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Research Report

Long-term increasing co-localization of SCN8A and ankyrin-G in rat hippocampal cornu ammonis 1 after pilocarpine induced status epilepticus

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ARTICLE INFO

Article history:
Accepted 3 March 2009
Available online 21 March 2009

Keywords:
Epilepsy
Pilocarpine kindling
Hippocampus
Voltage-gated sodium channel
Ankyrin-G

ABSTRACT

Voltage-gated sodium channels (VGSC) are important determinants of neuronal excitability which are implicated in the pathogenesis of epilepsy. Ankyrin-G contributes to the distribution and regulation of VGSC. Here we investigated the alterations of the two α-subunits SCN8A and SCN1A and their adapter ankyrin-G in the hippocampal cornu ammonis 1 (CA1) of rats after pilocarpine induced status epilepticus (PISE), compared to the sham-control group (C1) and blank-control group (C2). Significant increase of SCN8A mRNA (41.08% increase compared to C1, $P<0.001$; 30.88% increase compared to C2, $P=0.011$) was detected 60 days after PISE. At D1 SCN8A mRNA reduced but no significant changes were detected when compared to controls (one-way ANOVA, $F=1.232$, $P=0.276$). After measuring the optical density of Western blot, we detected significant differences between the levels of SCN8A protein in different groups but no difference between the protein levels of SCN1A at D1 and D60 after pilocarpine treatment compared to the control. At D60 the relative copies of ankyrin-G mRNA on internal control β-actin in PISE group increased significantly compared to C1 and C2 (one-way ANOVA, $F=16.537$, $P<0.001$). Significantly increase of ankyrin-G immunoreactivity in Western blot from the PISE group 1 day and 60 days after PISE was observed, compared to the controls (one-way ANOVA, $F=24.255$ at D1, $P<0.001$; $F=29.280$ at D60, $P<0.001$). After analyzing the double-stained cells counting, we detected significant differences between the numbers of SCN8A+/ankyrin-G+ immunoreactive cells in different groups in acute and chronic period following PISE (two way-ANOVA, $F_{group}=37.905$, $P<0.001$; $F_{day}=45.310$, $P<0.001$). The data revealed that both SCN8A and ankyrin-G increased significantly in the CA1 subfield of the rat hippocampus 60 days following pilocarpine induced status epilepticus and co-localized with each other.

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Abbreviations: VGSC, Voltage-gated sodium channel; INaT, Transient Na+ currents; INaP, Persistent Na+ current; MB, Membrane-binding; CA1, Cornu ammonis 1; PISE, Pilocarpine induced status epilepticus; qrtPCR, Quantitative real-time PCR; MTLE, Mesial temporal lobe epilepsy

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doi:10.1016/j.brainres.2009.03.012

1. Introduction

The hallmark of epilepsy is the alteration of neuronal excitability. Voltage-gated sodium channels (VGSC) are important determinants of neuronal excitability which are implicated in the pathogenesis of epilepsy. Ankyrin-G has attracted special attentions because of its contribution to the distribution and regulation of membrane voltage-gated ion channels, such as VGSC.

VGSC are multimeric complexes consisting of a pore-forming α-subunit and two auxiliary β-subunits (Catterall et al., 2005). In excitable cells VGSC are responsible for action potential initiation with the fast, transient Na^+ currents (I_{NaT}) and enhancing neuronal repetitive firing capacity with persistent Na^+ currents (I_{NaP}) (Agrawal et al., 2003; Yue et al., 2005). It is generally believed that their abnormal functions may be critical in conditions leading to the alteration of neuronal excitability.

Recent genetic studies have implicated altered VGSC in the pathogenesis of epilepsy. *Scn1a*, the gene encoding α subunit $Na_V1.1$ has been associated with idiopathic epilepsy, such as generalized epilepsy with febrile seizures plus syndrome, severe myoclonic epilepsy of infancy, intractable childhood epilepsy with generalized tonic–clonic seizures and infantile spasms (Claes et al., 2001; Escayg et al., 2000; Fujiwara et al., 2003; Nabbout et al., 2003; Rhodes et al., 2005; Wallace et al., 2003). Moreover, VGSC are common targets of antiepileptic drugs, sharing by phenytoin, carbamazepine, oxcarbazepine and lamotrigine, potently on the same binding site at clinically relevant concentrations (Meldrum and Rogawski, 2007). Hence, VGSC modulations on intrinsic firing properties of pyramidal neurons are proposed to involve in epilepsy.

Alterations in the number, function or pharmacology of SCN1A in acquired epilepsy have been concerned. Although sufficient idiopathic epilepsy syndromes have been found mutations in SCN1A, no significant differences in the amount of SCN1A were detected in hippocampus (Bartolomei et al., 1997; Ellerkmann et al., 2003). There are inconsistent reports on *Scn8a*, the gene encoding α subunit $Na_V1.6$, mRNA expression in PISE model. Some studies revealed down-regulation of SCN8A mRNA expression (Ellerkmann et al., 2003), but a recent study showed a significant increase in the SCN8A mRNA level in the CA3 region of kindling rats (Blumenfeld et al., 2008). It is necessary to observe both mRNA and protein levels of SCN1A and SCN8A in acquired epilepsy models to explore the roles of the two subtypes in epileptogenesis.

Ankyrins are the multidomain adapter proteins and associate the spectrin/actin network to the cytoplasmic domains of trans-membrane proteins, such as ion channels and cell adhesion molecules (Bennett and Baines, 2001). The membrane-binding domain (MB domain) consists of a similar folding structure and the 24 consecutive repeats. MB domain plays a critical role in the direct interaction with VGSC and localization of VGSC at neuronal membrane. Ankyrin can also regulate the trans-membrane proteins in excitable cells (Bennett and Healy, 2008). A recent study in cardiocytes has demonstrated that mutation in ankyrin-B caused dominantly inherited type IV long-QT cardiac arrhythmia in humans (Mohler et al., 2003). In addition, ankyrin-B can regulate I_{NaP} and prolong the QT interval of cardiocytes in turn (Chauhan et al., 2000). Therefore, a similar mechanism may underlie excitability of neurons because neurons and cardiocytes share considerable similarity in excitability. Ankyrin-G might regulate neuronal excitability by modifying the sodium current of SCN8A (Shirahata et al., 2006). The role of ankyrin in chronic epilepsy remains an enigma.

Because of their distribution and function in hippocampus, SCN1A, SCN8A and ankyrin-G may exert an effect in hippocampus circuit by regulating neuronal excitability in chronic epilepsy. Here we investigated the alterations of the two α-subunits SCN8A and SCN1A and their adapter ankyrin-G in the hippocampal cornu ammonis 1 (CA1) of rats after pilocarpine induced status epilepticus (PISE).

2. Results

2.1. *Pilocarpine induced status epilepticus and subsequent epilepsy*

In the study we observed the changes of SCN8A, SCN1A and ankyrin-G in rats following PISE. For the purpose, we randomly divided the rats into three groups: PISE group ($n=30$), sham-control group (C1, $n=20$) and blank-control group (C2, $n=20$). Rats in C1 were injected with scopolamine methyl bromide and saline instead of pilocarpine and rats in C2 didn't receive any treatments. To investigate the alterations in different stages after PISE: the acute and chronic periods, we sacrificed rats at two time points after PISE. One day after pilocarpine treatments, 10 age-matched rats were sacrificed each in PISE,

Table 1 – Primers for PCR amplification of target SCN1A, SCN8A and ankyrin-G.

Target gene	Accession number	Primer sequences	Bases	Amplicon size	Annealing temperature
SCN1A	NM_030875	S Sense: 5′-TTGCTTTGGAATCACGCATCTC-3′ Antisense: 5′-GAGGTGCCTATGGTCTGCTTCTGTA-3′	1811– 1933	149 bp	56.7 °C
SCN8A	NM_019266	S Sense: 5′-TGGCATGTCCAACTTCGCATA-3′ Antisense: 5′-GTTCAGGATTGGCAGCAGCA-3′	5166– 5310	145 bp	58.6 °C
Ankyrin-G	NM_031805	S Sense: 5′-CTAGTGACGGTGACGTCAGTCCA-3′ Antisense: 5′-CACACTCCGCTCTTGCTAGAGATTC-3′	6328– 6432	105 bp	60.1 °C