Expression of HCN1 subunits is age-dependently regulated in developing hippocampus of rats

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Abstract

Hyperpolarization-activated, cyclic nucleotide-gated cation channels (HCN) encode hyperpolarization-activated cation current (I_h), playing regulatory roles in resting membrane properties, synaptic integration, and intrinsic excitabilities of hippocampal neurons. Expression patterns of HCN subtypes seem to be quietly variable to excitatory conditions of neurons, even though it has been previously observed that expression levels of HCN1, 2 and 4 are differentially regulated during developmental period. In the present study, we investigated expression patterns of HCN1 during an early developmental period within postnatal 3 weeks by using Western blot analysis. Inconsistent with previous reports, HCN1 expression was highest in neonatal and initial postnatal periods, while neurons in postnatal third week revealed lower levels. This result indicates that HCN1 proteins are age-dependently decreased during first postnatal 3 weeks. This finding suggests that CA1 neurons of early developmental hippocampi may have specific neuroprotective mechanisms correlated with HCN1 subunit against hyperexcitabilities or excitotoxic damages. (J Med Life Sci 2012;9:64-63)

Key Words : I_h Channel, HCN1, hippocampus, excitotoxicity

Introduction

It is well known that expression levels and gating properties of ion channels such as voltage-dependent K^+, Na^+, and hyperpolarization-activated cation currents (I_h) are dynamically and systemically changed in developmental mammalian neurons (Falk et al., 2003; Cingolani et al., 2002; Maletic-Savatic et al., 1995; Perney et al., 1992; Scheinman et al., 1989; Beckh et al., 1989). The hyperpolarization-activated, cyclic nucleotide-gated cation (HCN) channels encoding I_h contribute to determine the resting membrane potentials (RMPs), input resistance (R_{in}) and synaptic integration, and limits hyperpolarization and depolarization of cytosolic membranes in neurons (Magee, 1998; Poolos et al., 2002; Surges et al., 2004, Pape, 1996; Williams and Stuart, 2000). In addition, recent studies observed that HCN subunits play very important roles in regulation of overexcitation, which can induce neuronal disease such as seizures (Quardouz et al., 2010; Dyhrfjeld-Johnsen et al., 2008). In studies on molecular composition, HCN have four members of gene family (HCN1-4) and proteins encoded by each of these homomeric-form channels. The each subunit of HCN channels differently displays according to spatial and temporal developmental patterns of brains (Vasilyev and Barish, 2002; Bender et al., 2001; Surges et al., 2006). HCN1 subunits quickly activate and deactivate by hyperpolarization, compared with other subunits (Ludwig et al., 1998; Santoro et al., 1998; Jegla et al., 1999; Seifert et al., 1999) and strongly expressed in principal neurons and interneurons of hippocampi.

In this study, we tested the expression level of HCN1 during early postnatal 3 weeks in hippocampal neurons of SD rats. We classified neurons in this period into early developmental (ED) and late developmental (LD) neurons based on postnatal days (ED neurons: P6~8; LD neuron: P18~19). Consequently, HCN1 expression is age-dependently decreased at least within first 3 postnatal weeks, suggesting that ED neurons are non-sensitive to overexcitation than LD neurons.

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Methods

1.Tissue preparation

Hippocampi were prepared from embryo (n = 2) and pups (n = 20) during the first 3 postnatal weeks of Spraque-

Dawley (SD) rats. Experiments were approved by the Animal Care and Use Committee of Jeju National University. Brains were quickly extracted from decapitated rats and then hippocampus were rapidly dissected on ice in Ca2+-free normal tyrode solution containing (in mM): 140 NaCl, 5.4 KCl, 2.3 $MgCl_2$, 10 HEPES, 5 glucose (pH 7.4).

2. Western blot analysis

Hippocampi were homogenized on ice in lysis buffer (120 mM NaCl, 40 mM Tris pH 8.0, 0.1% NP 40) and lysed for 30min on ice. The lysate was then centrifuged at 13,000 × g for 15 min at 4 ℃. The supernatants were collected from the lysates and the protein concentrations were determined by using a protein assay kit (Bio-Rad, Hercules, CA, USA). An equal amount of protein was electrophoresed in 8% SDS-polyacrylamide gel and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). The membranes subsequently immunostained with primary antibodys, HCN1 antibody (1:1000, Millipore, Bedford, MA) for 24 hrs at 4 ℃ and β-actin(1:10000, Cell signaling, Laboratories, USA) for 2 hrs, at room temperature (RT), and then the membranes incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit or mouse immunoglobulin G (IgG) (1:5000, Cell signaling, Laboratories, USA) respectively, followed by exposure to X-ray film. The protein bands were detected using an enhanced chemiluminescence western blotting detection kit (Amersham, Little Chalfont, Buckinghamshire, UK).

3. Statistical analysis

Data analysis and statistical significance were performed by using Excel (Microsoft) software and then data were expressed as mean value±standard error of mean (SEM). The Student' s t-test was used, and the significance between groups was indicated for p values of<0.05.

Results

It has been reported that the expression levels of HCN1 age-dependently change in developing neurons within first 3 postnatal weeks. In some previous papers, the expression levels of HCN1 are increased during this developmental period (surges et al., 2006; Vasilyev and Barish, 2002). However, Bender et al. reported that expression levels of HCN1 in ED neurons were higher than in LD neurons (2001). We, therefore, confirmed the changes of HCN1 expression levels in this period. HCN1 protein levels were tested using Western blot analysis and measured by normalization with corresponding levels of β-actin. Figure 1 shows the HCN1 expression levels in neonatal and postnatal hippocampus of rats. In ED hippocampus, the expression levels of HCN1 of P6 were similar with E21 but age-dependent decreas in HCN1 protein levels was revealed (Figure 1, E21 = 0.82 ± 0.07, n = 2; P6 = 0.84 ± 0.07, n = 4; P7 = 0.81 ± 0.07, n = 4; P8 = 0.77 ± 0.04, n = 4). Moreover, HCN1 levels, in consistent with previous report (Bender et al., 2001), was significantly decreased in LD hippocampus (Figure 1, P18 = 0.66 ± 0.02, n = 4; P19 = 0.65 ± 0.03, n = 4, $p<0.05$). This resultindicates that the expression level of HCN1 is age-dependently decreased and this reflects that ED hippocampus is protected from cell damages by overexcitation in developmental stage.

Discussion

Our results indicate that ED hippocampi may be stable to excitatory stimulation because more HCN1 proteins express in ED hippocampi than in LD hippocampi. In the previous reports, expression levels of HCN 1, 2 and 4 subtypes are

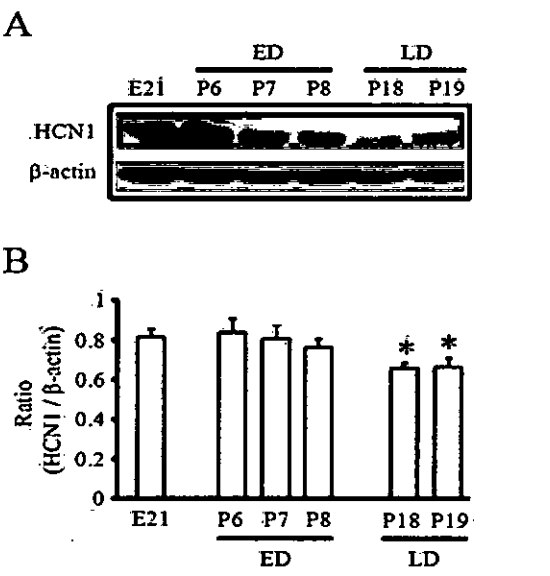

Figure 1. Age-dependent decrease in expression levels of HCN1 is observed in the developing hippocampus of rats. The expression levels of HCN1 were decreased with age, as shown in the film(A) and quantitative analysis(B). Proteins of HCN1 isolated from embryo(n=2) and postnatal rat hippocampus (n=4/age) and the expression levels of HCN1 were measured using Western blot analysis. Optical densities of individual bands were normalized to corresponding levels of β-actin

differently regulated during developmental periods in mammalian brains. Generally, HCN2 subtypes are increased while HCN4 subtypes are decreased (Surges et al., 2006; Brewster et al., 2007). However, it is not ensured whether expression levels of HCN1 are increased (Surges et al., 2006) or decreased (Bender et al., 2001) in this period. In the present study, we tested this issue by using western blot analysis to confirm change of HCN1 proteins during the first 3 postnatal weeks. Consequently, age-dependent increase in expression levels of HCN1 was observed during this period and HCN1 protein levels were not different between embrio-21 day and ED hippocampus (Fig. 1).

The HCN1 subtype plays important roles in the determination of membrane properties such as RMPs and R_{in} and participates in regulation of excitability of neurons. Recent studies reported that deficiency or downregulation of HCN1 subunits induces enhanced excitabilities and epileptogenesis because of the increased neuronal R_{in} and input summation (Huang et al., 2009; Jung et al., 2007; Kole et al,. 2007; Shah et al., 2004). Moreover, activation of Ih channels encoded by HCN1 induces the decreased neuronal excitabilities due to the decreased R_{in} (Fan et al., 2005; Poolos et al., 2002; Johnston, 2006). Therefore, our result suggests that ED hippocampi may be regulated by neurons but less in LD hippocampi.

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