Hyperpolarization-activated cation current \( I_h \) of dentate gyrus granule cells is upregulated in human and rat temporal lobe epilepsy

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Abstract

The hyperpolarization-activated cation current \( I_h \) is an important regulator of neuronal excitability and may contribute to the properties of the dentate gyrus granule (DGG) cells, which constitute the input site of the canonical hippocampal circuit. Here, we investigated changes in \( I_h \) in DGG cells in human temporal lobe epilepsy (TLE) and the rat pilocarpine model of TLE using the patch-clamp technique. Messenger-RNA (mRNA) expression of \( I_h \)-conducting HCN1, 2 and 4 isoforms was determined using semi-quantitative \textit{in-situ} hybridization. \( I_h \) density was \~1.8-fold greater in DGG cells of TLE patients with Ammon’s horn sclerosis (AHS) as compared to patients without AHS. The magnitude of somatodendritic \( I_h \) was enhanced also in DGG cells in epileptic rats, most robustly during the latent phase after status epilepticus and prior to the occurrence of spontaneous epileptic seizures. During the chronic phase, \( I_h \) was increased \~1.7-fold. This increase of \( I_h \) was paralleled by an increase in HCN1 and HCN4 mRNA expression, whereas HCN2 expression was unchanged. Our data demonstrate an epilepsy-associated upregulation of \( I_h \) likely due to increased HCN1 and HCN4 expression, which indicate plasticity of \( I_h \) during epileptogenesis and which may contribute to a compensatory decrease in neuronal excitability of DGG cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2012.02.133.
Keywords
Hippocampus; Rat pilocarpine model; Patch-clamp; h-Current; Human; Temporal lobe epilepsy

1. Introduction

The hyperpolarization-activated current $I_h$ is a slowly activating, non-inactivating depolarizing cationic current which is activated by hyperpolarization beyond $-50$ to $-70$ mV. These unique biophysical properties provide the basis for the diverse roles ascribed to this current, i.e. control of resting membrane potential, passive membrane properties, pacemaker activity, rebound burst firing in heart and brain, reduction of dendritic summation and the presence of certain types of resonance behavior in neurons [1-8].

The specific function of h-channels in each neuronal population depends strongly on the voltage- and time-dependent properties of $I_h$. An important molecular mechanism for generating functionally diverse $I_h$ is the differential expression of the four underlying HCN1-4 subunits. Homomeric channels formed by these HCN subunits display very different kinetics, steady-state voltage dependence and sensitivity to modulation by cAMP in heterologous expression systems [2]. Thus, regulation of the relative abundance of HCN subunit protein is likely to represent a key mechanism for plasticity of $I_h$. Importantly, the functional properties of $I_h$ and the expression of the corresponding subunits are differentially modulated during postnatal development as well as in both acquired and genetic forms of epilepsy in a region- and time-dependent manner in different in vitro and in vivo models [6,9-18]. An emerging “leitmotiv”, at least in CA1 hippocampal or neocortical neurons, seems to be the downregulation of the HCN1-subunit along with a decrease in somatodendritic $I_h$, followed, via an enhanced input resistance, by neuronal hyperexcitability in the chronic state.

Thus, a significant body of work has implicated alteration of HCN channels expression and altered magnitude and properties of $I_h$ in epilepsy [reviewed in 8,19]. In temporal lobe epilepsy (TLE), the large majority of existing studies has focused on animal models and on the hippocampal pyramidal cell layer [20]. Remarkably, an early study suggested that changes in HCN expression might arise also in the dentate gyrus [21]. Therefore, here, we focus on the DGG cells, and examine somatodendritic $I_h$ currents and the HCN expression levels in DGG cells of people with TLE and underlying Ammon’s horn sclerosis (AHS) and in the rat pilocarpine-model of TLE.

2. Material and methods

2.1. Pre- and postsurgical assessment of epilepsy patients

All patients had conventional scalp EEG recordings or intracranial recordings and brain MRI prior to neurosurgery. The histological grading of human hippocampus was performed according to Wyler and colleagues [22]. Investigation of human cerebral tissue was approved by the local ethics committee and written informed consent was obtained from each patient.

2.2. Pilocarpine-induced status epilepticus (SE)

Procedures on animals were performed in accordance with local guidelines and experiments were approved by the local animal care and use committee. Pilocarpine treatment of rats was carried out and animals were monitored as described in Supplementary material.
2.3. Semi-quantitative in situ hybridization (ISH) in rat hippocampus

ISH was carried out as described previously [10,21,23,24] on specimens obtained 30-60 days after SE. For details see Supplementary material.

2.4. Electrophysiology

Electrophysiological recordings of DGG cells were performed in both human and rat hippocampal slices using conventional patch clamp technique and established experimental procedures. See Supplementary material for details.

2.5. Data analysis

$I_h$ amplitudes in response to voltage steps were determined as the difference of the end of the instantaneous current component and the current amplitude in the steady-state. The reversal potential $V_{rev}$ was determined by analysis of tail currents and subsequent linear regression analysis (see Fig. 1C and Supplementary material). The conductance-voltage curves were fitted with a Boltzmann function of the form.

$$f(V) = 1 / \left(1 + e^{-(V - V_{50})/\text{slope}}\right)$$

with $V_{50}$ being the voltage of half-maximal activation.

The activation time course of $I_h$ was fit with exponential functions of the form.

$$f(t) = \sum A_i e^{-t/r_i} + c, i=1 \text{ or } 2.$$  (2)

Unpaired student’s t-test was used for statistical analyses. $P$ values <0.05 were considered as significant. All data are given as means ± SEM.

3. Results

3.1. $I_h$ recordings in human TLE

Properties of $I_h$ recorded in DGG cells from seven TLE patients with AHS (AHS-group) were compared to those from three patients without AHS (non-AHS). The clinical data of the patients are summarized in Supplementary Table 1. The intrinsic membrane properties of DGG cells were not significantly different between both groups (Supplementary Table 2). Gross morphology was not different between groups, as described previously [25].

However, upon a hyperpolarizing voltage step from $-50$ to $-90$ mV for 5 s, we observed slowly developing inward current which was more pronounced in the AHS-group (Fig. 1A1 and B1). These currents were blocked by the $I_h$-channel blocker ZD7288 (40 μM, $n = 8$) in neurons from both tissues (Fig. 1A2 and B2) and had similar reversal potentials (AHS: $-38.1$ mV, $n = 7$; non-AHS group: $-39.4$ mV, $n = 6$; Fig. 1C), consistent with reported reversal potentials of $I_h$ [5,13]. Application of 2 mM Cs⁺ in two recordings also blocked this current component (data not shown). Taken together, these findings indicate that the recorded time-dependent inward currents were a result of activation of $I_h$ channels.

The mean $I_h$ amplitudes measured with hyperpolarizing voltage steps from $-50$ to $-90$ mV ($p = 0.0056$, $n = 8$ non-AHS; $n = 7$ AHS; Fig. 1D) and $I_h$ current densities normalized to the cell capacitance were significantly higher in the AHS-group ($p = 0.0077$, $n = 8$ nonAHS; $n = 7$ AHS; Fig. 1D). The voltage-dependence of activation was significantly shifted to more depolarized potentials in the AHS-group (non-AHS group: $V_{50} = -85.9 \pm 2.3$ mV; AHS-group: $V_{50} = -78.2 \pm 2.7$ mV; $p = 0.0001$, Fig. 2A and D). The kinetics of $I_h$ activation...
contained a fast and a slow component, and was examined by fitting with a bi-exponential function. The time constants of activation did not differ between groups (Fig. 2B). Likewise, the relative contribution of the fast current component to the total current amplitude varied between 66% and 73% in a voltage range between −90 and −120 mV in both groups and was not significantly different (Fig. 2C).

### 3.2. \( I_h \) recordings in rat pilocarpine model of TLE

Similar to the human, a slowly activating, non-inactivating inward conductance was seen upon voltage steps to −103 mV which was completely blocked by ZD7288 (60 \( \mu \)M) both in control (\( n = 22 \)) and epileptic rats (\( n = 22 \), Fig. 3A, upper traces). Subtraction of the two traces allowed isolating \( I_h \) (Fig. 3A, lower trace).

We examined the magnitude of \( I_h \) in control and pilocarpine treated rats at two different time points: 7–9 days following SE (latent phase, no spontaneous generalized seizures) and in the chronic epileptic phase 35–63 days following SE (all spontaneous generalized seizures). \( I_h \) density was enhanced in both groups, but most pronouncedly in the latent period (Fig. 3B, \( p < 0.0001 \) and \( p = 0.0041 \) for the latent and chronic group, respectively). This increase could not be explained by a change in driving force, as the reversal potential of \( I_h \) did not differ between groups (controls: −35.9 ± 2.3 mV, \( n = 8 \); pilocarpine-treated rats: −36.8 ± 2.3 mV, \( n = 8 \)). The time course of activation was best fit with a mono-exponential equation with no significant differences between controls and pilocarpine-treated animals neither at early nor at late stages following SE (Fig. 3C).

To understand the neurobiological basis of the persistent increase in \( I_h \) in DGG cells of epileptic rats, we investigated the expression of the genes coding for the h-channels. We compared mRNA expression of HCN1, 2 and 4 channel isoforms in chronically epileptic, pilocarpine-treated rats versus the control group. Whereas HCN2 expression was not different in both groups (not shown), expression of HCN1 mRNA was significantly increased by 22% in the pilocarpine-treated (67.9 ± 4.0 nCi/gm) compared to control rats (55.4 ± 3.2 nCi/gm; \( p < 0.05 \), Fig. 4A). This effect was more pronounced for the HCN4 channel isoform. HCN4 mRNA level was enhanced by 52% in the granule cell layer of epileptic (70.75 ± 6.4 nCi/gm) relative to controls rats (46.75 ± 1.0 nCi/gm; \( p < 0.05 \); Fig. 4B).

### 4. Discussion

The principal findings of the current studies are that (1) \( I_h \) density is greater in granule cell of the dentate gyrus in TLE patients with AHS as compared to those without AHS. (2) Similar findings are observed in the rat pilocarpine-model of TLE, where \( I_h \) changes precede the onset of chronic epilepsy. (3) Voltage-dependence but not kinetics of \( I_h \) is altered in the epileptic state, which might result from augmented contribution of HCN1 and HCN4 to the total HCN channels pool that conducts \( I_h \).

#### 4.1. Molecular mechanism of \( I_h \) upregulation

The observed upregulation of \( I_h \) in both human and rat DGG cells is most likely due to an enhanced expression of HCN1 and, at least in rat hippocampus, HCN4 subunit, as shown in Fig. 4 and as found previously [21]. This is in line with the observed shift of voltage-dependence of \( I_h \) to more depolarized potentials in tissue from people with TLE due to AHS. In general, h-channels composed of HCN1 subunits display faster \( I_h \) activation than those composed of HCN2 and HCN4 subunits [13,26]. Strikingly, current kinetics was not faster in the AHS-group or in epileptic rats as compared to controls. This might be a result of the concomitant increase of HCN4 subunits. Furthermore, the properties of \( I_h \) in native...
hippocampus only partially overlap those of currents recorded in heterologous systems: in human and rat hippocampus, in addition to the intrinsic biophysical properties of each HCN isoform, a number of interacting molecules influence channel properties [18,27, reviewed in 28].

What are the molecular mechanisms and pathways involved in the upregulation of \( I_h \) and HCN channels in DGG cells? Although this question has not been directly addressed in the present work, a number of recent studies provide insights into regulation of HCN expression in association with seizures and epilepsy. In the kainic acid-induced SE model of TLE, downregulation of HCN1 expression in CA1 pyramidal region was caused by an upregulation of a repressing transcription factor, neuron-restrictive silencer factor (NRSF) which suppresses transcription of a number of genes, amongst them the \( HCN1 \) gene [20]. In addition, a reduction of HCN1 expression has been shown to involve transient \( Ca^{2+} \)-permeable AMPA receptor activation with subsequent activation of \( Ca^{2+}/calmodulin \)-dependent protein kinase II during in-vitro experiments in hippocampal slices [29]. Little is known, however, about mechanisms of upregulation of HCN expression. Reduction of NRSF levels might take place, or the enhanced expression might result from a myriad of cellular mechanisms that influence ion channel expression [30].

### 4.2. Significance for the pathophysiology of TLE and potential clinical implications

In previous studies, \( I_h \) expression appears to be differentially modulated in acquired and genetically caused epilepsies in a region- and time-dependent manner. For instance, in rat models of genetically caused absence epilepsies, impaired \( I_h \) with a concomitant decrease of HCN1 expression was found in cortical neurons [12], whereas thalamocortical neurons showed an increase in cAMP responsiveness, explained by an increase of HCN1 expression [31,32]. Both phenomena favor onset of absence seizures. In models of acquired TLE, \( I_h \) currents and expression of underlying HCN1 and HCN2 subunits were temporarily reduced in dendrites of the entorhinal cortex following kainate-induced SE [11]. A further study found sustained reduction in HCN1 and 2 expression following kainate-induced SE in the CA1 region [16]. Likewise, in the rat pilocarpine model of TLE, dendritic \( I_h \) of CA1 pyramidal cells was progressively reduced during epileptogenesis, because of permanent reduction of HCN1-subunit expression and a persistent alteration of \( I_h \)-modulating phosphorylation pathways [6,15,18]. The significance of HCN1 downregulation is further strengthened by the observation that a primary loss of the HCN1 subunit facilitates onset of seizures and of epilepsy [33,34]. In a model of seizure-inducing hypoxia, somatic \( I_h \) in rat CA1 pyramidal was decreased [14], and a complex region-specific regulation of HCN1 and 2 in an in vitro model of epilepsy has been described, with commensurate alteration of burst activity [17].

Taken together, downregulation of \( I_h \) seems to be a leitmotiv in the early phases of epileptogenesis and epilepsy. However, relatively few studies in animal models have explored potentially compensatory alterations in HCN isoform expression and \( I_h \) in the chronic and even ‘end stage’ of TLE. Even fewer yet are studies in human tissue. In human neocortical neurons of patients with TLE, Wierschke and co-workers reported on reduced \( I_h \) density in patients with higher seizure frequencies; these observations support the notion that \( I_h \) contributes importantly to the regulation of neuronal excitability [35].

Focusing on the dentate gyrus, a major gateway into the hippocampal formation, we report here a robust, epilepsy-associated upregulation of \( I_h \) in DGG of human and rat hippocampus. Our findings are in line with a recent study reporting enhanced \( I_h \) in DGG of people with TLE with severe AHS as compared to those with mild or moderate AHS [36]. Whereas the mechanisms leading to this increase of HCN1 expression and \( I_h \) density are not fully understood, the teleological purpose is intriguing: augmented \( I_h \) in the epileptic dentate...
gyrus might serve as an attempt to compensate for abnormal hyperexcitability in the circuit for example, by dampening dendritic summation [1]. Alternatively, in response to augmented inhibition (hyperpolarizing), the increase in $I_h$ might promote rebound depolarization and contribute to hyperexcitability of epileptic tissue [5,9]. Whereas other ion channels are also altered in epileptic DGG cells [37,38], the changes in $I_h$ reported here should contribute to neuronal firing behavior, and might provide a drug target to both existing and future medications (see below).

4.3. Temporal profile of $I_h$ upregulation

In human tissue, it is not possible to time the onset of HCN expression and $I_h$ changes, because tissue is only available from chronic epileptic tissue. Therefore, the current studies employed also tissue from animal models. In the rat pilocarpine model employed here, an $I_h$ upregulation was already present in the latent stage preceding the onset of spontaneous seizures, and may thus contribute to their onset. This increased $I_h$ was associated with, and hence is likely a result of, an upregulation of both HCN1 and HCN4 mRNA in neurons of the dentate gyrus. Given the importance of $I_h$ in attenuating postsynaptic potentials at dendrites and limiting their spread in pyramidal neurons of CA1 region and layer five of the somatosensory cortex [39,40], our data suggest that upregulation of $I_h$ might be a protective mechanism resulting in maintained dentate gyrus gating. Dendrites of DGG neurons in rats without epilepsy have no or little $I_h$ [41]. In view of the considerable increase of $I_h$, it is tempting to speculate that somatodendritic $I_h$ in DGG neurons plays a role in signal integration only under pathophysiological circumstances such as epileptogenesis and chronic epilepsy, but not under physiological conditions.

4.4. Implications for epilepsy therapy

$I_h$ is a target for the anticonvulsants acetazolamide, gabapentin and lamotrigine, all of which increase $I_h$ [42-44]. It is therefore also possible that the observed upregulation of $I_h$ renders its modulation by these antiepileptic drugs more effective, thereby dampening excitability. Such a mechanism operating in DGG cells may be particularly relevant in chronic epilepsy, when granule cells are one of the major preserved cell populations after the profound loss of neurons in the CA1, CA3 and CA4 subfields. Therefore, defining and understanding the changes in $I_h$ and in its ‘building blocks’, the HCN channels, both in humans and animal models is important. The knowledge is a prerequisite for using this important channel as target for epilepsy therapy and prevention in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

$I_h$ density is greater in DGG neurons of people with TLE due to AHS. $I_h$ is elicited by voltage steps from −50 to −90 mV in both DGG neurons from the non-AHS (A1) and AHS-group (B1). Scaling 10 pA/0.5 s. The inward current is blocked by ZD7288 in both groups (A2, B2). Scaling 50 pA/0.1 s. (C) Reversal potential was around −39 mV in both conditions (upper panel, tail currents indicated with an asterisk, Scaling 100 pA/0.5 s; bottom panel; filled symbols: non-AHS group; open symbols: AHS-group). (D) $I_h$ amplitudes (left panel) and current densities at −90 mV (right panel) were significantly greater in people with AHS.
Fig. 2. Activation properties of $I_h$ in human DGG neurons. (A) A family of current traces was elicited by stepping the voltage from $-50$ mV to more hyperpolarized potentials in 10 mV-steps (from $-60$ to $-120$ mV) in the non-AHS (upper panel) and AHS-group (lower panel). Scaling 100 pA/1 s. (B) Fast and slow time constants were not different in both conditions at different command potentials (filled symbols: non-AHS group; open symbols: AHS-group). (C) The amplitudes from the fast (the upper data points under each condition) and the slow time component (the lower data points under each condition) were derived from the bi-exponential fitting paradigms. There was no significant difference in the relative contributions of the fast and slow current component between both conditions. Symbols as in (B). (D) The voltage-dependence of activation was significantly shifted to more depolarized potentials in the AHS-group. Symbols as in (B).
Fig. 3.
$I_h$ density is upregulated in DGG cells of rat hippocampal slices in the latent and chronic phase following pilocarpine-injection. (A) Inward currents elicited upon a voltage step from −63 to −103 mV in the absence and presence of ZD7288 (upper panel, indicated by an arrow). Lower trace obtained after subtraction of traces in the absence and presence of ZD7288. Recordings from a control animal. Scaling 10 pA/0.5 s. (B) $I_h$ current densities during the latent and chronic phase (sham-control animals: black bars, SE-experienced animals: white bars). (C) Time constants at a potential of −103 mV were not different in controls and pilocarpine rats during latent ($p = 0.49$, $n = 9$ and 10 cells) and chronic phase ($p = 0.96$, $n = 13$ and 12 cells). Symbols as in (B).
Expression of both HCN1 and HCN 4 subunits is increased in epileptic animals following pilocarpine-induced SE. As depicted in the photomicrographs (A and B lower panels), HCN1 and HCN4 subunit mRNA signals were significantly increased in the granule cell layer (GCL) of the pilocarpine-group (A and B upper panels), with relatively little change in expression over the pyramidal cell layer of CA3. Brains from 4-9 animals were used per group. Asterisk indicates $p < 0.05$. 

**Fig. 4.**