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DYNAMIC TRAFFICKING OF HYPERPOLARIZATION ACTIVATED CYCLIC-NUCLEOTIDE GATED (HCN) CHANNELS IN HIPPOCAMPAL NEURONS: LIVE-IMAGING ANALYSIS AND RELEVANCE TO EPILEPSY

Yoav Noam,†, Qinqin Zha*, R. L. Wu*, L. Phan*, M. J. Vennat*, D. M. Chetkovich§, W. J. Wadman† and Tallie Z. Baram*,‡*
**Pediatrics, University of California, Irvine, Irvine, CA; †SILS – Center for NeuroScience, University of Amsterdam, Amsterdam, Netherlands; ‡Anatomy & Neurobiology, University of California, Irvine, Irvine, CA and §Neurology, Physiology, Northwestern University Medical School, Chicago, IL*

Rationale: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels mediate the non-selective cationic current Ih. These channels can influence excitability in pro- or anti-excitatory directions, depending on a number of factors including physiological context (Santoro & Baram 2003; Dhyfjeld-Johnson et al. 2008), and the subcellular distribution of the channels (Lorincz et al. 2002; Aponte et al. 2006; Bender et al. 2007). Long-lasting alterations in the expression and function of hippocampal HCN channels have been implicated in both seizures and epileptogenesis (e.g Chen et al. 2001;Brewster et al. 2005; Jung et al. 2007; Richichi et al. 2008). In contrast to the role of long-lasting changes in HCN channels, much less is known about the mechanisms and consequences of rapid changes in HCN1 channel localization and surface expression. The dynamics of HCN1 localization and membrane-trafficking may contribute to acute changes in neuronal excitability.

Methods: We studied the dynamics of HCN1 neuronal trafficking, using live imaging of hippocampal neurons transfected with two separate HCN1-GFP constructs.

Results: The HCN1-GFP constructs generated functional Ih with properties consistent with HCN1-type channels. The HCN1-GFP channels were distributed in a punctate and diffuse pattern, similar to the pattern of endogenous HCN1 channels in culture. Time-lapse imaging of live neurons revealed the movement of HCN1-containing puncta in dendrites. These puncta possessed varying degrees of motility (as assessed by velocity, directionality and total displacement), and their motility was affected by increased excitatory input. In addition, pilot data suggest that HCN1-GFP puncta co-localize with markers of several endocytic compartments, consistent with membrane recycling.

Conclusions: Live imaging of HCN1-GFP1 channels in hippocampal neurons provides a new tool for studying neuronal trafficking of HCN1 channels. The dynamics of HCN1 channels trafficking in neurons, especially during altered states of excitability, will enhance our understanding of HCN1 channel contribution to normal and deranged neuronal excitability.

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