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Oligodendrocyte myelin glycoprotein (OMgp) in rat hippocampus is depleted by chronic ethanol consumption

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Abstract

The hippocampal formation has been shown to be particularly vulnerable to the neurotoxic effects of chronic ethanol consumption. It was hypothesized that this damage was due to the disruption of the expression of neurotrophic factors and certain other proteins within the hippocampus. By using real-time reverse transcription-polymerase chain reaction (RT-PCR) techniques, this study aimed to determine whether chronic ethanol consumption could alter the mRNA expression level of brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), and oligodendrocyte myelin glycoprotein (OMgp) in the hippocampus. Wistar male rats received an unrestricted access to a liquid diet containing 5% (v/v) ethanol as the sole source of fluid from 10 to 29 weeks of age. Control rats had unlimited access to a liquid diet containing an isocaloric amount of sucrose. We found that chronic ethanol consumption did not cause significant changes in the levels of mRNA for BDNF and GDNF. However, OMgp mRNA showed a significant deficit in ethanol-treated animals. It is suggested that this deficit may be related to the demyelination that is commonly observed in human alcoholics and that this may contribute to the functional and cognitive deficits.
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Keywords: Chronic ethanol consumption; Hippocampus; BDNF; GDNF; OMgp; Real-time PCR

Alcohol dependence and abuse are serious global problems. It is now well known that even "uncomplicated" alcoholics, who have no specific neurological or hepatic problems, show signs of regional brain damage and cognitive dysfunction. Chronic ethanol ingestion has adverse effects on the central nervous system (CNS) [16]. Numerous studies on laboratory animals and humans have demonstrated that long-term ethanol consumption can result in an impairment of cognition, learning, and memory abilities [16]. The hippocampus, which plays important roles in learning and memory processing, is known as one of the more sensitive target sites for the neurotoxic effects of ethanol.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that is abundantly and widely distributed in the hippocampus [4]. BDNF is involved in the survival and maintenance of neuronal functions, and it acts as a neuroprotective agent against various adverse environmental conditions such as exposure to alcohol, epilepsy, ischemia, and physical trauma [9]. Glial-derived neurotrophic factor (GDNF) is a member of the transforming growth factor superfamily and is distributed through multiple brain regions, and it also has a neuroprotective function [6]. Oligodendrocyte myelin glycoprotein (OMgp) is a glycosylphosphatidylinositol-anchored protein localized on the myelin membrane and expressed on the surface of both neurons and oligodendrocytes in the CNS [20]. OMgp is known to be involved in the inhibition of neurite outgrowth and regeneration in adult CNS following injuries [20].

The purpose of the present study was to evaluate the changes in hippocampal BDNF, GDNF, and OMgp mRNA of rats following chronic ethanol consumption. In this study, a 19-week ethanol administration was performed in order to examine the fully developed alcoholic model in rats since a period of at least 2–4 months of ethanol exposure is required to cause structural and functional alterations in the rat brain [23]. Furthermore, to our knowledge, studies with fully developed alcoholic models

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in rats are limited [10,25]. We believe that the present study provides important insights into the mechanisms of brain damage induced by long-term alcohol consumption in human alcoholic patients.

Sixteen male Wistar rats obtained from CLEA Japan (Tokyo, Japan) were housed in individual cages in a temperature-controlled room (21.6 ± 0.5 °C) maintained on a 12/12 h light/dark cycle. They were divided into two groups, namely, ethanol-fed and pair-fed control. The experimental rats ($n = 8$) received unrestricted access to 5% (v/v) ethanol (99.5%, Wako, Osaka, Japan) containing liquid diet (Oriental yeast, Tokyo, Japan) as the sole fluid source for 19 weeks (beginning at 10 weeks of age). The pair-fed control rats ($n = 8$) were fed an identical liquid diet except that sucrose was substituted isocalorically for ethanol. This study was carried out in compliance with the guidelines for experimental use and care of laboratory animals set forth by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Kagawa University Animal Ethics Committee.

Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused intracardially with medical grade physiological saline (Otsuka, Tokyo, Japan). The brains were sectioned in a horizontal plane on a vibrotome to yield 1-mm-thick slices. The hippocampus was removed in chilled physiological saline with the aid of a dissection microscope. Total RNA was extracted from these hippocampal slices by homogenizing in TRIzol reagent (Invitrogen, Carisbad, USA). The concentration and purity of the extracted RNA were evaluated by optical density measurements at 260 nm and 280 nm using a spectrophotometer. These RNA samples were stored at −80 °C until use.

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) analysis was performed as described previously [8]. In brief, reverse transcription was carried out at 37 °C for 60 min, followed by 65 °C for 10 min with Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences, Piscataway, USA). For 33 μL of reaction mixture, the following regents were used: 1 μL of sample RNA, 29 μL of RNase-free water, Ready-To-Go You-Prime First-Strand Beads, and 3 μL of oligo dT primer (10 μM). Real-time PCR was performed using a LightCycler rapid thermal cycler system (Roche Diagnostics Ltd., Lewes, UK). Reactions were performed in a 20-μL volume with 2 μL of the cDNA diluted 10 times, 0.1 μM of primers and reagents included in the LightCycler-FastStart DNA Master SYBR Green I mix (Roche Diagnostics GmbH, Mannheim, Germany). The amplification protocol consisted of one cycle at 95 °C for 10 min followed by 30 cycles at 95 °C for 10 s, 65 °C for 10 s, 72 °C for 20 s, and 87 °C for 2 s. Detection of the fluorescent products was carried out at the end of the 87 °C extension period.

To assess an appropriate internal control, co-amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed in each sample. The following forward (F) and reverse (R) primers were used in the present study. For BDNF (gene accession number; X 67108), F: GAT GAG GAC CAG AAG GTT CG, R: GAT TGG GTA GTT CGG CAT TG; for GDNF (NM 019139), F: CCC GAA GAT TAT CCT GAC CA, R: TAG CCC AAA CCC AAG TCA GT; for OMgp (AY 250703), F: TCC GAT CCC CAC AAG A, R: GGC AGG CAG ACT TTC A; for GAPDH (AB 017801), F: GTA TTG GGC GCC TGG TCA CC, R: CGC TCC TGG AAG ATG GTG ATG G.

In general, during the designing process primers should be targeted to separate exons in order to reduce the possibility of amplifying contaminating genomic DNA [3]. However, the primer pairs of BDNF, GDNF, and GAPDH used in the present study are located on the same exons (exons IV, III, and III, respectively). The primer pair for OMgp is located on a different exon (F, exons I–II; R, exon II). To confirm amplification specificity, the PCR products from each primer pair were subjected to melting curve analysis and subsequent sequence analysis. To exclude genomic contamination, electrophoresis of the PCR products amplified from cDNA of BDNF, GDNF, OMgp, and GAPDH primers was carried out on 2% agarose gel and stained with ethidium bromide. A similar electrophoresis of the amplification product without reverse transcription (RT) was also performed for each sample as a negative control.

Statistical analysis of body and brain weights was performed by Student's *t*-test or Mann–Whitney *U*-test. The mRNA was quantified with the LightCycler analysis software. BDNF, GDNF, and OMgp mRNAs were expressed as the ratio of the mRNA for the housekeeping gene GAPDH. If the data was not normally distributed and/or the variances between groups were not homogeneous prior to analysis, it was first adjusted by the square root transformation using Student's *t*-test or Mann–Whitney *U*-test.

Table 1 shows the mean ± S.E.M. of body weight of ethanol-fed and pair-fed control rats. At the start of the ethanol treatment, both groups of rats had an average weight of about 295 g with no significant differences between groups. At 29 weeks of age, the ethanol-fed rats had a mean body weight of approximately 396 g, while that of the pair-fed controls was approximately 520 g; the difference was statistically significant.

The mean ± S.E.M. of brain (here, defined to include forebrain, brainstem, and cerebellum) weights of chronic ethanol-fed and pair-fed control rats at the end of the ethanol treatment (29 weeks of age) are shown in Table 2. The ethanol-fed rats were found to have a small but statistically significant deficit in brain weight as compared to that in the pair-fed controls.

Melting curve analyses of the PCR products amplified from BDNF, GDNF, OMgp, and GAPDH had a single and sharp transition. It was confirmed that a single PCR product was present, and primer–dimer formation was a rare occurrence within the

Table 1
Mean ± S.E.M. body weights (g) of chronic ethanol-fed and pair-fed control rats at 10 weeks of age and at the end of the 19-week ethanol exposure

Group	Beginning of ethanol treatment (10 weeks of age)	End of ethanol treatment (29 weeks of age)
Chronic ethanol-fed	294.6 ± 1.5	396.3 ± 17.6*
Pair-fed control	295.5 ± 2.5	520.6 ± 5.2

Number of animals examined in each group is eight.
Mann–Whitney *U*-test for the body weight data as the variances were not homogenous.
* $p < 0.001$.

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