Capsaicin-sensitive Innervation Modulates the Development of Apical Periodontitis

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Abstract

Introduction: Nociceptive neurons play a critical role in the detection of stimuli evoking actual or potential tissue injury. In addition, they are involved in neurogenic inflammation by the peripheral release of neuropeptides such as calcitonin gene-related peptide (CGRP). The dental pulp and periradicular tissues are innervated by capsaicin-sensitive neurons known to release CGRP. However, the role of these capsaicin-sensitive neurons in the development of apical periodontitis is largely unknown. The aim of this study was to evaluate the contribution of peptidergic neurons to the development of apical periodontitis. **Methods:** Neonatal Sprague-Dawley rats were injected with vehicle (control group) or a single subcutaneous capsaicin dose to cause the selective ablation of peptidergic neurons (neonatal capsaicin group). Ablation of capsaicin-sensitive neurons was verified with confocal microscopy, capsaicin-induced eye-wipe nocifensive behavior test, and by measurement of immunoreactive CGRP levels in the dental pulp. Five weeks after ablation, standardized pulp exposures were made in the mandibular left first molars. Mandibles were harvested at 7, 14, 21, and 28 days after pulp exposure and imaged with micro–computed tomography (μCT) to quantify apical lesion volume. Data were analyzed by using 2-way ANOVA analysis with Bonferroni post hoc test. **Results:** Rats in the control group displayed a robust capsaicin-induced nocifensive behavior, which was nearly abolished in the neonatal capsaicin group. In addition, the neonatal capsaicin group showed a significant depletion of susceptible neurons and CGRP in the dental pulp compared with control. Importantly, micro–computed tomography analysis showed larger periradicular lesions at 7 and 14 days after pulp exposure in the neonatal capsaicin group when compared with control. **Conclusions:** Results identify a protective role for capsaicin-sensitive neurons in the initial phase of apical periodontitis. Thus, interventions or disorders that alter activity of capsaicin-sensitive fibers are likely to alter the development of apical periodontitis. *(J Endod 2016;■:1–7)*

Key Words

Apical periodontitis, CGRP, endodontic infection, microCT, neurogenic inflammation, peptidergic nociceptors, TRPV1

Significance

This study demonstrated that at early stages of injury, a subclass of pain-sensing neurons delays the development of apical periodontitis. Thus, the clinical presentation of pulpal pain represents an early defense mechanism against infection and the development of apical periodontitis.

The goal of endodontic therapy is the prevention and treatment of apical periodontitis. Dental caries, trauma, developmental defects, and defective dental restorations provide portals of entry for microorganisms to infect the pulp (1, 2), resulting in the subsequent development of apical periodontitis. The inflammatory process associated with the development of apical periodontitis leads to several changes in the periradicular tissues including activation and sensitization of primary afferent nociceptors leading to periradicular pain (3) and activation of osteoclasts leading to demineralization of surrounding bone. To date, most studies on the pathogenesis of apical periodontitis have used a rodent model where pulp exposure and subsequent infection from the oral environment (1, 4–7) lead to apical periodontitis. The progression of apical periodontitis in this model is typically evaluated by the changes in the periradicular bone structure as accomplished with the use of periradicular radiographs (8). Recently, 3-dimensional (3D) micro–computed tomography (μCT) was introduced as a more accurate method that allows for the quantification of periradicular bone destruction (9, 10). Many studies have described the cellular and inflammatory components of the innate and acquired immune response with the use of the pulp exposure model (4, 7, 11). However, the role played by specific subsets of sensory neurons implicated in neurogenic inflammation in the initiation and progression of apical periodontitis is not completely understood and represents an important gap in knowledge.

The dental pulp and periradicular tissues are innervated by peptidergic as well as non-peptidergic sensory nerve fibers (3, 12). Calcitonin-gene related peptide (CGRP) and substance P (SP) are among many neuropeptides known to be expressed and released from peripheral primary afferent peptidergic nerve terminals (13). Dental pulp injury that is due to caries, trauma, or restorative procedures results in substantial sprouting of CGRP-containing nerve fibers (14–16). Importantly, *in vitro* activation of primary afferent fibers leads to the robust release of both CGRP and SP from the human dental pulp (17, 18), resulting in tissue edema (19) and an infiltration of inflammatory cells, a process known as neurogenic inflammation (20).

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.06.009

Neuropeptides such as CGRP and SP are co-expressed with the transient receptor potential vanilloid-1 (TRPV1), an ionotropic channel expressed in a major subpopulation of trigeminal sensory neurons that respond to noxious stimuli (21). Capsaicin, a potent activator of TRPV1 channels, evokes the influx of cations into the nociceptor terminal, leading to depolarization and the release of vasoactive peptides through a calcium-dependent mechanism (22). Importantly, prolonged activation of TRPV1 in neonatal animals may lead to supraphysiological accumulation of calcium, resulting in neurotoxicity and neuron death (23). Therefore, systemic administration of capsaicin in newborn rodents triggers the pharmacologic ablation of TRPV1-expressing neurons (24, 25). This approach has been used extensively in pain research to investigate the role of peptidergic neurons and neurogenic inflammation in peripheral pain mechanisms. However, the role of TRPV1-expressing neurons in regulating apical periodontitis is largely unknown. Therefore, the purpose of this study was to evaluate the role of capsaicin-sensitive neurons in the development of pulpal and periradicular disease.

Materials and Methods

Animals and Neonatal Capsaicin Treatment

Eighteen female Sprague-Dawley rats with timed pregnancies were housed for 5–7 days at the University of Texas Health Science Center at San Antonio according to the guidelines established by our Institutional Animal Care and Use Committee, the National Institutes of Health Guide for the care and use of laboratory animals, and conforming to the ARRIVE guidelines of the National Center for the Replacement and Reduction of Animals in Research. A total of 185 pups were obtained from the timed-pregnant rats. The pups were assigned randomly to either the vehicle or capsaicin group.

At day 2 after birth, pups received a single subcutaneous injection of capsaicin (neonatal capsaicin group: 50 mg/kg, 5 μL/g; Sigma-Aldrich, St Louis, MO) or vehicle (control group: 10% ethanol, 10% Tween 80 in isotonic saline) into the dorsal interscapular region (25). Immediately after the injections, the pups were placed on a heating pad and monitored until they regained normal reflexes. The pups were then transferred back to the cage with their respective mothers and maintained on a 12-hour light/dark schedule with *ad libitum* access to food and water. Rats were weaned 3 weeks after birth by moving them into a new cage with 2 other animals from the same group. At weaning, 95% (n = 85) survived in the neonatal capsaicin group, and 99% (n = 94) survived in the control group.

Capsaicin-evoked Nocifensive Behavior

Five weeks after neonatal capsaicin treatment, the eye-wipe nocifensive behavior test was used to verify the effectiveness of the pharmacologic ablation of the capsaicin-sensitive innervation (26). A solution of 0.01% (w/v) capsaicin was instilled onto 1 eye of freely moving animals in both groups (n = 80/group). The time spent grooming or closing the affected eye was recorded for a total of 2 minutes, with observers unaware of treatment allocation.

Measurement of CGRP Levels in the Dental Pulp

Measurement of CGRP levels in the dental pulp was used to verify the ablation of peptidergic capsaicin-sensitive neurons. Briefly, randomly selected animals were killed by decapitation (n = 6 rats/group), and the mandibular molars were extracted. The dental pulp was extracted from the 4 molars of each animal by using a surgical forceps and placed in NP40 cell lysis buffer (Life Technologies, Carlsbad, CA) in presence of protease inhibitors (Complete Protease Inhibitor Cocktail tablets; Roche, Indianapolis, IN). The dental pulp tissue was homogenized in TissueLyser LT (Qiagen, Valencia, CA). Next, samples were centrifuged at 13,000 rpm for 5 minutes in a refrigerated microcentrifuge (Savant, Holbrook, NY). The supernatant was collected, and total protein was quantified by using the BCA protein assay (Thermo Scientific, Rockford, IL). Tissue level of rat CGRP was measured with enzyme-linked immunoassay following manufacturer's instructions (Cayman Chemical, Ann Arbor, MI).

Apical Periodontitis Model

A total of 64 rats in the neonatal capsaicin group that demonstrated complete lack of capsaicin-evoked nocifensive responses were included with the same number of vehicle-treated littermates (n = 128 total).

Rats from both groups were anesthetized by intraperitoneal injection of ketamine (75 mg/kg)/Dexdomitor (0.5 mg/kg). Animals were then placed on a custom jaw retraction apparatus, and pulpal exposure of the lower left first molar of each rat was performed by using a sterile size #1/2 round bur rotated by a low-speed handpiece to the depth of the diameter of the bur with the aid of a surgical microscope (Zeiss, Ontario, CA). Pulp exposure was verified by inserting #8 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into pulp canals. Anesthesia was reversed by intraperitoneal injection of Antisedan (Zoetis, Florham Park, NJ) (0.5 mg/kg). Teeth were left open to the oral environment for 7, 14, 21, and 28 days. Animals had free access to acetaminophen (2 mg/mL) in drinking water for postoperative pain management for a period extending from 2 days before pulp exposure to 2 days after exposure (4 days total). The body weight of animals in both groups was measured weekly after pulp exposure.

Tissue Collection

Sixteen rats from each group were killed at 7, 14, 21, and 28 days after pulp exposure. The animals were killed with Euthasol (Virbac Corporation, Fort Worth, TX) (100 mg/kg by intraperitoneal injection of a solution containing 390 mg/mL pentobarbital sodium and 50 mg/mL phenytoin sodium) followed by decapitation. The mandible containing the tooth with the pulp exposure and its contralateral control were dissected free of soft tissues and fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB) for 2 hours, rinsed, and stored in 0.1 mol/L PB buffer at 4°C until the mandibles were subjected to μCT.

Micro–Computed Tomography

Fixed mandible samples were scanned in a μCT system (Bruker Skyscan1172, Aartselaar, Belgium). Each specimen was placed in a vial, submerged in 0.1 mol/L PB, and positioned with the incisor oriented upward. Samples were scanned with the following settings: 60 kV, 167 μA, 0.7° rotation step, 4 frame averaging, 2000 × 1336 charge coupled device, 700 millisecond exposure, and 10 micrometer voxel size. A 0.5-mm aluminum filter was used during scans, and a polynomial correction was also used to reduce beam-hardening effects during reconstructions. The total scan time for each sample was 22 minutes. The volume of interest (VOI) included the void space of periradicular destruction and/or periodontal ligament space, encompassing all roots of the first molar as a measure of apical lesion volume. The VOI started at the first transaxial slice, where the molar was completely encapsulated by crestal bone and continued toward the root apex, ending at the last slice where the void space was seen (Fig. 1). An automated script was used to isolate the void space, and this volume was analyzed and used to determine the effect of treatment.

Immunohistochemistry

Randomly selected contralateral hemi-mandibles of rats at 8 weeks after capsaicin or vehicle injection were prepared for