Neurobiology of Disease 41 (2011) 261–269

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

# *Scn1a* missense mutation causes limbic hyperexcitability and vulnerability to experimental febrile seizures

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ARTICLE INFO

*Article history:*
Received 17 May 2010
Revised 15 August 2010
Accepted 19 September 2010
Available online 25 September 2010

*Keywords:*
Febrile seizure
$Na_v1.1$ channel
Scn1a
Missense mutation
Rat model

ABSTRACT

Mutations of the voltage-gated sodium ($Na_v$) channel subunit SCN1A have been implicated in the pathogenesis of human febrile seizures including generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy in infancy (SMEI). Hyperthermia-induced seizure-susceptible (Hiss) rats are the novel rat model carrying a missense mutation (N1417H) of Scn1a, which is located in the third pore-forming region of the $Na_v1.1$ channel. Here, we conducted behavioral and neurochemical studies to clarify the functional relevance of the Scn1a mutation *in vivo* and the mechanism underlying the vulnerability to hyperthermic seizures. Hiss rats showed markedly high susceptibility to hyperthermic seizures (mainly generalized clonic seizures) which were synchronously associated with paroxysmal epileptiform discharges. Immunohistochemical analysis of brain Fos expression revealed that hyperthermic seizures induced a widespread elevation of Fos-immunoreactivity in the cerebral cortices including the motor area, piriform, and insular cortex. In the subcortical regions, hyperthermic seizures enhanced Fos expression region—specifically in the limbic and paralimbic regions (e.g., hippocampus, amygdala, and perirhinal–entorhinal cortex) without affecting other brain regions (e.g., basal ganglia, diencephalon, and lower brainstem), suggesting a primary involvement of limbic system in the induction of hyperthermic seizures. In addition, Hiss rats showed a significantly lower threshold than the control animals in inducing epileptiform discharges in response to local stimulation of the hippocampus (hippocampal afterdischarges). Furthermore, hyperthermic seizures in Hiss rats were significantly alleviated by the antiepileptic drugs, diazepam and sodium valproate, while phenytoin or ethosuximide were ineffective. The present findings support the notion that Hiss rats are useful as a novel rat model of febrile seizures and suggest that hyperexcitability of limbic neurons associated with Scn1a missense mutation plays a crucial role in the pathogenesis of febrile seizures.



## Introduction

Febrile seizures (FS) are the most common type of seizures in childhood, often occurring between 6 months and 5 years of age. Although most FS are generally benign, one-third are complex and exhibit persistent and/or recurrent seizures (Fetveit, 2008; Scantlebury and Heida, 2010; Shinnar, 2003). Specifically, patients of generalized epilepsy with febrile seizures plus (GEFS+) exhibit FS in childhood progressing to generalized epilepsy in adults and, in some cases, accompany variable symptoms including tonic–clonic, absence, and/or myoclonic seizures (Scheffer and Berkovic, 1997). Severe myoclonic epilepsy in infancy (SMEI) is more severe usually beginning within the first 6 months after birth, followed by progressive worsening of seizures associated with ataxia and mental decline (Dravet et al., 2005; Incorpora, 2009). Although familial or twin studies have shown that genetic predisposition contributes to the etiology of FS (Baulac et al., 2004; Baulac and Baulac, 2009; Nakayama, 2009), the causative genes and pathogenic mechanisms underlying human FS are still elusive.

Voltage-gated sodium ($Na_v$) channels mediate the generation and propagation of action potentials in electrically excitable cells such as neurons and muscles. They are classified into 9 subtypes encompassing $Na_v1.1$ to $Na_v1.9$ and have a common structure composed of 4 homologous domains, each of which contains voltage-sensor and pore-forming regions. Among these subtypes, $Na_v1.1$ channels have been implicated in the pathogenesis of multiple types of FS (Meisler and Kearney, 2005; Ragsdale, 2008). Specifically, more than 200 mutations in human $Na_v1.1$ channel α subunit SCN1A have been reported in patients with FS

*Abbreviations:* ACF, artificial cerebrospinal fluid; FS, febrile seizure; GEFS+, generalized epilepsy with febrile seizures plus; $Na_v$, voltage-gated sodium; PBS, phosphate-buffered saline; IR, immunoreactivity; SMEI, severe myoclonic epilepsy in infancy.

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doi:10.1016/j.nbd.2010.09.013

including GEFS+ and SMEI (Meisler and Kearney, 2005; Ragsdale, 2008). The 13 SCN1A mutations found in patients with GEFS+ are known to be missense mutations, while about half of the SMEI mutations are missense and the remaining are truncated mutations (e.g., nonsense or frame-shift mutations) (Meisler and Kearney, 2005; Mulley et al., 2005). In addition, recent studies have shown that haploinsufficiency of $Na_v1.1$ channel causes hypersusceptibility to hyperthermic seizures and sporadic spontaneous seizure in mice, which accompanied a marked reduction in sodium currents in inhibitory GABAergic neurons (Yu et al., 2006; Oakley et al., 2009; Martin et al., 2010). All these findings indicate a close relationship between the $Na_v1.1$ channel function and the etiology of FS. Nonetheless, due to the diversity of functional changes of $Na_v1.1$ channels in patients with FS (Meisler and Kearney, 2005; Catterall et al., 2010), the precise mechanism and functional relevance of the SCN1A mutations underlying the pathogenesis of FS remain to be clarified.

Using the gene-driven *N*-ethyl-*N*-nitrosourea mutagenesis, we have recently generated a rat model which carries a missense mutation (N1417H) in the third pore-forming region of Scn1a (Mashimo et al., 2010). We designated them hyperthermia-induced seizure-susceptible (Hiss) rats because of their high susceptibility to hyperthermia-induced seizures. Since missense mutations in the pore-forming region of $Na_v1.1$ channels are frequently reported in patients with SMEI or GEFS+ (Meisler and Kearney, 2005; also see Fig. 1), Hiss rats may serve as a useful FS model with a genetic background similar to that of SMEI or GEFS+. Indeed, the mutation site in Hiss rats is very close to one (V1428A) of the GEFS+ mutations (Sugawara et al., 2001). In our previous studies (Mashimo et al., 2010), Hiss rats at a very young age (1–5 weeks old) exhibited a high susceptibility to hyperthermia-induced seizures. Electrophysiological analysis of the N1417H mutant channels or hippocampal neurons dissociated from Hiss rats revealed that N1417H mutation causes a hyperpolarized shift in the voltage dependency of $Na_v1.1$ channel inactivation and a slight increase in persistent leak current. In addition, hippocampal bipolar neurons (i.e., GABAergic interneurons) exhibited a significant decrease in spike amplitude, indicating reduced function of $Na_v1.1$ channels. These changes in $Na_v1.1$ functions were specifically observed in the biplolar neurons but not in the pyramidal neurons (Mashimo et al., 2010). Thus, our findings suggest that the Scn1a missense mutation impairs functions of the hippocampal GABAergic neurons to confer FS susceptibility. Nonetheless, the functional relevance of the Scn1a missense mutation *in vivo* and its mechanism underlying the vulnerability of animals to hyperthermic seizures remain to be determined.

In the present study, therefore, we further conducted behavioral and neurochemical studies to clarify pathophysiological mechanisms underlying the seizure vulnerability in Hiss rats. In addition, responses of hyperthermic seizures in Hiss rats to antiepileptic drugs were also evaluated to assess their clinical relevance as a human FS model. The present results show that the N1417H mutation of Scn1a markedly enhances the excitability of the corticolimbic neural circuit and the limbic vulnerability to hyperthermic seizures. Hiss rats seem to be a useful rat model for understanding the etiology of FS and also for searching new drugs to treat FS.

## Materials and methods

### *Animals*

Hiss rats (F344-*Scn1a*$^{Kyo811/Kyo811}$) were obtained from the National BioResource Project for the Rat (NBRPR#0455) in Japan. As reported previously (Mashimo et al., 2010), Hiss rats carry homozygous

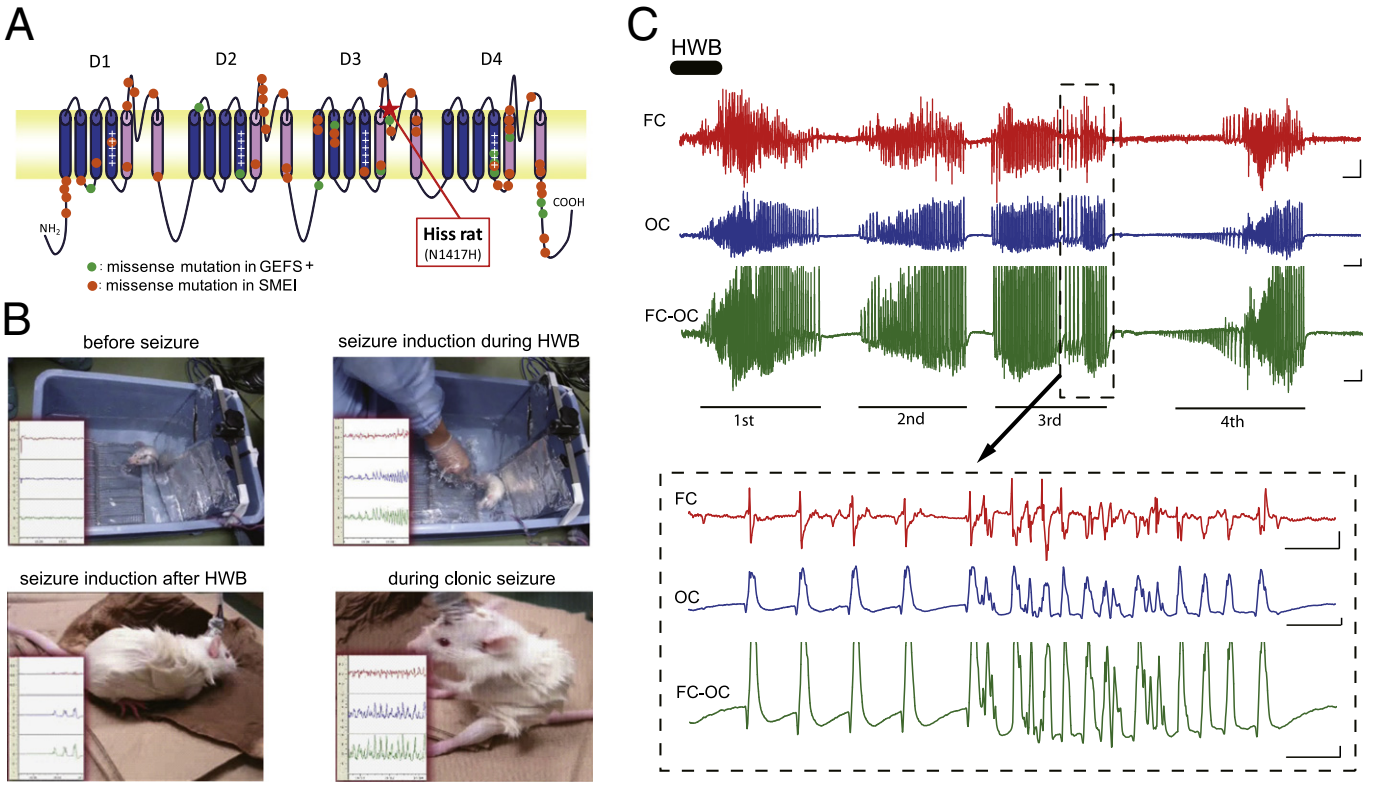


**Fig. 1.** Hyperthermic seizures and EEG in Hiss rats. (A) Structure of Scn1a and location of the missense mutation N1417H in Hiss rats. Scn1a consists of 4 homologous domains (D1–D4), each of which contains voltage-sensor and pore-forming regions. As a reference, missense mutation sites reported in GEFS+ and SMEI are also shown (Meisler and Kearney, 2005). (B) Photos illustrating induction of hyperthermic seizures and simultaneously monitored EEG in Hiss rats. Hiss rats were immerged in a hot water bath (HWB: 45 °C) for a maximum of 5 min or until a seizure occurred. Cortical EEG was simultaneously monitored under freely-moving conditions. All Hiss rats mainly developed clonic seizures (barely tonic–clonic seizures), which usually repeated even after HWB. (C) Typical paroxysmal discharges in Hiss rats. Solid lines (1st~4th) under EEG chart indicate the period of clonic seizures. Calibration: upper panel, 100 μV and 2 sec; lower panel, 100 μV and 1 sec.