Cellular distribution of HCN2 ion channel immunoreactivity in the spinal dorsal horn of rats in control and inflammatory pain conditions

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SUMMARY

Utilizing immunocytochemical methods, here we demonstrated that – in addition to perikarya – hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channel protein isoform 2 (HCN2) is also expressed by central axon terminals of some population of dorsal root ganglion (DRG) neurons in laminae I-IIo of the spinal dorsal horn of rats. With precise neurochemical identification of primary afferents terminating in laminae I-IIo, we demonstrated that HCN2 immunoreactivity is shown by peptidergic nociceptive primary afferent terminals. With further characterization, we also demonstrated that HCN2-positive axon terminals display also a strong immunoreactivity for substance P. Investigating the postsynaptic targets of HCN2-positive nociceptive primary afferents, it was revealed that these afferents primarily form close appositions with dendrites and perikarya of putative excitatory interneurons that are immunoreactive for neurokinin1 receptor, calbindin, GluR2 subunits of AMPA type glutamate receptors and μ-opioid receptor, but they sparsely come also into contact with GAD65-positive inhibitory interneurons. Our electrophysiological studies confirmed that HCN2 ion channels expressed by axon terminals of nociceptive primary afferents are functional and their activation increase the reliability of synaptic transmission from nociceptive primary afferents to secondary sensory neurons and thus may play an important role in the presynaptic modulation of nociceptive synaptic transmission.

In chronic inflammatory pain state evoked by intraplantar injection of complete Freund's adjuvant into the hindpaw of experimental animals, the number of peptidergic nociceptive primary afferent terminals immunoreactive for HCN2 channel protein was substantially increased. It was also found that in the majority of axon terminals, showing elevated HCN2 channel protein expression in chronic inflammation, substance-P immunoreactivity was also enhanced. This finding suggests that presynaptic mechanisms modulated by I_h currents may primarily play a role in synaptic mechanisms mediated by substance-P release.

With the description of spinal expression of HCN2 ion channels and their role in nociceptive transmission, our results contributed to a more exhaustive understanding of the morpho-functional properties of nociceptive neural circuits in the spinal dorsal horn and may initiate further studies that may lead to the development of analgesic drugs inhibiting HCN ion channel mechanisms.

Keywords: hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN), spinal cord, inflammatory pain

Kulcsszavak: hiperpolarizáció által aktiválódó ciklikus nukleotid-függő kation csatorna (HCN), gerincvelő, gyulladásos fájdalom