available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Acute ethanol administration decreases GAP-43 and phosphorylated-GAP-43 in the rat hippocampus

*Hyun Joon Kim, Kyung Mi Choi, Bo Mi Ku, Jihye Mun, Yeon Joo, Jae Yoon Han, Young Hee Kim, Gu Seob Roh, Sang Soo Kang, Gyeong Jae Cho, Wan Sung Choi**

Department of Anatomy and Neurobiology, Institute of Health Sciences, College of Medicine, Gyeongsang National University, 92 Chilam-dong, Jinju, Kyungnam 660-751, Korea

ARTICLE INFO

Article history:
Accepted 4 July 2006
Available online 14 August 2006

Keywords:
Ethanol
Hippocampus
GAP-43
p-GAP-43
LTP

Abbreviations:
BALs, blood alcohol levels
CA3p, pyramidal cells of CA3
CA3sr, stratum radiatum of CA3
dg, dentate gyrus
GAP-43, growth-associated protein 43
i.p., intraperitoneally
Iml, inner molecular layer of gentate gyrus
p-GAP-43, phosphorylated growth-associated protein 43

ABSTRACT

Acute alcohol ingestion is well known to have deleterious effects on memory and also known to inhibit long-term potentiation, a putative cellular substrate of memory. In this study, we for the first time revealed that growth-associated protein 43 (GAP-43), which is well known as a presynaptic substrate of protein kinase C and one of the major synaptic plasticity-related genes, was down regulated by single ethanol administration (2.5 g/kg, 15% in saline, i.p.) in the rat hippocampus. Using real-time PCR, we confirmed that GAP-43 mRNA level is significantly decreased 2 h after ethanol administration. GAP-43 and p-GAP-43 (Ser^{41}) immunoreactivities in the hippocampus were also reduced 4 h after ethanol administration. Immunohistochemical study showed that the reduction of GAP-43 and p-GAP-43 expression was associated with the perforant and mossy fibers pathways. These results suggest that the reduction of GAP-43 in the hippocampus might be, at least in part, a cause of memory impairment after acute ethanol ingestion.

1. Introduction

Ethanol affects various functions of the CNS and peripheral organs, resulting in CNS depression, intoxication, addiction, cardiac arrhythmia and hepatic and pancreatic dysfunctions (Givens et al., 2000). The rapidity of ethanol's effects is due to its complete solubility in water, which results in its rapid absorption by the blood and distribution throughout the

* *Corresponding author*. Fax: +82 55 759 0779.
E-mail address: choiws@gsnu.ac.kr (W.S. Choi).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.07.018

highly vascularized brain (Charness, 1993). Because alcohol dehydrogenase activity is lower in the brain than in the liver and because ethanol easily penetrates into brain tissue through the blood brain barrier, equilibrium of the ethanol between the blood and brain occurs rapidly after injection (Alling et al., 1993).

Acute ethanol administration has been reported to alter the excitability of neurons and to modulate synaptic transmission. In central neurons, ethanol has been shown to act on various membrane receptors, such as the NMDA or $GABA_A$-receptors, as well as on voltage-gated ion channels, especially Ca^{2+} channels (Little, 1991; Weight, 1992). Ethanol has diverse effects on the expression of various mRNAs within the CNS, including ion channels, neuropeptides, membrane receptors and many immediate early genes (Crews et al., 1996).

In addition, acute ethanol administration impairs performance in many cognitive tasks that are dependent on hippocampal functions. For example, acute ethanol administration produces dose-dependent impairments in spatial learning. Ethanol also decreases the spatial specificity of hippocampal place cells (Matthews et al., 1995). Such findings raise the possibility that ethanol affects learning and memory by altering, either directly or indirectly, neuronal activity in the hippocampus and related structures (Silvers et al., 2003).

These deleterious effects of acute ethanol on hippocampal memory functions are closely related to the disturbance of long-term potentiation (LTP). According to the original definition of Bliss and Lomo (1973), LTP is a potentiation of excitatory postsynaptic potentials that is sustained for at least 30 min after titanic stimulation. Previous studies reported that the LTP in the rat hippocampus can be significantly reduced by acute ethanol at concentrations as low as 5 mM, a level attainable following ingestion of a single alcoholic drink (Blitzer et al., 1990). However, the detailed mechanism by which acute ethanol exposure reduces LTP remains unclear.

The growth associated protein 43 (GAP-43) plays a role in axonal growth during development, sprouting and regeneration and participates in intracellular changes that allow the growing axon to adapt to the changes in its environment (Benowitz and Routtenberg, 1997). GAP-43 can be phosphorylated on Ser^{41} by protein kinase C (PKC). Phosphorylated-GAP-43 (p-GAP-43) is the active form that carries out various intracellular functions. The time course of GAP-43 phosphorylation correlates with the enhancement of neurotransmitter release during the induction of LTP (Ramakers et al., 1995). Not only is this process susceptible to the same inhibitors and activators of LTP (Linden et al., 1987, 1988; Lovinger et al., 1987; Leahy et al., 1993), but changes in the levels of p-GAP-43 can also affect the establishment of LTP (Benowitz and Routtenberg, 1997).

These findings lead us to speculate that there is an intimate relationship between the changes in LTP and GAP-43 expression caused by acute alcohol ingestion. Thus, we first investigated the GAP-43 mRNA expression pattern after ethanol injection. We also analyzed the amount of GAP-43 and p-GAP-43 protein by Western blot. In addition, using immunohistochemistry, we identified regions where GAP-43 and p-GAP-43 were decreased after acute ethanol injection.

2. Results

2.1. *Single acute ethanol injection increases BALs and decreases GAP-43 mRNA*

To verify whether our alcohol regimen is suitable for evaluating alcohol's deleterious effects on hippocampal functions related to learning and memory, we assayed BALs after single acute ethanol administration using a commercial alcohol kit. BALs were rapidly increased after the injection and reached maximum level (250±30 mg/dl) within 1 h (Fig. 1A). BALs above 200 mg/dl were maintained until 2 h after injection and could

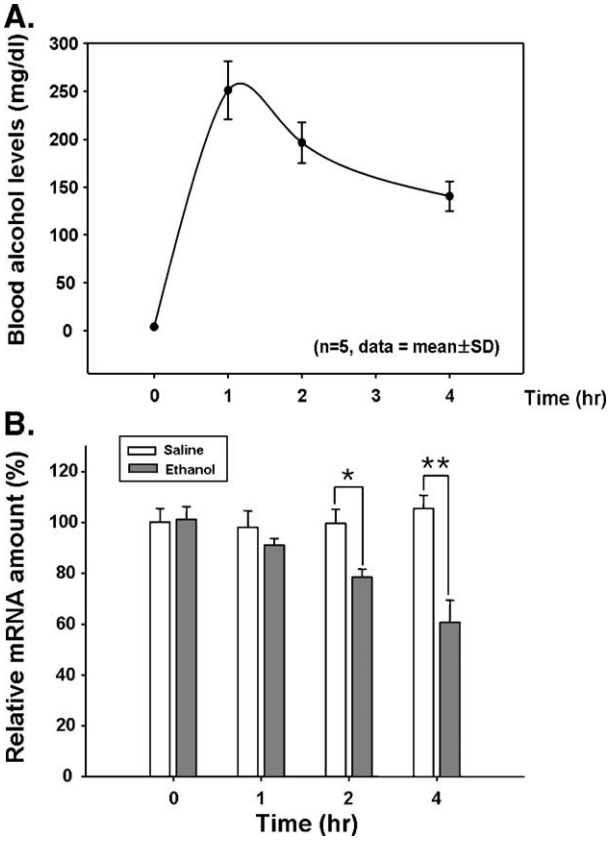

Fig. 1 – (A) Blood alcohol levels (BALs) after an acute ethanol injection (15% in saline, 2.5 g/kg, i.p.). Note that 4 h after the injection the BAL level was above 100 mg/dl. Results represent the means±SD (n=5). (B) Real-time PCR analysis of GAP-43 between ethanol and saline injected group. The relative amounts of GAP-43 mRNA are represented as arbitrary units (percentages compared with '0' time point) obtained from the value of relative quantification using automatically calculated Ct values for GAP-43 and normalized by GAPDH Ct values of each sample in LC4 (version 4.0, Roche) software. Results represent the means±SEM (n=5). $^{*}P<0.05$; $^{**}P<0.01$ between saline and ethanol injected group.