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Differential regulation of HCN channel isoform expression in thalamic neurons of epileptic and non-epileptic rat strains

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ABSTRACT

Hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels represent the molecular substrate of the hyperpolarization-activated inward current (I_h). Although these channels act as pacemakers for the generation of rhythmic activity in the thalamocortical network during sleep and epilepsy, their developmental profile in the thalamus is not yet fully understood. Here we combined electrophysiological, immunohistochemical, and mathematical modeling techniques to examine HCN gene expression and I_h properties in thalamocortical relay (TC) neurons of the dorsal part of the lateral geniculate nucleus (dLGN) in an epileptic (WAG/Rij) compared to a non-epileptic (ACI) rat strain. Recordings of TC neurons between postnatal day (P) 7 and P90 in both rat strains revealed that I_h was characterized by higher current density, more hyperpolarized voltage dependence, faster activation kinetics, and reduced cAMP-sensitivity in epileptic animals. All four HCN channel isoforms (HCN1-4) were detected in dLGN, and quantitative analyses revealed a developmental increase of protein expression of HCN1, HCN2, and HCN4 but a decrease of HCN3. HCN1 was expressed at higher levels in WAG/Rij rats, a finding that was correlated with increased expression of the interacting proteins filamin A (FilA) and tetratricopeptide repeat-containing Rab8b-interacting protein (TRIP8b). Analysis of a simplified computer model of the thalamic network revealed that the alterations of I_h found in WAG/Rij rats compensate each other in a way that leaves I_h availability constant, an effect that ensures unaltered cellular burst activity and thalamic oscillations. These data indicate that during postnatal developmental the hyperpolarizing shift in voltage dependency (resulting in less current availability) is compensated by an increase in current density in WAG/Rij thereby possibly limiting the impact of I_h on epileptogenesis. Because HCN3 is expressed higher in young versus older animals, HCN3 likely does not contribute to alterations in I_h in older animals.

Introduction

A number of brain rhythms are controlled by HCN channels, the molecular substrate of the pacemaker current, I_h (Biel et al., 2009; Kaupp and Seifert, 2001; Robinson and Siegelbaum, 2003; Santoro and Baram, 2003). The HCN gene family is comprised of four pore-forming subunits (HCN1-4) that assemble as homo- or heteromers thereby forming functional channels (Brewster et al., 2005; Chen et al., 2005; Much et al., 2003). Cyclic AMP (cAMP) rapidly regulates channel opening of HCN2, HCN4, and, to a lesser extent, HCN1 (Biel et al., 2009; Robinson and Siegelbaum, 2003; Wainger et al., 2001). The HCN3 isoform appears to be inhibited by cAMP (Mistrik et al., 2005; Stieber et al., 2005). The expression pattern of HCN isoforms in the mammalian brain displays activity-, age-, region-, and species-dependent differences (Bender and Baram, 2008; Bender et al., 2001; Fan et al., 2005; Kanyshkova et al., 2009; Monteggia et al., 2000; Moosmang et al., 1999; Narayanan et al., 2010; Noam et al., 2010; Notomi and Shigemoto, 2004; Shin and Chetkovich, 2007; van

Abbreviations: ACI, August Copenhagen Irish rat; ACSF, artificial cerebrospinal fluid; ATP, adenosine triphosphate; BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; BSA, bovine serum albumin; C-terminal, carboxy-terminal; cAMP, cyclic adenosine monophosphate; cDNA, complementary deoxyribonucleic acid; Cy2/Cy3, cyanine dyes 2/3; DIC-IR, infrared differential interference contrast; dLGN, dorsal part of the lateral geniculate nucleus; ECL, enhanced chemiluminescence; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FilA, filamin A; GABA, γ-amminobutyric acid; GAERS, Genetic Absence Epilepsy Rats from Strasbourg; g_h, I_h conductance; GTP, guanosine triphosphate; GST, glutathione-S-transferase; HCN, hyperpolarization-activated cyclic nucleotide-gated cation channel; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IgG, immunoglobulin G; I_h, hyperpolarization-activated inward current; I_T, T-type Ca^{2+} current; LE, Long Evans rat; LTS, low threshold Ca^{2+} spike; MirP1, Mink-related peptide 1; NEC, non-epileptic control rat; NHS, normal horse serum; P, postnatal day; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PFA, paraformaldehyde; RE, reticular thalamic; SD, Sprague Dawley rat; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM, standard error of mean; SWD, spike-and-wave discharge; TBS, Tris-buffered saline; TC neuron, thalamocortical relay neuron; TRIP8b, tetratricopeptide repeat-containing Rab8b-interacting protein; VB, ventrobasal thalamic complex; V_h, half-maximal activation voltage; WAG/Rij, Wistar Albino Glaxo rats from Rijswijk.

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Welie et al., 2004). Epileptic activity results in altered electrophysiological properties of I_h and influences the ratio of HCN1 and HCN2 expression in the hippocampus and entorhinal cortex (Bender et al., 2003; Brewster et al., 2002; Chen et al., 2001; Shah et al., 2004). Furthermore, β-subunits seem to influence the surface expression and electrophysiological properties of HCN channels (Lai and Jan, 2006; Pongs and Schwarz, 2010). Along these lines, the K^+ channel ancillary subunit Mink-related peptide 1 (MirP1), also termed KCNE2, enhances expression and speeds activation of HCN1, HCN2, and HCN4 (Decher et al., 2003; Yu et al., 2001). The cytoplasmic scaffolding protein filamin A (FilA) interacts with HCN1 and influences its membrane expression and localization (Gravante et al., 2004). Recent evidence indicates a central role for TRIP8b in the complex regulation of HCN channels in the brain (for review see: Braun, 2009). All TRIP8b isoforms produce a hyperpolarizing shift in voltage-dependency and antagonize the cAMP-induced enhancement of HCN channels, while splice variants produce either an increase or a decrease in the cell surface expression of HCN channels (Lewis et al., 2009; Santoro et al., 2009; Zolles et al., 2009).

In the thalamocortical system slow rhythmic synchronized activity during slow-wave sleep and absence epilepsy depends on HCN channel activation, and regulation of the voltage-dependence of I_h through the cAMP system is one important mechanism for the control of this activity mode (Lüthi and McCormick, 1998; Pape, 1996; Pape et al., 2005). TC neurons of the dLGN, a thalamic area known to be critically involved in physiological sleep (McCormick and Pape, 1990) and pathological epileptic rhythms (Guyon et al., 1993), have been frequently investigated as prototypical sensory relay cells. Alterations in the postnatal HCN isoform expression profiles in dLGN were accompanied by changes in I_h properties in TC neurons and the maturation of sleep-related slow oscillations (Kanyshkova et al., 2009). Evidence suggests a role for thalamic and cortical HCN channel dysfunction (for review see: Poolos, 2006) in the generation of epileptic discharges in the thalamocortical system. Epileptic activity appears as spike-and-wave discharges (SWD) which are initiated in cortical areas (Crunelli and Leresche, 2002; Pinault and O'Brien, 2007; van Luijtelaar et al., 2011). Studies in rat models of absence epilepsy, the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo rats from Rijswijk (WAG/Rij), reveal abnormal regulation of I_h. In both epileptic strains, TC neurons in different sensory thalamic nuclei exhibit reduced responsiveness to cAMP, associated with a selective increase in the expression of the relatively cAMP-insensitive HCN1 isoform (Budde et al., 2005; Kuisle et al., 2006). Based on the parallel early postnatal maturation of I_h and sleep oscillations (Kanyshkova et al., 2009), the gradual manifestation of SWD during ontogeny (Depaulis and Van Luijtelaar, 2006), and the observation that SWD are preceded by physiological sleep oscillations (van Luijtelaar et al., 2011), we hypothesize that alterations in the normal developmental expression profiles of HCN channels contribute to the occurrence of abnormal oscillatory behavior in the thalamocortical system. Therefore we conducted a quantitative study of the expression of all HCN channel isoforms, and selective HCN channel interacting proteins in the thalamus of epileptic WAG/Rij in comparison to non-epileptic ACI rats. We utilized a combination of electrophysiological, immunofluorescence and biochemical studies to correlate I_h current density, voltage-dependence and cAMP-sensitivity with HCN channel subunit expression. Mathematical modeling demonstrated the importance of I_h for rhythmic activity in the thalamic network in epileptic and non-epileptic animals.

Materials and methods

Preparation

All animal work has been approved by local authorities (review board institution: Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen; approval ID: 8.87-51.05.2010.117, 8.87-51.04.2010.A322). Rats (P7–P90) were sacrificed and in rapid sequence, after surgically removing a skull cap caudal to the bregma, a block of brain tissue containing the thalamus was removed from the cranial vault and submerged in ice-cold aerated (O_2) saline containing (in mM): sucrose, 200; PIPES, 20; KCl, 2.5; NaH_2PO_4, 1.25; $MgSO_4$, 10; $CaCl_2$, 0.5; dextrose, 10; pH 7.35, with NaOH. Thalamic slices were prepared as coronal sections on a vibratome. Before recording, slices were kept submerged in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 125; KCl, 2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 24; $MgSO_4$, 2; $CaCl_2$, 2; dextrose, 10; pH adjusted to 7.35 by bubbling with carbogen (95% O_2 and 5% CO_2).

Whole-cell patch clamp

Recordings were performed on TC neurons of the dLGN at controlled room temperature (22 ± 1 °C) in a solution containing (in mM): NaCl, 120; KCl, 2.5; NaH_2PO_4, 1.25; HEPES, 30; $MgSO_4$, 2; $CaCl_2$, 2; dextrose, 10; pH 7.25 was adjusted with HCl. In some experiments the temperature of the recording chamber was set to controlled values of 30 °C and 35 °C (In-Line Solution Heater, Harvard Apparatus, March, Germany). Individual cells were visually identified by infrared differential interference contrast video-microscopy (DIC-IR). Inspection of dLGN slices with DIC-IR videomicroscopy readily revealed two morphologically distinct types of neurons: neurons possessing a multipolar cell body (15–25 μm in diameter) giving rise to three to six dendrites, and smaller neurons (about 10 μm in diameter) of a fusiform or oval shape with dendrites emerging from opposite poles of the soma which represent TC neurons and interneurons, respectively (Munsch et al., 1997). TC neurons from different rat strains labeled with 50 μM Alexa Fluor® 594 (Figs. S1A–C) revealed no differences in the number of primary dendrites (Fig. S1D) and the diameter of the soma (Fig. S1E). In addition TC neurons are characterized by robust burst firing, a feature that is less pronounced or lacking in interneurons (Broicher et al., 2007). Membrane currents were measured with glass microelectrodes pulled from borosilicate glass capillaries (GC150T-10; Clark Electromedical Instruments, Pangbourne, UK) and filled with (in mM): K-gluconate, 95; K_3-citrate, 20; NaCl, 10; HEPES, 10; $MgCl_2$, 1; $CaCl_2$, 0.5; BAPTA, 3; Mg-ATP, 3; Na_2-GTP, 0.5. The internal solution was set to a pH of 7.25 with KOH and an osmolality of 295 mOsm/kg. The electrodes were connected to an EPC-10 amplifier (HEKA Elektronik, Lamprecht, Germany) with a chlorided silver wire. Electrode resistances were in the range of 2–3 MΩ, with access resistances in the range of 5–20 MΩ. Series resistance compensation of >30% was routinely applied. Voltage-clamp experiments were controlled by the software Pulse or PatchMaster (HEKA Elektronik) operating on an IBM-compatible personal computer. Measurements were corrected for a liquid junction potential of 10 mV.

The protocol used to assess I_h was designed in order to increase the stability of whole-cell recordings and account for increasingly fast activation kinetics of I_h (see Fig. 1A left inset). Therefore the pulse length was shortened by 1500 ms with increasing hyperpolarization (3.5 s pulse length at −130 mV) (Kanyshkova et al., 2009). Including an additional step to −140 mV (2 s pulse length) did not produce further activation. Steady-state activation of I_h, p(V), was estimated by normalizing the mean tail current amplitudes (I) 50–100 ms (see Fig. 1A, right inset) after stepping to a constant potential from a variable amplitude step using the following equation:

$$p(V) = (I - I_{min})/(I_{max} - I_{min}),$$

with I_{max} being the tail current amplitude for the voltage step from −130 mV to −100 mV and I_{min} for the voltage step from −40 mV to −100 mV, respectively. I_h activation was well accounted for by a Boltzmann equation of the following form:

$$p(V) = 1/(1 + \exp((V - V_h)/k)),$$