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INCREASED EXPRESSION OF HCN2 CHANNEL PROTEIN IN L4 DORSAL ROOT GANGLION NEURONS FOLLOWING AXOTOMY OF L5- AND INFLAMMATION OF L4-SPINAL NERVES IN RATS

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Abstract—A hallmark of peripheral neuropathic pain (PNP) is chronic spontaneous pain and/or hypersensitivity to normally painful stimuli (hyperalgesia) or normally nonpainful stimuli (allodynia).This pain results partly from abnormal hyperexcitability of dorsal root ganglion (DRG) neurons. We have previously shown, using a modified version of the lumbar 5 (L5)-spinal nerve ligation model of PNP (mSNA model involving L5-spinal nerve axotomy plus loose ligation of the lumbar 4 (L4)-spinal nerve with neuroinflammation-inducing chromic-gut), that L4 DRG neurons exhibit increased spontaneous activity, the key characteristic of neuronal hyperexcitability. The underlying ionic and molecular mechanisms of the hyperexcitability of L4 DRG neurons are incompletely understood, but could result from changes in expression and/or function of ion channels including hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which are active near the neuron's resting membrane potential, and which produce an excitatory inward current that depolarizes the membrane potential toward the threshold of action potential generation. Therefore, in the present study we used the mSNA model to investigate whether: (a) expression of HCN1–HCN3 channels is altered in L4 DRG neurons which, in the mSNA model, are essential for transmission of the evoked pain, and which contribute to chronic spontaneous pain, and (b) local (intraplantar) blockade of these HCN channels, with a specific blocker, ZD7288, attenuates chronic spontaneous pain and/or evoked pain in mSNA rats. We found 7 days after mSNA: (1) a significant increase in HCN2-immunoreactivity in small (<30 μm) DRG neurons (predominantly IB4-negative neurons), and in the proportion of small neurons expressing HCN2 (putative nociceptors); (2) no significant change in HCN1- or HCN3-immunoreactivity in all cell types; and (3) attenuation, with ZD7288 (100 μM intraplantar), of chronic spontaneous pain behavior (spontaneous foot lifting) and mechanical, but not, heat hypersensitivity. The results suggest that peripheral HCN channels contribute to mechanisms of spinal nerve injury-induced PNP, and that HCN channels, possibly HCN2, represent a novel target for PNP treatment.

Key words: dorsal root ganglion, HCN, neuropathic pain, pain hypersensitivity, spontaneous pain, primary sensory neurons.

INTRODUCTION

Chronic peripheral neuropathic pain (PNP) is characterized in humans by chronic spontaneous pain and/or hypersensitivity to normally painful stimuli (hyperalgesia) and normally non-painful stimuli (allodynia), as well as and dysesthesias and/or parasthesias (Bonica, 1990). Preclinical studies using animal models of nerve injury-induced PNP suggest that PNP is due, at least in part, to abnormal hyperexcitability of dorsal root ganglion (DRG) neurons (Costigan et al., 2009). We have previously shown, using the modified spinal nerve axotomy (mSNA) model (a modified version of the widely used spinal nerve ligation (SNL) model of PNP (Kim and Chung, 1992) that involves lumbar 5 (L5)-spinal nerve axotomy plus loose ligation of the lumbar 4 (L4)-spinal nerve with neuroinflammation-inducing chromic-gut), that L4 DRG neurons exhibit increased spontaneous activity (SA) (Djouhri et al., 2006), the key characteristic of neuronal hyperexcitability. The underlying ionic and molecular mechanisms of the hyperexcitability of L4 DRG neurons are incompletely understood, but could result from changes in expression and/or function of ion channels including hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which are active near resting membrane potential, and which produce an excitatory inward current (termed I_h in neurons) that depolarizes the membrane potential toward the threshold of action potential generation (for reviews see Biel et al., 2009 and Dunlop et al., 2009).

HCN channels have been implicated in development of PNP on the basis that: (a) I_h current density and the rate of activation were increased in injured DRG and trigeminal ganglion neurons after nerve injury (Chaplan

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Abbreviations: CCI, chronic constriction injury; DAPI, 4′,6-diamidino-2-phenylindole; DRG, dorsal root ganglion; HCN, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels; HWL, paw withdrawal latency to a noxious heat stimulus; IB4, isolectin-B4; L4, lumbar 4; L5, lumbar 5; mSNA, modified spinal nerve axotomy; MWT, paw withdrawal threshold to a mechanical stimulus; PBS, phosphate-buffered saline; PNP, peripheral neuropathic pain; SA, spontaneous activity; SFL, spontaneous foot lifting; SNA, spinal nerve axotomy; SNL, spinal nerve ligation.

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et al., 2003; Yao et al., 2003; Kitagawa et al., 2006); (b) pharmacological blockade of HCN channels with ZD7288 or ivabradine significantly attenuated mechanical hypersensitivity in the SNL and chronic constriction injury (CCI) models of PNP (Chaplan et al., 2003; Lee et al., 2005; Luo et al., 2007; Jiang et al., 2008b; Young et al., 2014) and (c) ZD7288 reduced SA in A-fiber neurons in these models of PNP (Chaplan et al., 2003; Lee et al., 2005; Sun et al., 2005; Jiang et al., 2008b; see also Dunlop et al., 2009 and Jiang et al., 2008a for reviews).

Of the four isoforms of the HCN channels (HCN1–4), HCN2 is believed to play a pivotal role in chronic pain on the basis that: (a) deletion of HCN2 in nociceptive neurons prevented the development of pain behaviors in the CCI model and inflammatory pain models (Emery et al., 2011; Schnorr et al., 2014, see also Emery et al., 2012 for review) and (b) HCN2 expression was increased in small DRG neurons following chronic hindlimb inflammation (Weng et al., 2012).

HCN channels are expressed throughout the whole peripheral pain pathway including the peripheral sensory terminals (Luo et al., 2007), the central terminals of sensory afferents in the spinal cord, and the somata and axons of DRG neurons (reviewed in Herrmann et al., 2015). Since most of the proteins that are expressed in the somata of primary afferent (DRG) neurons are also expressed in their free nerve endings and in their central and peripheral axonal branches (see Basbaum et al., 2009), it is possible that changes in HCN expression in the somata of DRG neurons may also occur in their peripheral terminals. Changes in expression and/or function of HCN channels at peripheral nerve terminals may result in a lowered threshold and/or a greater gain of the stimulus–response relationship (Schnorr et al., 2014) and may also contribute to SA generation and thereby to PNP.

In the present study we used the mSNA model of PNP to examine, immunohistochemically, whether expression of HCN1–HCN3 channels is altered in L4 DRG neurons which are essential for transmission of the evoked pain in the mSNA model, and which contribute to chronic spontaneous pain (Djouhri et al., 2006), the primary complaint of patients with PNP (Backonja and Stacey, 2004). We used the mSNA model because, unlike the classical SNL model, it shows significant spontaneous foot lifting (SFL), a behavioral sign of spontaneous pain that is believed to be driven, at least partly, by SA in L4 C-fiber nociceptive DRG neurons (Djouhri et al., 2006). We also investigated whether local (intraplantar) blockade of HCN channels, with a specific blocker ZD7288, attenuates SFL and/or pain hypersensitivity (evoked pain) in mSNA rats. Blockade of HCN channels with both local (intraplantar) and systemic (i.p.) administration of ZD7288 has been shown to reverse acute spontaneous pain measured 1 h after mild thermal injury (Luo et al., 2007), but the effect of ZD7288 on nerve injury-induced chronic spontaneous pain (whose underlying mechanisms are likely to differ from those of acute spontaneous pain) has not been explored. We used intraplantar rather than systemic administration of ZD7288 to target HCN channels in skin nerve terminals (see Luo et al., 2007) where the axon peripheral terminals of the L4 DRG neurons terminate, and where SA in L4 C-nociceptors is believed to originate (at least partly) following nerve injury (Wu et al., 2001). Furthermore intraplantar administration of ZD7288 reduced tactile allodynia more significantly and more rapidly than systemic (i.p.) administration of ZD7288 (Luo et al., 2007). We have previously shown, using ATF3 immunostaining, that 20–40% of L4 DRG neurons were damaged in the mSNA model (see Djouhri et al., 2006). Thus most of the L4 DRG neurons in the mSNA model presumably have conducting/uninterrupted sensory nerve fibers, conducting through the damaged nerve alongside axotomized/degenerating fibers (see Djouhri et al., 2012).

EXPERIMENTAL PROCEDURES

Experimental animals

Young, female Wistar rats (180–280 g, Charles River, UK) were used throughout. Animals were housed in a room maintained at room temperature between 20 and 26 °C which is optimum for rats (Yamauchi et al., 1981) while under a 12-hour (h) dark and light cycle, with soft bedding and access to food and water and libitum. All experimental procedures were reviewed by the University of Liverpool Ethical review group and complied with the 1986 UK Scientific Procedures Animals Act. All rats appeared healthy throughout the experiments based on previously established criteria of exploratory activity and weight gain (Deacon, 2006).

Animal model of PNP

We used a modified version of the SNL model (Kim and Chung, 1992) that was described previously (Djouhri et al., 2006, 2012). We have referred to the model as mSNA that involves, in addition to L5 spinal nerve zaxotomy (SNA), loose ligation of the L4 spinal nerve with chromic-gut. This model was introduced by Lee et al. (2003) although chromic-gut ligatures were originally used in the CCI model of PNP (Bennett and Xie, 1988). We used the mSNA model, because it shows, unlike the SNA/SNL models, significant SFL, a behavioral sign of spontaneous/ongoing pain (see section ‘SFL’ below). The effectiveness of chromic-gut ligatures in inducing neuropathic pain-related behavior has been attributed to induction of neuroinflammation (Maves et al., 1993), although the physical presence of the ligature may cause some mechanical damage (Djouhri et al., 2006, 2012; Lee et al., 2003).

In the present study we used two approaches: (1) immunohistochemistry to examine whether expression of HCN1–HCN3 channel subunits is altered in L4 DRG neurons in the mSNA model and (2) behavioral testing to investigate the effects of local (intraplantar) blockade of HCN channels, with a specific blocker ZD7288, on chronic spontaneous pain and pain hypersensitivity (evoked pain) in mSNA rats. The behavioral studies were carried out after we have established,