Please cite this article in press as: Eroli F et al. Hyperpolarization-activated current I_h in mouse trigeminal sensory neurons in a transgenic mouse model of familial hemiplegic migraine type-1. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.03.033

Neuroscience xxx (2017) xxx–xxx

HYPERPOLARIZATION-ACTIVATED CURRENT I_h IN MOUSE TRIGEMINAL SENSORY NEURONS IN A TRANSGENIC MOUSE MODEL OF FAMILIAL HEMIPLEGIC MIGRAINE TYPE-1

FRANCESCA EROLI,[a] SANDRA VILOTTI,[a] ARN M. J. M. VAN DEN MAAGDENBERG[b,c] AND ANDREA NISTRI[a]*

[a] *Neuroscience Department, International School for Advanced Studies (SISSA), Trieste, Italy*

[b] *Department of Neurology, Leiden University Medical Centre, Leiden, Netherlands*

[c] *Department of Human Genetics, University Medical Centre, Leiden, Netherlands*

Abstract—Transgenic knock-in (KI) mice that express $Ca_V2.1$ channels containing an R192Q gain-of-function mutation in the α_{1A} subunit known to cause familial hemiplegic migraine type-1 in patients, exhibit key disease characteristics and provide a useful tool to investigate pathophysiological mechanisms of pain transduction. Previously, KI trigeminal sensory neurons were shown to exhibit constitutive hyperexcitability due to up-regulation of ATP-gated P2X3 receptors that trigger spike activity at a more negative threshold. This implies that intrinsic neuronal conductances may shape action potential generation in response to ATP, which could act as a mediator of migraine headache. Here we investigated whether the hyperpolarization-activated conductance I_h, mediated by hyperpolarization-activated cyclic nucleotide-gated channel (HCN) channels, contributes to sub-threshold behavior and firing in wild-type (WT) and KI trigeminal ganglia (TG) neurons. Whereas most WT and KI trigeminal neurons expressed I_h current, blocked by the specific inhibitor ZD7288, it was smaller in KI neurons despite similar activation and deactivation kinetics. HCN1 and HCN2 were the most abundantly expressed subunits in TG, both *in situ* and in culture. In KI TG neurons, HCN2 subunits were predominantly present in the cytoplasm, not at the plasma membrane, likely accounting for the smaller I_h of such cells. ZD7288 hyperpolarized the membrane potential, thereby raising the firing threshold, and prolonging the spike trajectory to generate fewer spikes due to P2X3 receptor activation. The low amplitude of I_h in KI TG neurons suggests that down-regulation of I_h current in sub-threshold behavior acts as a compensatory mechanism to limit sensory hyperexcitability, manifested under certain stressful stimuli.

Key words: trigeminal ganglion, excitability, P2X3, ZD7288, CGRP, HCN.

INTRODUCTION

Familial hemiplegic migraine type-1 (FHM1) is a rare monogenic subtype of migraine with aura (Headache Classification Committee of the International Headache Society (IHS), 2013) that is caused by specific missense mutations in the *CACNA1A* gene, which encodes the pore-forming α_{1A} subunit of neuronal voltage-gated $Ca_V2.1$ (P/Q-type) calcium channels (Ophoff et al., 1996; Ferrari et al., 2015; Tolner et al., 2015). A transgenic knock-in (KI) mouse model of FHM1 expressing the R192Q missense mutation shows a gain-of-function phenotype of mutated $Ca_V2.1$ channels with increased neurotransmission and susceptibility to cortical spreading depression (van den Maagdenberg et al., 2004; Eikermann-Haerter et al., 2009; Tottene et al., 2009), heightened trigeminal sensory neuron firing (Hullugundi et al., 2014; Marchenkova et al., 2016a), and head pain (Chanda et al., 2013, p. 20). In particular, these KI mice exhibit intense photophobia and unilateral head pain only under stressful conditions in analogy to human attacks of migraine (Chanda et al., 2013). The issue of what controls trigeminal sensory excitability in between migraine attacks remains unclear.

Trigeminal ganglia (TG) of R192Q KI mice show a selective potentiation of neuronal P2X3 receptor-mediated currents (Nair et al., 2010), which results in a lower firing threshold and a larger number of spikes in response to P2X3 activation by ATP agonists, whereas capsaicin-sensitive transient receptors potential vanilloid-1 (TRPV1) receptors (Julius and Basbaum, 2001; North, 2003) are not facilitated (Nair et al., 2010). Furthermore, an enhanced release of the neuropeptide calcitonin gene-related peptide (CGRP; (Ceruti et al., 2011), which is believed to be a mediator that triggers headache attacks (Olesen et al., 2004; Messlinger et al., 2011), was observed in R192Q KI TG that may con-

*Corresponding author. Address: SISSA, Via Bonomea 265, 34136 Trieste, Italy. Fax: +39-040-3787702.
E-mail addresses: feroli@sissa.it (F. Eroli), vilotti@sissa.it (S. Vilotti), A.M.J.M.van_den_Maagdenberg@lumc.nl (A. M. J. M. van den Maagdenberg), nistri@sissa.it (A. Nistri).

Abbreviations: α,β-meATP, α,β-methylene-ATP; AP, action potential; DRG, dorsal root ganglia; CGRP, calcitonin gene-related peptide; FHM1, familial hemiplegic migraine type-1; HCN, hyperpolarization-activated cyclic nucleotide-gated channel; I_h, hyperpolarization-activated current; KI, knock-in; MF, multiple-firing; NS, non-spiking; P2X3R, purinergic P2X3 receptor; SS, single-spike; TEA, tetraethylammonium chloride; TG, trigeminal ganglion; TRPV1, transient receptor potential vanilloid-1; V_{rev}, reversal potential; WT, wildtype.

http://dx.doi.org/10.1016/j.neuroscience.2017.03.033
0306-4522/

tribute to the observed up-regulation of P2X3 receptors (Fabbretti et al., 2006; Hullugundi et al., 2013).

The KI phenotype, therefore, recapitulates two functional changes in TG activity: up-regulation of $Ca_V2.1$ channels and P2X3 receptors. Both targets are expected to operate in a range of membrane potentials below the threshold for neuronal firing. Below threshold excitability is regulated by a combination of (de) activation of certain neuronal conductances (Bean, 2007) ultimately responsible for the speed and extent of depolarization that a chemical signal like ATP (which acts on P2X3 receptors) might produce. Within this framework, the hyperpolarization-activated current (I_h), a mixed cationic conductance that is activated by membrane hyperpolarization, may be viewed as a key regulator of cellular excitability and electrical responsiveness of cells (reviewed in Biel et al., 2009). I_h channels belong to the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel superfamily (Robinson and Siegelbaum, 2003; Hofmann et al., 2005). Four subunits of mammalian HCN channels (HCN1–4) have been identified (Ludwig et al., 1998; Santoro et al., 1998) that have distinct properties (Moosmang et al., 2001; Stieber et al., 2003). Functional HCN channels can be assembled as homomeric or heteromeric tetramers, the latter being HCN1 and HCN2 as observed *in vivo* (Much et al., 2003). In the peripheral nervous system, all four HCN subtypes are expressed, HCN1 and HCN2 being most abundantly expressed (Chaplan et al., 2003; Kouranova et al., 2008; Hatch et al., 2013). In dorsal root ganglia (DRG) and TG, HCN1 is found mainly in medium- to large-sized non-nociceptive neurons (Tu et al., 2004; Kouranova et al., 2008; Hatch et al., 2013), apart from a small subpopulation of cold-sensitive neurons (Momin et al., 2008; Orio et al., 2009). HCN2 subunits are expressed in DRG and TG neurons of all sizes (Tu et al., 2004; Matsuyoshi et al., 2006; Kouranova et al., 2008; Hatch et al., 2013), especially in small nociceptive neurons, and play a critical role in inflammatory and neuropathic pain (Emery et al., 2011, 2012). The distribution of HCN3 and HCN4 subunits in sensory neurons is less clear and only a relatively small proportion of TG sensory neurons express these proteins. Consistent with this, I_h is differentially expressed in a subpopulation of primary sensory neurons (Scroggs et al., 1994) in which it exerts a prominent role in shaping the electrical behavior (Momin et al., 2008; Orio et al., 2009; Cho et al., 2011). HCN channels play a role in generating hyperexcitability of peripheral nerve fibers and DRG neurons in various pain models (Chaplan et al., 2003; Tu et al., 2004; Emery et al., 2011; Weng et al., 2012). In particular, in chronic and inflammatory pain, I_h current density and the rate of activation are increased in TG and DRG cells (Chaplan et al., 2003; Yao et al., 2003; Tsuboi et al., 2004; Kitagawa et al., 2006). The selective HCN blocker ZD7288 is known to depress pain behavior and ectopic neuronal firing both *in vivo* and *in vitro* (Chaplan et al., 2003; Lee et al., 2005; Emery et al., 2011).

The aim of the present study was to compare the expression of I_h in wild-type (WT) and R192Q KI mice TG neurons and assess its impact on neuronal excitability elicited by either activation of P2X3 receptors with the selective agonist α,β-methylene-ATP (α,β-meATP) or TRPV1 receptors by capsaicin.

EXPERIMENTAL PROCEDURES

Animals and primary TG cultures

All experimental procedures were carried out in accordance with guidelines of the Italian Animal Welfare Act and approved by the Scuola Internazionale Superiore di Studi Avanzati (SISSA) ethics committee (prot. 3599, 28 May 2012). All efforts were made to minimize the number of animals used for the experiments and their suffering. Homozygous $Ca_V2.1$ R192Q KI and WT mouse littermates (van den Maagdenberg et al., 2004) of either sex were used for the study. Genotyping was performed by PCR as previously reported (van den Maagdenberg et al., 2004). Trigeminal ganglia (TG) primary cultures were obtained from mice at postnatal day 14 (P14) rapidly decapitated under i.p. urethane-anesthesia (10% solution, 0.1 mL injection) as previously described (Simonetti et al., 2006), and incubated (37 °C, 5% CO_2) for 24 h before use. Ganglion tissue samples and cultures were collected and processed in parallel for R192Q KI and WT mice.

Western blot

Mouse TG ganglia or cultures were homogenized in ice-cold lysis buffer containing 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% Nonidet P (NP)-40, 0.1% sodium deoxycholate, 0.1% SDS, 2 mM EDTA plus protease inhibitors mixture (Complete, Roche Applied Science, Basel, Switzerland). The procedure was essentially the same as described earlier (Fabbretti et al., 2004). The following polyclonal antibodies were used: anti-HCN1 (1:1000, rabbit #APC-056), anti-HCN2 (1:1000, rabbit #APC-030), anti-HCN3 (1:500, rabbit #APC-057), anti-HCN4 (1:1000, rabbit #APC-052), all purchased from Alomone Laboratory (Jerusalem, Israel) and isoform-specific anti-β-actin (1:5000; A5441, Sigma Milan, Italy), and anti-β-tubulin III (1:3000; T5076, Sigma). Secondary antibodies were conjugated with horseradish peroxidase and their reaction was visualized with the ECL detection system (Amersham Biosciences, Piscataway, NJ, USA) and recorded with the Alliance 4.7 (UVITEC, Cambridge, UK) digital imaging. Data were normalized with respect to levels of β-tubulin III or β-actin. The amount of loaded proteins was in the 20–50 μg/mL range. The specificity of the antibodies used in this study was previously validated (Han et al., 2002; Chaplan et al., 2003; Much et al., 2003; Cho et al., 2011; Acosta et al., 2012). Specificity of the HCN antibodies was further confirmed by Western blots of mouse tissue (for details see Fig. 7). Olfactory bulb was used as a positive control tissue for HCN1-3 (Notomi and Shigemoto, 2004). Heart was used as positive control for HCN4 (Moosmang et al., 2001). Liver was used as negative control tissue, where no HCN isoforms were detected as previously reported (Arroyo et al., 2006). To remove N-glycosylation moiety of the protein, lysates were incubated with Peptide-N-Glycosidase

Please cite this article in press as: Eroli F et al. Hyperpolarization-activated current I_h in mouse trigeminal sensory neurons in a transgenic mouse model of familial hemiplegic migraine type-1. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.03.033