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Short communication

Tooth injury increases expression of the cold sensitive TRP channel TRPA1 in trigeminal neurons

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ABSTRACT

Objective: Transient receptor potential (TRP) channels, a family of structurally related proteins have been implicated in the sensation of pain and hyperalgesia caused by exogenous and endogenous agonists, as well as touch, pH, and temperature. The objective of this study was to determine the effects of tooth injury on the expression of the cold sensitive channel TRPA1, in the trigeminal ganglion, the primary source of sensory and nociceptive innervation of teeth.

Design: We analyzed TRPA1 expression in a rodent model of tooth injury, by Western blot analyses of proteins extracted from trigeminal ganglia.

Results: We found that TRPA1 was selectively increased in trigeminal ganglia innervating injured teeth when compared to TRPA1 expression in trigeminal ganglia innervating healthy teeth.

Conclusions: Our results provide the first evidence of increased expression of a cold-sensitive TRP channel in trigeminal ganglia after pulp exposure, and are consistent with the possibility that increased expression and function of TRPA1 in trigeminal neurons contributes to hyperalgesia and allodynia following tooth injury.

1. Introduction

The suppression of pain is of major clinical concern for a variety of conditions caused by disease or injury to tissues, including those within the oral cavity. Pain associated with tooth decay or trauma is frequently associated with an increased sensitivity to heat, cold, and other environmental stimuli. The dental pulp, the vital component of teeth, is densely innervated by free nerve endings which originate from neurons in the trigeminal ganglia (TG).[1,2] Nerve endings within the pulp are equipped with a variety of ion channels, receptors and neuropeptides known to modulate nociception.[3–6] TG neurons are therefore important cellular components in pain perception in the orofacial region. There is evidence that tooth injury is associated with increased expression of nociceptive ion channels in rodent TG neurons, suggesting that activation of pain pathways can induce global changes in trigeminal expression of nociceptive molecules[7,8]; however, a detailed understanding of the molecules and mechanisms involved in pain associated with tooth injury is lacking.

Recent studies have shown that temperature-sensitive channels of the transient receptor potential (TRP) family play a major role in sensing hot and cold temperatures and in pain perception associated with temperature.[6,9] TRPV1 is a capsaicin-sensitive channel that is activated by elevated (>42 °C) temperature.[10] TRPM8 and TRPA1 channels are responsive to reduced temperatures when expressed in heterologous cells (<25 °C and <18 °C, respectively), and are selectively

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responsive to ligands such as menthol, and icillin.[11–13] In contrast, the role of TRPA1 in cold sensation is less clear *in vivo*. Cellular and behavioural analyses of TRPA1-deficient mice showed that TRPA1 is not essential for acute responses to cold[14]; however, suppression of TRPA1 by antisense nucleotides reduces cold allodynia in rat models of inflammation and nerve injury.[15] Other results also support the idea that TRPM8 is important for acute cold sensation under normal physiological conditions and that TRPA1 is a mediator of cold hypersensitivity in pathological conditions associated with inflammation.[16,17]

In addition to a role in noxious cold sensation, there is increasing evidence in support of a role for TRPA1 channels in a variety of nociceptive pathways. TRPA1 channels are activated by environmental irritants, oxidative stress, inflammatory peptides, and mechanical stress.[18–21] The sensitivity of TRPA1 to tetrahydrocannabinol (THC) and morphine indicates that there may be other endogenous modulators of TRPA1 function.[22,23] TRPA1-deficient mice exhibit pronounced deficits in bradykinin-evoked nociceptor excitation and pain hypersensitivity,[24,25] suggesting that TRPA1 function is an important component of signalling pathways by which inflammatory mediators produce pain and hyperalgesia. There is further evidence that the bradykinin-induced inflammatory response occurs by sensitization of TRPA1 via phospholipase C and PKA mediated intracellular signalling pathways.[26,27] Interestingly, bradykinin-induced hypersensitivity to heat, which is diminished in TRPV1-deficient mice, is also absent in TRPA1-deficient mice, indicating that TRPA1 and TRPV1 are interdependently regulated.[15] Similarly, TRPA1 response to mustard oil, a TRPA1 agonist, is diminished in trigeminal neurons from TRPV1-deficient mice compared to controls.[28] Together, the results suggest that in sensory neurons co-expressing TRPA1 and TRPV1, the activities of both channels are modulated via direct or indirect interactions between them. The exact nature of this interdependence remains to be investigated.

In humans, tooth injuries that expose the dentine and pulp generally produce pain, and are associated with an enhanced intensity of pain sensation or with the abnormal perception of pain from non-noxious stimuli. There is evidence that nociceptive ion channel expression increases in pulpal axons, and in cell bodies of TG after tooth injury.[4,7,29] One explanation for the increased hypersensitivity to cold temperatures is that the density of functional TRP ion channels in the membranes of nociceptive neurons is increased after injury, thereby sensitizing the injured tooth to a variety of noxious stimuli. In rodent models of nerve injury, cold allodynia was associated with increased expression of TRPM8 in nociceptive afferent neurons[30,31]; however, TRPM8 expression was decreased in human teeth with irreversible pulpitis and cold hyperalgesia.[32] Cultured rodent dental sensory afferents express transcripts encoding the cold-sensing TRPM8 and TRPA1 channels, and are activated by changes in temperature and by thermo-TRP channel ligands such as menthol, and icillin.[3] Electrophysiological, immunohistochemical, and single-cell RT-PCR evidence suggests that dental primary afferents have electrophysiological characteristics of nociceptors and express several nociceptor-specific ion channels, including the temperature-sensitive TRPV1, TRPA1, and TRPM8 channels.[33] Changes in expression levels, activity or regulation of TRPA1 channels could therefore contribute significantly to tooth pain. Here, we show that TRPA1 channel proteins are transiently increased in TG isolated from rats subjected to a clinically relevant model of tooth injury compared to TG innervating uninjured teeth.

2. Materials and methods

All surgical and experimental procedures on animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and were performed in accordance with Federal guidelines.

2.1. *Pulp exposure*

Adult male Sprague-Dawley rats (~250 g; Hilltop Lab Animals) were anaesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg). The mouth was held open with a micro-dissecting retractor and the mesial cusps of the left, mandibular first and second molars were prepared with a high-speed handpiece and a 1/4 round bur (Brasseler, Savannah, GA) until the pulp chamber was exposed. Verification of pulp exposure was made by tactile 'give' of tooth structure and the visualization of blood. Experimental (pulp-exposed) animals and controls were allowed to recover with softened food *ad libitum*. In one group of rats ($n = 4$), pulp exposures were performed on the first and second left molars. The left (injured side) and right (uninjured side) TG from these animals were isolated and stored at −80 °C on day 4 after pulp injury. In a second group of rats ($n = 12$), at 1, 4 and 7 days after pulp exposure, experimental animals ($n = 3$) and a control were anaesthetized with pentobarbital (80 mg/kg), and decapitated. Rats used as controls in this group received anaesthesia, but no pulp exposure. Left and right TG from each animal were isolated and stored at −80 °C.

2.2. *Preparation of protein extracts*

Individual trigeminal ganglia were rinsed, and homogenized in a buffered saline solution (20 mM Tris pH 7.4, 150 mM NaCl) containing a protease-inhibitor cocktail (Thermoscientific), using a Biomasher device (Daigger). Membrane proteins were extracted using the Pierce MEM-PER membrane protein extraction kit and prepared for gel electrophoresis using the Pierce SDS- PAGE sample preparation kit according to the manufacturer's directions (Thermoscientific). An aliquot of each sample was saved for protein assays and the remainder was diluted in Laemmli sample buffer for electrophoresis and Western blotting using standard techniques. Briefly, membrane extracts from individual TG were separated on either 10% or 4–15% gradient gels by denaturing polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (BioRad), blocked with 1% casein in TBS-T (20 mM Tris–HCl, 500 mM NaCl, 0.1%Tween 20, pH 7.5) for 2 h at room temperature, and incubated overnight at 4 °C with a rabbit anti-TRPA1 antibody (Alomone Labs, 1:100 dilution). Blots were washed with TBS-T, and incubated with HRP-labelled donkey anti-rabbit secondary antibody (GE Healthcare, 1:1000 dilution) in TBS-T for 1 hour at room temperature. After