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Research report

Inhibition of hyperpolarization-activated current by ZD7288 suppresses ectopic discharges of injured dorsal root ganglion neurons in a rat model of neuropathic pain

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Abstract

Peripheral nerve injury causes ectopic discharges of different firing patterns, which may play an important role in the development of neuropathic pain. The molecular mechanisms underlying the generation of ectopic discharges are still unclear. In the present study, by using in vivo teased fiber recording technique we examined the effect of ZD7288, a specific blocker of hyperpolarization-activated current (I_h), on the ectopic discharges in the dorsal root ganglion (DRG) neurons injured by spinal nerve ligation. We found that ectopic discharges of all three firing patterns (tonic, bursting and irregular) were dose- and time-dependently inhibited by local application of ZD7288. Interestingly, the extent of suppression was negatively related to frequency of firing prior to application of ZD7288. We also observed that ZD7288 could alter the firing patterns of the ectopic discharges. At 100 μM, tonic firing pattern was gradually transformed into bursting type whereas at 1 mM, it could be transformed to integer multiples firing. These results indicate that I_h might play a role in the generation of various forms of ectopic discharges in the injured DRG neurons and may thus be a possible target for neuropathic pain treatment.

Theme: Sensory system
Topic: Pain modulation: anatomy and physiology

Keywords: Neuropathic pain; Ectopic discharge; I_h; HCN channel; ZD7288; Teased fiber recording

1. Introduction

Peripheral nerve injury causes ectopic discharges originating from injured sites or dorsal root ganglion (DRG) neurons [1,13,20,26]. These ectopic discharges are widely believed to be major contributors to the development of chronic neuropathic pain following peripheral nerve injury (Refs. [20,32], but see also Refs. [10,18,38]). The mechanisms underlying ectopic discharge generation are still unclear, but changes in certain ion channels in the DRG neurons has been suggested to play a role [17]. For example, the up-regulation of $Na_v1.3$ channel in injured DRG neurons has been shown to be involved in the generation of ectopic discharges [8,16,17,37].

Interestingly, ectopic discharges from both the injured sites and the DRG neurons have strong rhythmic components. The spontaneity of different firing patterns strongly suggests that a pacemaker element underlying the generation of ectopic discharges [7]. Hyperpolarization-activated I_h current (the name of current of hyperpolarization activated, cyclic nucleotide-gated cation channels, or HCN channels, in neuron) have previously been observed in DRG neurons with patch-clamp recording and immunocytochemistry in our experiments [36] as well as in other laboratories [6,30,41]. Several characteristics of I_h, such as contributing to resting membrane potential [7,28,31] and participating in pacemaker currents [23,27,28], indicate that it may be one

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of the candidates for the ectopic discharge generation. Furthermore, ectopic discharges were found to originate mainly in medium to large-sized DRG neurons [20]. Our previous study and other investigations have demonstrated that the current amplitudes and densities of I_h in large DRG neurons are significantly larger than those in the small neurons [7,36]. Thus, the distribution of I_h in DRG neurons parallels the origin of ectopic discharges. Moreover, recent studies have reported that the amplitudes of I_h in DRG neurons were significantly increased following spinal nerve ligation [7] or chronic compression of the DRG [42], especially in the medium to large-sized neurons, implicating that I_h might contribute to the hyperexcitability of injured DRG neurons. Our recent work [35] has found that almost all ectopic discharges could be divided into three different firing patterns based on their interspike interval (ISI): tonic, bursting and irregular. In the present study, using in vivo teased fiber recording technique, we try to examine the effect of an I_h blocker, ZD7288, on the different firing patterns of ectopic discharges.

2. Materials and methods

2.1. Animals and surgery

Male Sprague–Dawley rats weighing 200–250 g were used in the present study. They were provided by the Department of Experimental Animal Sciences, Health Science Center, Peking University and were habituated for 7 days before experiments. The animals had free access to food and water during the experiments and were maintained on natural day/night cycles. All experimental protocols have been approved by the Animal Use and Care Committee of Peking University.

Ligation of the left L4 or L5 spinal nerve was performed as described by Kim and Chung [15]. Briefly, the rats were anesthetized with 10% chlorohydrate (0.3 ml/100 g body weight), and placed in a prone position. An incision was made left of the spine at the L4-S2 levels. The left L4 or L5 spinal nerves were then carefully isolated and tightly ligated with 6–0 silk suture 5–10 mm distal to the DRG, and cut approximately 2 mm distal to the suture.

2.2. Extracellular electrophysiological recording of ectopic discharges in vivo

Three to eight days after ligation and cut of L4 or L5 spinal nerve, rats were anesthetized with urethane (1.5 g/kg, i.p.). A tracheotomy was performed. ECG and heart-rate were monitored and body temperature was maintained at 36–37 °C using a feedback-controlled radiant heater. No paralytic agents were used. L4 or L5 dorsal root was exposed by a lower lumbar laminectomy and covered with warmed paraffin oil (36 °C) in a pool formed of skin flaps. The teased fiber recording method was used to evaluate the ectopic afferent discharges entering the spinal cord along the dorsal root. Most of the dorsal muscles supplied by the dorsal ramus of the L4 or L5 spinal nerve were removed during the laminectomy. The dorsal roots were carefully examined and any communicating branches between them and neighboring dorsal roots were cut to eliminate any afferent firing from normal receptive fields. Nevertheless, residual dorsal ramus fibers, identified by their receptive fields on the lower back, were occasionally encountered in the dorsal root. These were excluded from the present study.

Fine axon bundles (microfilaments) were teased from dorsal root using specially honed No. 5 jewelers forceps (Fine Science Tools, Swiss). Microfilaments, cut centrally but in continuity with the DRG distally, were separated from the dorsal root near its point of entry into the spinal cord, 25–30 mm central to the DRG. The cut end of the microfilament was placed on a platinum recording electrode referenced to a nearby indifferent electrode. Each microfilament was observed passively for ≥30 s. If during this period any spontaneous action potentials were noted, observation was extended and the frequency and patterns of firing were registered. Spike discrimination was performed by a window discriminator and controlled by means of an electronic delay unit. The numbers of spontaneously active nerve fibers in each microfilament, and their firing patterns were measured by observing the different spike heights in the ectopic discharges. Data was captured and analyzed with Micro1401 mk II and Spike 2 software (Cambridge Electronic Design, UK) as in our previous paper [40].

2.3. Application of ZD7288

ZD7288 (molecular weight 292.81, Tocris Cookson, UK) was dissolved and diluted in sterile 0.9% saline to reach desired concentrations (1–1000 μM) and administered topically to the DRG. The reagent was maintained at 36 °C before administration.

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. The suppression of ectopic discharges by ZD7288 application was shown by the ratio. Ratio = frequency of firing after drug/frequency of firing before drug. Repeated measures analysis of variance (ANOVA) followed by the Dunnett's test and the Student's *t*-test were used for data analysis. *p*-value less than 0.05 was considered to be statistically significant.

3. Results

Topically applying ZD7288 to DRG neurons injured by spinal nerve ligation significantly suppressed ectopic discharges (Fig. 1). ZD7288 (1–1000 μM) produced a dose-dependent decrease of the frequency of ectopic discharges in DRG neurons. A significant suppression of