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DOSE-DEPENDENT INFLUENCE OF SHORT-TERM INTERMITTENT ETHANOL INTOXICATION ON CEREBRAL NEUROCHEMICAL CHANGES IN RATS DETECTED BY *EX VIVO* PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Abstract—The aim of this study was to quantitatively assess the effects of short-term intermittent ethanol intoxication on cerebral metabolite changes among sham controls (CNTL), low-dose ethanol (LDE)-exposed, and high-dose ethanol (HDE)-exposed rats, which were determined with *ex vivo* high-resolution spectra. Eight-week-old male Wistar rats were divided into three groups. Twenty rats in the LDE (n = 10) and the HDE (n = 10) groups received ethanol doses of 1.5 and 2.5 g/kg, respectively, through oral gavage every 8 h for 4 days. At the end of the 4-day intermittent ethanol exposure, one-dimensional *ex vivo* 500-MHz ^{1}H nuclear magnetic resonance spectra were acquired from 30 samples of the frontal cortex region (from the three groups). Normalized total *N*-acetylaspartate (tNAA: NAA + NAAG [*N*-acetyl-aspartyl-glutamate]), GABA, and glutathione (GSH) levels were significantly lower in the frontal cortex of the HDE-exposed rats than that of the LDE-exposed rats. Moreover, compared to the CNTL group, the LDE rats exhibited significantly higher normalized GABA levels. The six pairs of normalized metabolite levels were positively (+) or negatively (−) correlated in the rat frontal cortex as follows: tNAA and GABA (+), tNAA and aspartate (Asp) (+), myo-Inositol (mIns) and Asp (−), mIns and alanine (+), mIns and taurine (+), and mIns and tNAA (−). Our results suggested that short-term intermittent ethanol intoxication might result in neuronal degeneration and dysfunction, changes in the rate of GABA synthesis, and oxidative stress in the rat frontal cortex. Our *ex vivo* ^{1}H high-resolution magic angle spinning nuclear magnetic resonance spectroscopy results suggested some novel metabolic markers for the dose-dependent influence of short-term intermittent ethanol intoxication in the frontal cortex.

Key words: intermittent ethanol intoxication, brain, metabolites, frontal cortex, high-resolution spectra.

INTRODUCTION

Alcohol is the most commonly used intoxicating substance worldwide and in developing countries, and it ranks high as a cause of disability (Saraceno, 2002; Little et al., 2008). Binge alcohol consumption (heavy consumption of alcohol over a short period) can cause various adverse consequences, including an increased risk of developing alcohol dependence and diverse systemic effects on various organs (Kim and Shukla, 2006; Lowery-Gionta et al., 2012). A number of studies have suggested that excessive alcohol abuse can cause various brain disorders such as changes in brain structure/volume, neurological dysfunction, functional abnormalities, and neurochemical alterations (Obernier et al., 2002b; Kelso et al., 2011; Welch et al., 2013).

Numerous studies have shown that binge ethanol-exposed rats exhibit significant metabolic abnormalities,

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Abbreviations: Ala, alanine; ANOVA, analysis of variance; Asp, aspartate; BALs, blood-alcohol levels; CNTL, controls; Cr, creatine; D_2O, deuterium oxide; Eth, ethanol; GAD, glutamic acid decarboxylase; Gln, glutamine; Glu, glutamate; Glx, glutamine complex; GPC, glycerophosphocholine; GSH, glutathione; HDE, high-dose-ethanol; HR-MAS, high-resolution magic angle spinning; HSD, honestly significant difference; Lac, lactate; LDE, low-dose ethanol; mIns, myo-Inositol; MRS, magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; NAAG, *N*-acetyl-aspartyl-glutamate; NMR, nuclear magnetic resonance; PCh, phosphocholine; PCr, phosphocreatine; SD, standard deviation; Tau, taurine; tNAA, total *N*-acetylaspartate; TSP, trimethylsilyl propionate.

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functional impairments, and neuronal changes such as cerebral metabolite changes (Zahr et al., 2010), cognitive deficits (Cippitelli et al., 2010), and neuronal dysfunction and degeneration/recovery (Crews et al., 2000; Crews and Nixon, 2009) in the hippocampus (Zahr et al., 2010; Kelso et al., 2011), temporal (entorhinal/perirhinal) cortex (Crews et al., 2000; Crews and Braun, 2003; Crews and Nixon, 2009), and olfactory bulb (Cippitelli et al., 2010). To date, to the best of our knowledge, studies on the neurochemical effects of binge alcohol intoxication in the rat frontal cortex are scarce. Moreover, information and studies on the dose-dependent effects of binge alcohol intoxication are also lacking. Here, we have created a model of binge-like alcohol intoxication with a 4-day binge protocol (Majchrowicz, 1975; Zahr et al., 2010). The binge protocol described by Majchrowicz is extensively used as a model of alcoholism because of the continuously sustained blood-alcohol levels (BALs) due to intragastric ethanol exposure (Zahr et al., 2010). Therefore, the Majchrowicz binge protocol is appropriate for the assessment of dose-dependent effects on the neurochemical changes induced in intermittent ethanol-intoxicated rats.

In vivo magnetic resonance spectroscopy (MRS) provides a noninvasive approach for the biochemical identification and quantification of specific organs (Batouli et al., 2012). However, quantification of the *in vivo* MRS technique has been severely limited by overlapping peaks in the narrow chemical shift range (Lee et al., 2013). The potential of high-field nuclear magnetic resonance (NMR) spectroscopy in providing biologically detailed neurochemical profiles on the basis of increased spectral resolution and improved signal-to-noise ratios has been demonstrated in previous reports (Gruetter et al., 1998; Tkac et al., 2009). *Ex vivo* proton (^{1}H) high-resolution magic angle spinning (HR-MAS) NMR spectroscopy is widely used in biological applications (Sitter et al., 2010). The HR-MAS is a powerful tool for observing cerebral neurochemical changes and allows high-resolution spectra to be harvested directly from biopsy tissues (Opstad et al., 2010; Llorente et al., 2012). Moreover, the HR-MAS technique can provide narrow line-widths of metabolite peaks by reducing the line-broadening effects in semi-solid tissues through rapid sample spinning at a magic angle (54.7°) against the magnetic field (Beckonert et al., 2010).

To date, the influence of dose effects of binge alcohol intoxication on cerebral metabolite changes of the frontal cortex region of the rats has not been experimentally investigated using ^{1}H *in vivo* MRS or *ex vivo* NMRS. Therefore, the first goal of this study was to determine the influence of the dose-dependent effects of intermittent ethanol intoxication on cerebral metabolite changes among sham controls and low- and high-dose ethanol-exposed rats with *ex vivo* high-resolution spectra. The second goal of this study was to determine the correlations between the metabolite-metabolite levels (pairs of metabolite levels) from all of the individual data from the frontal cortex of the intermittent ethanol-intoxicated rats. We hypothesized that the high-dose ethanol-exposed rats would exhibit significantly lower levels of total acetylaspartate (tNAA; *N*-acetylaspartate [NAA] + *N*-acetylaspartyl-glutamate [NAAG]), GABA, and glutathione (GSH) in the region of the frontal cortex because of the greater neurochemical damages from the ethanol toxicity compared to those of the sham controls and the low-dose ethanol-exposed rats. In addition, we hypothesized that the pairs of cerebral metabolites would significantly correlate with the pairs of metabolite levels among the sham-control rats and the intermittent low- and high-dose ethanol-exposed rats. In order to test these hypotheses, we compared the cerebral neurochemical levels and the pairs of metabolite levels in a dose-dependent manner in the intermittent ethanol-exposed rats.

EXPERIMENTAL PROCEDURES

Ethics statement

The animal experiments were approved by the Institutional Animal Care and Use Committee at The Catholic University of Korea, College of Medicine (IACUC Number: 2012-0084-02). The animals were maintained according to the 'Guide for the Care and Use of Laboratory Animals' (NIH Publications No. 80-23) issued by ILAR, USA.

Animals

Eight-week-old male Wistar rats (mean body weight, 314.7 g; range, 295.0–329.0 g; $n = 30$; Central Lab. Animal, Inc., Seoul, Republic of Korea) were divided into three groups (control rats [CNTL]: $n = 10$; low-dose [1.5 g/kg] ethanol [LDE] group: $n = 10$; and high-dose [2.5 g/kg] ethanol [HDE] group: $n = 10$). All animals were individually housed in standard plastic cages and maintained on a 12-h light–dark cycle at ambient temperature (24–25 °C). Before the start of the experiments, the rats were allowed free access to food and water for a week.

Intermittent ethanol intoxication

The design of the intermittent ethanol intoxication model has been previously described (Majchrowicz, 1975; Zahr et al., 2010). For the initial exposure on the first day (day 1; at 18:00 h), the 20 rats in the LDE and HDE groups received an initial dose of 5.0 g/kg (30% w/v solution) through oral gavage, and the rats then received additional doses of 1.5 g/kg and 2.5 g/kg (25% w/v solution), respectively, every 8 h (at 02:00 10:00, and 18:00 h) for 4 days. The 10 rats in the sham CNTL group received an equivalent volume (about 2.66 mL) of distilled water at comparable times (at 03:00, 11:00, and 19:00 h). Oral gavage ethanol was administered according to body weight as mentioned in the Majchrowicz binge alcohol protocol (Majchrowicz, 1975). The LDE- and the HDE-exposed rats showed signs of intoxication, including sedation and ataxia, after intermittent ethanol injections. The body weights of the rats in the CNTL, LDE, and HDE groups were recorded daily for 5 days; the initial body weights before ethanol