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Interleukin-10 down-regulates voltage gated sodium channels in rat dorsal root ganglion neurons

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ABSTRACT

The over-expression of voltage-gated sodium channels (VGSCs) in dorsal root ganglion (DRG) neurons following peripheral nerve injury contributes to neuropathic pain by generation of the ectopic discharges of action potentials. However, mechanisms underlying the change in VGSCs' expression are poorly understood. Our previous work has demonstrated that the pro-inflammatory cytokine TNF-α up-regulates VGSCs. In the present work we tested if anti-inflammatory cytokine IL-10, which had been proven to be effective for treating neuropathic pain, had the opposite effect. Western blot and immunofluorescence results showed that IL-10 receptor was localized in DRG neurons. Recombinant rat IL-10 (200 pg/ml) not only reduced the densities of TTX-sensitive and Nav1.8 currents in control DRG neurons, but also reversed the increase of the sodium currents induced by rat recombinant TNF-α (100 pg/ml), as revealed by patch-clamp recordings. Consistent with the electrophysiological results, real-time PCR and western blot revealed that IL-10 (200 pg/ml) down-regulated VGSCs in both mRNA and protein levels and reversed the up-regulation of VGSCs by TNF-α. Moreover, repetitive intrathecal administration of rrIL-10 for 3 days (4 times per day) attenuated mechanical allodynia in L5 spinal nerve ligation model and profoundly inhibited the excitability of DRG neurons. These results suggested that the down-regulation of the sodium channels in DRG neurons might contribute to the therapeutic effect of IL-10 on neuropathic pain.

Introduction

Neuropathic pain, characterized by allodynia and hyperalgesia, usually arises from peripheral nerve injury. It has been well established that the hypersensitivity of the primary sensory neurons in the dorsal root ganglion (DRG) plays a critical role in the development of neuropathic pain (Stemkowski and Smith, 2012; White et al., 2007). Although previous studies have demonstrated that increased expression of voltage-gated sodium channels is associated with the hyperexcitability of DRG neurons (Black et al., 2004; Chahine et al., 2005; Rogers et al., 2006), the signals linking the nerve injury to the up-regulation of voltage-gated sodium channels are not fully understood.

Recent studies have evidenced that the inflammatory cytokines, such as TNF-α, IL-1β and IL-6, were dramatically elevated in cerebrospinal fluid (CSF), lumbar spinal cord and DRG from neuropathic pain animal models (Chen et al., 2011; Murphy et al., 1995; Watkins and Maier, 2003; Watkins et al., 2001), and IL-1β and IL-6 increased significantly in CSF from patients suffering from complex regional pain syndrome (Alexander et al., 2005). Administration of TNF-α, IL-1β or IL-6 induced neuropathic pain behaviors in rats, whereas blockade of these pro-inflammatory cytokines alleviated neuropathic pain in animal models (DeLeo et al., 1996; Gabay et al., 2011; Ramer et al., 1998; Wei et al., 2007; Wolf et al., 2006; Xu et al., 2006; Zelenka et al., 2005). Moreover, mice lacking TNF-α receptor 1 or IL-1α or IL-1β displayed reduced duration and magnitude of mechanical allodynia (Chen et al., 2011; Honore et al., 2006; Nadeau et al., 2011). These findings suggest that pro-inflammatory cytokines play an important role in the induction and maintenance of neuropathic pain, and that suppression of the production and the activity of pro-inflammatory cytokines is an effective strategy to prevent chronic pain.

IL-10, one of the most important anti-inflammatory cytokines, cannot only reduce the production of pro-inflammatory cytokines but also inhibit their actions (Moore et al., 2001). Thus, IL-10 is a promising candidate to antagonize the actions of pro-inflammatory cytokines and to depress neuropathic pain. Indeed, this has been proven in several animal models. Intrathecal administration of recombinant IL-10 could reverse IL-1 mediated mechanical allodynia induced by dynorphin (Laughlin et al., 2000) or phospholipase A2 (Chacur et al., 2004). IL-10 also blocked the development of mechanical allodynia and thermal hyperalgesia in a sciatic nerve chronic constriction injury (CCI) animal model (Milligan et al., 2006a). Moreover, intrathecal delivery of adenoviral vectors containing IL-10 cDNA could effectively attenuate the mechanical allodynia induced by sciatic nerve inflammation and CCI (Milligan et al., 2005a,b, 2006a,b).

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Although previous results have validated that IL-10 is a powerful suppressor of neuropathic pain, the mechanisms underlying its beneficial effects remain elusive. In the present study, we reported for the first time that IL-10 receptor 1 (IL-10R1) was expressed in DRG neurons and IL-10 inhibited the expression and the function of VGSCs in DRG neurons. Moreover, intrathecal injection of rrIL-10 decreased the excitability of DRG neurons, thus alleviating mechanical allodynia induced by L5 spinal nerve ligation in rats.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 150–180 g were used. Animals were housed in separated cages at a room temperature kept at 24 ± 1 and 50–60% humidity, under a 12:12-h light/dark cycle. They had access to food and water ad libitum. Experimental procedures were approved by the Animal Care Committee of Sun Yat-Sen University and were carried out in accordance with the guidelines of the National Institutes of Health on animal care. All rats were maintained in a specific pathogen-free environment.

Surgical procedures and drug delivery

Spinal nerve ligation was done following the procedures described before (Kim and Chung, 1992). Briefly, under chloral hydrate (10%) anesthesia, the left L5 spinal nerve was isolated adjacent to the vertebral column and tightly ligated with 6–0 silk sutures distal to the dorsal root ganglion and proximal to the formation of the sciatic nerve. The L5 spinal nerves of sham-operated rats were identically exposed but not ligated. For intrathecal delivery of rrIL-10 or saline, rats were implanted with i.t. catheters during the same surgery time, according to the method described previously (Wei et al., 2007). Briefly, a sterile PE-10 tube filled with saline was inserted through L5/L6 intervertebral space, and the tip of the tube was positioned at the lumbosacral spinal level. Any rats with hind limb paralysis or paresis after surgery were excluded. For rats without movement disorders, lidocaine (2%, 7 μl) was administered through the catheter to verify an intraspinal location, and this was performed 6 days after catheterization. An immediate bilateral hind limb paralysis of the animals (within 15 s) lasting for 20–30 min confirmed the correct catheterization. Animals without these features were not used in the following experiments. rrIL-10 (500 ng) or vehicle was administered one time per day for 3 consecutive days, or 4 times per day for 3 days in a volume of 10 μl followed by a flush of 15 μl of saline to ensure that drugs are delivered into the subarachnoid space.

Behavioral tests

The rats were accommodated to the testing environment by placement within testing chambers for 15–20 min on three separate days just prior to pre-operative testing. Mechanical sensitivity was assessed using von Frey hairs as described previously (Chaplan et al., 1994). Briefly, rats were placed under three different transparent Plexiglas chambers positioned on a wire mesh floor. Fifteen minutes were allowed for habituation. Each stimulus consisted of a 5–6 s application of the von Frey hair to the sciatic innervation area of the foot with a 5 min interval between stimuli. Brisk withdrawal or licking of the paw following the stimulus was considered as positive response. The experimenter who conducted the behavioral tests was blinded to all treatments.

Culture of DRG neurons

DRG neurons were dissociated using enzyme digestion as previously described (Chen et al., 2011). Briefly, ganglia from the cervical to the lumbar level from five rats (or L4 and L5 DRG from the ipsilateral side of operation group and sham group) were excised, freed from their connective tissue sheaths and broken into pieces with a pair of sclerotic scissors in DMEM/F12 medium (GIBCO, USA) under low temperature (in a mixture of ice and water). After enzymatic and mechanical dissociation, DRG neurons were seeded on cover slips coated with Poly-L-Lysine (Sigma, USA) in a humidified atmosphere (5% CO_2, 37 °C) overnight and then used for patch-clamp investigation or processed for immunocytochemical study, western blot and RT-PCR.

Drug application and experimental procedures

Recombinant rat TNF-α and IL-10 (rrTNF-α and rrIL-10; R&D systems, Inc.) was stored as a stock solution of 10 μg/ml and 50 μg/ml respectively at −80 °C, diluted to 100 pg/ml and 200 pg/ml respectively in medium. After being attached for 4 h in normal medium, neurons were treated with rrIL-10 for 12 h or rrTNF-α for 12 h, or rrIL-10 for 12 h after pretreatment with rrTNF-α for 4 h.

Immunofluorescence

The cultured neurons were fixed with 4% paraformaldehyde for 15 min and washed in PBS for three times (5 min each) at room temperature. Then the fixed cells were blocked with 3% donkey serum in 0.3% Triton X-100 for 1 h at room temperature and then incubated overnight at 4 °C with rabbit anti-IL-10R1 (PL Laboratories, Inc.) at 1:50, rabbit anti-Nav1.3, rabbit anti-Nav1.6 or rabbit anti-Nav1.8 antibody (Alomone labs) at 1:200. After rinsing with PBS, the cells were incubated with Cy3-conjugated donkey anti-rabbit IgG (1:400; Jackson ImmunoResearch, USA) for 1 h at room temperature. For double immunofluorescence staining, cells were incubated with a mixture of anti-IL-10R1 antibody and NeuN (FITC conjugated, Sigma, St. Louis, USA) overnight at 4 °C. For DRG tissue, cryostat sections (16 μm) were cut and processed for immunohistochemical staining, sections were blocked with 3% donkey serum in 0.3% Triton X-100 for 1 h at the room temperature, and then incubated overnight at 4 °C with anti-IL-10R1 antibody and mouse anti-neurofilament-200, an A-fiber neuronal marker (NF-200, 1:200, Chemicon), mouse anti-isolectin B4, a C-fiber neuronal marker (IB4, 1:200, Sigma) or glial fibrillary acidic protein, a satellite cell marker (mouse anti-GFAP, 1:2000, Chemicon). After rinsing three times with PBS, sections were incubated in donkey anti-rabbit IgG secondary antibody labeled with Cy3 (1:500, Jackson) or a mixture of IgG secondary antibody labeled with Cy3 and FITC respectively (1:500, Jackson) for 1 h at a room temperature. The stained cells and sections were examined with a fluorescence microscope attached to a CCD spot camera (LEICA DFC350FX/DMIRB) and processed with LEICA IM50 software (Germany). To confirm the specificity of the primary antibody, control groups were incubated without primary antiserum.

Electrophysiological recordings

Whole-cell patch-clamp recordings from DRG neurons were performed using an EPC-10 amplifier and the PULSE program (HEKA Electronics, Lambrecht, Germany) as previously described (Chen et al., 2011). The membrane capacitance was read from the amplifier by software PULSE for determining the size of cells. Patch pipettes with 1 to 3 MΩ resistance were fabricated from borosilicate glass capillaries (Sutter Instruments, Novato, CA) using a Sutter P-87 puller (Sutter Instruments, Novato, CA). Membrane currents were filtered at 2 kHz and sampled at 5 kHz. Voltage errors were minimized by using 80–90% series resistance compensation and linear leak subtraction was used for all recordings. For voltage clamp experiments, the extracellular solution contained (in mM): 30 NaCl, 20 TEA-Cl, 90 choline-Cl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, and 0.1