Neuroscience 284 (2015) 632–642

DEVELOPMENTAL INCREASE IN HYPERPOLARIZATION-ACTIVATED CURRENT REGULATES INTRINSIC FIRING PROPERTIES IN RAT VESTIBULAR GANGLION CELLS

R. YOSHIMOTO,[a] S. IWASAKI,[b]* H. TAKAGO,[c] T. NAKAJIMA,[d] Y. SAHARA[e] AND K. KITAMURA[a]

[a] *Department of Otolaryngology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan*

[b] *Department of Otolaryngology, Faculty of Medicine, University of Tokyo, Tokyo, Japan*

[c] *Department of Rehabilitation for Sensory Functions, Research Institute, National Rehabilitation Center for Persons with Disabilities, Saitama, Japan*

[d] *Department of Circular Physiology, Faculty of Medicine, University of Tokyo, Tokyo, Japan*

[e] *Department of Physiology, Iwate Medical University, School of Dentistry, Iwate, Japan*

Abstract—The primary vestibular neurons convey afferent information from hair cells in the inner ear to the vestibular nuclei and the cerebellum. The intrinsic firing properties of vestibular ganglion cells (VGCs) are heterogeneous to sustained membrane depolarization, and undergo marked developmental changes from phasic to tonic types during the early postnatal period. Previous studies have shown that low-voltage-activated potassium channels, Kv1 and Kv7, play a critical role in determining the firing pattern of VGCs. In the present study, we explored the developmental changes in the properties of hyperpolarization-activated current (I_h) in rat VGCs and the role played by I_h in determining the firing properties of VGCs. Tonic firing VGCs showed a larger current density of I_h as compared to phasic firing VGCs, and tonic firing VGCs became phasic firing in the presence of ZD7288, an I_h channel blocker, indicating that I_h contributes to control the firing pattern of VGCs. The amplitude of I_h increased and the activation kinetics of I_h became faster during the developmental period. Analysis of developmental changes in the expression of hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels revealed that expression of HCN1 protein and its mRNA increased during the developmental period, whereas expression of HCN2–4 protein and its mRNA did not change. Our results suggest that HCN1 channels as well as Kv1 channels are critical in determining the firing pattern of rat VGCs and that developmental up-regulation of HCN1 transforms VGCs from phasic to tonic firing phenotypes.

Key words: hyperpolarization-activated current, vestibular ganglion, development.

INTRODUCTION

The vestibular end organs in the inner ear provide a sense of balance and orientation. Specifically, three semicircular canals on each side of the body measure how the head rotates in three-dimensional space (i.e., yaw, pitch and roll), and two otolith organs measure how the body translate in space and how it is positioned relative to gravity (Baloh and Honrubia, 2001). The vestibular system works to transmit information about head tilt and head motion to reflex pathways that control gaze, head position, and body posture.

Primary vestibular neurons transmit information from the vestibular end organs in the inner ear to vestibular nuclei and cerebellum. In mammals, primary afferents of the vestibular nerve are distinctive in their firing rate, response dynamics and the range of spike timing (Goldberg, 1991; Eatock and Songer, 2011). Regular neurons, which show small variability in interspike intervals of spontaneous firing, have lower sensitivity and tonic response dynamics to angular and linear accelerations acting on the head. On the other hand, irregular neurons, which show larger variability in interspike intervals, have greater sensitivity and phasic response dynamics to accelerations. Most neurons are irregularly discharging at birth, whereas the number of regularly discharging neurons increases during early postnatal periods (Curthoys, 1979).

We have previously examined the intrinsic firing properties of rat vestibular ganglion cells (VGCs) and have shown that VGCs have heterogeneous firing properties during sustained membrane depolarization (Iwasaki et al., 2008). We classified the firing properties of VGCs into the following three types: (1) phasic types, which exhibit a strong adaptation generating just a single or a few spikes, (2) intermediate types, which exhibit

*Corresponding author. Present address: Department of Otolaryngology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel: +81-3-5800-8655; fax: +81-3-3814-9486.

E-mail address: iwashin-tky@umin.ac.jp (S. Iwasaki).

Abbreviations: AHP, afterhyperpolarization; AP, action potential; cDNA, complementary DNA; EGTA, ethylene glycol tetraacetic acid; HCN, hyperpolarization-activated cyclic nucleotide-gated cation; HEPES, 10*N*-[2-hydroxyethyl]piperazine-*N*-[2-ethanesulfonic acid]; I_h, hyperpolarization-activated current; I_{max}, maximum tail current; I_{min}, minimum tail current; P, postnatal days; PBS-T, phosphate-buffered saline with 0.1% Tween 20; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-polymerase chain reaction; $V_{1/2}$, half-activation potential; V_{test}, test potential; VGCs, vestibular ganglion cells.

http://dx.doi.org/10.1016/j.neuroscience.2014.10.034
0306-4522/

moderate adaptation, and (3) tonic types, which exhibit tonic firing during membrane depolarization. At postnatal day (P) 5–7, the majority of VGCs (66%, 173 of 261 cells) belonged to phasic type. The intermediate type and tonic type composed 18% (47 of 261 cells) and 16% (41 of 261 cells) (Iwasaki et al., 2008). Previous studies have shown that the low-voltage-activated potassium channels, Kv1 and Kv7, play an important role in determining the patterns of firing properties in VGCs since phasic discharging neurons turned into tonic firing neurons in the presence of the Kv1 and/or Kv7 channel antagonists (Iwasaki et al., 2008; Kalluri et al., 2010).

The firing properties of rat VGCs undergo developmental changes during early postnatal periods (Curthoys, 1979; Iwasaki et al., 2008). Tonic discharging VGCs were rare at birth, but increased to make up 60% of neurons by around postnatal day (P) 14. We demonstrated that expression of Kv1.6 protein is down-regulated together with expression of Kv1.6 mRNA after P7 in rat VGCs whereas expression of Kv1.1 and Kv1.2 proteins and their mRNA did not change during the same period (Iwasaki et al., 2012). These results suggest that the down regulation of Kv1.6 has an association with developmental changes in the firing properties of VGCs.

The hyperpolarization-activated cation current (I_h) is a slowly activated current during hyperpolarization and is carried by Na^+ and K^+ (Pape, 1996; Robinson and Siegelbaum, 2003; He et al., 2014). Four isoforms of I_h channels have been identified with different rates of activation and different sensitivities to cyclic nucleotides, and are termed as hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels (Ludwig et al., 1998; Santoro et al., 1998).

The HCN channels are present in many excitable cells (Pape, 1996; Santoro et al., 2000) and all subtypes are present in VGCs (Almanza et al., 2012; Horwitz et al., 2014). The HCN channels have been implicated in maintaining the resting potential and input resistance (Surges et al., 2004), rhythmogenesis (McCormick and Pape, 1990), and control of synaptic transmitter release (Beaumont and Zucker, 2000). In sensory neurons, HCN channels contribute to neuronal excitability, where activation of I_h at resting and more negative membrane potentials counterbalances hyperpolarization and brings the membrane toward action potential (AP) threshold after an afterhyperpolarization (AHP) (Masuda et al., 2006; Momin et al., 2008).

Postnatal changes in HCN channel protein expression and I_h have been observed in the central nervous system (Surges et al., 2006), cardiac neurons (Hogg et al., 2001) and trigeminal ganglion cells (Cho et al., 2011). In vestibular ganglion neurons, it has recently been reported that the current density of I_h increases and activation kinetics become faster during postnatal periods (Almanza et al., 2012). However, the functional role of the developmental increase of I_h in the firing properties of VGCs remains unclear.

In the present study, we investigated developmental changes in the properties of I_h in rat VGCs and their role in the firing properties of VGCs. The results showed that the current density of I_h increases and its activation kinetics become faster during the postnatal period and that these changes in I_h influence the developmental changes in the firing properties of VGCs. Furthermore, we examined the developmental changes in the expression of each isoform of the HCN channel at protein levels using western blotting as well as at mRNA levels assayed using quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR), and found that an increase in the expression of HCN1 is primarily responsible for the developmental changes in I_h in rat VGCs.

EXPERIMENTAL PROCEDURES

All procedures were approved by the Animal Care Committee of the University of Tokyo and the Animal Care and Use Committee of Tokyo Medical and Dental University.

Cell isolation

Rats were killed by decapitation in accordance with the Japanese Animal guidelines of the National Center of Neurology and Psychiatry. The superior vestibular ganglia, which innervate the utricular macula and the horizontal and anterior cristae, were isolated from neonatal (postnatal days (P) 3 or 5) or juvenile (P10 or 14) Wistar rats. The dissected vestibular ganglia from rats up to P10 were incubated in Hank's solution (Gibco, Gaithersburg, MD, USA) with papain (20 U/ml; Worthington Biochemical, Freehold, NJ, USA) at 37 °C for 15 min. The ganglia from rats older than P10 were incubated in Hank's solution with a combination of papain (20 U/ml) and collagenase IA (0.5 mg/ml; Sigma) at 37 °C for 15 min.

Cells were dissociated by trituration using a sterile Pasteur pipette, and subsequently plated onto poly-L-lysine-pretreated 35-mm culture dishes. The plating medium contained Leibovitz's L-15 solution (Gibco BRL, Grand Island, NY, USA), 10% fetal calf serum, 26 mM $NaHCO_3$ and 30 mM D-glucose. Cells were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The cells were used for recording $\geqslant$6 h after plating to minimize the effect of papain treatment (Armstrong and Roberts, 1998; Kimitsuki et al., 2005). In our experiments, VGCs did not show any APs within 3 h after plating; we were able to record APs and K^+ currents between 6 and 12 h after plating.

Electrophysiological recordings

Whole-cell recording was carried out with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA) at room temperature (23–27 °C). Cells were visualized under phase contrast on an inverted microscope (Olympus BX50, Tokyo, Japan) or under a 40× water immersion lens (Olympus Optical) attached to an upright microscope (Axioskop, Zeiss, Oberkochen, Germany). VGCs were identified by the morphological features described by Desmadryl et al. (1997), i.e., spherical shape with refringent cytoplasm, and a larger diameter (> 15 μm). Pipettes for whole-cell recording contained