American Pain Society
RESEARCH
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TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 16, No 1 (January), 2015: pp 67-76
Available online at www.jpain.org and www.sciencedirect.com

Peripheral Group I Metabotropic Glutamate Receptor Activation Leads to Muscle Mechanical Hyperalgesia Through TRPV1 Phosphorylation in the Rat

Man-Kyo Chung, Jongseok Lee, John Joseph, Jami Saloman, and Jin Y. Ro

Department of Neural and Pain Sciences, Program in Neuroscience, University of Maryland School of Dentistry, Baltimore, Maryland.

Abstract: Elevated glutamate levels within injured muscle play important roles in muscle pain and hyperalgesia. In this study, we hypothesized that protein kinase C (PKC)–dependent TRPV1 phosphorylation contributes to the muscle mechanical hyperalgesia following activation of Group I metabotropic glutamate receptors (mGlu1/5). Mechanical hyperalgesia induced by (*R*,*S*)-3,5-dihydroxyphenylglycine (DHPG), an mGlu1/5 agonist, in the masseter muscle was attenuated by AMG9810, a specific TRPV1 antagonist. AMG9810 also suppressed mechanical hyperalgesia evoked by pharmacologic activation of PKC. DHPG-induced mechanical hyperalgesia was suppressed by pretreatment with a decoy peptide that disrupted interactions between TRPV1 and A-kinase–anchoring protein (AKAP), which facilitates phosphorylation of TRPV1. In dissociated trigeminal ganglia, DHPG upregulated serine phosphorylation of TRPV1 (S800), during which DHPG-induced mechanical hyperalgesia was prominent. The TRPV1 phosphorylation at S800 was suppressed by a PKC inhibitor. Electrophysiologic measurements in trigeminal ganglion neurons demonstrated that TRPV1 sensitivity was enhanced by pretreatment with DHPG, and this was prevented by a PKC inhibitor, but not by a protein kinase A inhibitor. These results suggest that mGlu1/5 activation in masseter afferents invokes phosphorylation of TRPV1 serine residues including S800, and that phosphorylation-induced sensitization of TRPV1 is involved in masseter mechanical hyperalgesia. These data support a role of TRPV1 as an integrator of glutamate receptor signaling in muscle nociceptors.

***Perspective:** This article demonstrates that activation of mGlu1/5 leads to phosphorylation of a specific TRPV1 residue via PKC and AKAP150 in trigeminal sensory neurons and that functional interactions between glutamate receptors and TRPV1 mediate mechanical hyperalgesia in the muscle tissue.*

***Key words:** Peripheral, trigeminal, sensory neurons, muscle pain.*

TRPV1 is a nonselective cationic channel that can be activated by capsaicin, proton, and noxious heat. TRPV1 mediates cutaneous thermal hyperalgesia during inflammation and tissue injury.[7,13] Under pathophysiologic conditions, multiple inflammatory mediators invoke the activation of multiple kinases that phosphorylate TRPV1, enhancing its functionality. Increased heat sensitivity of TRPV1 following phosphorylation underpins mechanisms of thermal hyperalgesia.[13,33] Recently, the causative association between phosphorylation of TRPV1 and cutaneous thermal hyperalgesia has been demonstrated in studies that broke the association of TRPV1 with A-kinase–anchoring protein (AKAP), which facilitates TRPV1 phosphorylation.[9] TRPV1 is expressed not only in skin but also in nociceptors projecting to deep tissues such as gut, joint, and muscle.[7] In contrast to its contribution to thermal hyperalgesia in skin, accruing evidence suggests that TRPV1 in deep tissue afferents contributes to sensitization mediated by mechanical stimuli. Inflammation or injury to deep tissues induces enhanced responses to mechanical stimuli, and these enhanced responses in deep tissues are attenuated by pharmacologic or genetic inhibition of TRPV1.[1,16,26,30,36] Therefore, TRPV1

Received May 28, 2014; Revised October 2, 2014; Accepted October 21, 2014.

This study was supported by National Institutes of Health grants R01 DE016062 (J.Y.R.) and R01 DE023846 (M.-K.C). The authors disclose no conflict of interest in respect to this work.

Address reprint requests to Jin Y. Ro, PhD, Department of Neural and Pain Sciences, University of Maryland School of Dentistry, 650 W. Baltimore St., 8-South, Baltimore, MD 21201. E-mail: jro@umaryland.edu

1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2014.10.008

represents a candidate target not only for treating cutaneous thermal hyperalgesia but also for treating mechanical hyperalgesia in deep tissues.

Glutamate is one of the substances released upon muscle injury and is a major contributor to muscle hypersensitivity.[4,34] Activation of ionotropic or metabotropic glutamate receptors (mGluRs) induces mechanical hyperalgesia in masseter muscles.[4,21,22] Interestingly, mechanical hyperalgesia induced by masseteric injection of *N*-methyl-D-aspartate (NMDA) depends not only on peripheral NMDA receptors but also on TRPV1,[21] demonstrated by the fact that an antagonist of TRPV1 prevents the development of NMDA-induced masseter hypersensitivity. NMDA enhances capsaicin-evoked responses in a subset of primary afferents and increases phosphorylation of TRPV1 in primary afferents, which further supports the functional involvement of TRPV1 in NMDA-induced masseter hypersensitivity.[20] We previously demonstrated that masseteric injection of (*R*,*S*)-3,5-dihydroxyphenylglycine (DHPG), an agonist of Group I mGluR (mGlu1/5), induced masseter hypersensitivity.[22] It is unclear, however, whether masseter hypersensitivity induced by the activation of mGlu1/5 also involves TRPV1.

Activation of NMDA receptors induces masseter hypersensitivity in a protein kinase C (PKC)- and TRPV1-dependent manner.[21] Pharmacologic inhibition of PKC or knockdown of AKAP150 decreases NMDA-induced phosphorylation of TRPV1 in primary afferents.[20] Because masseter hypersensitivity induced by mGlu1/5 activation is primarily dependent on PKC,[22] it is possible that mGlu1/5 is also linked to TRPV1 through PKC. However, PKC-dependent interactions between mGlu1/5 and TRPV1 in primary afferents have not been reported. Activation of mGlu1/5 was reported to attenuate capsaicin-induced desensitization of TRPV1 through protein kinase A (PKA) via de novo synthesis of prostaglandin E_2.[11] Activation of mGluR was also suggested to generate diacylglycerol, which directly activates TRPV1 through a PKC-independent mechanism.[18] Therefore, in this project, we tested the hypothesis that PKC-dependent phosphorylation of TRPV1 contributes to masseter hypersensitivity following the activation of mGlu1/5. This hypothesis was tested by a combination of biochemical and electrophysiologic analyses and behavioral assays in a rodent model of masseter hypersensitivity.

Methods

Animals

Adult male Sprague Dawley rats (150–350 g; Harlan Laboratories, Indianapolis, IN) were used. All animals were housed in a temperature-controlled room under a 12:12 light-dark cycle with access to food and water ad libitum. All procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*[10] and under a University of Maryland–approved Institutional Animal Care and Use Committee protocol.

Drugs and Peptide Preparation for Behavioral Experiments

DHPG was dissolved in phosphate-buffered saline (PBS; 1 μmol in 100 μL). AMG9810, a specific antagonist of TRPV1, was dissolved in a vehicle containing 5% dimethyl sulfoxide (DMSO), 10% Tween-80, and 85% PBS (1 or 100 nmol in 10 μL). Phorbol 12-myristate 13-acetate (PMA), an activator of PKC, was dissolved in PBS (300 nmol in 30 μL), and forskolin, an activator for PKA, was dissolved in PBS (50 nmol in 20 μL). A peptide spanning residues 736 to 745 of A-kinase–anchoring protein 150 (AKAP150) fused to the TAT sequence (transactivator of transcription of HIV) (735-757-TAT: KDDYRWCFRVYGRKKRRQRRR; TAT sequence is underlined) that interferes with the interaction of TRPV1 with AKAP150 was synthesized from the published sequences.[9] As a control, a scramble-TAT peptide (RFVCWDKYRDYGRKKRRQRRR) was also synthesized. Both peptides contained the TAT sequence to mediate transmembrane transport. Purity of the peptides was >95% in mass spectroscopic analysis (Genscript). Both 736-745-TAT (10 and 30 μM) and scramble-TAT (30 μM) were dissolved in PBS.

Behavioral Assays—Assessment of Mechanical Sensitivity in Masseter Muscle

Noxious chemical or mechanical stimulation of the masseter muscle evokes characteristic shaking of the ipsilateral hind paw in lightly anesthetized rats. We have previously described the use of this behavior for testing mechanical sensitivity of the masseter muscle.[21,22,30] This lightly anesthetized rodent model allows the delivery of a calibrated and reliable mechanical stimulus to the masseter muscle or temporomandibular joint before and after pharmacologic manipulations, which is difficult to accomplish in awake animals. Initially, rats (250–350 g) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A level of light anesthesia was determined by providing a noxious pinch to the tail or the hind paw with a serrated forceps. Animals typically responded to the noxious pinch on the tail with an abdominal contraction and with a withdrawal reflex to the noxious pinch of a hind paw within 30 minutes after the initial anesthesia. Once the animal reached this level, a metal clip calibrated to produce 600 g of force was applied 5 consecutive times to the tail, and experiments were continued only after the animals showed reliable reflex responses to every clip application. A tail vein was connected to an infusion pump (Pump 11; Harvard Apparatus, Holliston, MA) for continuous infusion of pentobarbital. The rate of infusion was adjusted to maintain a relatively light level of anesthesia throughout the duration of the experiment (3–5 mg/h). A baseline mechanical threshold for evoking the nocifensive response was determined 15 minutes before drug injection using the electronic von Frey anesthesiometer (IITC Life Science,