Neuroscience 228 (2013) 325–333

DIFFERENT FUNCTIONS OF HYPERPOLARIZATION-ACTIVATED CATION CHANNELS FOR HIPPOCAMPAL SHARP WAVES AND RIPPLES *IN VITRO*

S. A. KRANIG,[†] N. DUHME[†] C. WALDECK A. DRAGUHN, S. REICHINNEK AND M. BOTH[*]

Universität Heidelberg, Institut für Physiologie und Pathophysiologie, Heidelberg, Germany

Abstract—Hyperpolarization-activated currents (I_h) affect multiple neuronal functions including membrane potential, intrinsic firing properties, synaptic integration and frequency-dependent resonance behavior. Consistently, I_h plays a key role for oscillations at the cellular and network level, including theta and gamma oscillations in rodent hippocampal circuits. Little is known, however, about the contribution of I_h to a prominent memory-related pattern of network activity called sharp-wave-ripple complexes (SPW-R). Here we report that pharmacological suppression of I_h induces specific changes in SPW-R in mouse hippocampal slices depending on the specific drug used and the region analyzed. Spontaneous generation of the events was reduced by blocking I_h whereas the amplitude was unaffected or increased. Interestingly, the superimposed ripple oscillations at ~200 Hz persisted with unchanged frequency, indicating that I_h is not critical for generating this rhythmic pattern. Likewise, coupling between field oscillations and units was unchanged, showing unaltered recruitment of neurons into oscillating assemblies. Control experiments exclude a contribution of T-type calcium channels to the observed effects. Together, we report a specific contribution of hyperpolarization-activated cation currents to the generation of sharp waves in the hippocampus.

Key words: high-frequency oscillations, HCN channels, I_h, hippocampus, sharp wave, ripple.

INTRODUCTION

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are important regulators of cellular excitability and synaptic signal integration (Biel et al., 2009). The underlying subunits HCN1–HCN4 are widely expressed in the mammalian brain, with distinct expression patterns depending on region (Moosmang et al., 1999; Notomi and Shigemoto, 2004), developmental stage (Bender et al., 2001), neuronal subtype (Bender et al., 2001; Aponte et al., 2006) and subcellular compartment (Magee 1998a,b; Williams and Stuart, 2000; Lörincz et al., 2002). As indicated by their name, these channels have two peculiar functional properties: (i) they open upon hyperpolarization, rather than depolarization, of the membrane potential and (ii) their gating is modulated by direct binding of cyclic nucleotides to the cytosolic domain. This behavior distinguishes HCN-channels from most other voltage-dependent membrane currents and gives them important functions for the regulation of membrane potential at the cellular level. These functions include the stabilization of resting membrane potential (Biel et al., 2009), amplification of distal synaptic inputs (Magee 1998a,b; Williams and Stuart, 2000; Lörincz et al., 2002), and support of memory-related changes in synaptic weights. I_h contributes to working memory (Wang et al., 2007), motor learning (Nolan et al., 2003) and spatial memory formation (Nolan et al., 2004). Finally, voltage-dependence and gating kinetics provide resonance properties to neurons (Hutcheon and Yarom, 2000). As a result, HCN channels are critically involved in several oscillating systems, including the sinoatrial pacemaker of the heart (Mangoni and Nargeot, 2008; DiFrancesco, 2010), rhythmogenic thalamocortical networks (Pape and McCormick, 1989; McCormick and Bal, 1997) and many others (Gauss and Seifert, 2000; Biel et al., 2009).

The hippocampus expresses a variety of state-dependent network oscillations which serve functions in the formation and consolidation of declarative or spatial memories (Buzsaki and Draguhn, 2004; Klausberger and Somogyi, 2008). Several lines of evidence point toward a prominent role of HCN channels in these highly coordinated activity patterns. Mice with forebrain-specific deletion of HCN1 show increased power of theta oscillations (Nolan et al., 2004), and HCN channels contribute specifically to intracellular signal propagation during such oscillations (Hu et al., 2009). By tonically depolarizing dendrites of pyramidal cells, HCN channels do also contribute to faster oscillations in the gamma domain (Fisahn et al., 2002). Finally, changes in expression and activation of HCN channels contribute to the generation of hypersynchronous states during epilepsy (Chen et al.,

*Corresponding author. Address: Universität Heidelberg, Institut für Physiologie und Pathophysiologie, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany. Tel: +49-6221-544139; fax: +49-6221-546364.

E-mail address: mboth@physiologie.uni-heidelberg.de (M. Both).

[†] These authors contributed equally to this work.

Abbreviations: ACSF, artificial cerebro-spinal fluid; fEPSP, field excitatory postsynaptic potential; HCN, hyperpolarization-activated cyclic nucleotide-gated channels; I_h, hyperpolarization-activated currents; PS, population spikes; SPW-R, sharp-wave-ripple complexes.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.10.050

2001, 2002; McCormick and Contreras, 2001; Steriade, 2005).

Little is known, however, about the contribution of HCN channels to another class of fast hippocampal network oscillations, sharp-wave-ripple oscillations (SPW-R). These events occur during quiet wakefulness or slow-wave sleep and are characterized by waves of synaptic activity which propagate along the hippocampal output loop and are superimposed by very fast (~200 Hz) network oscillations (O'Keefe and Dostrovsky, 1971; Buzsaki, 1986; Ylinen et al., 1995). SPW-R have been implicated in memory consolidation (Wilson and McNaughton, 1994) and thereby constitute a link between synaptic plasticity and coordinated network oscillations, two functions well compatible with properties of HCN channels. We therefore tested the contribution of I_h in an *in vitro* model of SPW-R (Maier et al., 2003; Both et al., 2008). Application of three different HCN-blocking drugs reduced the frequency of sharp waves while the amplitude of the events was increased or unchanged, depending on the drug used and the region analyzed. Superimposed fast oscillations, on the other hand, became more prominent. These differential and specific effects suggest a specific role of HCN channels in the generation of coherent increases in activity, but not in the associated high-frequency synchronization of neurons.

EXPERIMENTAL PROCEDURES

Experiments were approved by the state government of Baden–Württemberg and were performed on male C57BL/6 mice aged 4–8 weeks. Mice were deeply anesthetized with CO_2 (or, in some experiments, with ether). Immediately after losing muscle tone, animals were decapitated, the brain was removed, and transferred into cooled (1–4 °C) artificial cerebro-spinal fluid (ACSF) containing (in mM): 124 NaCl, 3.0 KCl, 1.8 $MgSO_4$, 1.6 $CaCl_2$, 10 glucose, 1.25 NaH_2PO_4, 26 $NaHCO_3$, saturated with 95% O_2/5% CO_2, pH 7.4 at 37 °C. Horizontal brain slices were cut using a Leica Vibratome (VT1000 S), after removal of the cerebellum and frontal brain structures. Slices were transferred to a Haas-type interface recording chamber at 34 ± 1 °C and were allowed to recover for at least 2 h before recording.

Extracellular field potentials were recorded with glass electrodes (tip diameter > 5 µm; filled with ACSF) from CA1 and CA3 pyramidal layer, and from the stratum radiatum of CA1. Potentials were amplified 100 times with an EXT 10-2F amplifier (npi Electronic), low-pass-filtered at 2 kHz, and digitized at 5–10 kHz for offline analysis (1401 interface; CED). Electrical stimulation was performed with bipolar platinum/iridium wire electrodes that were located in the Schaffer collaterals (75 µm tip distance, 100 kOhm at 1 kHz, Science Products Trading, Hofheim, Germany). Monopolar square pulses of 100 µs duration were delivered at a strength calibrated to evoke 60% of the maximal action potential firing.

ZD 7288 (Tocris, Bristol, UK), Cilobradine, Ivabradine, Ethosuximide, and Mibefradil (Sigma Aldrich GmbH, Steinheim, Germany) were dissolved in ACSF at concentrations indicated in the results section and were applied via bath perfusion for 15 min (ZD 7288) or 60 min (Cilobradine, Ivabradine, Ethosuximide, Mibefradil), respectively.

Signals were sampled with the Spike-2 program (CED) and analyzed off-line using custom routines written in Matlab (The MathWorks). Sharp waves were detected from low-pass-filtered raw data (50 Hz) as local maxima with amplitudes exceeding baseline noise by four standard deviations (0.15–0.2 mV) within 30-ms time windows (Both et al., 2008). This procedure yields stable and reliable detection of SPW-R, as confirmed by visual inspection of traces and detected events. High-frequency ripple oscillations are particularly prominent in CA1. Therefore, superimposed fast oscillations were analyzed in CA1 with continuous wavelet transform using the complex Morlet wavelet (Both et al., 2008), starting 33 ms before and ending 67 ms after the peak of the detected sharp wave. From this wavelet spectrogram (50–300 Hz divided into 81 bins on a log scale), we computed the leading ripple frequency (the most prominent frequency > 140 Hz) and its energy (the integral of the spectrogram at this frequency).

Coupling strength of pyramidal cell firing to the field ripple in CA1 was computed from phase analysis between spikes and ripple troughs. Each spike was assigned to a vector within the ripple oscillation phase (cycle length ~5 ms; unit circle). Vector-encoded spikes were then averaged for the whole period of analysis. The direction of the resulting vector resembles the dominant phase angle of unit activity, and the length resembles the strength of coupling between spikes and a given ripple phase, with a maximum value of one.

Quantitative results are given as mean ± SEM. Parametric tests were used if groups passed a normality test (Kolmogorov–Smirnov test), and otherwise nonparametric statistics were used. A p-value < 0.05 was regarded as significant.

RESULTS

In order to assess the role of HCN on neuronal network oscillations we recorded field potentials from mouse hippocampal slices in the absence and presence of HCN-blocking drugs. Extracellular recordings from stratum pyramidale revealed spontaneously occurring sharp waves with superimposed fast ripple oscillations (SPW-R; Fig. 1) in all slices (N = 49 from 30 mice). The basic properties of these events were similar to previous observations (Maier et al., 2003; Nimmrich et al., 2005; Weiss et al., 2008; Both et al., 2008). In brief, sharp waves were generated in CA3 at 2.4 ± 0.1 Hz and traveled along the Schaffer collateral toward CA1. Amplitude of the events was 0.28 ± 0.03 mV in CA3 and 0.33 ± 0.02 mV in CA1. Superimposed ripples had a mean frequency of 204 ± 3 Hz. Following registration of baseline activity for at least 10 min we perfused the slices with one of three different HCN channel-blockers, ZD 7288 (10 µM, example trace in Fig. 1), Cilobradine (10 µM) or Ivabradine (30 µM), respectively (Fig. 2). ZD 7288 and Cilobradine enhanced the amplitude of SPW-R in CA3 (from 0.21 ± 0.01 to 0.34 ± 0.06 mV, N = 7, and from 0.52 ± 0.12 to 0.89 ± 0.17 mV, N = 8) and ZD 7288 also in CA1 (from 0.30 ± 0.04 to 0.40 ± 0.06 mV, N = 13; p < 0.05, paired t-test; Fig. 2). Ivabradine induced an intermittent small increase in SPW amplitudes which returned to baseline values (p > 0.05, paired t-test; Fig. 2). At the same time, the frequency of occurrence of SPW-R events was strongly reduced, reaching ~20–70% of baseline values (Fig. 2). This effect was significant for all three drugs in both regions examined (p < 0.05 for all values, paired t-test). Specifically, SPW frequency was reduced from 1.9 ± 0.2 to 1.4 ± 0.2 Hz in CA3 (N = 7) and from 2.2 ± 0.2 to 0.8 ± 0.1 Hz in CA1 for ZD 7288 (N = 13), from 3.0 ± 0.6 to 1.9 ± 0.5 Hz in CA3 and from 2.8 ± 0.3 to 1.1 ± 0.1 Hz in CA1 for Cilobradine