, B2 receptor, and prodynorphin) to obtain a ΔCT value. The control sample ΔCT value was then subtracted from the treated animal ΔCT value to obtain the ΔΔCT. The relative fold change from our control was expressed by a calculation of $2^{-\Delta\Delta CT}$ for each sample. The level of expression of each target gene is converted to the copy number of that same target gene, which is relative to 500,000 copies of β-actin.

Visceral Pain Behavioral Measurements

Behavioral responses to light tactile stimuli were determined by probing with von Frey filaments applied to the skin to establish visceral pain hypersensitivity.[29–31] Animals were placed in a suspended plastic chamber with a wire mesh platform and allowed to habituate to the environment for 30 to 60 minutes. Measurements were taken before and after DBTC injection and after administration of drug or vehicle. A 4-g filament was applied from underneath, through the mesh floor, to different points of the midabdomen. Each trial consisted of 10 applications of the filament applied, with a 10-second interval between each application. Behavioral responses to visceral stimuli were determined before DBTC injection, again before drug injection and 15, 30, 45, and 60 minutes after drug administration. A positive response was defined as either abdominal withdrawal from the filament, immediate licking or scratching of the tested site, or jumping. The mean occurrence of withdrawal events in each of the trials was defined as the total number of responses out of 10 applications to the abdomen.

Pancreatic Histology

Animals treated with DBTC or vehicle were killed in a CO_2 chamber, and pancreatic tissue was collected for confirmation of pancreatitis. Pancreata were fixed in 4% paraformaldehyde overnight followed by 30% phosphate-buffered saline/sucrose. Tissues were then embedded in OCT compound (Tissue-Tek Optimal Cutting Temperature Compound; Sahura, Torrance,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Calif), and 10-μm-thin sections were cut with a cryostat maintained at −20°C. Sections of pancreatic tissue were routinely stained with hematoxylin and eosin to visualize pancreatic inflammation. Images were captured using a Nikon E800 (Nikon Inc, Melville, NY) fluorescence microscope and a Hamamatsu C5810 color CCD camera and its proprietary Image Processor software (Hamamatsu Photonic System, Bridgewater, NJ).

Data Analysis

The results are presented as the means and SEM. Statistical analysis was performed with JFlashCalc (www.u.arizona.edu/~michaelo). For comparison of multiple means to a baseline value, we used 1-way analysis of variance (ANOVA) followed by Fisher least significant difference post hoc test. When only 2 means were compared to each other, then Student *t* test was used. Significance was established at $P = 0.05$.

RESULTS

Pancreatic Morphology Analysis

On day 7 after injection of DBTC, the pancreas was harvested, and sections were cut, mounted, and stained with hematoxylin and eosin. In the DBTC-treated rats, signs of inflammation including edema, inflammatory cell infiltration, and acinar cell atrophy were observed. (Fig. 1A). No significant morphological changes were observed in control rats (Fig. 1B).

Systemic B2, But Not B1, Receptor Antagonist Reverses Abdominal Hypersensitivity Induced by DBTC

To investigate the contribution of visceral BK receptors to abdominal hypersensitivity in DBTC-induced pancreatitis, receptor-selective BK antagonists were injected intraperitoneally on day 7 after DBTC injection. Behavioral signs of DBTC-induced pancreatitis pain, manifested by abdominal mechanical hypersensitivity, were well established and predominant on day 7 after DBTC injection.

On day 7 after DBTC injection, the mean frequency of abdominal withdrawal to probing with von Frey filaments in rats significantly ($P < 0.0001$) increased from a pre-DBTC baseline of 0.26 ± 0.09 to 7.71 ± 0.31, indicating abdominal hypersensitivity (Fig. 2A). To investigate the potential role of BK in pancreatitis-induced abdominal hypersensitivity, rats were given B2 antagonist HOE 140 (0.1, 0.3, 1, and 3 μmol/kg) by intraperitoneal injection on day 7 after induction of pancreatitis, and data were gathered at the time of peak effect, which was 15 minutes after injection. Systemic administration of the B2 antagonist HOE 140 produced a significant ($P < 0.0001$), dose-dependent reversal of the abdominal mechanical hypersensitivity, whereas injection of a vehicle was without effect (Fig. 2A). In contrast, systemic intraperitoneal administration of the B1 antagonist DALBK at doses 1, 10, and 100 μmol/kg did not produce a ($P = 0.2041$) reversal of abdominal hypersensitivity (Fig. 2B).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bradykinin B1 and B2 Receptor mRNA Expression in the Pancreas After DBTC Injection

Pancreatic tissue was harvested from rats treated with DBTC or vehicle on day 7 after for quantitative RT-PCR analysis of B1-R and B2-R mRNA expression. A significant upregulation of B2-R mRNA expression was seen in the pancreata of rats treated with DBTC compared with the pancreatic tissue obtained from the vehicle-treated rats ($P < 0.05$). B1-R expression did not change at the same time point examined compared with the vehicle-treated group (Fig. 3).

Spinal B2, But Not B1, Receptor Antagonist Reverses the Abdominal Hypersensitivity Induced by DBTC

Pancreatitis-induced pain was evaluated by probing the abdomen with von Frey filaments on day 7 after injection of DBTC. Response to von Frey stimulation was significantly ($P = 0.00024$) increased from a mean baseline value of 0.24 ± 0.12 to 8.33 ± 0.38, indicating the presence of abdominal hypersensitivity (Figs. 4A and B). Randomly assigned groups of rats received 1 of several intrathecal doses of HOE 140 (3, 10, and 30 pmol) (Fig. 4A), and abdominal withdrawal frequency to mechanical stimulation was measured. Spinal administration of HOE 140 produced a significant dose-dependent reversal of abdominal hypersensitivity ($P < 0.0001$). At the highest dose tested (30 pmol), withdrawal frequency decreased to 3.4 ± 1.17 (Fig. 4A). In contrast, spinal administration of the B1 antagonist DALBK (10, 50, and 100 nmol) did not attenuate abdominal hypersensitivity indicative of DBTC-induced pancreatitis pain over the dose range tested ($P = 0.9689$) (Fig. 4B).

Bradykinin B1 and B2 Receptor mRNA Expression in DRG and Spinal Cord After DBTC Injection

To determine if pancreatitis-induced pain may be due to upregulation of BK receptors, in the DRG and the spinal cord, levels of B1 and B2 receptor mRNA were quantified in the thoracic DRGs and the spinal cord from rats 7 days following vehicle or DBTC injection (Fig. 5A). The mRNA expression of both B1 and B2 receptors in the DRG of rats with DBTC compared with DRGs obtained from vehicle-treated rats was significantly ($P < 0.05$) upregulated. Basal levels of both B1 and B2 receptor mRNA expression were found in the dorsal spinal cord taken from vehicle-treated animals (Fig. 5B). Dibutyltin dichloride–induced pancreatitis did not induce any significant changes in mRNA expression for either BK B1 or B2 receptor in the spinal cord at this time point (Fig. 5B).

Spinal Dynorphin Antiserum Reverses Abdominal Hypersensitivity Induced by DBTC

On day 7 after intravenous DBTC injection, abdominal withdrawal frequency was significantly ($P < 0.05$) increased from a pre-DBTC value of 0.33 ± 0.33 to 8.5 ± 0.43, indicating the presence of abdominal hypersensitivity (Fig. 6). Randomly assigned groups of rats received either intrathecal dynorphin antiserum (200 μg) or control serum. Spinal administration of antiserum to dynorphin (200 μg) produced reversal of abdominal hypersensitivity ($P = 0.00081$), whereas administration of a control serum was without effect (Fig. 6).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

DISCUSSION

The present study explored the role of BK receptors in DBTC-induced model of experimental pancreatitis. Rats with DBTC-induced pancreatitis displayed an increased number of abdominal withdrawal events to probing with von Frey filaments, indicating the presence of abdominal hypersensitivity. We demonstrated that intraperitoneal administration of HOE 140, a B2 antagonist, produced a dose-dependent reversal of abdominal hypersensitivity in this model of pancreatitis, whereas [*des*-Arg^9-Leu^8]BK, a B1 receptor antagonist, had no effect. These data suggest that activation of B2 receptor is the mechanism that contributes to the pain behaviors seen in animals with pancreatitis. This hypothesis is further supported by the observation that BK B2, but not B1, receptor is upregulated in the pancreata of animals treated with DBTC. The effect of the B2 antagonist may therefore be attributed to the blockade of B2 receptors in the pancreas.

The enhancement of behavioral responses to noxious (hyperalgesia) and innocuous (allodynia) stimulation during persistent inflammatory conditions is consistent with sensitization of primary afferent neurons supplying the tissue. Bradykinin, which is generated during the inflammatory process and tissue injury and which may produce peripheral sensitization through the activation of B2 receptors, is among the most potent algogenic inflammatory mediators and regulators of peripheral nociceptors.[32,33] Bradykinin both activates and sensitizes primary afferent neurons.[9–12] Moreover, BK sensitizes nociceptors to mechanical and thermal stimuli to the actions of most inflammatory mediators or pronociceptive neuropeptides.[21,32,33] A number of previous investigations demonstrated that pharmacological blockade of B2 receptors with selective antagonists alleviated hyperalgesia in acute and chronic inflammatory models,[19,20,22,23,34] further supporting the critical role of BK in nociception.

Several mechanisms exist through which BK may promote enhanced nociception in pancreatitis. Previous studies suggest that the pancreas is one of the organs with the highest expression of tissue kallikrein, and the role of the kinin-kallikrein system in acute pancreatitis has been extensively investigated for several decades.[35] It has been demonstrated that kallikrein-like activities and kininogen levels in the pancreatic tissue are significantly increased during acute pancreatitis.[36] Clinical evidence suggests that activation of the kallikrein-kinin system contributes to some symptoms of acute pancreatitis, because serum BK and kallikrein levels in ascites are increased during the course of the disease.[18,37,38] Furthermore, it has been shown that selective blockade of B2 receptors reduces the severity of experimental pancreatitis, emphasizing the important role that BK, acting through the B2 receptor, plays in the maintenance of pancreatic inflammation.[24–27] Whereas these studies focused on the biochemical and inflammatory parameters indicative of pancreatitis, the present investigation extends these observations to include possible referred pain in the form of hypersensitivity to light touch. Application of BK directly to the surface of the pancreas in rats has been shown to activate nociceptive afferent neurons via B2 receptors,[39,40] and the possibility exists that the release of endogenous kinins may be sufficient to account for the severe abdominal pain observed in pancreatitis. Together with the evidence in the present study, showing increased expression of B2 receptors and the behavioral consequences of application of the B2 antagonist, it is suggested that activation

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of pancreatic B2 receptors by BK may mediate the neurogenic inflammation and pain of pancreatitis.

In the present study, intrathecal injection at the thoracic spinal level of HOE 140 also elicited a dose-related inhibition of pancreatitis pain, whereas the B1 antagonist had no effect. Dibutyltin dichloride–induced pancreatitis induced an increased level of both B1 and B2 receptors in the thoracic DRG but no changes in BK receptor expression in the spinal cord. These data demonstrate that activation of B2 receptors at the spinal level also contributes to pain behavior in animals with pancreatitis. Although the role of BK receptors in activating and sensitizing nociceptors in the periphery is well known, our understanding of its activity at central sites is still emerging. Recently, we have demonstrated that the pronociceptive actions of spinal dynorphin in the central nervous system may be the result of a novel interaction with BK receptors.[41–43] Dynorphin was found to induce calcium influx via voltage-sensitive calcium channels in sensory neurons by activating BK receptors and to bind to BK receptors with relatively low affinity.[43] Blockade of BK receptors reversed nerve injury-induced tactile and thermal hyper-algesia, but only at times at which spinal levels of dynorphin were elevated.[42,43] Finally, enhanced nociception induced by spinal BK was abolished by B2 antagonists.[42,43] This evidence indicates that pathologically elevated levels of spinal dynorphin produces pronociceptive effects through the BK receptor. Previous work from our laboratory showed that, in the model of pancreatitis induced with DBTC injection, the referred abdominal mechanical hypersensitivity correlated with significant up-regulation of dynorphin in the thoracic spinal cord.[30] Our result here that spinal administration of antiserum to dynorphin also produced a significant reversal of abdominal hypersensitivity in DBTC-induced pancreatitis model is in agreement with our previous observations.[30] These findings provide strong evidence for the pronociceptive effect of spinal dynorphin in DBTC-induced pancreatitis. It is noteworthy that treatment with both a B2 antagonist and dynorphin antiserum showed a significant antihypersensitivity effect with spinal administration. Therefore, together with the evidence in the present study, it seems likely that increased dynorphin in DBTC-induced pancreatitis may produce a pronociceptive effect through the B2 receptor in the spinal cord.

In conclusion, our findings suggest that both systemic and spinal administration of the BK B2 receptor antagonist HOE 140 is effective in attenuating abdominal hypersensitivity arising from pancreatic inflammation. Systemic effects of HOE 140 could occur at peripheral sites and may be due to blocking the interaction of BK with the B2 receptor in the pancreas. The BK B2 receptors in the pancreas have a role in the generation of pancreatic pain as demonstrated by their increased expression on the same days we observe increased pain behaviors. This time course also corresponds with the upregulation of spinal dynorphin previously reported in response to DBTC injection.[30] These observations, along with our finding that spinal injection of dynorphin antiserum blocked hypersensitivity, suggest that upregulated dynorphin in the spinal cord during pancreatitis acts on BK receptors to maintain the abdominal hypersensitivity. Although it might be hypothesized that activation of kinin receptors could lead to upregulation of spinal dynorphin, this mechanism is unlikely, because BK has not been identified in the spinal cord.[41–43] Therefore, selective B2 antagonists that can penetrate the central nervous system may therefore have therapeutic potential against pancreatitis pain through both peripheral and central sites.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

References

1. Andren-Sandberg A, Hoem D, Gislason H. Pain management in chronic pancreatitis. Eur J Gastroenterol Hepatol. 2002; 14(9):957–970. [PubMed: 12352215]
2. Bonica, JJ., Loeser, JD., Chapman, CR., et al. The Management of Pain. Philadelphia, PA: Lea & Febiger; 1990. Causalgia and other reflex sympathetic dystrophies; p. 220-243.
3. DiMagno EP. Toward understanding (and management) of painful chronic pancreatitis. Gastroenterology. 1999; 116(5):1252–1257. [PubMed: 10220520]
4. Gachago C, Draganov PV. Pain management in chronic pancreatitis. World J Gastroenterol. 2008; 14(20):3137–3148. [PubMed: 18506917]
5. Di Sebastiano P, Fink T, Weihe E, et al. Immune cell infiltration and growth-associated protein 43 expression correlate with pain in chronic pancreatitis. Gastroenterology. 1997; 112(5):1648–1655. [PubMed: 9136844]
6. Woolf CJ, Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. Proc Natl Acad Sci U S A. 1999; 96(14):7723–7730. [PubMed: 10393888]
7. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science. 2000; 288(5472): 1765–1769. [PubMed: 10846153]
8. Di Sebastiano P, di Mola FF, Bockman DE, et al. Chronic pancreatitis: the perspective of pain generation by neuroimmune interaction. Gut. 2003; 52(6):907–911. [PubMed: 12740353]
9. Kanaka R, Schaible HG, Schmidt RF. Activation of fine articular afferent units by bradykinin. Brain Res. 1985; 327(1–2):81–90. [PubMed: 3986521]
10. Kumazawa T, Mizumura K, Minagawa M, et al. Sensitizing effects of bradykinin on the heat responses of the visceral nociceptor. J Neurophysiol. 1991; 66(6):1819–1824. [PubMed: 1667414]
11. Manning DC, Raja SN, Meyer RA, et al. Pain and hyperalgesia after intradermal injection of bradykinin in humans. Clin Pharmacol Ther. 1991; 50(6):721–729. [PubMed: 1752117]
12. Neugebauer V, Schaible HG, Schmidt RF. Sensitization of articular afferents to mechanical stimuli by bradykinin. Pflugers Arch. 1989; 415(3):330–335. [PubMed: 2622760]
13. Marceau F, Bachvarov DR. Kinin receptors. Clin Rev Allergy Immunol. 1998; 16(4):385–401. [PubMed: 9926287]
14. Barlas A, Okamoto H, Greenbaum LM. T-kininogen—the major plasma kininogen in rat adjuvant arthritis. Biochem Biophys Res Commun. 1985; 129(1):280–286. [PubMed: 4004880]
15. Barlas A, Okamoto H, Greenbaum LM. Increased plasma level of T-kininogen in rats treated with Freund's adjuvant. Adv Exp Med Biol. 1986; 198(pt A):61–68. [PubMed: 3812097]
16. Bianchi G, Salvati P, Barlassina C, et al. The kidney in essential hypertension. Contrib Nephrol. 1985; 49:173–178. [PubMed: 3830565]
17. Tsurufuji S, Kumakura S. Role of bradykinin in exudative reactions of inflammation in rats. Dermatologica. 1989; 179(suppl 1):64–67.
18. Ruud TE, Aasen AO, Kierulf P, et al. Studies on the plasma kallikrein-kinin system in peritoneal exudate and plasma during experimental acute pancreatitis in pigs. Scand J Gastroenterol. 1985; 20(7):877–882. [PubMed: 2413521]
19. Asano M, Hatori C, Inamura N, et al. Effects of a nonpeptide bradykinin B2 receptor antagonist, FR167344, on different in vivo animal models of inflammation. Br J Pharmacol. 1997; 122(7): 1436–1440. [PubMed: 9421292]
20. Calixto JB, Cabrini DA, Ferreira J, et al. Kinins in pain and inflammation. Pain. 2000; 87(1):1–5. [PubMed: 10863040]
21. Dray A, Perkins M. Bradykinin and inflammatory pain. Trends Neurosci. 1993; 16(3):99–104. [PubMed: 7681240]
22. Perkins MN, Campbell E, Dray A. Antinociceptive activity of the bradykinin B1 and B2 receptor antagonists, *des*-Arg9, [Leu8]-BK and HOE 140, in two models of persistent hyperalgesia in the rat. Pain. 1993; 53(2):191–197. [PubMed: 8393171]
23. Rupniak NM, Boyce S, Webb JK, et al. Effects of the bradykinin B1 receptor antagonist *des*-Arg9[Leu8]bradykinin and genetic disruption of the B2 receptor on nociception in rats and mice. Pain. 1997; 71(1):89–97. [PubMed: 9200178]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

24. Bloechle C, Kusterer K, Kuehn RM, et al. Inhibition of bradykinin B2 receptor preserves microcirculation in experimental pancreatitis in rats. Am J Physiol. 1998; 274(1 pt 1):G42–G51. [PubMed: 9458772]
25. Griesbacher T, Lembeck F. Effects of the bradykinin antagonist, HOE 140, in experimental acute pancreatitis. Br J Pharmacol. 1992; 107(2):356–360. [PubMed: 1384910]
26. Griesbacher T, Tiran B, Lembeck F. Pathological events in experimental acute pancreatitis prevented by the bradykinin antagonist, HOE 140. Br J Pharmacol. 1993; 108(2):405–411. [PubMed: 8448591]
27. Kanbe T, Naruse S, Kitagawa M, et al. Effects of a bradykinin receptor antagonist (HOE140) on taurocholate-induced acute pancreatitis in rats. Pancreas. 1996; 13(3):283–288. [PubMed: 8884850]
28. Sparmann G, Merkord J, Jaschke A, et al. Pancreatic fibrosis in experimental pancreatitis induced by dibutyltin dichloride. Gastroenterology. 1997; 112(5):1664–1672. [PubMed: 9136846]
29. Vera-Portocarrero LP, Lu Y, Westlund KN. Nociception in persistent pancreatitis in rats: effects of morphine and neuropeptide alterations. Anesthesiology. 2003; 98(2):474–484. [PubMed: 12552208]
30. Vera-Portocarrero LP, Yie JX, Kowal J, et al. Descending facilitation from the rostral ventromedial medulla maintains visceral pain in rats with experimental pancreatitis. Gastroenterology. 2006; 130(7):2155–2164. [PubMed: 16762636]
31. Winston JH, Toma H, Shenoy M, et al. Acute pancreatitis results in referred mechanical hypersensitivity and neuropeptide up-regulation that can be suppressed by the protein kinase inhibitor k252a. J Pain. 2003; 4(6):329–337. [PubMed: 14622690]
32. Juan H, Lembeck F. Proceedings: Influence of prostaglandin E1, indomethacin, calcium and potassium on the action of nociceptive substances. Naunyn Schmiedebergs Arch Pharmacol. 1974; 282 suppl 282(0):R242.
33. Regoli D, Barabe J. Pharmacology of bradykinin and related kinins. Pharmacol Rev. 1980; 32(1): 1–46. [PubMed: 7015371]
34. Griesbacher T, Amann R, Sametz W, et al. The nonpeptide B2 receptor antagonist FR173657: inhibition of effects of bradykinin related to its role in nociception. Br J Pharmacol. 1998; 124(6): 1328–1334. [PubMed: 9720808]
35. Chao J, Chao L. Biochemistry, regulation and potential function of kallistatin. Biol Chem Hoppe Seyler. 1995; 376(12):705–713. [PubMed: 9072045]
36. Griesbacher T, Rainer I, Tiran B, et al. Mechanism of kinin release during experimental acute pancreatitis in rats: evidence for pro- as well as anti-inflammatory roles of oedema formation. Br J Pharmacol. 2003; 139(2):299–308. [PubMed: 12770935]
37. Satake K, Rozmanith JS, Appert H, et al. Hemodynamic change and bradykinin levels in plasma and lymph during experimental acute pancreatitis in dogs. Ann Surg. 1973; 178(5):659–662. [PubMed: 4748305]
38. Satake K, Rozmanith JS, Appert HE, et al. Hypotension and release of kinin-forming enzyme into ascitic fluid exudate during experimental pancreatitis in dogs. Ann Surg. 1973; 177(4):497–502. [PubMed: 4691869]
39. Holzer-Petsche U. Blood pressure and gastric motor responses to bradykinin and hydrochloric acid injected into somatic or visceral tissues. Naunyn Schmiedebergs Arch Pharmacol. 1992; 346(2): 219–225. [PubMed: 1448184]
40. Houghton AK, Wang CC, Westlund KN. Do nociceptive signals from the pancreas travel in the dorsal column? Pain. 2001; 89(2–3):207–220. [PubMed: 11166477]
41. Luo MC, Chen Q, Ossipov MH, et al. Spinal dynorphin and bradykinin receptors maintain inflammatory hyperalgesia. J Pain. 2008; 9(12):1096–1105. [PubMed: 18976961]
42. Lai J, Luo MC, Chen Q, et al. Pronociceptive actions of dynorphin via bradykinin receptors. Neurosci Lett. 2008; 437(3):175–179. [PubMed: 18450375]
43. Lai J, Luo MC, Chen Q, et al. Dynorphin A activates bradykinin receptors to maintain neuropathic pain. Nat Neurosci. 2006; 9(12):1534–1540. [PubMed: 17115041]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

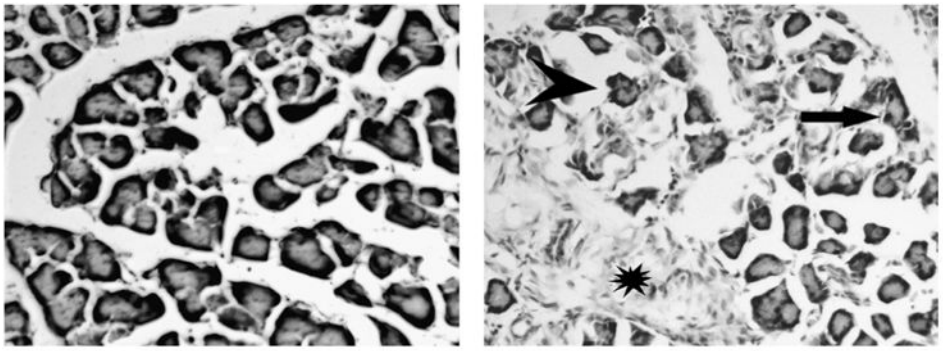

FIGURE 1.

Histological sections of pancreata stained with hematoxylin and eosin from rats injected with vehicle (left) or DBTC (right) on day 7 after administration. The pancreata from DBTC-treated rats showed signs of inflammation including edema (arrowhead), inflammatory cell infiltration (star), and acinar cell atrophy (arrow) (right).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

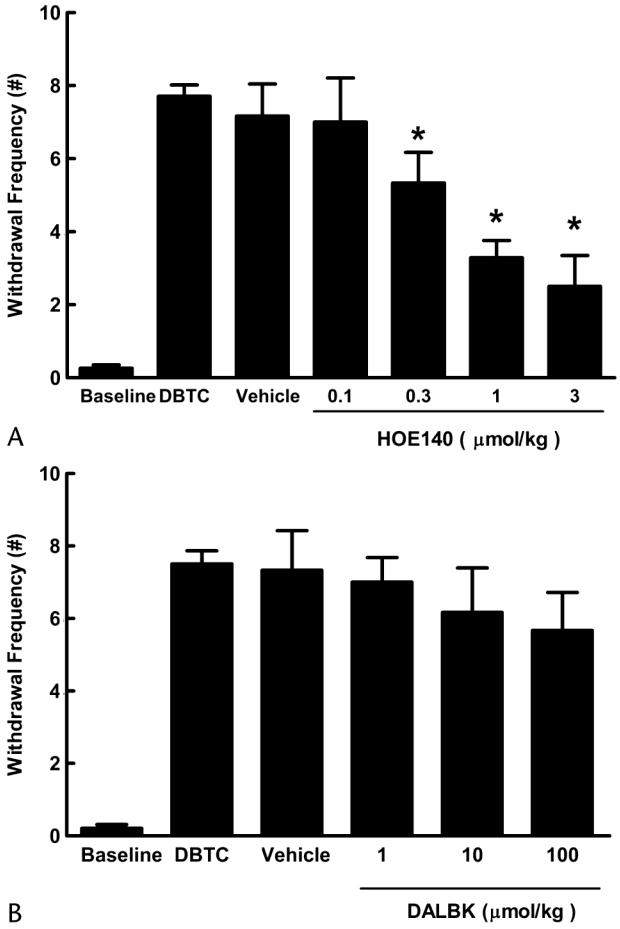

FIGURE 2.

Effects of 0.1 to 3 μmol/kg HOE 140 (A) and 1 to 100 μmol/kg DALBK (B) on mechanical hypersensitivity 15 minutes after intraperitoneal administration are shown. The experiments were done on day 7 after induction of pancreatitis with intravenously administered DBTC. Increased mechanical hypersensitivity is shown in animals after treatment with intravenously administered DBTC. One-way ANOVA, $*P < 0.05$; n = 5–6.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

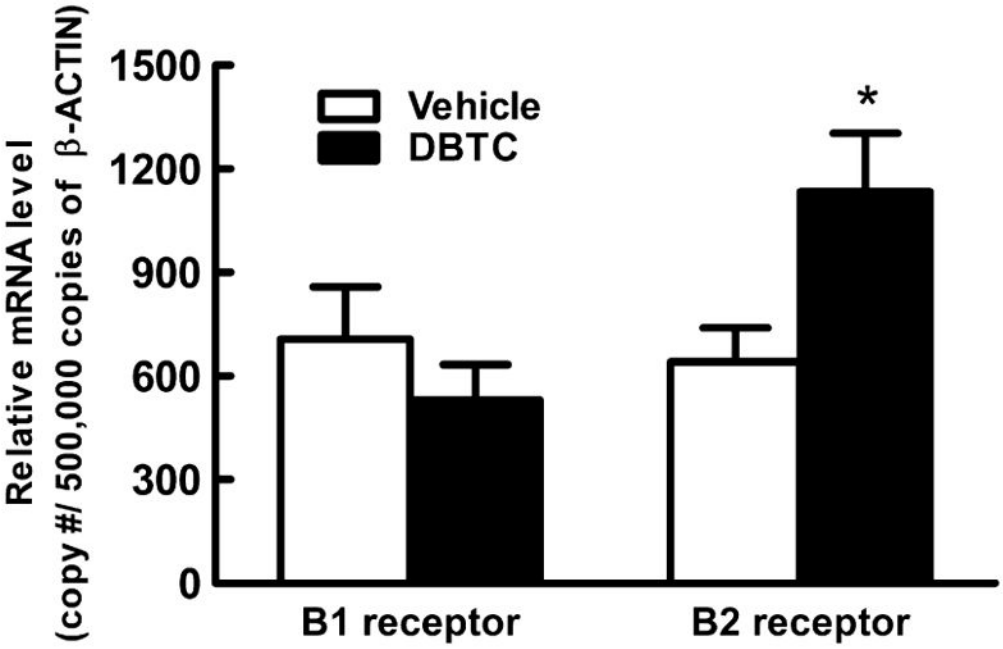

FIGURE 3.

Reverse transcription–PCR analysis of B1-R and B2-R mRNA expression in the pancreas at day 7 after DBTC injection is shown. Student *t* test, $*P < 0.05$.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

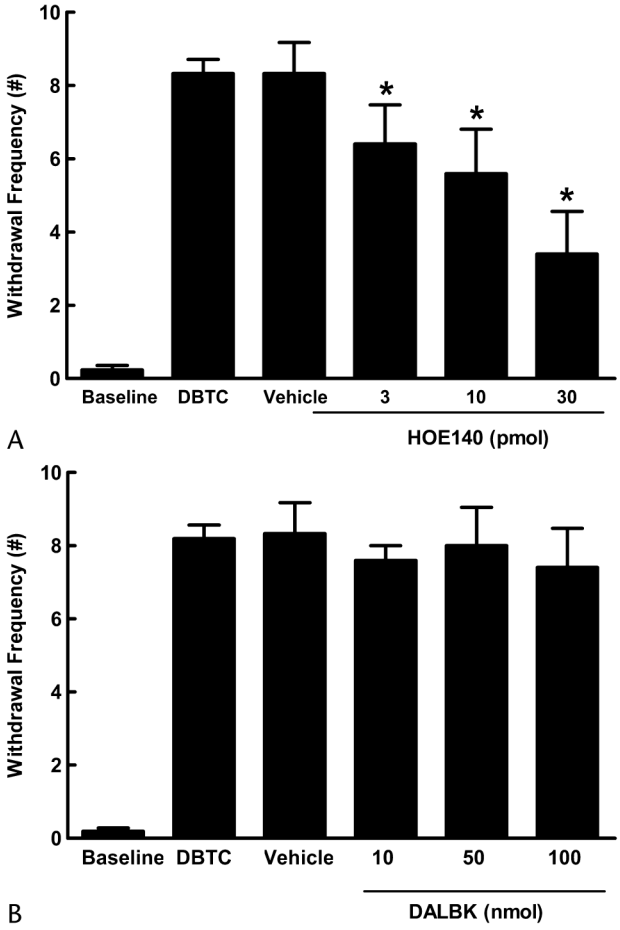

FIGURE 4.

Effects of spinal HOE 140, 3 to 30 pmol (A) and spinal DALBK 30 to 100 nmol (B) on mechanical hypersensitivity 15 minutes after intrathecal administration are shown. The spinal injections were made 7 days after intravenous administration of DBTC. One-way ANOVA, $*P < 0.05$; n = 5–6.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

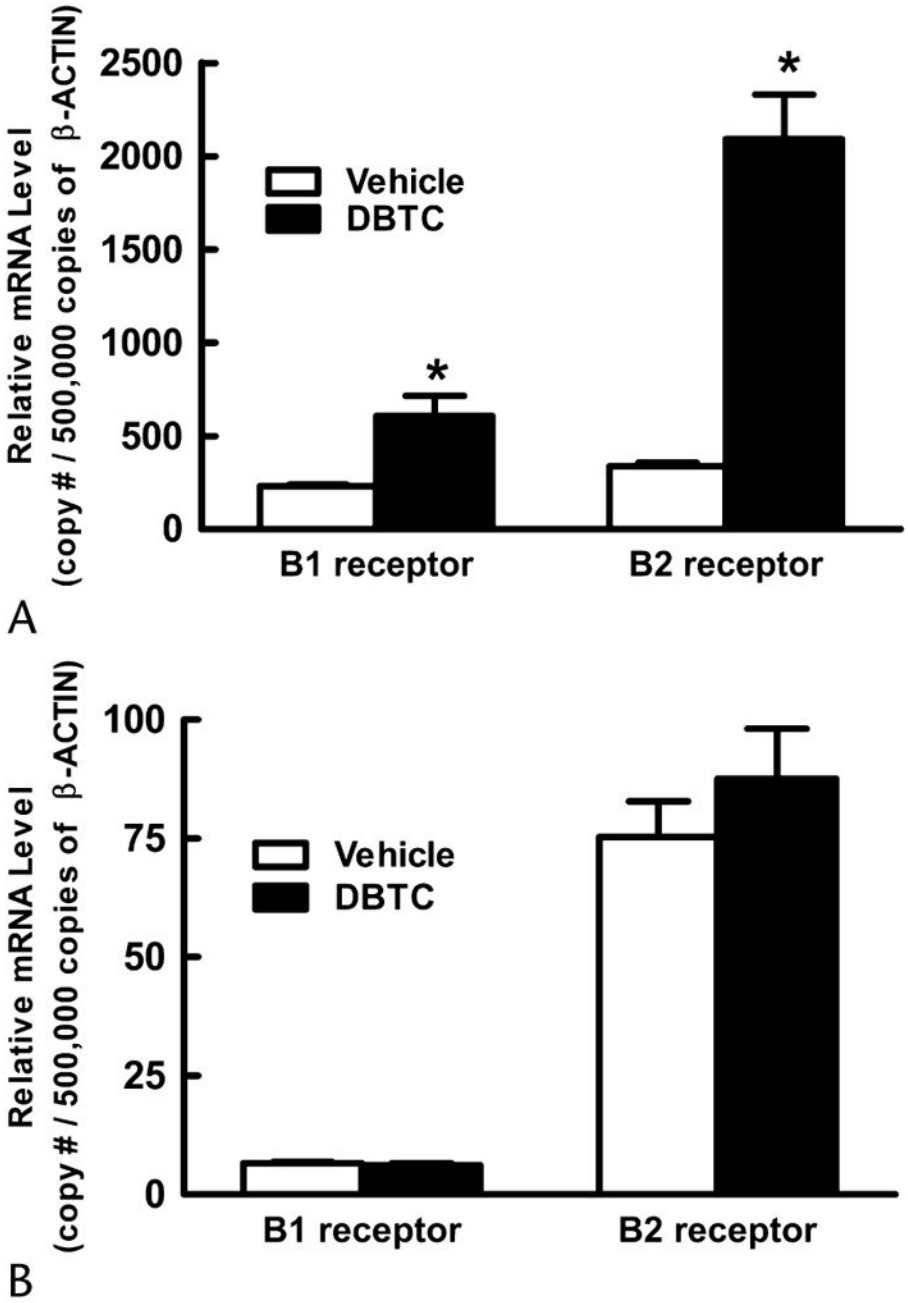

FIGURE 5.

Reverse transcription–PCR analysis of B1-R and B2-R mRNA expression in the thoracic DRG (A) and spinal cord (B) on day 7 after DBTC injection. Student *t* test, $*P < 0.05$.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

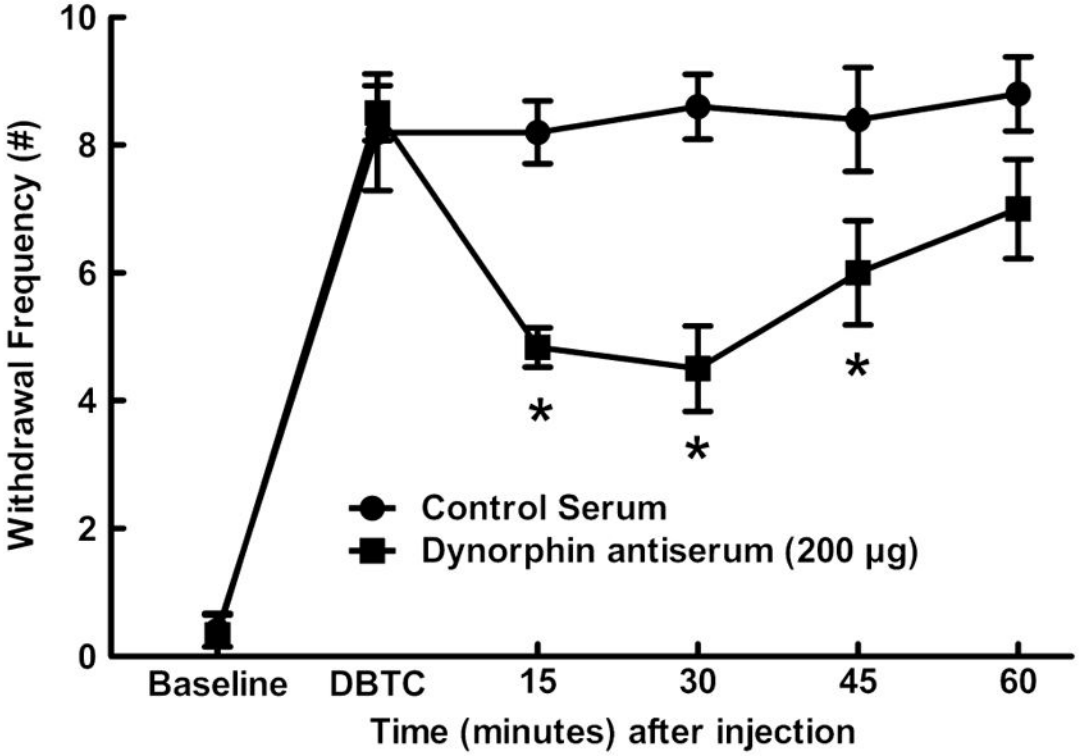

FIGURE 6.

Effects of intrathecal administration of dynorphin antiserum on abdominal hypersensitivity on day 7 after DBTC injection are shown. One-way ANOVA, $*P < 0.05$; n = 5–6.