*Neuroscience* 247 (2013) 65–74

# IDENTIFICATION OF THE SENSORY NERVE FIBER RESPONSIBLE FOR LYSOPHOSPHATIDIC ACID-INDUCED ALLODYNIA IN MICE

**M. OHSAWA, Y. MIYABE, H. KATSU, S. YAMAMOTO AND H. ONO** *

*Laboratory of CNS Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan*

**Abstract—Lysophosphatidic acid (LPA) has been considered one of the molecular culprits for neuropathic pain. Understanding how LPA changes the function of primary afferent fibers might be an essential step for clarifying the pathogenesis of neuropathic pain. The present study was designed to identify the primary afferent fibers (Aβ, Aδ, or C) participating in LPA-induced allodynia in ddY mice. Mechanical allodynia and thermal hyperalgesia were evaluated by the von Frey filament test and thermal paw withdrawal test, respectively. Sensory nerve fiber responsiveness was measured using a Neurometer. Daily repeated intrathecal treatment with LPA led to a decrease in the mechanical, but not thermal nociceptive threshold, and a reduction in the threshold for paw withdrawal induced by 2000-Hz (Aβ fiber) and 250-Hz (Aδ fiber), but not 5-Hz (C fiber) sine-wave electrical stimulation. When the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor agonist resiniferatoxin (RTX) was administered subcutaneously before the start of LPA treatment, LPA-induced mechanical allodynia and Aβ and Aδ fiber hypersensitivity demonstrated by neurometry were not affected, indicating that TRPV1-expressing nerve fibers (possibly C fibers) might not be essential for LPA-induced allodynia. LPA-induced allodynia was reversed by treatment with RTX at 7 days after the start of LPA treatment. Expression of TRPV1 on myelinated nerve fibers after repeated intrathecal LPA treatment was observed in the dorsal root ganglion. These results suggest that sensitization of Aβ and Aδ fibers, but not C fibers, contributes to the development of intrathecally administered LPA-induced mechanical allodynia. Moreover, increased or newly expressed TRPV1 receptors in Aβ and Aδ fibers are considered to be involved in the maintenance of LPA-induced allodynia.**
**© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.**

**Key words: lysophosphatidic acid (LPA), mechanical allodynia, TRPV1, myelinated A fiber, Neurometer test.**

## INTRODUCTION

Injury to peripheral nerves often leads to neuropathic pain, which is thought to result from both abnormal excitability of damaged primary afferents and pathological changes within the central nervous system. Investigations using animal models of neuropathic pain have indicated that many cellular and molecular events are involved. Recently, lysophosphatidic acid (LPA) was identified as an important endogenous substance participating in neuropathic pain (Inoue et al., 2004). LPA is a potent biologically active lipid mediator that exerts a wide range of cellular responses, including cell adhesion, cell motility, cytoskeletal changes, proliferation, angiogenesis, process retraction, and cell survival (Rivera and Chun, 2008). It has been reported that intrathecal LPA administration induces a neuropathic pain-like state (Ma et al., 2009a). Moreover, mice lacking the $LPA_1$ receptor gene do not develop neuropathic pain, demyelination or upregulation of pain-related gene/protein expression after partial sciatic nerve injury (Inoue et al., 2004, 2006).

Primary afferent fibers have been classified into three major types: unmyelinated C, myelinated thin Aδ, and myelinated Aβ fibers. The nociceptors of C fibers and Aδ fibers conduct noxious chemical, mechanical or thermal stimuli, which in turn cause nociceptive responses. On the other hand, stimulation of Aβ fibers is thought to induce mostly an innocuous tactile sensation. Therefore, the function of primary afferent fibers needs to be evaluated individually. The Neurometer® can selectively activate sensory neurons using sine-wave pulses of different frequencies without affecting the nociceptors. It has been reported that frequencies of 5, 250 and 2000 Hz activate C, Aδ and Aβ fibers, respectively. This stimulus-dependent selective activation of each type of primary afferent fiber has been confirmed by electrophysiological (Koga et al., 2005), pharmacological, and immunohistochemical (Matsumoto et al., 2006, 2008) experiments. Because of this selective activation of primary afferent fibers, the Neurometer test is widely used clinically to evaluate sensory function in patients with peripheral neuropathic pain (Pitei et al., 1994; Katims, 1997; Lengyel et al., 1998).

A relatively common symptom of neuropathic pain is tactile allodynia, where innocuous mechanical stimuli are perceived as painful. This can be mediated by low-threshold mechanoreceptive Aβ afferents (Campbell et al., 1988; Koltzenburg et al., 1994). Because

*Corresponding author. Tel/fax: +81-532-836-3676.
E-mail address: hiono@phar.nagoya-cu.ac.jp (H. Ono).
*Abbreviations:* DRG, dorsal root ganglion; LPA, lysophosphatidic acid; PBS, phosphate-buffered saline; RTX, resiniferatoxin; TRPV1, transient receptor potential cation channel subfamily V member 1; TRPV1-ir, TRPV1 immunoreactivity.

0306-4522/13 $36.00 © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2013.05.014

stimulation of these afferents does not normally result in pain, it is assumed that a central mechanism must be involved (Woolf, 1997). It has been postulated that the action potentials conducted along Aβ fibers might be transmitted to nociceptive primary afferent neurons, such as C and/or Aδ fibers, in neuropathic pain. One plausible explanation is cross-excitation or ephapses between Aβ and nociceptive primary afferent fibers (Bridges et al., 2001; Schoffnegger et al., 2008; Sandkühler, 2009). Since most C fibers and some Aδ fibers express the transient receptor potential cation channel subfamily V member 1 (TRPV1), resiniferatoxin (RTX) destroys these TRPV1-expressing nerve fibers (Pan et al., 2003). Therefore, using RTX treatment, it is possible to identify which nociceptive primary afferent fibers are involved in LPA-induced mechanical allodynia. The present study was therefore designed to identify the primary afferent fibers responsible for LPA-induced allodynia in mice.

## EXPERIMENTAL PROCEDURES

All of the experimental protocols used in the present study were approved by the Animal Care and Use Committee of Nagoya City University, and carried out in accordance with the guidelines of the National Institutes of Health and the Japanese Pharmacological Society.

### Animals

ddY mice weighing 20–25 g were used at the beginning of this study. The animals were housed five per cage in a room maintained at 23 ± 2 °C with an alternating 12-h-light–dark cycle. Food and water were available *ad libitum*. Animals were used only once in all experiments.

### Assessment of thermal hypersensitivity

Thermal hypersensitivity was assessed using the plantar test (Ugo Basile, Comerio, Italy) following a modification of the method of Hargreaves et al. (1988). Mice were placed in a clear plastic chamber with a glass floor and allowed to acclimate to their environment before testing. During this time, the mice initially demonstrated exploratory behavior, but subsequently stopped exploring and stood quietly with occasional bouts of grooming. A mobile radiant heat source located under the glass floor was focused onto the plantar surface of the right hindpaw, and paw withdrawal latencies were recorded. The intensity of radiant heat was adjusted to give a 7- to 8-s withdrawal latency in naïve mice. A cutoff latency of 15 s was imposed to avoid any tissue damage. Paw withdrawal latencies were measured in duplicate for the right hindpaw of each animal, and the mean of the two values was used for analysis.

### Assessment of mechanical allodynia

Mice were placed in individual transparent Perspex cubicles with a wire mesh bottom, and a series of calibrated von Frey filaments (Semmes–Weinstein monofilaments; Stoelting, Wood Dale, IL, USA) was used to determine the 50% likelihood of a paw withdrawal response (50% threshold) by the up–down method of Dixon (1980). Eight von Frey filaments, with approximately equal logarithmic incremental bending forces, were chosen (0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, and 1.4 g). Testing was initiated with the 0.16-g hair, and each hair was applied perpendicularly to the plantar surface of the hindpaw, with sufficient force to bend the filament, for 3–4 s. Lifting of the paw indicated a positive response and prompted the use of the next weaker (i.e. lighter) filament. Absence of a paw withdrawal response prompted the use of the next stronger (i.e. heavier) filament. This paradigm was continued until four measurements had been obtained after an initial change in behavior, or until four consecutive positive scores (score of 0.02 g) or five negative scores (score of 1.4 g) had been obtained. The resulting scores were used to calculate the 50% threshold (Chaplan et al., 1994).

### Electrical stimulation-induced paw withdrawal test

A pair of ball-shaped electrodes (2 mm in diameter) were fastened to the right plantar surface and instep of the mice. Transcutaneous nerve stimuli with each of the three kinds of sine-wave frequency (5, 250, and 2000 Hz) were applied using the Neurometer CPT/LAB (Neurotron Inc. Baltimore, MD, USA). The minimum intensity (microampere) at which each mouse withdrew its paw and/or vocalized was defined as the stimulus threshold. Stimuli were applied at 10-min intervals. All behavioral experiments were carried out by investigators blinded to the drug treatment.

### Immunohistochemistry

Mice were anesthetized with pentobarbital (60 mg/kg, i.p) and intracardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The spinal cords were removed and thick coronal sections containing L4, L5 and L6 were initially dissected. The L4, L5 and L6 dorsal root ganglia were dissected out. These tissues were post-fixed in the same fixative for 2 h, immersed in 30% sucrose/PBS solution overnight, and then frozen in an embedding compound (Sakura Finetechnical, Tokyo, Japan). Frozen 8-μm-thick coronal sections were cut with a cryostat (CM1900, Leica Microsystems, Heidenberg, Germany) and thaw-mounted on poly-L-lysine-coated glass slides.

The lumbar spinal cord and dorsal root ganglion (DRG) sections were blocked in 10% normal goat serum in 0.01 M PBS for 2 h at room temperature. Each primary antibody was diluted in 0.01 M PBS containing 0.5% bovine serum albumin and 0.4% Triton-X 100 [1:2000 rabbit polyclonal antibodies against TRPV1 (Alomone Labs., Jerusalem, Israel)] and incubated for 2 days at 4 °C. The sections were then rinsed and incubated with the secondary antibody conjugated with Alexa 592 for 2 h at room temperature. The slides were then coverslipped with fluorescence mounting medium (Vector Laboratories, Burlingame, CA, USA). Fluorescence of immunolabeling was detected using a

Download English Version:

https://daneshyari.com/en/article/6274842

Download Persian Version:

https://daneshyari.com/article/6274842

Daneshyari.com