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Effects of colchicine-induced microtubule depolymerization on TRPV4 in rats with chronic compression of the dorsal root ganglion

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HIGHLIGHTS

- Colchicine administration results in a reduction in CCD-induced allodynia.
- Colchicine administration induces decreases in TRPV4 mRNA and protein expression.
- Colchicine results in reduction and advance of TRPV4 currents.
- Intrathecal administration of colchicine attenuates allodynia.
- TRPV4 contributes to the colchicine-induced attenuation of allodynia in CCD rats.

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ABSTRACT

The aim of this study was to investigate the effect of colchicine-induced microtubule depolymerization on allodynia in rats with chronic compression of the dorsal root ganglion (DRG) (CCD) and the effect of colchicine on transient receptor potential vanilloid 4 (TRPV4). Intrathecal administration of the anti-microtubule agent, colchicine, resulted in a dose-dependent and partial reduction in CCD-induced mechanical and thermal allodynia. The reduction of allodynia was associated with significant and dose-dependent decreases in the levels of both TRPV4 mRNA and protein expression in CCD rats. In addition, colchicine resulted in reduction and advance of TRPV4 currents in both DRG neurons and HEK293-TRPV4 cells. The current–voltage (*IV*) relation in HEK293-TRPV4 cells that were exposed to colchicine displayed a typical outward rectification characteristic of TRPV4 with the reversal potential shifted toward a more positive voltage. In conclusion, intrathecal administration of colchicine attenuated allodynia and TRPV4 contributed to the colchicine-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. Rats with CCD show ipsilateral spontaneous pain as well as mechanical and thermal allodynia. After CCD surgery, both gene and protein expression levels of transient receptor potential vanilloid subtype 4 (TRPV4) in DRGs are increased, and calcium responses to hypotonic stimuli and 4α-phorbol 12,13-didecanoate (4α-PDD) are enhanced [24]. Furthermore, intrathecal treatment with TRPV4 antisense oligodeoxynucleotide (ODN) leads to a reduction in mechanical allodynia in CCD rats [24], suggesting a significant role of TRPV4 in CCD-induced allodynia.

TRPV4 is a member of the TRP superfamily of Ca^{2+}-permeable cation channels. TRPV4 has been shown to interact directly with microtubule [15], while microtubule dynamics play an important role in the regulation of TRPV4 activity. Taxol, a microtubule stabilizer [14], could reduce Ca^{2+}-influx via TRPV4 [15]. The interplay between TRPV4 and microtubule also appears at the behavioral level. Taxol-induced microtubule polymerization contributes to the

Abbreviations: DRG, dorsal root ganglion; CCD, chronic compression of the dorsal root ganglion; TRPV4, transient receptor potential vanilloid subtype 4; ODN, oligodeoxynucleotide; CCI, loose ligation of the sciatic nerve.

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painful peripheral neuropathy, and TRPV4$^{-/-}$ knockout mice show reduced nociceptive responses to taxol-induced neuropathic pain [2]. Colchicine binds to the subunit of tubulin heterodimers to form a tubulin–colchicine complex that subsequently inhibits the polymerization of microtubule. Recent evidence shows that colchicine could relieve the hyperalgesia in rats with loose ligation of the sciatic nerve (CCI) [23]. However, the effect of colchicine on the allodynia in CCD rats has not been explored and the effect of colchicine on TRPV4 has not been defined. In this study, we thus assessed the effect of colchicine, an anti-microtubule agent, on allodynia in CCD rats. The potential involvement of TRPV4 in the effect of colchicine on allodynia was also investigated in CCD rats.

2. Methods

2.1. Animals and surgical procedure

This study complied with the Chinese Institutional Animal Care Committee and performed in accordance with the Helsinki declaration. Efforts were made to minimize the suffering and the number of animals. Adult male Wistar rats, weighing 180–220 g (Shandong University Lab Animal Center, Jinan, China), were housed in a controlled environment with free access to food and water. Rats were anesthetized using sodium pentobarbital (Nembutal, 50 mg/kg i.p.), then two stainless steel rods were implanted unilaterally into the intervertebral foramen at L4 and L5 according to the procedure that has been described previously [11,24]. The correct placement of the rods was confirmed by behavioral testing and ganglia harvest. Sham operations were not considered necessary because of the absence of behavioral or other differences between control group and sham group [22].

2.2. Behavioral testing

Mechanical withdrawal threshold (MWT) was measured with a von Frey hair monofilament (BME-403, Biomedical Engineering Institute of Chinese Academy of Medical Sciences) with logarithmically incremental stiffness (0.09–17.30 g) [24]. Thermal allodynia was assessed using the paw withdrawal latencies (PWLs) in response to radiant heat (BME-410C) [11,22]. The behavioral testing was carried out in the ipsilateral hind paw of the animals prior to surgery and on postoperative days 4, 6, 7, 14 and 28. The effect of colchicine on CCD-induced allodynia was tested 0.5–8 h post injection.

2.3. DRG neurons culture

L4 and L5 ganglia were removed from the operated side of the animals 7 days post-surgery and the corresponding side of the normal animals. DRGs were incubated with collagenase and trypsin (Sigma–Aldrich, St. Louis, MO) and cultured in Neurobasal medium (Invitrogen, Carlsbad, CA) that had been supplemented with N_2, NGF and glutamine (Gibco Invitrogen, Grand Island, NY) [7]. The neurons were cultured 4 days for the cell viability, whole-cell patch-clamp and immunofluorescence microscopy tests.

2.4. HEK293 cells culture and transfection

HEK293 cells were cultured in DMEM containing 10% fetal bovine serum and transfected with TRPV4-GFP plasmids (kindly supported by Prof. Yu Xiao from the Physiology Department of Shandong University) using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) [7]. After transfection for 24–48 h, HEK293-TRPV4 cells were used to investigate TRPV4 currents and cell viabilities.

2.5. Cell viability assay

Cell viability was measured via 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (the MTT test). The cell viability of the group that was incubated in the normal medium was taken to represent 100% viability. Cells (colchicines group) were all incubated with colchicines for 2 h to investigate cell viabilities, TRPV4 currents, and TRPV4 immunofluorescence.

2.6. Whole-cell current recording

TRPV4 currents were recorded from DRG neurons and fluorescent HEK293-TRPV4 cells. 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 capacitance and series resistance were compensated by at least 80%. Compensation for liquid junction potentials was established prior to patching. The bath solution contained the following compounds (in mM) [3]: 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 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): 140 CsCl, 2 NaCl, 3 $MgCl_2$, 10 HEPES, 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 initiated by a voltage step to the holding potential of −60 mV for 20 ms, followed by a 400 ms ramp from −100 to +110 mV with sampling interval of 0.2 ms, and returning to −60 mV.

2.7. Western blot analysis

Seven days post-surgery, animals were intrathecally injected with colchicine (312.5–1250 μg/kg) for 0.5–8 h. Then L4 and L5 ganglions from the operated side were harvested. After incubation overnight at 4 °C in a polyclonal anti-TRPV4 preparation (1:1000, Abcam, Cambridge, UK), the polyvinyl fluoride membranes were incubated with an anti-rabbit peroxidase (HRP)-conjugated secondary antibody (1:10,000, Zhongshan Goldenbridge, Beijing, China) for 1 h. The binding was detected with a chemiluminescent HRP method (Millipore, Billerica, MA, USA). The protein level was expressed as a ratio of the density of the detected band relative to the β-actin (1:1000, Cell Signaling Technology Inc.) [6,16].

2.8. Real-time quantitative RT-PCR

L4 and L5 ganglions were harvested in the same manner as described above. Fragments of TRPV4 or β-actin were amplified with the following primers: TRPV4 (forward, 5′-AAGTGGCGTAAGTTCGGG-3′; reverse, 5′-TAAGGGTAGGGTGGCGTG-3′) and β-actin (forward, 5′-AGACCTTCAACACCCCAG-3′; reverse, 5′-CACGATTTCCCTCTCAGC-3′). Instrument control, automated data collection, and data analysis were all performed using the LightCycler software program, version 4.0. The $2^{-\Delta\Delta CT}$ method was used to analyze the data [24].

2.9. Immunofluorescence microscopy

Paraffin-embedded DRG tissues and DRG neurons were incubated with rabbit anti-TRPV4 (1:200, Abcam, Cambridge, UK) antibodies overnight at 4 °C. Then slides 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).