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Research article

Role of actin filaments in allodynia induced by chronic compression of the dorsal root ganglion

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HIGHLIGHTS

- Disruption of actin filaments results in reduction in CCD-induced allodynia.
- Disruption of actin filaments results in inhibition of TRPV4-mediated currents.
- Disruption of actin filaments inhibits the plasma membrane-associated TRPV4.
- Intact actin filaments are necessary for CCD-induced allodynia.
- TRPV4 contributes to the relief of allodynia induced by disruptors' treatment.

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ABSTRACT

The role of actin filaments in allodynia induced by chronic compression of the dorsal root ganglion (DRG) (CCD) and the effects of microfilaments dynamics on transient receptor potential vanilloid 4 (TRPV4) were investigated in this study. Anti-microfilaments agents resulted in dose-dependent and partial reduction in CCD-induced allodynia, which could be prevented by the prior stabilizer administration. In association with the reduction of allodynia by microfilaments' disruption, TRPV4-mediated currents were inhibited by disruptors. In addition, plasma membrane-associated TRPV4 was also depressed by disruptors. The time courses for the changes of TRPV4 activity and distribution in vitro were similar to the time courses for the attenuation of allodynia in vivo. Phalloidin, the stabilizer of microfilaments, did not affect the allodynia in CCD rats. However, phalloidin resulted in reduction and delay of TRPV4 current, which was not consistent with the effect of phalloidin on CCD-induced allodynia. In accordance with the inhibition of TRPV4 activity, the reversal potentials shifted toward more positive voltages and the plasma membrane-associated TRPV4 was depressed by phalloidin. In conclusion, intact actin filaments were necessary for CCD-induced allodynia, and disruptors of microfilaments attenuated CCD-induced allodynia. However, stabilizer of actin filaments did not affect allodynia in CCD rats. Further, TRPV4 contributed to the disruptors-induced attenuation of allodynia in CCD rats.

1. Introduction

Chronic compression of the dorsal root ganglion (DRG) (CCD) in rats is a typical model of neuropathic pain. CCD rats show ipsilateral spontaneous pain, mechanical and thermal allodynia. Several findings suggested that cytoskeletal elements were involved in the mechanical transduction in sensory neurons and cytoskeletons may play a role in the development of chronic pain [11]. Results of our prior study showed that intact microtubules contributed to CCD-induced allodynia and disruption of microtubules could attenuate the allodynia in CCD rats. In addition, intact actin filaments were reported to be necessary for inflammatory pain. Disruptors of the microfilaments markedly attenuated the hyperalgesia in rat paws by injection of epinephrine or its downstream mediators [5]. However, the effects of microfilaments dynamics on CCD-induced allodynia have not been determined. Thus, microfilaments agents and anti-microfilaments agents were used to investigate the role of actin in CCD-induced allodynia in this study.

Abbreviations: DRG, dorsal root ganglion; CCD, chronic compression of the dorsal root ganglion; ODN, oligodeoxynucleotide.

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Transient receptor potential vanilloid subtype 4 (TRPV4) plays an important role in CCD-induced allodynia. In CCD rats, the gene and protein expression levels of TRPV4 in DRGs were both increased, calcium responses to hypotonic stimuli and phorbol ester 4α-phorbol 12, 13-didecanoate (4α-PDD) were enhanced [20]. Furthermore, intrathecal treatment with TRPV4 antisense oligodeoxynucleotide (ODN) led to a reduction in mechanical allodynia. TRPV4 physically interacts with actin filaments [8]. Actin cytoskeleton could regulate the TRPV4 channel activity as disruption of microfilaments impaired the TRPV4-mediated currents [15] and Ca^{2+}-influx in TRPV4 expressing cells in response to hypotonic shock [3]. Therefore, we hypothesized that TRPV4 may be involved in the effects of microfilaments dynamics on CCD-induced allodynia.

2. Methods

2.1. Animals and surgical procedure

This study complied with the Chinese Institutional Care Committee for the use of animals and the tests were performed in accordance with the Helsinki declaration. Concerted efforts were made to minimize both the suffering and the number of animals that were used. Adult male Wistar rats were randomly divided into CCD groups and sham groups. In CCD rats, under pentobarbital sodium anesthesia (Nembutal, 50 mg/kg i.p.), the transverse process and intervertebral foramina of the L4 and L5 vertebrae were exposed unilaterally [6,20]. Two hollow, stainless steel, L-shaped rods (0.66 mm diameter and 4 mm length), connected to silicon tubing (0.51 mm ID, 0.94 OD, 30–40 mm length), were inserted into L4 and L5 foramen respectively, to compress the DRG. Chemicals and saline were all injected by the silicon tubing according to the study of Song [21]. Sham surgery involved identical surgical procedures but without insertion of the rods.

2.2. Behavioral testing

Behavioral tests were conducted on the 7th day post-surgery [18]. Cytochalasin B (CB) and phalloidin (PHA) were injected in several doses (100 μg, 200 μg and 300 μg) to confirm that the appropriate doses were used. Effects of CB and PHA on CCD-induced allodynia were tested 1, 2, 4, 6, 8, and 24 h post injection. In order to investigate whether phalloidin could prevent the effect of CB on CCD-induced allodynia, phalloidin was injected 1 h before CB (both 300 μg) next.

Mechanical stimuli were applied to the plantar surface using a series of von Frey monofilaments (BME-403) in an ascending order [9]. According to the protocol of Villetti et al. [16,18], thermal allodynia was assessed using the paw withdrawal latency (PWL) in response to radiant heat (BME-410C).

2.3. DRG neurons culture

L4 and L5 ganglia were removed from the operated sides of rats on 7 days after surgery. DRG neurons were cultured in Neurobasal medium (Invitrogen, Carlsbad, CA) that had been supplemented with N_2, NGF and glutamine (Gibco Invitrogen, Grand Island, NY). The neurons were cultured for 4 days prior to whole-cell current recording, immunofluorescence microscopy and cell viability test.

2.4. Whole-cell current recording

The effects of dynamic changes of microfilaments on TRPV4-mediated channel activity were tested by patch-clamp technique. DRG neurons were incubated with CB, CD (both 10^{-4} M) and phalloidin (10^{-5} M) for 2 h before recording the phorbol ester 4α-phorbol 12, 13-didecanoate (4α-PDD)-induced TRPV4 currents. Currents were digitized at a sampling rate of 5 kHz and filtered at 1 kHz for analysis (Axon 700B amplifier with pCLAMP software; New York, NY, USA).

The bath solution contained the following compounds (in mM) [2]: 124 NaCl, 5 KCl, 1.2 KH_2PO4, 1.3 $MgCl_2$, 2.4$CaCl_2$, and 26 $NaHCO_3$ (310 mOsm), pH adjusted to 7.35 with NaOH. The pipette solution contained the following compounds (in mM): 140CsCl, 2 NaCl, 3 $MgCl_2$, 10Hepes, and 5 EGTA (290 mOsm), pH adjusted to 7.25 with CsOH. The resistance was 3 6 MΩ. The current–voltage relationship was determined using a voltage ramp protocol that was initiated by a 20 ms voltage step to −60 mV and a subsequent 400 ms ramp from −100 mV to + 110 mV with a 0.2 ms sampling interval. The measurement procedure was completed by returning to the holding potential of −60 mV.

2.5. Immunofluorescence microscopy

DRG neurons were incubated with CB (10^{-4} M) and phalloidin (10^{-5} M) 2 h before immunofluorescence microscopy. DRG neurons were incubated with rabbit anti-TRPV4 (1:200, Abcam, Cambridge, UK) antibodies overnight at 4 °C. Then DRG neurons were incubated with FITC-conjugated goat anti-rabbit IgG (1:100, Zhongshan Goldenbridge, Beijing, China) for 1 h at room temperature. Cell nuclei were counterstained with DAPI prior to analysis under a confocal laser scanning microscope (LSM710, Zeiss, Jena, Germany) [10,18].

2.6. Cell viability assay

Cell viabilities of cultured DRG neurons were measured via 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (the MTT test). DRG neurons were all incubated with chemicals for 2 h to investigate cell viabilities.

2.7. Chemicals and reagents

The following chemicals were used: 4α-PDD (TRPV4 synthetic agonist, Sigma), CB (Anti-Microfilaments Agent, Merck), CD (Anti-Microfilaments Agent, Merck) and phalloidin (Microfilaments Stabilizer, Merck). All chemicals were dissolved in ethanol, and the final experimental dilutions were prepared in either saline or bath solution. Both the doses and schedules were selected on previous reports [14] and on pilot experiments.

2.8. Data analysis

Data were analyzed by Sigmaplot software. A two-way repeated measures ANOVA was used to analyze between-groups differences in the thermal withdrawal latencies. Student's *t*-tests were used to compare the differences in the distribution of TRPV4, as well as the values in the electrophysiological studies. Values in the text and figures were expressed as means ± standard errors of the means (SEMs). The significance level was set at $P < 0.05$.

3. Results

3.1. Effects of microfilaments dynamics on CCD-induced allodynia

All rats walked normally after the CCD surgery, indicating that CCD surgery did not injure the motor behavior. Both of two disrupting agents (CB and CD) resulted in attenuation of CCD-induced mechanical and thermal allodynia ($n = 9$ in each group, both $P < 0.01$; Fig. 1A and B). CB was injected in several doses to confirm that the appropriate doses were used. Compared with the