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Expression of background potassium channels in rat DRG is cell-specific and down-regulated in a neuropathic pain model

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ABSTRACT

Neuropathic pain is associated with hyperexcitability of DRG neurons. Despite the importance of leakage potassium channels for neuronal excitability, little is known about their cell-specific expression in DRGs and possible modulation in neuropathic pain. Multiple leakage channels are expressed in DRG neurons, including TASK1, TASK3, TRESK, TRAAK, TWIK1, TREK1 and TREK2 but little is known about their distribution among different cell types. Our immunohistochemical studies show robust TWIK1 expression in large and medium size neurons, without overlap with TRPV1 or IB4 staining. TASK1 and TASK3, on the contrary, are selectively expressed in small cells; TASK1 expression closely overlaps TRPV1-positive cells, while TASK3 is expressed in TRPV1- and IB4-negative cells. We also studied mRNA expression of these channels in L4–L5 DRGs in control conditions and up to 4 weeks after spared nerve injury lesion. We found that TWIK1 expression is much higher than TASK1 and TASK3 and is strongly decreased 1, 2 and 4 weeks after neuropathic injury. TASK3 expression, on the other hand, decreases 1 week after surgery but reverts to baseline by 2 weeks; TASK1 shows no significant change at any time point. These data suggest an involvement of TWIK1 in the maintenance of the pain condition.

Introduction

Neuronal signal transmission is mediated by action potentials generated by voltage-gated ion channels (Hodgkin and Huxley, 1952). The diverse functional properties of voltage-gated channels lead to cell-to-cell variability in action potential phenotype, including differences in threshold, width, frequency and spiking pattern (McCormick, 2004). However, leakage channels are also critically involved in the modulation of neuronal excitability (Bayliss and Barrett, 2008). Particularly, work over the last 15 years has led to the characterization of KCNK, a family of leakage potassium channels abundantly expressed in neuronal and non-neuronal tissues (reviewed by Lesage, 2003; Lesage and Lazdunski, 2000). KCNK channels influence cell excitability by setting the resting potential and by setting neuronal membrane potential and input resistance (Berg and Bayliss, 2007; Taverna et al., 2005) and are modulated by a vast array of transmitters and modulators (Lesage and Lazdunski, 2000; Talley et al., 2000). These channels also regulate several other electrophysiological properties; for instance, in thalamocortical neurons, KCNK blockade shifts the firing mode from bursting to tonic (Meuth et al., 2003), while, in cortical basket cells, TASK3 channels regulate the maximum firing frequency (Goldberg et al., 2011).

Dorsal root ganglia neurons (DRGs) are the primary sensory neurons that convey information from the skin, muscles and joints to the spinal cord. Nociceptive fibers can be myelinated A fibers, originating from large DRGs, or unmyelinated C fibers originating from small and medium DRGs. Unmyelinated fibers are classified (Julius and Basbaum, 2001) into the substance P expressing peptidergic population and another population, which does not express substance P, but binds the lectin IB4 (Stucky and Lewin, 1999). Both these populations are further divided into TRPV1-positive and negative (Aoki et al., 2005; Michael and Priestley, 1999).

Under normal conditions sensory signals originate in DRG's peripheral endings, where the transducers are located, whereas somatic initiation of firing is sporadic and has minor sensory consequences (Devor, 1999). In the presence of nerve injury or pain, however, ectopic (somatic) DRG firing is strongly enhanced and is believed to represent a major cause of neuropathic pain (Nordin et al., 1984; Sukhotinsky et al., 2004); additionally, nociceptors become intrinsically firing and their intrinsic firing frequency correlates with spontaneous pain (Djouhri et al., 2006). Much work has been performed to investigate changes in the expression of voltage-gated sodium channels correlated with nerve injury; these studies show a complex scenario of up- and down-regulation of different channel subunits (reviewed by Rogers et al., 2006; Waxman, 1999). In addition, nerve injury has been shown to induce downregulation in the expression of voltage gated potassium channels; in particular, downregulation of several members of the shaker-homologous class (Kv 1.1,1.2, Kv1.4) has been reported after nerve injury (Kim et al., 2002; Rasband et al., 2001; Yang et al., 2004). However, far less is known about neuropathic injury-associated changes in leakage channel expression in DRG neurons. TASK channels were shown to regulate excitability in sensory neurons from humans with neuropathic pain (Baumann et al., 2004). Accordingly, DRG expression of several members of the KCNK family has been found to be down regulated in the first

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4 days following an inflammatory response (Marsh et al., 2012) while expression of TRESK was diminished 3 weeks after injury in a rat neuropathic injury model (Tulleuda et al., 2011). Yet, a characterization of KCNK subunit expression among the various DRG neuron subtypes is still lacking, as is any knowledge of their long-term regulation in neuropathic pain. Because several studies have demonstrated changes in extracellular pH due to inflammation following nerve injury (Reeh and Steen, 1996; Steen and Reeh, 1999) we focused on expression of TASK1 and TASK3 subunits, which are sensitive to even small changes of pH in the physiological range (Berg et al., 2004) and on TWIK1, which represents an intriguing molecule whose expression has not been previously investigated in DRGs. We investigated the expression of these subunits in different DRG neuron subclasses and its regulation up to 4 weeks after neuropathic injury.

Results

Immunochemical analysis was performed to study cell-specific expression of TASK1, TASK3 and TWIK1, three KCNK family members in adult rat L4-L5 DRGs. Additionally, the temporal profile of gene expression of these channel subunits was examined in DRGs in the spared nerve injury model of neuropathic pain at 1, 2 and 4 weeks after the peripheral injury.

For these experiments, DRGs ipsilateral and contralateral to the nerve lesion were collected as well as DRGs from sham controls and mRNA expression of the gene of interest was quantified relative to GAPDH using real-time PCR.

Relative abundance of background channels

1 month old naïve rats were used to determine the relative abundance of transcripts of the background channels of interest in L4–L5 DRGs (Fig. 1B). Kv1.2 was included in the analysis because its expression has previously been characterized in DRGs and it was also shown to be reduced after nerve injury (Kim et al., 2002; Rasband et al., 2001; Yang et al., 2004). Interestingly, TWIK1 transcripts showed the highest expression, approximately 80% higher than Kv1.2, which had the second highest expression level. TASK1 was approximately 3 fold higher than TASK3, which had the lowest transcript expression. These measurements confirmed that TASK1, TASK3 and TWIK1 genes are expressed in rat DRGs but did not provide any information concerning the cells in which the genes are expressed. Immunohistochemical staining was thus performed to answer this question.

KCNK channel expression is cell-type specific

Immunohistochemical staining was performed on 30 μm-thick slices obtained from L4-L5 DRG of 1 month old naïve rats. Distribution of TASK1, TASK3 and TWIK1 protein was assessed in parallel with IB4 and TRPV1, two widely used cellular markers of C-fiber nociceptive neurons. In agreement with previous reports, IB4 and TRPV1 stained partially overlapping populations of small DRG neurons (Aoki et al., 2005; Stucky and Lewin, 1999).

TWIK-1 expression is abundant in large neurons and does not overlap with IB4 and TRPV1

PCR data showed an abundant expression of TWIK1 in DRG tissue. Immunostaining confirmed that expression is robust and showed that it predominates in large and medium size neurons (Fig. 2A,E) in agreement with the outcome of a previous in-situ characterization (Talley et al., 2001). TWIK1 expression was seldom found in small size neurons (2 of 28 cells Fig. 2I). Interestingly, co-staining studies showed no overlap of TWIK1 with IB4 and TRPV1, even in the few cases when expression was detected in small size neurons (D,H,L). In 12 z-stacks

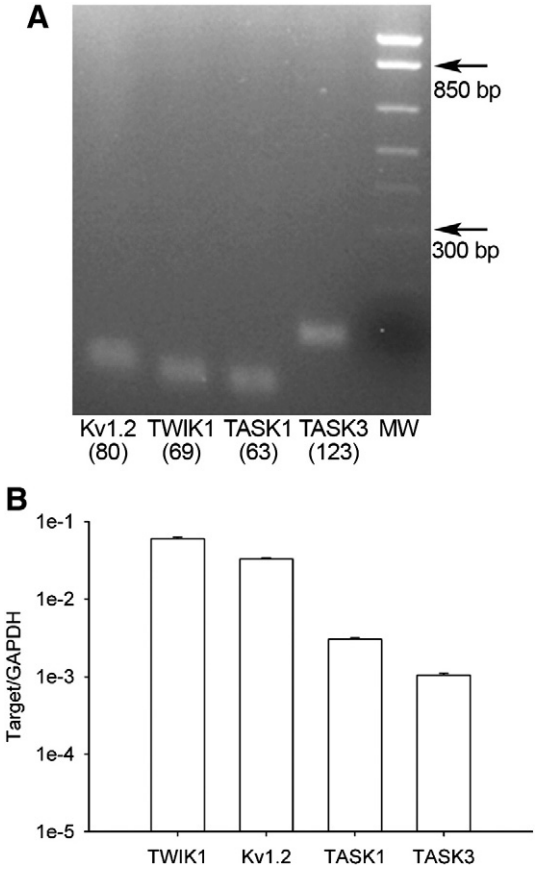

Fig. 1. Relative abundance of TWIK1, TASK1, TASK3 and Kv1.2 in rat DRG. A, PCR products run on a 1.8% agarose gel demonstrated a single amplification product for each gene of interest. B, Relative abundance of genes of interest detected in naïve animals at 1 month of age. Because no difference was observed between ipsi- and contra-lateral DRGs, the data were pooled for this bar chart. Genes of interest were normalized to GAPDH and plotted on a logarithmic scale. Note the very high expression level for TWIK1 and Kv1.2, while TASK1 and TASK3 have lower expression levels. Bar chart represents average and SEM. Data are from 5 naïve rats.

encompassing 28 TWIK1 positive cells, no overlap with IB4 or TRPV1 was detected.

TASK1 expression overlaps with IB4 and TRPV1 positive cells

Our data showed that TASK1 expression was largely limited to small size (<25 μm) neurons (Fig. 3). Because preliminary studies revealed some overlap of TASK1 with IB4 and TRPV1, a more detailed co-staining and cell counting study was undertaken, as detailed in Table 2. 93% of TASK1 expressing cells also expressed TRPV1, while 43% of TRPV1 positive cells expressed TASK1 (Fig. 3C and Table 2). In a separate staining 58% of TASK1 positive cells co-localized with cells positive for IB4, while 45% of the IB4 population expressed TASK1 (Fig. 3G and Table 2). Thus TASK1 showed very high selective expression in TRPV1-positive cells and substantial overlap with the IB4 population within the DRG.

TASK3 expression is sparse and limited to a subpopulation of small cells

In keeping with the PCR data that showed much lower expression of TASK3 compared to TASK1 in DRG tissue, TASK3 staining was sparse. Similar to TASK1, TASK3 expression was mostly limited to small (presumably C-fiber) DRGs (Fig. 4). Contrary to TASK1, however, TASK3 expression was almost exclusively limited to the TRPV1- and IB4-negative population. Indeed, only 6% (5/85) of the TASK3-positive cells expressed TRPV1 (Fig. 4C and Table 2) and 4% (3/86) expressed IB4