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Short communication

Role of $GABA_A$ receptors in the ethanol-mediated inhibition of extracellular signal-regulated kinase

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Abstract

In the present study, we demonstrate the involvement of $GABA_A$ receptors in the ethanol-mediated modulation of extracellular signal-regulated kinases (ERK). Intraperitoneal (i.p.) administration of ethanol (3.5 g), flurazepam (75 mg) or (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cycloheptane-5,10-iminemaleate (MK-801) (0.4 mg/kg body weight) decreased, while picrotoxin (10 mg/kg body weight) increased, the phosphorylation of ERK following 10 min of their injection. However, the picrotoxin-induced phosphorylation of ERK was inhibited by ethanol, but was not affected by MK-801. These results indicate that ethanol's inhibitory effect on ERK phosphorylation may involve the modulation of $GABA_A$ receptor function.

Keywords: Ethanol; GABA (γ-amino butyric acid); MAP (mitogen-activated protein) kinase

1. Introduction

Signal transduction has been identified as a major target of ethanol's action in neural and nonneural tissues. Ethanol affects protein kinase A (Dohrman et al., 1996), protein kinase C (Messing et al., 1991), tyrosine kinases (Miyakawa et al., 1997) and a variety of other signaling pathway(s) that are implicated in the growth, development and maturation of the central nervous system. Recent observations from several laboratories have demonstrated the regulation of mitogen-activated protein (MAP) kinase phosphorylation by ethanol in a variety of preparations (Hendrickson et al., 1998; Arabi et al., 1999; Kalluri and Ticku, 2000). Extracellular signal-regulated kinase(s) are members of mitogen-activated protein kinase family that are involved in the transduction of extracellular signals into intracellular responses. Stimulation by growth factors (Yamada et al., 1997), neurotransmitters (Kurino et al., 1995), phorbol esters (Zhang et al., 1998), electroconvulsive shock (Barban et al., 1993) and depolarization (Rosen et al., 1994) induces the phosphorylation of MAP kinase(s). Although several reports have shown the modulation of MAP kinase by ethanol (Seiler et al., 2001; Kalluri and Ticku, 2002), the mechanism of ethanol's modulatory effect is not known. In this report, we investigated the mechanism of ethanol's action on the MAP kinase phosphorylation in mouse brain by using phospho-specific antibodies for MAP kinase (phospho-p44/42), and normalized the data using MAP kinase (p44/42) (Pan) antibodies. We used MAP kinase/extracellular signal-regulated kinase (ERK) 1/2 interchangeably.

2. Materials and methods

2.1. Animals and tissue preparation

C57BL/6CR mice were purchased from Harlan (Indianapolis, IN) and housed in a room maintained at a constant temperature of 22 ± 1 °C with free access to food and water and kept on a 12-h light/12-h dark cycle. Briefly, absolute ethanol was diluted in saline (25% w/v) and injected (3.5 g/kg body weight) intraperitoneally (i.p.) into mice. Likewise, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cycloheptane-5,10-iminemaleate (MK-801) (0.4 mg/kg), flurazepam (75 mg/kg) and/or picrotoxin (10 mg/kg) were dissolved in water and injected into mice. Mice were sacrificed 10 min following drug administration and their cerebral cortices were homogenized in lysis buffer (20 mM Na_2HPO_4, pH 7.0, 50 mM NaF, 10 mM $Na_4P_2O_7$, 150 mM NaCl, 5 mM

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EGTA, 5 mM EDTA, 2% Triton X-100, 1 mM Na_3VO_4, 0.5% deoxycholate, 1 mM phenyl methyl sulfonyl fluoride and 1 mg/ml bacitracin), centrifuged at 13,000 rpm in an Eppendorf centrifuge for 10 min to clarify lysate. All of the experimental procedures were performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Electrophoresis and immunoblotting

Thirty micrograms of protein (Lowry et al., 1951) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) and transferred to polyvinylidene difluoride membrane (Towbin et al., 1979) followed by blocking in 5% milk in Tris-buffered saline containing 0.1% Tween 20 and overnight incubation with primary antibody phospho-ERK1/ERK2 (1:2000) or p42/44 MAP kinase (1:1000) (New England Biolabs). After several washes, peroxidase-coupled secondary antibody (anti-mouse IgG/anti-rabbit IgG; New England Biolabs) was added and incubated for 1 h. The membrane was washed and specific bands were visualized using super signal detection reagents (Pierce, Rockford, IL). Immunoblot band intensity was measured by NIH image system. Data was analyzed by analysis of variance (ANOVA) supplemented with Duncan's multiple range test. Differences were considered to be statistically significant when $P < 0.05$. The values are represented as means ± S.E.

3. Results

Intraperitoneal injection of ethanol or flurazepam decreased the activity and the animals were sedated. In

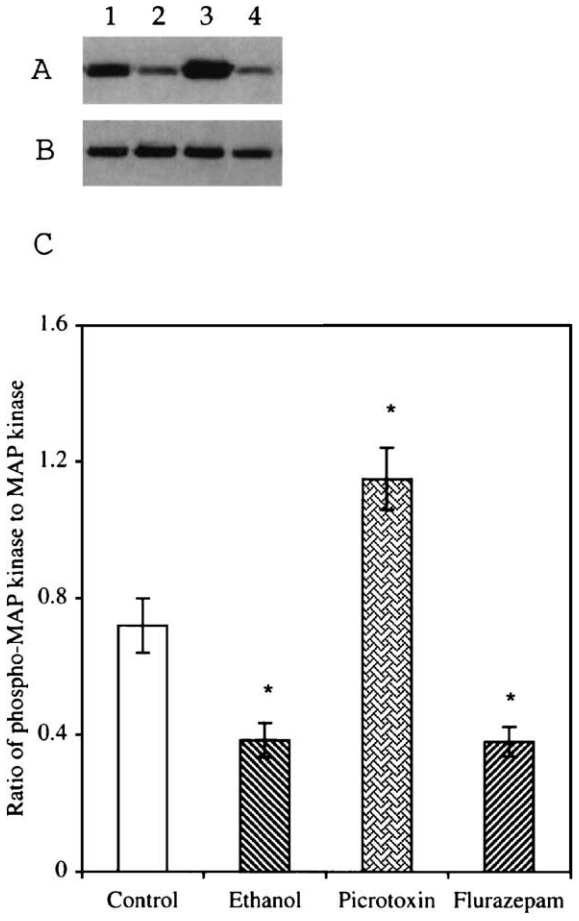

Fig. 1. The effect of ethanol, flurazepam and picrotoxin on the phosphorylation of MAP kinase. Ethanol (3.5 g/kg), flurazepam (75 mg/kg) or picrotoxin (10 mg/kg) were injected into mice, which were sacrificed after 10 min and whose cortices were processed as described in Section 2. Thirty micrograms of lysate was subjected to Western blotting and probed with phospho-MAP kinase and MAP kinase antibodies. The data are represented as means ± S.E. of three experiments and analyzed by ANOVA. * $P < 0.05$ as compared to control. (A) Phospho-MAP kinase. (B) MAP kinase. (C) Ratio of phospho-MAP kinase to MAP kinase. (1) Control. (2) Ethanol. (3) Picrotoxin. (4) Flurazepam.

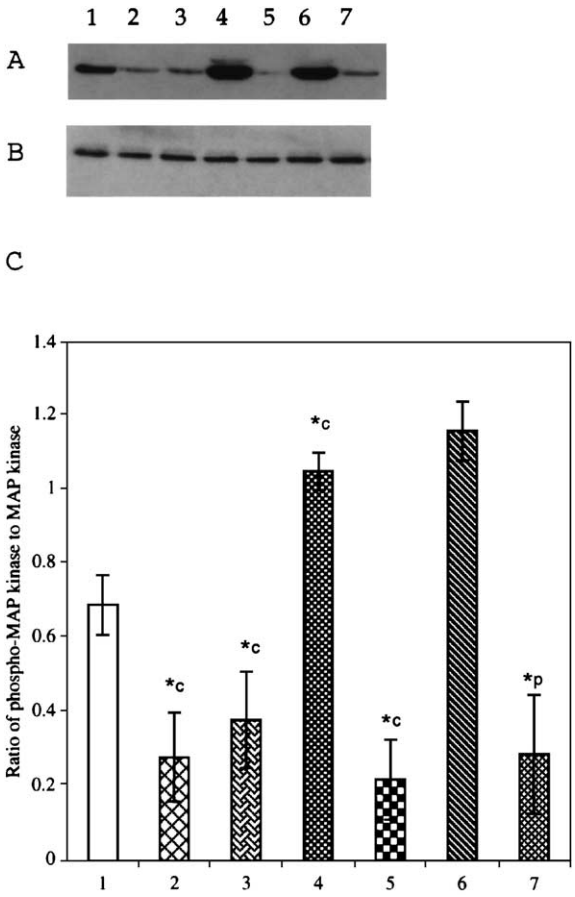

Fig. 2. The effect of ethanol or MK-801 administration on the picrotoxin-induced phosphorylation of MAP kinase. Ethanol (3.5 g/kg) and MK-801 (0.4 mg/kg) were intraperitoneally injected into mice alone or in conjunction with picrotoxin. Mice were sacrificed after 10 min and their cortices were processed as described in Section 2. The data shown are representative means ± S.E. of three individual experiments and analyzed by ANOVA. * c $P < 0.05$ as compared to control. * p $P < 0.01$ as compared to picrotoxin. (A) Phospho-MAP kinase. (B) MAP kinase. (C) Ratio of phospho-MAP kinase to MAP kinase. (1) Control. (2) Ethanol. (3) MK-801. (4) Picrotoxin. (5) Ethanol + MK-801. (6) MK-801 + Picrotoxin. (7) Ethanol + Picrotoxin.

contrast, picrotoxin induced seizures within 10 min following its administration. However, administration of MK-801 did not show any behavioral symptoms at the dose tested. Concomitant administration of ethanol and picrotoxin delayed the onset of seizures, which is consistent with earlier findings (Rastogi and Ticku, 1986; Kulkarni et al., 1990). Immunoblot analysis revealed the inhibition of MAP kinase phosphorylation by ethanol or flurazepam, whereas picrotoxin induced the phosphorylation of MAP kinase (Fig. 1). Administration of ethanol in conjunction with either MK-801 or picrotoxin decreased the phosphorylation of MAP kinase while MK-801 could not alter the picrotoxin-induced phosphorylation of MAP kinase (Fig. 2).

4. Discussion

It is well documented that acute ethanol can inhibit *N*-methyl-D-aspartate (NMDA) receptors and potentiate GABA receptor function. Studies showing the activation of MAP kinase by Ca^{2+} via NMDA receptors or voltage-dependent calcium channels have been very well documented (Orban et al., 1999; Rosen et al., 1994; Xia et al., 1996). However, both these channels are sensitive to voltage, hence, their gating can also be regulated by GABA receptors, whose activity hyperpolarizes neurons. Since ethanol potentiates GABA receptors, it is reasonable to speculate their role in the modulatory effect of ethanol. Recently, we have demonstrated a decrease in the phospho-MAP kinase staining by ethanol, MK-801 and flurazepam following their administration (Kalluri and Ticku, 2002). Since ethanol also decreased the phosphorylation of MAP kinase, we speculated a role for either NMDA receptors or GABA receptors. Hence, in the present work, we used MK-801 and ethanol in conjunction with picrotoxin to elucidate the mechanism of ethanol's action on the phosphorylation of MAP kinase in the cerebral cortex of mouse brain. Intraperitoneal injection of MK-801 and ethanol together decreased the phospho-MAP staining. Co-administration of ethanol and picrotoxin decreased the phosphorylation of MAP kinase following 10 min of their administration, while MK-801 could not alter the picrotoxin-induced phosphorylation of MAP kinase (Fig. 2). These data indicate that ethanol may inhibit MAP kinase phosphorylation by activating GABA receptors rather than inhibiting NMDA receptors. In cultured cortical neurons, the MAP kinase activation by picrotoxin was demonstrated to be due to the synaptic activation of the neurons (Fiore et al., 1993). Hence, our results indicate that the inhibition of MAP kinase phosphorylation by ethanol could be due to the inhibition of synaptic activity following the potentiation of GABA receptor function. However, it is not clear whether ethanol's action on MAP kinase is due to its potentiation of GABA receptor function or due to some other effect on MAP kinase signaling.

These results demonstrate, for the first time, that potentiation of GABA receptor activity following acute ethanol ingestion may have a negative regulatory role on the MAP kinase pathway.

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