Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Icilin reduces voltage-gated calcium channel currents in naïve and injured DRG neurons in the rat spinal nerve ligation model

T. Hagenacker, M. Lampe, M. Schäfers*

Department of Neurology, University Hospital Essen, Hufelandstr. 55, 45147 Essen, Germany

ARTICLE INFO

Article history:
Accepted 11 February 2014
Available online 18 February 2014

Keywords:
Neuropathic pain
Patch clamp
Cold allodynia
Voltage gated calcium channel current
Electrophysiology

ABSTRACT

Recently, the transient receptor potential (TRP) channels TRPM8 and TRPA1 have been identified as molecular sensors for cold, and it has been suggested that they play a crucial role in allodynia by modulating voltage-gated calcium channel currents ($I_{Ca(V)}$). The aim of this study was to analyze the modulation of $I_{Ca(V)}$ by the TRPM8-agonist icilin in vitro and to investigate the analgesic effect of icilin in a neuropathic pain model in vivo. Whole cell patch-clamp recordings were performed on isolated naïve and injured rat dorsal root ganglia (DRG) neurons, and the analgesic efficacy of icilin applied topically to the paws or intrathecally was tested in rats after spinal nerve ligation (SNL). $I_{Ca(V)}$ (depolarization from −80 to 0 mV) in naïve DRG neurons was reduced dose dependently (0.002–200 μM) by icilin (18–80%). Subtype isolation of calcium channels show a marked reduction of L-type channel currents compared to N-type channel currents. The effects of icilin on $I_{Ca(V)}$ were not significantly different in non-injured and SNL-injured DRG neurons. In vivo, neither topical (10–200 μM) nor intrathecal application of icilin (0.1 nM to 1 μM) affected tactile allodynia or thermal hyperalgesia after SNL, but it increases cold allodynia 6 h after application. We conclude that the icilin-induced modulation of $I_{Ca(V)}$ in DRG neurons is unlikely to mediate analgesic effects or contribute directly to the pathogenesis of cold allodynia in the rat SNL model, but it is a potential mechanism for the analgesic effects of icilin in other pain models.

1. Introduction

Neuropathic pain derived from peripheral nerve injury is a disorder that adversely affects patients' quality of life. Current therapies for neuropathic pain are focused on voltage-gated channels and transmitter homeostasis and are limited in specificity and efficacy. Cold allodynia and cold hyperalgesia lead to pain and discomfort at temperatures

Abbreviations: TRPM8, transient receptor potential melastatin 8; TRPA1, transient receptor potential ankyrin 1; SNL, spinal nerve ligation; CCI, chronic constriction injury; DRG, dorsal root ganglion; VGCC, voltage gated calcium channel; EPSC, excitatory postsynaptic current; $I_{Ca(V)}$, VGCC currents; IV-curve, current–voltage relation

*Corresponding author. Fax: +49 201 7235901.
E-mail address: tim.hagenacker@uk-essen.de (T. Hagenacker).

http://dx.doi.org/10.1016/j.brainres.2014.02.022
0006-8993

that are normally perceived as being innocuously cool, and they are frequently reported in neuropathies, CRPS and post-stroke pain (Greenspan et al., 2004; Kemler et al., 2000; Verdugo and Ochoa, 1992). Clinical trials show beneficial effects of cooling agents in chronic lower back pain or post-operative pain (Sauls, 1999). To date, the underlying mechanisms of the enhanced sensitivity to cold or the analgesic effects of cooling are unknown.

The isolation of transient receptor potential (TRP) channels in sensory neurons was central to improving the understanding of thermal sensing. TRPM8 is expressed by a small population of ~10–15% of dorsal root ganglion (DRG) neurons (Abe et al., 2006; Peier et al., 2002) and has generated great interest in its role in cold sensing, cold allodynia and cooling-induced analgesia (McKemy et al., 2002; Peier et al., 2002). Mouse knockout studies have demonstrated that TRPM8 is required for cold sensation after both innocuous and noxious cold temperatures (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007). Activation of TRPM8 by innocuous temperatures (cool temperatures of approximately 18–19 °C) or by menthol or icilin (McKemy et al., 2002), which act as selective but not very specific agonists, mainly evokes calcium-driven inward currents through TRPM8. In addition, the TRP family member TRPA1 contributes to cold-sensing and cold allodynia in neuropathic pain (Katsura et al., 2006; Obata et al., 2005).

Several studies have examined the expression of TRPM8 after nerve injury, with conflicting results: mRNA was reported to be weakly upregulated in DRG neurons (Frederick et al., 2007), and the number of cells expressing TRPM8 is increased after chronic constriction injury of the sciatic nerve (CCI) (Proudfoot et al., 2006; Su et al., 2011; Xing et al., 2007). Furthermore, the development of cold allodynia paralleled the expression of TRPM8 after CCI in trigeminal nerve lesions (Rossi et al., 2012). In the spinal nerve ligation (SNL) model, TRPM8 mRNA was reported to be downregulated in injured adjacent ganglia (Caspani et al., 2009) and unchanged in uninjured adjacent ganglia (Obata et al., 2005; Persson et al., 2009). In addition to the changes in expression of TRPM8 after nerve injury, the modulation of TRPM8, by either agonists or antagonists, has been shown to be effective in the treatment of neuropathic pain. Topical menthol reduced mechanical withdrawal thresholds at higher concentrations, while it has a biphasic effect on cold avoidance in low and high concentrations of menthol (Klein et al., 2010), and the topical application or intrathecal injection of the TRPM8 agonists icilin and menthol elicits analgesia after CCI (Proudfoot et al., 2006; Su et al., 2011), but this could not be replicated in further studies (Caspani et al., 2009). In contrast, icilin inhibited mechanically evoked responses after intraplantar injection in the SNL model (Brignell et al., 2008), and the TRPM8 antagonist PBMC reduces cold hypersensitivity after CCI but not in the oxaliplatin pain model (Knowlton et al., 2011). Intrathecal TRPM8 antisense oligonucleotides attenuate cold hyperalgesia but have no effect on mechanical allodynia or thermal hyperalgesia after CCI (Katsura et al., 2006; Su et al., 2011). The interpretation of the data for the relevance of TRPM8 in neuropathic pain such as cold allodynia is further complicated by the finding that menthol inhibits voltage-gated sodium and calcium channels (VGCCs) (Haeseler et al., 2002; Swandulla et al., 1987), though the effects of icilin on voltage-gated channels were not investigated. The application of menthol to DRG-dorsal horn co-cultures leads to a decrease in the EPSC frequency, suggesting the involvement of presynaptic mechanisms, possibly of presynaptic localized VGCCs (Tsuzuki et al., 2004). The modulation of the function and expression of VGCCs is an essential mechanism in the generation of neuropathic pain. After nerve lesions, VGCC currents were reduced in DRG neurons (Abdulla and Smith, 2001; Baccei and Kocsis, 2000; Hogan et al., 2000; McCallum et al., 2003, 2006). Ligation of the sciatic nerve decreases the currents of high-voltage-activated calcium channels in small DRG neurons, especially N-type and L-type calcium currents (Baccei and Kocsis, 2000; Kim et al., 2001), while L-type channel expression is upregulated in the spinal cord. Both correlate with the change in biophysical properties of sensory neuron membranes during action potential generation, suggesting that neuropathic pain may be partially mediated by that decrease (Fossat et al., 2010; McCallum et al., 2006). Although these results support the "loss" of calcium channels in neuropathic pain, several studies have shown the effectiveness of calcium channel antagonists. N-type calcium channels, especially, are highly concentrated in sensory structures such as spinal dorsal horn neurons and DRG cell bodies and their central terminals (Catterall and Few, 2008; Westenbroek et al., 1992). Several small peptide inhibitors of N-type channels have been shown to be effective in the treatment of pain, possibly by blocking the synaptic transmission of pain signals to the central nervous system (Feng et al., 2001; Yarotskyy and Elmslie, 2009), and the Omega-Conotoxin MVIIA (Ziconotide) has been approved for the management of severe pain (Miljanich, 2004). Therefore, recent studies in the development of analgesics have focused on interactions with VGCCs (Vink and Alewood, 2012). Other previous studies have shown that, in addition to menthol, TRP modulators frequently interact with voltage-gated channels. The TRPV1 agonist capsaicin differentially modulates VGCCs in DRG (Hagenacker et al., 2005; Hagenacker and Busselberg, 2007; Sugimoto et al., 2008) and the TRPV1 antagonist capsazepine blocks VGCCs (Docherty et al., 1997). Of TRPM8 modulators, only menthol has been described to block VGCCs, but other TRPM8 modulators have not yet been investigated.

In light of these observations, we reasoned that TRPM8 modulators are capable of modulating VGCC currents ($I_{Ca(V)}$) and that this may be a mechanism for the modulation of pain-associated behavior after nerve injury. We therefore measured the effects of icilin on $I_{Ca(V)}$ in naïve and SNL-injured DRG neurons and investigated effect of icilin on pain-associated behavior in the SNL model of neuropathic pain in rats.

Consistent with our hypothesis, our study shows for the first time that icilin dose-dependently reduces $I_{Ca(V)}$ in naïve and injured DRG neurons. However, icilin has no analgesic effect after SNL, suggesting that the reduction of $I_{Ca(V)}$ in DRG neurons by icilin has no relevant inhibitory effects on excitation but possibly promotes cold allodynia in this pain model.

2. Results

Depolarization of the neurons from holding potential to 0 mV resulted in an inward current ($I_{Ca(V)}$) that inactivates during