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Involvement of protein isoprenylation in neuropathic pain induced by sciatic nerve injury in mice

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HIGHLIGHTS

- Partial sciatic nerve injury elicited neuropathic pain.
- Phosphorylation of spinal MARCKS was increased in nerve ligation.
- Neuropathic pain was attenuated by geranylgeranyl transferase inhibitor.
- MARCKS protein phosphoryation was mediated by protein isoprelylation.
- Inhibition of protein isoprenylation could relive neuropathic pain.

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ABSTRACT

Isoprenylation is crucial step for activating many intracellular signaling. The present study examined whether inhibition of the protein isoprenylation could affect neuropathic pain in partial sciatic nerve-ligated mice. Intrathecal treatment with a geranylgeranyl transferase I inhibitor GGTI-2133, but not with a farnesyl transferase inhibitor FTI-277, dose-dependently blocked the thermal hyperalgesia in partial sciatic nerve-ligated mice. Intrathecal treatment with GGTI-2133 also attenuated the mechanical allodynia in partial sciatic nerve-ligated mice. Phosphorylated MARCKS expression was increased in the ipsilateral side of the spinal cord dorsal horn in partial sciatic nerve-ligated mice, and this increase was attenuated by GGTI-2133 but not by FTI-277. These results suggest that protein isoprenylation by geranylgeranyl transferase I is involved in the neuropathic pain.

1. Introduction

Isoprenylation is protein modification to attach the hydrophobic molecules. This protein isoprenylations are involved in the modulation of the functions of a large number of proteins. For example, this isoprenylation is essential for the translocation of small GTP-binding proteins, such as RhoA, from cytosol to plasma membrane and for the activation of subsequent intracellular signaling. Isoprenylation is catalyzed by farnesyl transferase and geranylgeranyl transferase which promote the attachment of a farnesyl and a geranylgeranyl moiety, respectively [1]. Recently, we have shown that intrathecal treatment with mevalonate, a precursor of isoprenoids elicited thermal hyperalgesia through the increase of spinal protein isoprenylation [2]. Treatment with statins has been shown to prevent the initiation and maintenance of inflammatory and neuropathic pain [3,4]. Since HMG-CoA reductase inhibition depletes isoprenoids, inhibition of isoprenylation might be involved in this pain prevention by HMG-CoA reductase inhibitors.

Myristoylated alanine-rich C-kinase substrate (MARCKS) is a substrate of Rho kinase that is downstream of RhoA. MARCKS has been implicated in neuronal functions such as synaptic vesicle trafficking and neurotransmitter release [5]. In nerve-transected and inflammatory pain model, the expression of phosphorylated MARCKS has been demonstrated to be increased in the spinal dorsal horn, which was attenuated by Rho kinase inhibition [6]. It is possible that inhibition of protein isoprenylation attenuates the expression of phosphorylated MARCKS in the neuropathic pain model.

The evidence suggesting contribution of small G proteins to aspects of hyperalgesia [2,4,6,7] would imply that blocking their functions through inhibition of either farnesylation or geranylgeranylation would influence hyperalgesia. We therefore examined

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the effects of selective inhibitors of isoprenylation on thermal and mechanical sensitivity in partial sciatic nerve-ligated mice.

2. Materials and methods

The present study was conducted in accordance with the guiding principles for the care and use of laboratory animals, Kyushu University of Health and Welfare, which is accredited by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

2.1. Animals

Male ICR mice (Kyudo laboratory animals, Inc., Saga, Japan) weighing 20–30 g, were used in this study. Animals were housed five per cage in a room maintained at 23 ± 0.5° C with an alternating 12 h light-dark cycle. Food and water were available *ad libitum*.

For partial sciatic nerve-ligation surgery, the mice were deeply anesthetized with pentobarbital (60 mg/kg, i.p.). We produced a partial sciatic nerve injury by tying a tight ligature with a 8–0 silk suture around approximately one-third to one-half the diameter of the sciatic nerve on the right side (ipsilateral side) under a light microscope (Olympus, Tokyo, Japan) as described previously [8]. In sham-operated animals, the nerve was exposed without ligation. Mice were used only once in all experiments.

2.2. Hind paw withdrawal in response to a thermal stimulus

To measure withdrawal latency to radiant heat, mice were placed on a glass plate surrounded by a clear plastic chamber (model 336 Analgesia Meter; IITC Inc/Life Science Instruments, Woodland Hills, CA) [8]. A radiant heat stimulus was applied from underneath the glass floor with a high-intensity projector lamp bulb and the withdrawal latency was measured using an electronic timer. The heat stimulus was focused on the plantar surface of each hind paw. The intensity of the heat stimulus was adjusted to drive an average baseline paw withdrawal latency of approximately 9–12 s in naive mice. A 20 s cut-off was used to prevent tissue damage. Paw withdrawal latency was determined as the average of two measurements per paw. Left and right hind paws were tested alternately after more than 5 min.

2.3. Hind paw withdrawal in response to a mechanical stimulus

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 [9]. 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 hind paw, 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 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) of five negative scores (score of 1.4 g) had been obtained. The resulting scores were used to calculate the 50% threshold [10].

2.4. Immunohistochemistry

After the behavioral experiment, 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. These sections were removed, postfixed in the same fixative for 2 h, and immersed in 30% sucrose/PBS solution overnight. Coronal spinal cord sections of 50 μm were prepared with the use of a vibratome (VIB-1500). The spinal cord sections were blocked in 10% normal goat serum in 0.1 M PBS for 2 h at room temperature. Primary antibodies (4 °C, 2 days overnight) used were rabbit anti-phosphorylated myristoylated alanine-rich protein kinase C substrate (MARCKS) at Ser159 (1:2000, Cell Signaling Technology). Secondary antibodies (room temperature, 2 h) used were Alexa Fluor 488 conjugated anti-rabbit (1:2000). The slices were mounted on the slide and coverslipped with fluorescence mounting medium (Dako). Sections were examined on a laser scanning confocal microscope (LMS 510 META, Carl Zeiss) with excitation/emission wave length 488/520 nm. Quantifications of each protein expressions were done by the measurement of immunoreactivity on either side using Image J.

2.5. Drugs and intrathecal injection

Drugs used in the present study were GGTI-2133 and FTI-277 (Calbiochem, San Diego, CA). Other reagents used in the present study were molecular biology grade. GGTI-2133 was dissolved in a vehicle solution of 90% sterile saline, 5% dimethylsulfoxide (DMSO), and 5% cremophore EL (Sigma). FTI-277 was dissolved in physiological saline. All compounds were administered intrathecally 1 h before the nerve-injury and just after the measurement of thermal nociceptive threshold evaluated by the latency of paw withdrawal to thermal stimulation. Drugs and their vehicles repeatedly administered once daily for 7 days.

Intrathecal injection in a volume of 5 μl was performed according to the methods of Hylden and Wilcox [11]. The mouse was manually restrained and 30-gauge needle mated to 25 μl Hamilton syringe was inserted between L5 and L6 of the mouse spinal column.

2.6. Statistical analysis

Data are presented as the mean ± S.E.M. Differences between treatment groups were evaluated using Student's *t*-test (comparison of two groups) or analysis of variance (ANOVA) followed by the Bonferroni–Dunn test (comparison among multiple groups). At all times, a level of probability of 0.05 or less ($p < 0.05$) was considered significant.

3. Results

3.1. Effect of geranylgeranyl transferase I inhibitor on thermal hyperalgesia in nerve-ligated mice

Partial sciatic nerve ligation caused significant decrease of the paw withdrawal latency (Fig. 1), and the thermal threshold remained lower for 7 days after partial sciatic nerve ligation. Intrathecal treatment with geranylgeranyl transferase I inhibitor GGTI-2133 at doses of 0.001–0.1 nmol dose-dependently attenuated the thermal hyperalgesia in partial sciatic nerve-ligated mice (Fig. 1A). Intrathecal treatment with GGTI-2133 did not affect the thermal nociceptive threshold in the contralateral paw (Fig. 1B). In contrast to GGTI-2133, intrathecal treatment with farnesyl transferase inhibitor FTI-277 (30 nmol) showed no effect on the thermal hyperalgesia in partial sciatic nerve-ligated mice (Fig. 1C and D). Drugs and their vehicles repeatedly administered once daily for 7 days.