

Short communication

Involvement of hyperpolarization-activated, cyclic nucleotide-gated cation channels in dorsal root ganglion in neuropathic pain

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Dorsal root ganglion (DRG) neurons have peripheral terminals in skin, muscle, and other peripheral tissues, and central terminals in the spinal cord dorsal horn. Hyperpolarization-activated current (I_h) of the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels are present in the DRG. The genes encoding HCN channels have four subtypes named HCN1 to HCN4. HCN channels are permeable to both K^+ and Na^+ . They underlie the depolarization that modulates the rhythmic generations of action potentials (APs), contribute to the resting membrane potential, and modify the waveform of propagated synaptic and generator potentials.

Neuropathic pain is characterized by spontaneous pain, hyperalgesia and allodynia. After spinal nerve injury, the cell bodies of the primary sensory neurons in segmental DRG become hyperexcitable, characterized for some neurons by the presence of spontaneous firing (or ectopic discharge). In the following, we summarize our observations on the role of HCN channels in DRG neurons in neuropathic pain.

1 HCN subtypes and I_h in DRG neurons

Immunohistochemical staining revealed a subgroup of neurons in the DRG that were stained with rabbit polyclonal antibodies specific for HCN1, 2, 3 and 4. The most prominently expressed HCN subtype was HCN1. HCN1-positive cells in DRG were medium to large in size and doubly labeled with neurofilament-200 (NF-200), and were not labeled with isolectin B4 (IB4), a C fiber marker. In contrast, HCN2, 3 or 4 was expressed in all DRG neurons at a lower level. HCN4 was confined to small neurons.

DRG neurons expressed I_h . When membrane was hyperpolarized, the channel was activated, mediating a

slowly activated, inward current. I_h was distributed mainly in large and medium-sized DRG neurons.

2 Changes in expression of HCN in DRG after spinal nerve ligation

Western blotting was used to detect the changes in the expression of HCN subtypes in the DRG after spinal nerve ligation. HCN1 mRNA and protein were reduced in the DRG whose spinal nerve had been ligated. HCN1 expression was decreased to the lowest level at day 14 and restored at day 28 after spinal nerve ligation. HCN2 mRNA and medium molecular weight protein was also decreased in spinal-nerve ligated DRG. HCN3 and 4 in the same ganglion remained unchanged as evidenced by immunohistochemical staining, until day 28 when they became significantly decreased. HCN4 mRNA in DRG did not change, and protein expression slightly increased.

Interestingly, abundant axonal accumulation of HCN channel protein at the injured sites in chronic constriction injury (CCI) rats. Electron immunomicroscopy showed strong positive immunolabeling on the axolemma of myelinated thick axons.

3 Role of I_h in neuronal excitability and ectopic discharges after spinal nerve ligation

ZD7288, a specific I_h blocker, inhibited I_h in a time- and concentration-dependent manner. With patch-clamp recording on acutely isolated DRG neurons, it was found that ZD7288 perfusion resulted in a decrease of both I_h activity and the activation time constant. ZD7288 decreased the number of repetitive APs and caused an increase in AP rise time, accompanied by a small hyperpolarization of the

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membrane resting potential. The results demonstrated that I_h was involved in AP firing, and possessed the physiological functions to facilitate neuronal excitability and ectopic firing.

Extracellular electrophysiological recording from dorsal root fibers associated with the spinal nerve-ligated ganglion revealed three different firing patterns of ectopic discharges: tonic or regular, bursting and irregular. The average frequency of ectopic discharges and the proportions of active filaments also changed rapidly, both parameters reaching a peak within 24 h then declining gradually in the following days. It was also found that proportions of three different firing patterns changed dynamically over time. The tonic and bursting types were dominant patterns in the first 24 h, while the irregular became the only pattern at day 14.

We found that all three firing patterns (tonic, bursting and irregular) were dose- and time-dependently inhibited by local application of ZD7288 to DRG. The rate of suppression was negatively related to the frequency of firing prior to the application of ZD7288. We also found that, while the tonic firing pattern was gradually transformed to bursting type by application of 100 $\mu\text{mol/L}$ ZD7288, it could be transformed to integer multiples firing by 1 000 $\mu\text{mol/L}$ ZD7288.

4 Effects of administration of ZD7288 on mechanical allodynia after spinal nerve ligation or CCI

After spinal nerve ligation, i.t. injection of 30 μg ZD7288 significantly increased the 50% paw withdrawal threshold, ipsilateral to the ligated nerve. ZD7288 had no effect if the dose was lower than 15 μg , but resulted in motor deficits if the dose was higher than 60 μg . ZD7288 produced much better effects in the early stage (5 or 14 days after spinal nerve ligation) than that in the late stage (28 days after spinal nerve ligation). In CCI rats, ZD7288 application to the injured sited also significantly suppressed the ectopic discharges from injured nerve fibers with no effect on im-

pulse conduction. Moreover, mechanical allodynia was inhibited.

In conclusion, these results demonstrated that I_h participated in the development and maintenance of peripheral sensitivity associated with neuropathic pain and that it is a potential target for the design of novel analgesics in the future.

Key words: neuropathic pain; hyperpolarization-activated, cyclic nucleotide-gated cation channel; I_h ; dorsal root ganglion; rat

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