Molecular and Cellular Neuroscience xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Regionally specific expression of high-voltage-activated calcium channels in thalamic nuclei of epileptic and non-epileptic rats

Tatyana Kanyshkova [a,1], Petra Ehling [a,b,1], Manuela Cerina [a,b,1], Patrick Meuth [a,b], Mehrnoush Zobeiri [a], Sven G. Meuth [b], Hans-Christian Pape [a], Thomas Budde [a,*]

[a] Institut für Physiologie I, Robert-Koch-Str. 27a, D-48149 Münster, Germany
[b] Department für Neurologie, Klinik für Allgemeine Neurologie, Institut für Physiologie, Abteilung für Neuropathophysiologie, Albert-Schweitzer-Campus 1, Westfälische Wilhelms Universität, D-48149 Münster, Germany

ARTICLE INFO

Article history:
Received 23 October 2013
Revised 30 May 2014
Accepted 5 June 2014
Available online xxxx

Keywords:
HVA Ca^{2+} channels
L-type Ca^{2+} channels
Thalamus
Tonic firing
Genetic rat model of absence epilepsy

ABSTRACT

The polygenic origin of generalized absence epilepsy results in dysfunction of ion channels that allows the switch from physiological asynchronous to pathophysiological highly synchronous network activity. Evidence from rat and mouse models of absence epilepsy indicates that altered Ca^{2+} channel activity contributes to cellular and network alterations that lead to seizure activity. Under physiological circumstances, high voltage-activated (HVA) Ca^{2+} channels are important in determining the thalamic firing profile. Here, we investigated a possible contribution of HVA channels to the epileptic phenotype using a rodent genetic model of absence epilepsy. In this study, HVA Ca^{2+} currents were recorded from neurons of three different thalamic nuclei that are involved in both sensory signal transmission and rhythmic-synchronized activity during epileptic spike-and-wave discharges (SWD), namely the dorsal part of the lateral geniculate nucleus (dLGN), the ventrobasal thalamic complex (VB) and the reticular thalamic nucleus (NRT) of epileptic Wistar Albino Glaxo rats from Rijswijk (WAG/Rij) and non-epileptic August Copenhagen Irish (ACI) rats. HVA Ca^{2+} current densities in dLGN neurons were significantly increased in epileptic rats compared with non-epileptic controls while other thalamic regions revealed no differences between the strains. Application of specific channel blockers revealed that the increased current was carried by L-type Ca^{2+} channels. Electrophysiological evidence of increased L-type current correlated with up-regulated mRNA and protein expression of a particular L-type channel, namely $Ca_v1.3$, in dLGN of epileptic rats. No significant changes were found for other HVA Ca^{2+} channels. Moreover, pharmacological inactivation of L-type Ca^{2+} channels results in altered firing profiles of thalamocortical relay (TC) neurons from non-epileptic rather than from epileptic rats. While HVA Ca^{2+} channels influence tonic and burst firing in ACI and WAG/Rij differently, it is discussed that increased $Ca_v1.3$ expression may indirectly contribute to increased robustness of burst firing and thereby the epileptic phenotype of absence epilepsy.

Introduction

Ca^{2+} ions and Ca^{2+}-regulated processes play critical roles in the genesis of several forms of epilepsy (Heinemann et al., 1977). Under physiological conditions, voltage-gated Ca^{2+} (Ca_v) channels are of great importance in a wide variety of cellular functions (Lacinova, 2005; Lipscombe et al., 2004). Based on their electrophysiological properties these channels can be subdivided into high voltage-activated (HVA) Ca^{2+} channels, including L- ($Ca_v1.1$–1.4), N- ($Ca_v2.2$), P/Q- ($Ca_v2.1$), and R-type ($Ca_v2.3$), as well as low voltage-activated (LVA) or T-type ($Ca_v3.1$–3.3) Ca^{2+} channels (Ertel et al., 2000). During the last decade it was found that malfunction of Ca_v channels may contribute to different channelopathies, including absence epilepsy (Budde and Pape, 2009). Therefore, the identification of candidate channels was and still is a main focus of epilepsy research.

Slow wave sleep rhythms have been shown to depend on the activity of T-type Ca^{2+} channels, the key players of burst firing (Huguenard,

Abbreviations: AC, adenylyl cyclase; ACI rat, August Copenhagen Irish rat; ADRB2, β_2-adrenergic receptor; AP, action potential; BK_{Ca}, large conductance Ca^{2+}-activated K^+ channel; BSA, bovine serum albumin; cDNA, complementary DNA; Cy2/Cy3, cyanine dyes; dLGN, dorsal part of the lateral geniculate nucleus; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; fAHP, fast afterhyperpolarization; GABA, gamma-aminobutyric acid; GFAP, glial fibrillary acidic protein; GSTπ, glutathione S-transferase π; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HVA, high voltage-activated; Iba1, ionized calcium-binding adapter molecule 1; ISI, inter-spike-interval; I–V, current–voltage; LTS, low-threshold Ca^{2+} spike; LVA, low voltage-activated; MAP2, microtubule-associated protein 2; NHS, normal horse serum; NRT, reticular thalamic nucleus; P, postnatal day; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PDE, phosphodiesterase; PFA, paraformaldehyde; PKA, protein kinase A; TBS, Tris-buffered saline; TC neuron, thalamocortical relay neuron; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SWD, spike-and-wave discharge; VB, ventrobasal thalamic complex; WAG/Rij, Wistar Albino Glaxo rats from Rijswijk.

* Corresponding author at: Institut für Physiologie I, Westfälische Wilhelms-Universität Münster, Robert-Koch-Str. 27a, D-48149 Münster, Germany.
E-mail address: tbudde@uni-muenster.de (T. Budde).

[1] Equal contribution.

http://dx.doi.org/10.1016/j.mcn.2014.06.005
1044-7431/

1996; Perez-Reyes, 2003). Moreover, recent evidence indicated that $Ca_v2.3$ channels are critical for physiological oscillatory burst activity in neurons of the NRT in mice (Zaman et al., 2011). During wakefulness TC neurons are depolarized and T-type Ca^{2+} channels are largely inactivated. Under these conditions neurons fire tonic sequences of Na^+/K^+-mediated action potentials (APs) accompanied by robust activation of not only HVA Ca^{2+} channels but also a significant number of non-inactivated T-type Ca^{2+} channels (Budde et al., 2000; Deleuze et al., 2012). The role of increased T-type Ca^{2+} channel function in the generation of epileptic activity in absence epilepsy has been emphasized before (Cheong and Shin, 2013; Crunelli and Leresche, 2002; Khosravani and Zamponi, 2006; Zamponi et al., 2010). However, there is also evidence from humans and rodent disease models that both wild type as well as mutated HVA Ca^{2+} channels play a role in epileptogenesis. T- and L-type Ca^{2+} channel blockers show opposite effects on epileptic SWD. Whereas inhibition of T-type Ca^{2+} channels decreases the number of SWDs, blockade of L-type Ca^{2+} channels increases that number (van Luijtelaar et al., 1995, 2000). Evidence implicating that $Ca_v2.1$ channels are involved in the generation of SWD came from several mutant mouse strains that revealed absence-like seizure activity. Up-regulated T-type Ca^{2+} currents are observed in thalamic neurons of mice with mutations in $Ca_v2.1$ pore-forming or ancillary subunit genes (Fletcher and Frankel, 1999; Lorenzon et al., 1998; Noebels and Sidman, 1979; Zhang et al., 2002; Zwingman et al., 2001). In a rat model of absence epilepsy quantification of channel expression indicated that the development of SWD in WAG/Rij rats is concomitant with an increased expression of $Ca_v2.1$ channels in the NRT (van de Bovenkamp-Janssen et al., 2004). In humans, a point mutation in the $Ca_v2.1$ channel gene (C5733T, CACNA1A) has been associated with absence epilepsy (Jouvenceau et al., 2001). Another study reported on a family in which absence epilepsy combined with cerebellar ataxia could be linked to an amino acid substitution (E147K) in $Ca_v2.1$ channels resulting in impaired channel function (Imbrici et al., 2004).

Electrophysiological properties and expression profiles of T-type Ca^{2+} channels and Ca^{2+}-dependent mechanisms have been analyzed in rat models of absence epilepsy in comparison to an epilepsy-free strain in great detail (Broicher et al., 2007a,b, 2008; Budde et al., 2005; Ehling et al., 2012, 2013; Kanyshkova et al., 2012). However, a systematic analysis of HVA Ca^{2+} channels in different thalamic nuclei is still missing. Therefore we compared data obtained from the epileptic WAG/Rij strain to results derived from its corresponding control strain ACI (Depaulis and Van Luijtelaar, 2006; Inoue et al., 1990). Two thalamic nuclei which are known to be highly relevant in the pathophysiology of epilepsy (NRT; VB) and dLGN, a nucleus with a well-known critical role for L-type Ca^{2+} channels in the development of the visual pathway (Dilger et al., 2010; Lo et al., 2002), were investigated. We studied the gene expression pattern and basic electrophysiological properties of HVA Ca^{2+} channels in those nuclei and probed their contribution to thalamic firing modes in order to understand their possible contribution to epileptogenesis.

Results

Electrophysiological characterization of HVA Ca^{2+} currents

HVA Ca^{2+} currents were recorded from dLGN, VB and NRT neurons (Fig. 1A–C). After establishing the whole-cell configuration, currents were allowed to reach stable amplitudes for 10 min (Budde and White, 1998; Budde et al., 1998), and recordings were started thereafter. HVA Ca^{2+} currents were evoked by a series of voltage steps (−50 to +20 mV, 5 mV increments, 200 ms) from a holding potential of −40 mV. Under these conditions the I–V relationship of HVA Ca^{2+} currents revealed activation thresholds at membrane potentials between −40 and −30 mV and maximal current amplitudes at about −10 mV (Fig. 1D–F).

VB neurons of both WAG/Rij and ACI rats showed the largest HVA Ca^{2+} currents, followed by neurons in the dLGN and NRT (Fig. 1A–C). The I–V relationship obtained by plotting the peak current densities vs. the membrane potential revealed maximal values at −5 mV for dLGN neurons of ACI and WAG/Rij rats (Fig. 1D). For VB and NRT neurons of both strains, the maximum was at about −10 mV (Fig. 1E, F). Statistical analysis revealed significantly increased peak current densities in dLGN neurons of WAG/Rij rats at potentials between −15 and −5 mV (at −5 mV: −4.2 ± 0.2 pA/pF at −5 mV, n = 29) compared to densities recorded from control ACI rats (at −5 mV: −3.5 ± 0.2 pA/pF at −5 mV, n = 25; $p < 0.01$). The amplitudes of peak current densities in VB (at −10 mV: −7.9 ± 1.4 pA/pF for WAG/Rij, n = 7; −8.0 ± 0.8 pA/pF for ACI, n = 4) and NRT (at −10 mV: −2.2 ± 0.3 pA/pF for WAG/Rij, n = 7; −2.5 ± 0.2 pA/pF for ACI, n = 7) neurons were not significantly different at any membrane potential.

To characterize the difference in HVA Ca^{2+} currents between rat strains in dLGN in more detail, raw I–V relationships were fitted (see Fig. 1D–F) with a Boltzmann equation corrected for driving force (Bourinet et al., 1996; Budde and White, 1998). Fitting results revealed significantly different ($p < 0.05$) values for V_h (−16.2 ± 0.5 mV for WAG/Rij, n = 20; −13.6 ± 0.7 for ACI, n = 18) and G_{max} (−16.1 ± 0.8 nS for WAG/Rij, n = 20; −13.0 ± 0.9 nS for ACI, n = 18), while k (−4.7 ± 0.2 mV for WAG/Rij, n = 20; −5.3 ± 0.2 for ACI, n = 18) and E_{rev} (28.5 ± 1.7 mV for WAG/Rij, n = 20; 26.6 ± 1.5 mV for ACI, n = 18) were not distinguishable between rat strains. Additionally the conductance G was calculated from peak current amplitudes ($G = I / (V - E_{rev})$) and plotted as a function of voltage, the resulting activation curve reached plateau values (indicating activation of all available channels) at around 0 mV (Fig. S1A). Therefore experiments using current blockers and modulators focused on Ca^{2+} currents evoked by voltage steps from −40 to 0 mV, since all subtypes of HVA Ca^{2+} currents are completely activated at this membrane potential (Lipscombe et al., 2004).

Since the kinetics of inactivation of HVA Ca^{2+} currents appeared to be more pronounced in dLGN TC neurons from WAG/Rij rats (Fig. 1A), a mono-exponential function was fitted to the wave form of the total HVA Ca^{2+} current at −5 mV (Fig. S1B). In order to increase fit stability, maximal currents at −5 mV were analyzed. Current decay occurred significantly ($p < 0.05$) faster in TC neurons from epileptic animals (115.9 ± 9.2 ms for WAG/Rij, n = 20; 148.9 ± 7.9 ms for ACI, n = 18).

These findings demonstrate nucleus-specific I–V relationships in neurons from different thalamic nuclei. In dLGN TC neurons from WAG/Rij rats, an increase in HVA Ca^{2+} peak current density was accompanied by a more hyperpolarized activation curve and faster current decay.

Expression profile of the HVA Ca^{2+} channels

To identify the molecular basis of thalamic HVA Ca^{2+} currents we next examined the expression of HVA Ca^{2+} channel genes in dLGN, VB and NRT of WAG/Rij and ACI rats (P20–25). First, we analyzed the mRNA expression profile of HVA Ca^{2+} channel genes using conventional PCR (Fig. 2A–C). L-type Ca^{2+} channels can be formed by $Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$ or $Ca_v1.4$. Transcripts for $Ca_v1.2$ and $Ca_v1.3$ were detected in dLGN, VB and NRT, while both $Ca_v1.1$ and $Ca_v1.4$ isoforms were either not expressed or below detection limit. $Ca_v2.1$ and $Ca_v2.2$ Ca^{2+} channels revealed strong expression in all three thalamic regions of WAG/Rij and ACI rats, while $Ca_v2.3$ channel mRNA was solely detected in NRT. Further analysis using a quantitative real-time RT-PCR approach (Fig. 2D–F, n = 3 per group) revealed statistically significant differences between the rat strains only for $Ca_v1.3$ expression in dLGN (3.01 times increased expression in WAG/Rij; $\Delta\Delta C_t = -1.59 \pm 0.52$, $p < 0.05$; Fig. 2D). Given that some cortical areas of WAG/Rij rats show altered glial cell density (Sitnikova et al., 2011) and expression of the housekeeping gene β_2-microglobulin occurs in all nucleated cells, we compared threshold cycles (C_t) of β_2-microglobulin real time PCR signals between

Please cite this article as: Kanyshkova, T., et al., Regionally specific expression of high-voltage-activated calcium channels in thalamic nuclei of epileptic and non-epileptic rats, Mol. Cell. Neurosci. (2014), http://dx.doi.org/10.1016/j.mcn.2014.06.005