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h channel-dependent deficit of theta oscillation resonance and phase shift in temporal lobe epilepsy

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ABSTRACT

I_h tunes hippocampal CA1 pyramidal cell dendrites to optimally respond to theta inputs (4–12 Hz), and provides a negative time delay to theta inputs. Decreased I_h activity, as seen in experimental temporal lobe epilepsy (TLE), could significantly alter the response of dendrites to theta inputs. Here we report a progressive erosion of theta resonance and phase lead in pyramidal cell dendrites during epileptogenesis in a rat model of TLE. These alterations were due to decreased I_h availability, via a decline in HCN1/HCN2 subunit expression resulting in decreased h currents, and altered kinetics of the residual channels. This acquired HCN channelopathy thus compromises temporal coding and tuning to theta inputs in pyramidal cell dendrites. Decreased theta resonance *in vitro* also correlated with a reduction in theta frequency and power *in vivo*. We suggest that the neuronal/circuitry changes associated with TLE, including altered I_h-dependent inductive mechanisms, can disrupt hippocampal theta function.

Introduction

Hyperpolarizing-activated cyclic nucleotide-gated ion channels (HCN; I_h) provide the membrane with resonance properties, focusing responses within an optimal frequency range (Hutcheon and Yarom, 2000; Narayanan and Johnston, 2007). In addition to creating a bandpass filter, HCN channels also can alter the timing of the membrane response (Narayanan and Johnston, 2008). Below a certain frequency, changes in membrane potential appear to precede the current inputs (phase lead), whilst above this frequency, the membrane voltage response is delayed with respect to the current (phase lag). The biophysical properties and density of I_h currents optimize resonance and phase response within the theta (4–12 Hz) frequency band (Hu et al., 2002; Klink and Alonso, 1993; Narayanan and Johnston, 2008; Pike et al., 2000), coincident to theta rhythm, which is associated with numerous cognitive and memory processes (Buzsaki, 2006). Resonance at the theta bandwidth is prominent in layer V cortical cells, layer II and stellate entorhinal cortex cells, CA1 pyramidal cells and some GABAergic interneurons (Hu et al., 2002; Klink and Alonso, 1993; Nolan et al., 2007; Pike et al., 2000; Ulrich, 2002); possibly favouring the emergence of theta oscillations (Buzsaki, 2006). Phase response properties could influence temporal coding (Narayanan and Johnston, 2008). HCN channels might thus be central to function of theta oscillations (Rotstein et al., 2005).

The strongest theta resonance and phase shift is found in CA1 pyramidal cell distal dendrites (Narayanan and Johnston, 2007, 2008), where it is primarily I_h-dependent, reflecting the high density of HCN channels in this cell compartment (Magee, 1998). Consistent with this dendritic channel organization, the largest theta drive in the hippocampus is recorded in stratum lacunosum moleculare (Buzsaki, 2002). Resonance and phase response can be dynamically modified. Long-term potentiation is associated with an upregulation of I_h (Fan et al., 2005), which may underlie the concurrent widespread increased theta resonance and phase response in CA1 pyramidal cells (Narayanan and Johnston, 2007, 2008).

Downregulation (Jung et al., 2007) and mislocalization (Shin et al., 2008) of HCN1 have been reported in experimental temporal lobe epilepsy (TLE). Modelling studies predict that I_h downregulation would result in decreased resonance and phase response (Narayanan and Johnston, 2007, 2008), and experimental results show that mislocalization of h channels results in decreased resonance frequency in proximal dendrites in experimental TLE (Shin et al., 2008). To further evaluate the consequences of decreased I_h availability in distal dendrites, we measured at the same recording site I_h currents, resonance and phase response in control and

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experimental TLE animals. Since resonance and oscillation power co-vary (Maex and De, 2003), we further analysed hippocampal theta activity *in vivo* in the same experimental model.

Our results demonstrate a progressive erosion of theta resonance and phase response properties in CA1 pyramidal cell distal dendrites during both the latent and chronic epilepsy periods, which correlated with decreased availability of I_h. Theta rhythm recorded *in vivo* was also reduced initially during the latent period. This novel correlation may link hippocampus-dependent cognitive deficits found in TLE with cellular signaling abnormalities identified in individual CA1 pyramidal neurons.

Methods

Experiments were performed according to local INSERM guidelines.

Induction of pilocarpine model

Pilocarpine hydrochloride (340 mg kg^{-1}) was injected subcutaneously in 40 adult male Wistar rats (180–200 g) 30 min after the administration of N-methyl scopolamine (1 mg kg^{-1}). Diazepam (8 mg kg^{-1}) was administered to stop status epilepticus (SE) after forty minutes. Thirty animals experiencing SE were used for later electrophysiological analysis (SE group). Ten age-matched rats received saline injection instead of pilocarpine. Ten animals displayed stage 5 seizures, but did not develop SE (non-SE group). These animals do not become epileptic within 3 months and their depth EEG activity remains similar to control animals (El Hassar et al., 2007). Since *in vivo* and *in vitro* electrophysiological properties are similar between non-SE and saline-treated animals (El Hassar et al., 2007), data from both groups were pooled together (sham group). Ten and fourteen SE animals were used for the *in vitro* analysis of the seizure-free latent and chronic periods respectively, and six SE animals were used for *in vivo* recordings. Drugs were obtained from Sigma.

In vivo recordings

After one week handling, a separate group of ten adult male Wistar rats were anesthetized with a mixture of ketamine (1 mg kg^{-1}) and xylasine (0.5 mg kg^{-1}) and placed in a stereotaxic apparatus. One bipolar steel electrode (250 μm diameter) was stereotaxically implanted into the dorsal hippocampus (3.8 mm antero-posterior, 1.5 mm medio-laterally and 3.0 mm dorso-ventral from the bregma), and three stainless-steel cortical electrodes (2 mm depth) were screwed into the skull and partway into the brain (right and left frontal cortex, and the reference in the cerebellum). After one week recovery, hippocampal recordings were performed during one hour periods using an EEG system (Deltamed, Paris, France). These recordings used a bipolar montage, referencing the hippocampal electrodes to the cerebellar reference screw. During the electrical recording, a video-system (VideoTrack, France) was used to monitor rat behaviour, assessed during activity in a cylindrical open field. Rats then received pilocarpine injection as described above, and the same protocol was performed at 4 days, 10 days and 25 days after injection in six SE and four non-SE animals.

Animals that experienced SE became epileptic within 12–18 days after the pilocarpine injection, in keeping with a previous study (El Hassar et al., 2007). Non-SE animals did not show any sign of pathological behaviour or electrophysiological activity during the EEG recording periods. Latent period animals were used between 7 and 10 days following pilocarpine injection for *in vitro* electrophysiology.

Periods of exploration in the open field were analysed off-line, after selecting epochs with no significant interictal or ictal activity or movement artifacts on the EEG. Power spectral analysis of the hippocampal EEG was performed using 2 second epochs, digitized at 256 Hz with a fast Fourier transform (FFT). For each exploration period, we obtained the absolute power of the EEG at both an overall 1–100 Hz wide bandwidth and in the 4–12 Hz theta bandwidth, as well as the median frequency of hippocampal theta rhythm, after filtering the signal at 4–12 Hz, using AMADEUS software (Rennes, France).

Electrode positions were histologically verified. At the end of the electrophysiological experiments, the rats were deeply anesthetized with sodium pentobarbital injection (60 mg/kg, i.p.) and perfused intracardially with a fixative solution containing 4% paraformaldehyde in 0.12 M sodium phosphate buffer, pH 7.4 (PB). After perfusion, the brains were removed from the skull, postfixed in the same fixative for 1 h at room temperature (RT), and rinsed in 0.12 M PB for 1.5 h. Blocks of the forebrain containing the entire hippocampal formation were immersed in a cryoprotective solution of 20% sucrose in PB overnight at 4 °C, quickly frozen on dry ice, and sectioned coronally at 40 μm with a cryostat. Sections were stained with cresyl violet to verify electrode placement. Electrode position within the CA1 region of the hippocampus, usually close to stratum lacunosum moleculare, was confirmed in all reported animals (additional Fig. 2).

In vitro electrophysiology

Hippocampal slices (380 μm thick) were prepared from sham, latent and chronic animals, following an i.p. injection of chloral hydrate (800 mg kg^{-1}). Animals were perfused intracardially with cold ACSF in which NaCl was substituted with an equimolar concentration of choline. Animals were then killed by decapitation and slices were cut in modified ACSF using an oscillating slicer (Microm slicer, International GmbH, Germany). Slices were then transferred to a holding chamber at room temperature in normal ACSF. ACSF contained (in mM) NaCl 126, KCl 3.5, $CaCl_2$ 2, $MgCL_2$ 1.3, NaH_2PO_4 1.2, $NaHCO_3$ 26, D-glucose 10, and was continuously aerated with 95% O_2 and 5% CO_2. NBQX (1 μM), D-APV (50 μM) and bicuculline (10 μM)/picrotoxin (10 μM) were added to the perfusion solution to block AMPA, NMDA and $GABA_A$ receptors respectively. The recording temperature was kept at 34±1 °C (ALA Scientific instruments). Patch pipettes were filled with (in mM) $KMeSO_4$ 120, KCl 20, EGTA 0.2, $MgCl_2$ 2, HEPES 10, Na_2ATP 4, Tris GTP 0.3, Phosphocreatine 14, biocytin 0.4% and KOH to adjust to pH 7.3. Dendrites were recorded under visual control (Nikon FN1) with an Axopatch700A amplifier and Digidata 1322 interface (Axon Instruments). The distance between the recording site and the soma was determined both under visual control before performing dendritic recordings, and *post hoc* following morphological processing as previously described (Bernard et al., 2004). Neuronal input resistance was significantly increased during epileptogenesis (sham, 42±4 MΩ, $n=20$; latent, 51±5 MΩ, $n=8$, $p<0.01$; chronic, 58±4 MΩ, $n=17$, $p<0.01$), consistent with the downregulation of I_h. The I_h antagonist ZD7288 induced a hyperpolarization of the resting membrane potential by 15–20 mV. This hyperpolarization was compensated by current injection.

Analysis of I_h

Currents mediated by I_h were recorded in voltage-clamp mode by applying hyperpolarizing voltage steps (starting from a holding potential of −50 mV up to −140 mV). Data were acquired with the help of pCLAMP 10 and analysed offline using Clampfit 10.0 (Axon Instruments). The amplitude of I_h was determined by subtracting the instantaneous current at the beginning of the voltage step from the steady-state current at the end. The activation time constant was obtained using a double exponential fit. Boltzmann fits were made to obtain $V_{1/2}$, the midpoint activation voltage. Resonance and phase responses were analysed with software within the MATLAB environment.