



Repeated ethanol exposure influences key enzymes in cholesterol and lipid homeostasis via the AMPK pathway in the rat prefrontal cortex

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ARTICLE INFO

Article history:

Received 3 September 2019

Received in revised form

24 October 2019

Accepted 7 November 2019

Keywords:

ethanol

cholesterol homeostasis

HMG-CoAR

AMPK pathway

prefrontal cortex

ABSTRACT

Cholesterol homeostasis has been proposed to be implicated in the development of addiction. However, the effects of ethanol on cholesterol homeostasis within the brain are not well understood. One of the most important regulators of cholesterol homeostasis is HMG-CoA reductase (HMG-CoAR), the rate-limiting enzyme of cholesterol biosynthesis. We examined the phosphorylation of HMG-CoAR and the other key regulator of lipid synthesis, acetyl-CoA carboxylase (ACC), following acute or chronic treatment with ethanol (0.5, 1, or 2 g/kg) in the rat prefrontal cortex. The phosphorylation of AMP-activated protein kinase (AMPK), which regulates the HMG-CoAR activity, and its well-known upstream regulators, was also studied. The phosphorylation of HMG-CoAR and ACC were transiently increased by ethanol treatment only in animals previously treated chronically with ethanol. Acute administration to naïve animals did not induce the phosphorylation, regardless of dosage. Similarly, the phosphorylation of AMPK and the upstream regulators, LKB1 and CaMK4, were transiently increased only in chronically ethanol-treated animals. In naïve animals, a high dose (2 g/kg) of ethanol decreased phosphorylation. The phosphorylation of TAK1, another upstream kinase of AMPK, was increased only from 30 min to 24 h after the chronic treatment with ethanol. Together, these results indicate that repeated exposure is required for the activating effect of ethanol on HMG-CoAR and ACC. This effect seems to be mediated by the AMPK system, and may contribute to the long-lasting neuroadaptation involved in the development of alcohol dependence.

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Introduction

Repeated exposure to drugs of abuse affects neurons involved in the reward system (Olds & Milner, 1954). Ethanol is one of the most abused drugs. The action of ethanol is thought to be mediated by the activation of GABA-A receptors and the inhibition of glutamate receptors (Koob, Sanna, & Bloom, 1998; Ortiz et al., 1995), which are related to the depressant effects of ethanol in the central nervous system (CNS) (Mukherjee, 2013). Ethanol also increases dopamine levels in the mesocorticolimbic pathway that projects to the prefrontal cortex and nucleus accumbens, which is related to the addictive property of ethanol (Di Chiara & Imperato, 1988; Gessa, Muntoni, Collu, Vargiu, & Mereu, 1985; Morice, Denis, Giros, & Nosten-Bertrand, 2010; Wise, 1998).

Cholesterol is the major sterol found in the CNS (Martín, Pfrieger, & Dotti, 2014). Cholesterol homeostasis plays a

Abbreviations: ACC, acetyl-CoA carboxylase; ANOVA, analysis of variance; AMP, 5' adenosine monophosphate; AMPK, AMP-activated protein kinase; CaMK4, Ca²⁺/calmodulin-dependent protein kinase 4; DAT, dopamine transporter; HMG-CoAR, hydroxymethylglutaryl-CoA reductase; LKB1, liver kinase B1; PAG, periaqueductal gray; PKA, protein kinase A; TAK1, transforming growth-factor- β -activated kinase 1; OD, optical density.

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significant role in synaptic plasticity and thus may be involved in addiction, which is accompanied by plastic changes in the brain (Frank, Rufini, Tancredi, Forcina, Grossi, D & D'arcangelo, 2008; Koudinov & Koudinova, 2001; Lüscher & Malenka, 2011). The hydroxymethylglutaryl-CoA (HMG-CoA) reductase (HMG-CoAR) is a key enzyme in the process of cholesterol synthesis. It is the rate-limiting enzyme in cholesterol biosynthesis and thus plays an important role in cholesterol homeostasis (Geelen, Gibson, & Rodwell, 1986). The activity of HMG-CoAR is altered by ethanol consumption in the periaqueductal gray (PAG) (Koudinov & Koudinova, 2001), a brain region related to opioid signaling and addiction (Flores, Galan-Rodriguez, Ramiro-Fuentes, & Fernandez-Espejo, 2006; Morgan, Fossum, Levine, & Ingram, 2006). In microsomes of CHO–K1 cells, continuous ethanol treatment for 3 days increases the activity of HMG-CoAR (Sinensky & Kleiner, 1981). Additionally, chronic treatment with HMG-CoAR inhibitors, statins, reduces drug seeking for cocaine in the animal model of addiction (Chauvet, Nicolas, Lafay-Chebassier, Jaber, Thiriet & Solinas, 2016; Law & Rudnicka, 2006). Furthermore, cholesterol biosynthesis and trafficking-related gene transcripts are overexpressed in the cortex of the abusers of drugs including cocaine, cannabis, and phencyclidine (Lehrmann et al., 2006). These findings suggest that cholesterol homeostasis may play some role in alcohol and drug addiction.

HMG-CoAR is regulated by the 5' adenosine monophosphate-activated protein kinase (AMP)-activated protein kinase (AMPK), which also phosphorylates acetyl-CoA carboxylase (ACC), the rate-limiting enzyme of fatty acid biosynthesis (Goldstein & Brown, 1990; Ronnett, Kleman, Kim, Landree, & Tu, 2006). Regardless of cholesterol or fatty acid, lipid synthesis is regulated by the AMPK system. AMPK is known to be a metabolite-sensing kinase and is activated by increased intracellular AMP concentrations, which signify cellular energy depletion (Hardie, Hawley, & Scott, 2006; Young, Li, Baron, & Russell, 2005). In mammals, AMPK is also activated by liver kinase B1 (LKB1), Ca²⁺/calmodulin-dependent protein kinase (CaMK4), and TGF- β activated kinase-1 (TAK1) (Hawley et al., 2003; Woods et al., 2005; Xu et al., 2011). Our previous study demonstrated that ethanol regulated AMPK activity in the prefrontal cortex and dorsal striatum (Xu & Kang, 2019). Moreover, acute treatment with ethanol alters AMPK activity and lipid content in the developing brain (Saito et al., 2007). Based on these findings, we hypothesized that ethanol regulates HMG-CoAR phosphorylation through the AMPK pathway in the brain. Although the effects of ethanol on HMG-CoAR have been reported in the PAG, they have not been explored in other areas of the brain.

To determine whether ethanol affects cholesterol homeostasis in the mesocortical pathway, the present study examined the dose- and time-response effects of acute and chronic ethanol treatment on the phosphorylation of HMG-CoAR and ACC in the prefrontal cortex. The activities of AMPK and its up-stream kinases LKB1, CaMK4, and TAK1 were also examined to elucidate whether changes in the AMPK pathway were related to lipid metabolism following ethanol treatment.

Materials and methods

Animals and drugs

Male Sprague–Dawley rats (weight, 150–200 g; KoaTech, Gyeonggi-do, Korea) were housed under a 12/12-h light/dark cycle with food and water available *ad libitum*. Ethanol (Merck; Whitehouse Station, New Jersey, United States, cat. #1.00983.1011) was diluted to 20% (w/v) in 0.9% saline and administered via the intraperitoneal (i.p.) route; all drugs were administered in the animal's home cage. This study was approved by the Institutional

Animal Care and Use Committee at Seoul National University Hospital (SNUH-IACUC), and the animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International (#001169) in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition, National Research Council (NRC), 2010.

Drug treatment schedules

Two sets of experiments were conducted in the present study. The first set of experiments was designed to test the acute effects of ethanol. We examined the phosphorylation of AMPK α , and its up-stream and downstream molecules, in response to various doses of acute ethanol. Animals were randomly assigned to one of four treatment groups ($n = 6$ per group): saline, 0.5, 1.0, and 2.0 g/kg ethanol groups. They were dissected at 30 min after the treatment and the brains were removed. The second set of experiments was performed to measure the phosphorylation of AMPK α signaling pathway in response to different doses of chronic ethanol. Rats were randomly divided into four groups ($n = 6$ or 7 per group): saline, 0.5, 1.0, and 2.0 g/kg ethanol groups. According to our previous study (Roh, Cui, Kim, & Kang, 2011), the maximum blood ethanol concentration (BEC) after 4 g/kg was 40 mM (0.18%), and thus the BEC attained by the current study would be well in the clinically relevant range. Each group was chronically treated for 3 weeks (administered 15 times, 5 times per week from Monday to Friday between 10:00 and 16:00). After the final (15th) treatment, ethanol-treated groups were further divided into two subgroups and decapitated at 30 min or 24 h, and the dissected prefrontal cortices included the cingulate cortex, prelimbic cortex, and infralimbic cortex (Supplementary Fig. 1).

Immunoblot analysis

Brains were dissected on ice plates; the prefrontal cortex was homogenized in lysis buffer containing 50 mM Tris buffer (pH 7.4), 150 mM NaCl, 1 mM DTT, 4 mM EGTA, 10 mM EDTA, 100 mM β -glycerophosphate, 40 mM NaF, 4 mM sodium vanadate, 15 mM sodium pyrophosphate, 1 mM PMSF (Sigma–Aldrich), 0.2% NP40, and a protease inhibitor cocktail (Roche, Mannheim, Germany). This procedure has been described in detail previously (Xu & Kang, 2017). After centrifugation at 20,000 rpm for 20 min, supernatants were quantified using the Bradford protein assay method (Bio-Rad Laboratories, Hercules, California, United States) and boiled with Laemmli sample buffer. Equal amounts of protein were separated using SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane (Whatman, Dassel, Germany). The membrane was blocked using a buffer containing 5% skim milk in TBS-T (0.1% Tween 20 in TBS) for 1 h at room temperature and then incubated with primary antibodies overnight at 4 °C. The membranes were then incubated with secondary antibodies for 1 h at room temperature. Primary antibodies against AMPK α , p-Thr-172-AMPK α , TAK1, p-Thr-184/187-TAK1, CaMK4, LKB1, p-Ser-79-ACC, ACC (Cell Signaling Technology, Danvers, Massachusetts, United States), p-Thr-196-CaMK4, p-Ser-431-LKB1, HMG-CoAR (Santa Cruz Biotechnology, Santa Cruz, California), p-Ser-872-HMG-CoAR (Abcam, Cambridge, Massachusetts), and β -actin (Sigma–Aldrich, St. Louis, Missouri, United States) were used at dilutions of 1:500 to 1:30,000. Anti-rabbit or mouse IgG were used as secondary antibodies (Jackson Immuno Research Laboratories Inc., West Grove, Pennsylvania, United States) at dilutions of 1:1000 to 1:2000. Signals were detected using an enhanced chemiluminescence system (Pierce Biotechnology, Rockford, Illinois, United States). Signals on developed X-ray film were quantified using the TINA 2.10G software package (Raytest, Straubenhardt, Germany).

Statistical analysis

Immunoblot results are expressed as relative optical density units (ODs) and presented as mean $\pm$ SE. Statistical differences were analyzed using one-way or two-way analysis of variance (ANOVA) followed by *post hoc* Tukey tests. All tests were performed using the SPSS for Windows software package (ver. 17.0; SPSS Inc., Chicago, Illinois, United States). *p* values of <0.05 were used to indicate statistical significance.

Results

Dose-dependent effects of ethanol on the phosphorylation of AMPK α and its upstream LKB1, CaMK4, and TAK1 in the prefrontal cortex

The phosphorylation of AMPK at Thr-172 was measured as a surrogate variable of its enzymatic activity (Hawley et al., 1996). Acute treatment with ethanol significantly changed p-Thr-172-AMPK α level in the prefrontal cortex [$F(3,20) = 5.988$, $p = 0.006$; Fig. 1A]. Follow-up pairwise comparisons revealed that the lower doses (0.5 or 1 g/kg) did not affect phosphorylation ($p = 0.992$; $p = 0.787$, respectively), but the highest dose (2 g/kg) decreased phosphorylation compared to the control ($p = 0.009$). The amount of total AMPK α was not affected by ethanol treatment at any dose level [$F(3,20) = 0.437$, $p = 0.729$; Supplementary Fig. 2A].

The activities of LKB1, CaMK4, and TAK1 were estimated by Ser-431, Thr-196, and Thr-184/187 phosphorylation, respectively (Sapkota et al., 2001; Swilius & Waxham, 2008). Acute treatment with ethanol affected the phosphorylation of CaMK4 [$F(3,20) = 9.935$, $p = 0.001$; Fig. 1B] and LKB1 [$F(3,20) = 5.916$, $p = 0.006$; Fig. 1C] levels. Subsequent analysis revealed that p-CaMK4 and p-LKB1 levels were only decreased following the highest dosage ($p = 0.002$; $p = 0.006$, respectively). The phosphorylation of TAK1 was not altered, regardless of dosage [$F(3,20) = 0.725$, $p = 0.552$; Fig. 1D]. Ethanol did not alter the amounts of CaMK4 [$F(3,20) = 0.318$, $p = 0.812$; Supplementary Fig. 2B], LKB1 [$F(3,20) = 0.877$, $p = 0.469$; Supplementary Fig. 2C], and TAK1 [$F(3,20) = 0.738$, $p = 0.542$; Supplementary Fig. 2D]. These data suggest that the change in p-AMPK level was mediated by LKB1 and CaMK4, but not by TAK1.

Dose-dependent effects of ethanol on the phosphorylation of AMPK α -downstream molecules, ACC, and HMG-CoAR in the prefrontal cortex

ACC activity was determined by Ser-79 phosphorylation (Mantovani & Roy, 2011; Ronnett, Ramamurthy, Kleman, Landree, & Aja, 2009). HMG-CoAR activity was determined by Ser-872 phosphorylation (Goldstein & Brown, 1990). Acute treatment with ethanol did not alter the p-Ser-79-ACC [$F(3,20) = 0.423$, $p = 0.739$; Fig. 2A] or the p-Ser-872-HMG-CoAR [$F(3,20) = 0.229$, $p = 0.875$; Fig. 2B] in the prefrontal cortex, regardless of dosage. Ethanol did not alter the amounts of ACC and HMG-CoAR [$F(3,20) = 0.702$, $p = 0.562$; $F(3,20) = 0.568$, $p = 0.643$, respectively; Supplementary Fig. 2E and 2F].

Dose- and time-dependent effects of chronic ethanol administration on the phosphorylation of AMPK α , LKB1, CaMK4, and TAK1 in the prefrontal cortex

Chronic treatment with ethanol altered p-Thr-172-AMPK levels dose- and time-dependently in the prefrontal cortex [$F(3,40) = 6.112$, $p = 0.002$; Fig. 3A]. Pairwise comparisons revealed that p-AMPK level was increased at 30 min after the last injection, regardless of dosage (0.5 g/kg: $p = 0.007$; 1 g/kg: $p = 0.001$; 2 g/kg: $p = 0.001$), but not at 24 h. These data indicate that in chronically ethanol-treated animals, further treatment with ethanol transiently increased p-AMPK level, but this effect vanished at 24 h. The amount of AMPK α was not affected by any dose of ethanol treatment at any time point [$F(3,40) = 0.521$, $p = 0.671$; Supplementary Fig. 3A].

Changes in the phosphorylation levels of CaMK4 [$F(3,40) = 7.752$, $p = 0.001$; Fig. 3B] and LKB1 [$F(3,40) = 6.092$, $p = 0.002$; Fig. 3C] were similar to those of AMPK itself. Pairwise comparisons revealed that p-CaMK4 (0.5 g/kg: $p < 0.001$; 1 g/kg: $p < 0.001$; 2 g/kg: $p = 0.005$) and p-LKB1 (0.5 g/kg: $p = 0.003$; 1 g/kg: $p = 0.001$; 2 g/kg: $p < 0.001$) were increased at 30 min after the last administration of ethanol. However, these differences were not observed at 24 h. In contrast, the phosphorylation of TAK1 was increased by chronic treatment with ethanol [$F(3,40) = 16.426$, $p < 0.001$; Fig. 3D] and the increased level was maintained even

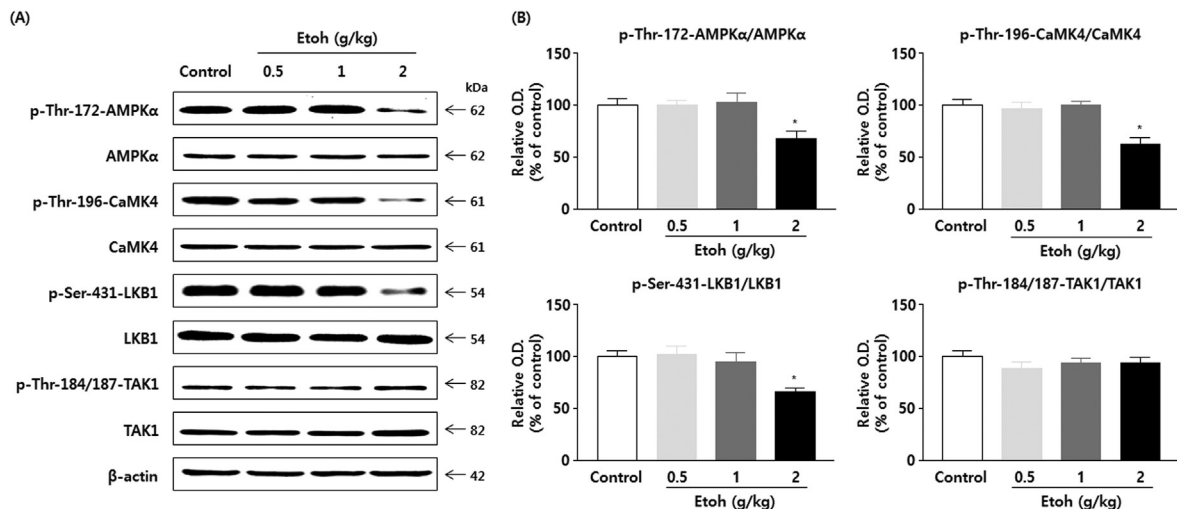


Fig. 1. Phosphorylation of AMPK and its upstream kinases CaMK4, LKB1, and TAK1 in the prefrontal cortex with acute ethanol treatment. (A) Representative immunoblots at 30 min following an acute treatment with saline or ethanol (0.5, 1, or 2 g/kg). (B) Quantification of the immunoblot data were expressed as the relative optical densities (ODs), which were normalized against their corresponding total protein signals. All data are presented as mean $\pm$ SE ($n = 6$ rats/group) and were analyzed using one-way analysis of variance (ANOVA). * significant difference compared to the control group.

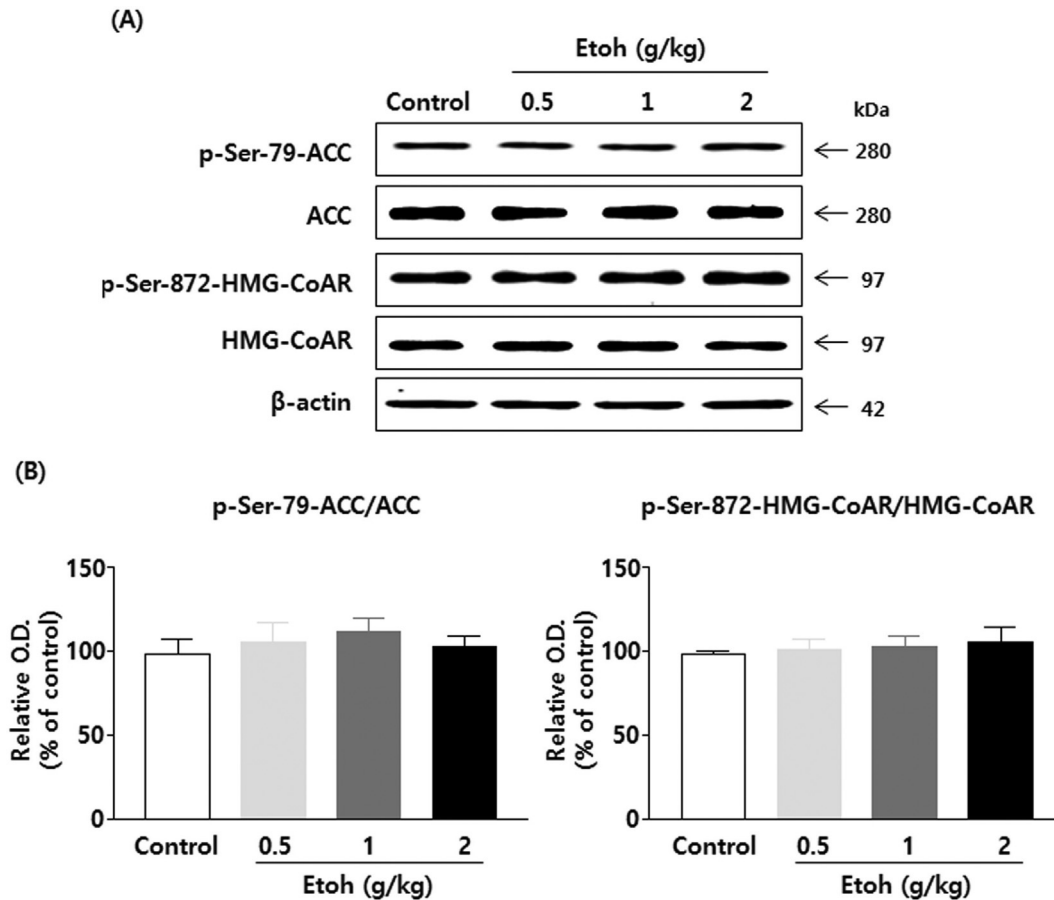


Fig. 2. Phosphorylation of AMPK downstream molecules ACC and HMG-CoAR in the prefrontal cortex with acute ethanol treatment. **(A)** Representative immunoblots at 30 min after the acute injection with ethanol (0.5, 1, or 2 g/kg). **(B)** Quantified immunoblot data were presented as mean $\pm$ SE ($n = 6$ rats/group) and were analyzed using one-way analysis of variance (ANOVA). * significant difference compared to the control group.

24 h after the last treatment (0.5 g/kg: $p = 0.002$; 1 g/kg: $p < 0.001$; 2 g/kg: $p < 0.001$ for 24 h). The amounts of CaMK4 [$F(3,40) = 0.687$, $p = 0.565$; [Supplementary Fig. 3B](#)], LKB1 [$F(3,40) = 0.685$, $p = 0.567$; [Supplementary Fig. 3C](#)], and TAK1 [$F(3,40) = 0.177$, $p = 0.912$; [Supplementary Fig. 3D](#)] did not differ across the subgroups. Therefore, p-Thr-184/187-TAK-1 seems to be continuously increased, as compared to the naïve state, under the daily treatment schedule. In short, the level of p-AMPK paralleled those of LKB1 and CaMK4 under the chronic treatment.

Dose- and time-dependent effects of chronic ethanol administration on downstream molecules Ser-79-ACC and Ser-872-HMG-CoAR in the prefrontal cortex

The levels of p-Ser-79-ACC [$F(3,40) = 6.598$, $p = 0.002$; [Fig. 4A](#)] and p-Ser-872-HMG-CoAR [$F(3,40) = 5.131$, $p = 0.005$; [Fig. 4B](#)] in the prefrontal cortex were altered in exactly the same manner as those of p-AMPK α and its upstream kinases, LKB1 and CaMK4, following chronic treatment with ethanol. Pairwise comparisons revealed that p-ACC (0.5 g/kg: $p < 0.001$; 1 g/kg: $p < 0.001$; 2 g/kg: $p < 0.001$) and p-HMG-CoAR (0.5 g/kg: $p < 0.001$; 1 g/kg: $p < 0.001$; 2 g/kg: $p = 0.002$) levels increased at 30 min after the last administration, but returned to control level at 24 h. The amounts of ACC and HMG-CoAR were not affected by any dose of ethanol treatment until 24 h [$F(3,40) = 1.539$, $p = 0.219$; $F(3,40) = 0.856$, $p = 0.472$, respectively; [Supplementary Fig. 3E and F](#)]. These

findings suggest that, in a chronic treatment schedule, ethanol could affect the phosphorylation of ACC and HMG-CoAR through AMPK signaling.

Discussion

Our findings revealed that chronic treatment (15 injections) with either low (0.5 or 1 g/kg) or high (2 g/kg) doses of ethanol increased the phosphorylation of Thr-172-AMPK in the prefrontal cortex. Unlike TAK1 phosphorylation, which was constantly elevated after chronic ethanol treatment, no long-lasting phosphorylation of AMPK was observed after chronic treatment. However, chronic treatment with ethanol made AMPK reactive to an additional acute treatment. In this state, each dosing of ethanol transiently phosphorylated the AMPK. We previously observed this phenomenon accompanied by locomotor sensitization ([Xu & Kang, 2019](#)). AMPK phosphorylation was not induced by ethanol in naïve animals but was induced in chronically ethanol-treated animals 30 min after an ethanol challenge. In that report, we tried only the lowest dose (0.5 mg/kg), because higher doses were not effective in inducing the locomotor sensitization. However, our current findings indicate that the sensitizing effect of ethanol to AMPK phosphorylation is maintained at higher dose ranges; this seems to be incompatible with the role of the AMPK system in terms of locomotor sensitization ([Xu & Kang, 2019](#)). Some independent inhibitory effects of ethanol on locomotor sensitization

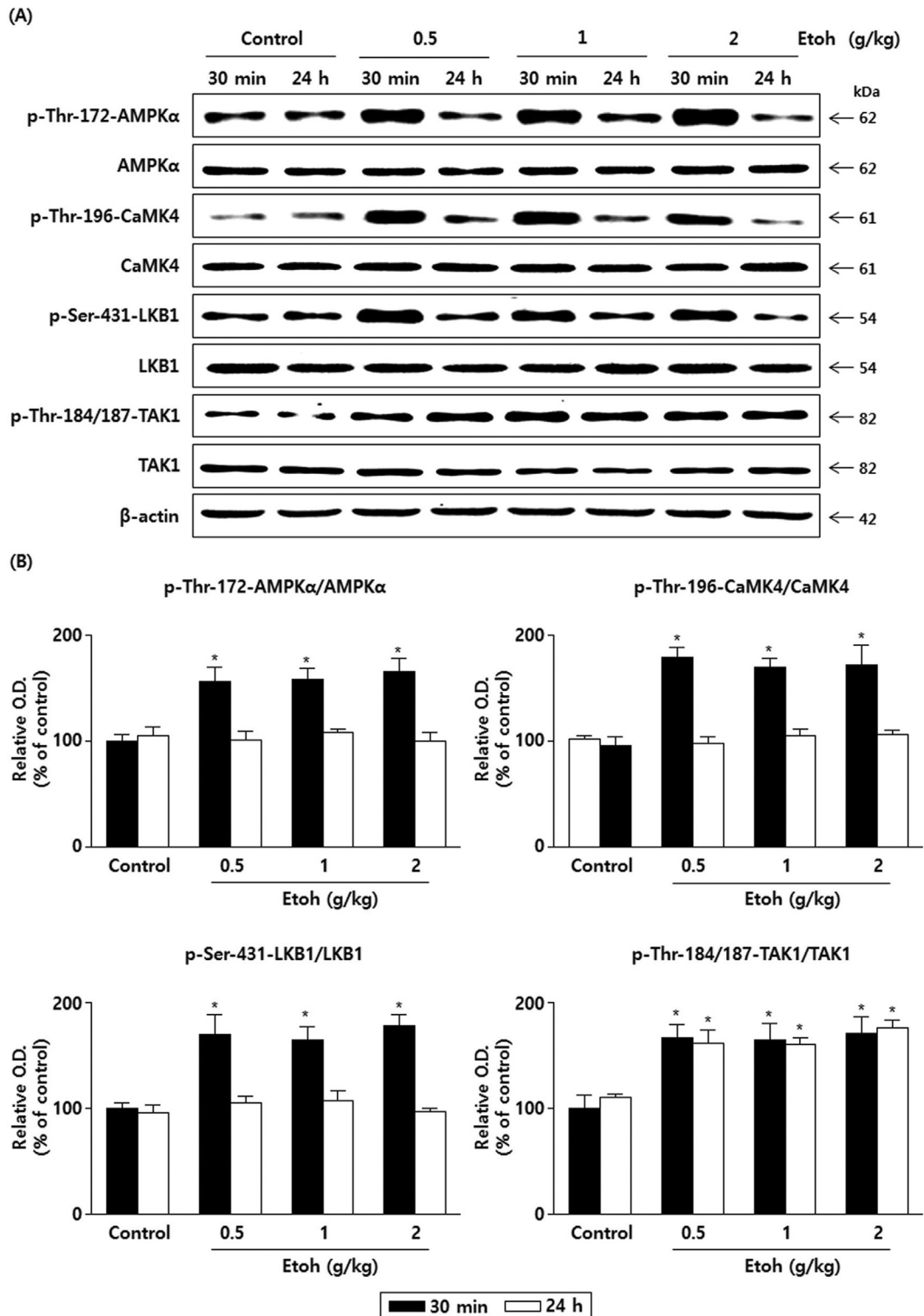


Fig. 3. Dose- and time-dependent changes in the phosphorylation of AMPK, CaMK4, LKB1, and TAK1 in the prefrontal cortex with chronic ethanol treatment. (A) Representative immunoblots at 30 min and 24 h following the administration of saline or ethanol (0.5, 1, or 2 g/kg) in chronically ethanol-treated animals. (B) Quantified immunoblot data were presented as mean $\pm$ SE ($n = 6$ rats/group) and were analyzed using one-way analysis of variance (ANOVA). * significant difference in each immunoreactivity value compared to the control group.

may occur at higher doses of ethanol, which is related to the depressant effect of ethanol. In terms of AMPK phosphorylation, the important factor was not the dosage but, rather, the duration of treatment.

A single administration of ethanol decreased the phosphorylation of Thr-172-AMPK at the high dose (2 g/kg) but not at the lower doses (0.5 or 1 g/kg). Acute treatments with high doses (2 or 4 g/kg) of ethanol attenuate the evoked dopamine release in the prefrontal

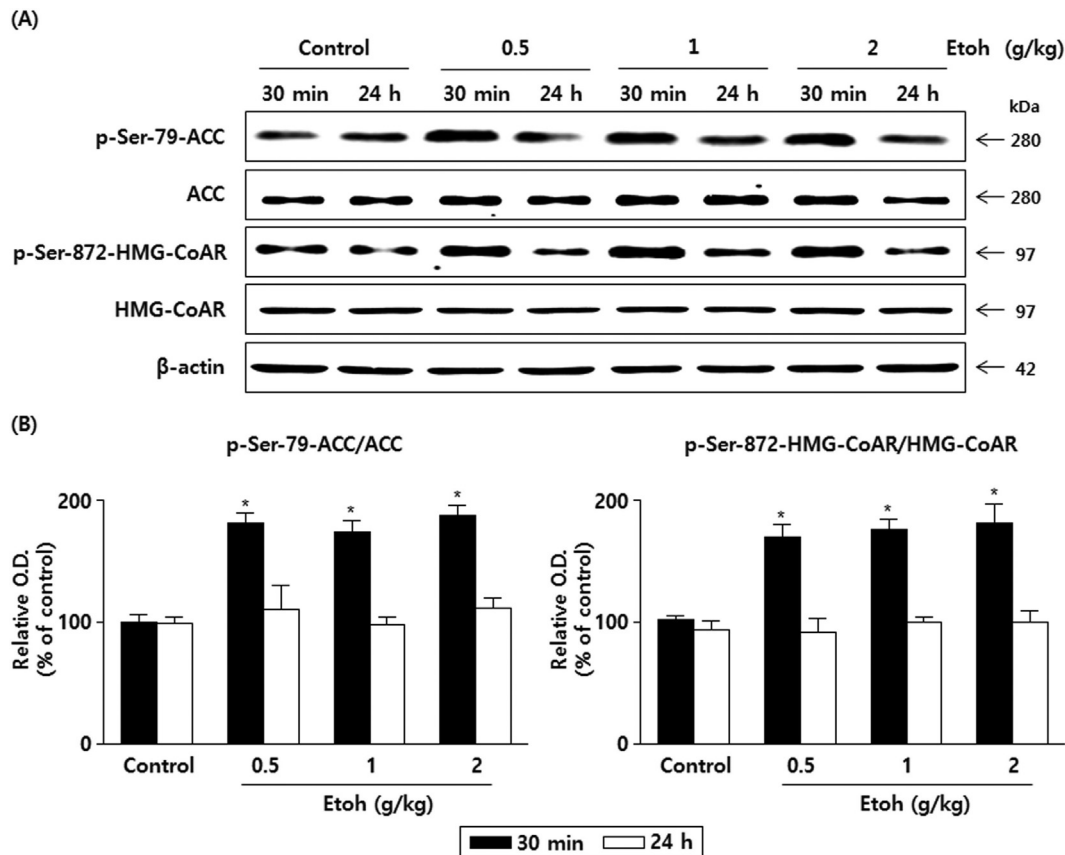


Fig. 4. Dose- and time-dependent changes in the phosphorylation of ACC and HMG-CoAR in the prefrontal cortex with chronic ethanol treatment. **(A)** Representative immunoblots at 30 min and 24 h following the administration of saline or ethanol (0.5, 1, or 2 g/kg) in chronically ethanol-treated animals. **(B)** Quantified immunoblot data were presented as mean $\pm$ SE ($n = 6$ rats/group) and were analyzed using one-way analysis of variance (ANOVA). * significant difference in each immunoreactivity value compared to the control group.

cortex (Shnitko, Kennerly, Spear, & Robinson, 2014). The phosphorylation of the AMPK system is indirectly affected by dopamine. In the prefrontal cortex, where dopamine D₁ receptors are abundant relative to dopamine D₂ receptors, the dopamine reuptake inhibitor, cocaine, increased the phosphorylation of AMPK (Xu & Kang, 2016). Therefore, attenuated dopamine signaling seems to be related to the decreased phosphorylation of AMPK system molecules by high-dose ethanol, which has depressant properties in the prefrontal cortex. Although chronic ethanol also decreases DA release in the medial prefrontal cortex (Fadda, Mosca, Colombo, & Gessa, 1989; Okamoto, Harnett, & Morikawa, 2006), it is accompanied by a reduction of D₂ receptor function (Kroener et al., 2012; Trantham-Davidson et al., 2014), and the net effect is increased cyclic-AMP (cAMP) and protein kinase A (PKA)-mediated signaling. The phosphorylation of LKB1 and CaMK4 are mediated by PKA- or cAMP-dependent pathways (Sapkota et al., 2001; Swulius & Waxham, 2008).

Ethanol suppresses the phosphorylation of AMPK and ACC in the developing mouse brain (Saito et al., 2007). However, in our paradigm of adult animals chronically receiving ethanol, AMPK and ACC were activated. ACC is generally considered to be the rate-limiting enzyme in fatty acid biosynthesis (Carling, Zammit, & Hardie, 1987; Ronnett et al., 2006), and the profile of ACC activity suggests that brain lipid homeostasis is affected by ethanol only under the condition of chronic use. Very recently, it has been reported that the hippocampal AMPK-ACC pathway is activated by chronic nicotine use but is normalized at 24 h after cessation of nicotine (Brynnildsen, Lee, Perron, Jin, Kim & Blendy, 2018), which is a pattern similar to

the alterations in prefrontal AMPK-ACC pathway activity with ethanol or cocaine treatments in our previous (Xu & Kang, 2019) and current studies. The increased phosphorylated AMPK levels were suggested to be associated with reduction of negative affect (e.g., anxiety) following nicotine withdrawal (Brynnildsen et al., 2018). More precise determinations on the effects of ethanol-induced alterations in prefrontal AMPK activity on withdrawal or other addictive symptoms would be an interesting goal for future studies.

Previous research suggests that cholesterol may modulate neurotransmitter uptake or release in the addicted brain. For example, cholesterol depletion significantly reduces dopamine transporter (DAT) functioning (Adkins et al., 2007; Foster, Adkins, Lever, & Vaughan, 2008; Jones, Zhen, & Reith, 2012) and, thus, may enhance dopaminergic signaling and, theoretically, make the subject vulnerable to the development of addiction (Kahlig & Galli, 2003; Zhu & Reith, 2008). Consumption of ethanol could reduce the availability of dopamine transporter (Yen, Shih, Cheng, Ma, Lu, & Huang, 2016). Our findings demonstrated that when subjects were chronically treated with ethanol, each dosing of ethanol transiently increased HMG-CoAR activity, thereby increasing cholesterol biosynthesis. Thus, the present findings are compatible with the hypothesis that increased AMPK and HMG-CoAR activities serve as processes of neuroadaptation that compensate for dysfunction in dopamine transporter.

In conclusion, our experiments demonstrated that repeated treatment with ethanol at either low or high doses significantly increased the phosphorylation of HMG-CoAR and ACC. These

increases were accompanied by a parallel increase in the phosphorylation of the upstream molecule AMPK. Among the upstream kinases of AMPK, the phosphorylation of LKB1 and CaMK4, but not of TAK1, paralleled the phosphorylation of AMPK, HMG-CoAR, and ACC. These findings suggest that chronic ethanol treatment affects the regulatory systems of lipid metabolism via the AMPK pathway in the rat prefrontal cortex.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant (NRF-2019R1C1C1008342 to S.X.) funded by Ministry of Science and ICT, South Korea. Support was also provided by the KBRI basic research program (19-BR-02-05 to J.W.K.) and the Brain Research Program and Biomedical technology development program (NRF-2017M3C7A1048089 to J.W.K.) funded by Ministry of Science and ICT, South Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alcohol.2019.11.004>.

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