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Sustained Expression of Neuritin mRNA After Repeated Electroconvulsive Stimulations in the Rat Hippocampal Formation

Kun Woo Park[2], Il Hwan Kim[1], Woong Sun[1] and Hyun Kim[1]*

[1]Department of Anatomy, Brain Korea 21, [2]Department of Neurology, College of Medicine, Korea University, Seoul 136-705, Korea

ABSTRACT

In the present study, we compared the expression patterns of *neuritin* mRNA in the dentate gyrus of hippocampal formation following single (1×ECS) or repeated elctroconvulsive seizures (8×ECS) treatments to the rat. The expression of *neuritin* mRNA was transiently increased, and returned to basal level within 12 hours after 1×ECS. Whereas initial induction of *neuritin* mRNA was similarly seen, these increased mRNA level was maintained for 24 hours in 8×ECS group. In contrast, induction profile of BDNF was similar following 1× and 8×ECS. These results suggest that regulation of *neuritin* expression may be plastically changed by repeated ECS treatment.

Key words: *neuritin*, elctroconvulsive seizure (ECS), neuronal plasticity, dentate gyrus

INTRODUCTION

neuritin (also called cpg15) is an activity-regulated, growth promoting protein exhibiting activity to promote neuronal development and regeneration (Nedivi et al., 1998; Fujimo et al., 2008). During the development, *neuritin* expression peaks at active neuritogenic/axonal remodeling periods in the brain (Nedivi et al., 1996; Harwell et al., 2005), and contributes to the neuromuscular synaptogenesis (Javeherian and Cline, 2005). In adult brains, various neural stimulations such as kainic acid, brainderived neurotrophic factor and light can induce the *neuritin* expression (Nedivi et al., 1993; Nedivi et al., 1996; Gall et al., 1997; Naeve et al., 1997), suggesting that the induction of *neuritin* is also related to the activity-induced structural changes in adult brain.

Although the expression of *neuritin* has been well known in chemically induced epilepsy model (Naeve et al., 1997), single KA treatment also induces neuronal death of several brain regions including hippocampal formation. Therefore, it is yet uncertain whether induction of *neuritin* is directly related to the KA-induced neuronal plasticity or neuronal death *in vivo*. Therefore, here we took advantage of direct electrical stimulation model. Single electroconvulsive stimulation (ECS) has been shown to cause plastic alternations of nervous system without significant neuronal death. In addition, ECS is known to induce the expression of activity-related genes including transcription factors, signaling molecules, and brain-derived neurotrophic factor (BDNF) (Kim et al., 1994; Nibuya et al., 1995; Sun et al., 2007). On the other hand, it is believed that mul-

*To whom correspondence should be addressed.
TEL: 82-2-920-6153, FAX: 82-2-929-5696
e-mail: kimhyun@korea.ac.kr
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tiple treatments of ECS is required for the alteration of neuronal circuit including plastic change of mossy fiber sprouting in the hippocampus (Nibuya et al., 1995; Vaidya et al., 1997; Scott et al., 2000). In this paper, we examined whether *neuritin* expression is also induced by direct electric stimulation of the brain, and whether repeated vs. single electrical stimulation evoke different responses in terms of *neuritin* expression.

MATERIALS AND METHODS

Animals and treatments

Adult male Sprague-Dawley rats with weight of 200～250 g were stimulated 130 V electrical shocks for 0.5 sec through the bilateral ear clips using the Medicraft B-24 (Kim et al., 1994). They were divided into single and repeated ECS groups. Repeated ECS group was received single electrical shock per day for 8 consecutive days. All stimulated rats showed generalized tonic clonic seizure for 5～10 sec. Single ECS group were sacrificed 0.5 hr, 1 hr, 3 hr, 6 hr, 12 hr, 24 hr after ECS and repeated ECS group 0.5 hr, 1 hr, 3 hr, 6 hr, 12 hr, 24 hr, 48 hr, 72 hr after the last ECS.

In situ hybridization

The frozen sections of rat brain were taken from the 4 rats each group and used for *In situ* hybridization histochemistry. The 12 μm thick frozen sections were cut coronally. The transferred tissues to gelatin-coated slide were post-fixed with 4% paraformaldehyde and treated with 0.1 M triethanol amine/0.9% NaCl (pH 8.0) and 0.25% acetic anhydride for 10 minutes. Following these treatments, the tissues were dehydrated by ascending ethanol density and then delipolinosis with chloroform for 5 minutes, rinsed with ethanol and dried. *neuritin* cDNA (kind gift from Prof Young Sung Lee at Hanyang University) and BDNF cDNA corresponds to 2100～3136 bases of rat cDNA sequence (Genbank ID AA9262 56) were subcloned in pGEM vector (Promega) and ^{35}S-labeled riboprobes were prepared by *in vitro* transcription with T7 RNA polymerase of cDNA. The 60 μl of labeled riboprobe (1×10^7 cpm/ml) solution was added each slide. The slides were kept warm at 35 C for 16 hours, rinsed with SSC, dried and exposed to beta-max hyperfilm (Amersham) for 7 days. The autographs were analyzed and pseudocolored using the automatic analyzer of NIH image version 1.60. Optical densities were changed to nominal disintegration per minute (dpm) compared to simultaneous expressed ^{14}C standard. Statistical significance of the data was analyzed with ANOVA followed by post hoc test (Dunnett's test).

RESULTS

Following 1×ECS, we observed a rapid induction of BDNF in the dentate gyrus (DG) as early as 30 min, and this induction was maintained until 12 hr

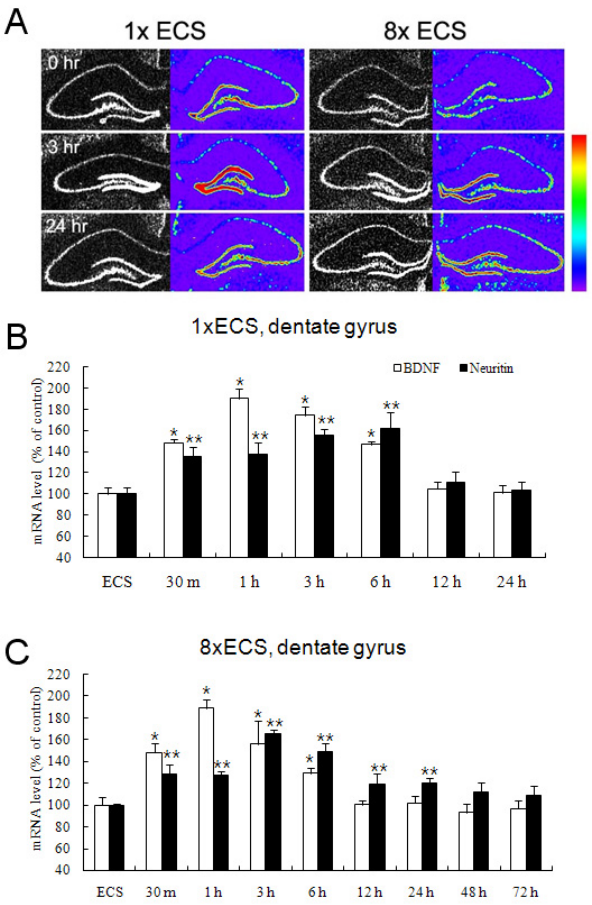

Fig. 1. (A) *In situ* hybridization of the *neuritin* mRNA expression after single and repeated ECS in the rat hippocampus. The different intensity of neuritin mRNA signal was pseudocolored with blue (weaker) to red (stronger) colors (right of autoradiograms). (B, C) Histogram demonstrating the effect of 1×ECS (B) or 8×ECS (C) on the abundance of BDNF mRNA (white bar) and *neuritin* mRNA (black bar) in rat dentate gyrus region. Values are mean±S.D. from 4 rats in each group. *$p<0.001$ ANOVA with Dunnett's t-test, in BDNF mRNA; **$p<0.001$ ANOVA with Dunnett's t-test, in *neuritin* mRNA.

after 1×ECS (Fig. 1B). Similar time-course changes in the BDNF mRNA expression was also observed following 8×ECS treatment (Fig. 1C). However, the expression of *neuritin* mRNA following 1×ECS vs. 8×ECS appeared to be different. Fig. 1A shows typical autoradiographs of *neuritin* mRNA signals and pseudocolored images exhibiting relative mRNA levels in the hippocampal formation region. Quantification of the autoradiograph intensities in the dentate gyrus (DG) revealed that the expression of *neuritin* mRNA was also increased 0.5 hr (35%), 1 hr (38%), 3 hr (56%), and 6 hr (62%) after 1× ECS with a peak on 3 hr (Fig. 1B). By 12 hours after 1×ECS, *neuritin* mRNAs levels were returned to basal. On the other hand, the abundance of the *neuritin* mRNA was maximally detected in 3 hours (66%) after last 8×ECS, and the significantly increased level of *neuritin* mRNA was sustained until 24 hr after last 8×ECS.

DISCUSSION

Neuronal plasticity requires abilities to change their functional and structural responsiveness to the stimuli. *neuritin* is proposed to be closely related to neuronal plasticity in dentate gyrus (Nedivi et al., 1993). Based on the results from kainite induced seizure model, Neave and colleagues proposed that *neuritin* could be directly induced by neuronal activation induced by glutamateric stimulation, neuronal depolarization or an induction of activity-related neurotrophic factors such as BDNF (Nedivi et al., 1998). In consistent with their suggestions, here we also found that *neuritin* mRNA expression is increased after direct electrical stimulations *in vivo.*

Most interesting finding in this study is a prolonged expression of *neuritin* following repeated ECS treatments. The induction of *neuritin* expression was prolonged for 24 hours after repeated ECS, whereas the induction of *neuritin* mRNA expression by ECS returned to basal level within 12 hours after single ECS. The repeated ECS-evoked prolonged *neuritin* expression appears to be specific, because the time-course changes in the BDNF expression was similar after single and repeated ECS. Because BDNF level was returned to basal level far earlier than the reduction of *neuritin* mRNA after repeated ECS, these results suggest that other mechanism(s) may be involved in the sustained *neuritin* expression. Although exact mechanism underlying this intriguing phenomenon is required to be identified, these results suggest that the regulation of the stimulation pathway of the *neuritin* may be changed by repeated ECS. It has been demonstrated that administration of repeated, but not single, ECS leads to mossy fiber sprouting in the hippocampus (Nibuya et al., 1995; Smith et al., 1997; Vaidya et al., 1999). In depression patients, the therapeutic effect of ECS was only sustained after repeated electroconvulsive therapy (Nibuya et al., 1995; Vaidya et al., 1999). These results suggest that repetitive electrical activation may promote axonal growth via *neuritin* expression *in vivo*, which is required to be elucidated.

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