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Involvement of metabotropic glutamate receptor 5 in ethanol regulation of NMDA receptor activity in rat substantia gelatinosa neurons

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ABSTRACT

Aims: Glutamatergic receptors are important targets of ethanol. Intake of ethanol may produce analgesic effects. The present study examined the effects of ethanol on the activity of ionotropic glutamate receptors in spinal cord substantia gelatinosa (SG) neurons, critical neurons involved in nociceptive transmission.

Main methods: Whole-cell recordings were made from SG neurons of the lumbar spinal cord slices from 15 to 20-day-old rats. Ethanol and glutamate receptor agonists or antagonists were applied by superfusion.

Key finding: Ethanol (50 and 100 mM) applied by superfusion for 5 min dose-dependently decreased the amplitude of evoked excitatory postsynaptic potential in SG neurons. Superfusion of ethanol (100 mM) for 15 min consistently inhibited NMDA- or AMPA-induced depolarizations in SG neurons. Ethanol (100 mM) also inhibited the depolarizations induced by glutamate. However, ethanol inhibition of glutamate-induced responses significantly decreased at 10–15 min following continuous superfusion, suggesting the development of acute tolerance to the inhibition during prolonged exposure. Application of MPEP hydrochloride (an antagonist of metabotropic glutamate receptor [mGluR] 5) or GF109203X (a protein kinase C [PKC] inhibitor), together with ethanol significantly blocked the tolerance. The inhibition by ethanol of the NMDA-induced, but not AMPA-induced, depolarizations significantly decreased at 15 min during continuous superfusion while ACPD (a mGluR agonist) was co-applied with ethanol.

Significance: The results suggest that (1) ethanol exposure may inhibit ionotropic glutamate receptor-mediated neurotransmission; (2) regulation of NMDA receptor function by mGluR5/PKC pathways may be involved in the development of the tolerance to ethanol inhibition of glutamate-induced responses during prolonged exposure in SG neurons.

1. Introduction

The amino acid glutamate is the primary neurotransmitter mediating fast excitatory neurotransmission in the central nervous system. Glutamate receptors divided into two major categories: ionotropic glutamate receptors (iGluRs) such as NMDA and AMPA receptors are cation channels; metabotropic glutamate receptors (mGluRs) are linked to cytoplasmic enzymes through G proteins. Ethanol is a central nervous system depressant. Many studies have reported that iGluRs and mGluRs are important target of ethanol and it regulates the function of glutamate receptors *in vivo* and *in vitro* [1–3]. Among all of the glutamate receptors, NMDA receptors are the most prominent targets to ethanol because of their crucial role in synaptic plasticity [4]. Acute application of ethanol at pharmacologically relevant concentrations inhibited NMDA receptor function in many brain regions [5–7]. However, chronic ethanol treatment may result in increases in NMDA receptor function, which is thought to be involved in the development of ethanol tolerance and subsequent withdrawal hyperexcitability [7,8]. Besides, several studies *in vitro* and *in vivo* showed a reduction in ethanol inhibition of NMDA receptor function during or after acute ethanol exposure in the neurons of spinal cord, medulla, hippocampus, and locus coeruleus [6,9–12]. This phenomenon is known as acute tolerance or tachyphylaxis and might represent a short-term adaptation of receptor function to the effects of ethanol. Activation of kinases during prolonged ethanol leading to changes in the phosphorylation status of NMDA receptor subunits may participate in the development of acute tolerance to ethanol inhibition of NMDA-mediated responses in central sympathetic neuron [13,14].

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Evidence indicates that mGluRs especially group I mGluRs such as mGluR5, play an important role in ethanol addiction and ethanol-induced neurobiological effects [15]. Electrophysiological studies have demonstrated that activation of Group I mGluR potentiated NMDA-mediated responses in cultured neurons or brain slices [16,17]. Thus, Group I mGluRs and the underlying signaling cascades may play a role in ethanol regulation of iGluR function during ethanol exposure. An electrophysiological study showed that a non-specific mGluR antagonist decreased acute tolerance to alcohol inhibition of glutamate-evoked currents in spinal cord motor neurons *in vitro* [18], suggesting the involvement of mGluRs in ethanol regulation of the function of iGluRs.

Studies on several animal nociceptive models and humans have demonstrated the analgesic effects of ethanol [19,20]. Neurons of the superficial spinal dorsal horn particularly those on substantia gelatinosa (SG) play an important role in nociceptive transmission. Glutamate, released from primary afferent fibers, is the fast transmitter mediating the activity of SG neurons by acting on iGluRs [21]. Besides, group I mGluRs such as mGluR5 are involved in the regulation of the synaptic transmission and plasticity in the spinal dorsal horn [22]. As far as we know, no documents examined the effect of ethanol on the function of iGluRs in SG neurons. This study aimed to examine the effects on ethanol on the function of iGluRs and the role of mGluRs, which is coupled to activation of PKC, on ethanol effects in SG neurons.

2. Materials and methods

2.1. Animals

A breeding colony of Sprague–Dawley rats purchased from BioLASCO Co., LTD. (Taipei, Taiwan) was established at the Laboratory Animal Center, Tzu Chi University. Immature rats (14–20 day old) of either sex were selected from the colony for use in the present study. All experimental procedures were carried out under the guidelines of the Institutional Animal Care and Use Committee of Tzu Chi University.

2.2. Slice preparations and whole-cell recording techniques

The procedures used in obtaining transverse spinal cord slices from immature rats and in the whole-cell recording were similar to those described previously [23,24]. Coronal 500-μm sections from spinal cord lumbar segments were prepared with a Vibratome (1000, Ted Pella, Inc., Redding, CA, U.S.A.). The slices were incubated in a holding chamber containing Krebs solution at room temperature for at least 2 h. The Krebs solution consists of (in mM) 117 NaCl, 2.0 KCl, 1.2 KH_2PO_4, 2.3 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$, and 10 glucose, which was saturated with 95% O_2 and 5% CO_2. A slice was transferred to a recording chamber (RC-22, Warner Instrument Corp., Hamden, CT, U.S.A.) and was continuously perfused with Krebs solution saturated with 95% O_2 and 5% CO_2. The flow rate of the Krebs solution was kept constant at 2–3 ml/min. Recording electrodes, filled with the following intracellular solution (in mM): 130 K^+ gluconate, 1 $MgCl_2$, 2 $CaCl_2$, 4 ATP, 10 EGTA, and 10 HEPES, had a resistance of 3–5 MΩ. The pH of the intracellular solution was adjusted to 7.2 with KOH.

Whole-cell recordings were made from neurons located in the area of superficial dorsal horn under current-clamp mode with the use of an Axoclamp 2B at room temperature (25 ± 1 °C). Hyperpolarizing current pulses (−0.01 nA) of constant amplitude with 200 ms duration were injected into the neurons recorded at a frequency of 0.2 Hz. Signals were displayed on Tektronix digital oscilloscope (TDS 360). The potentials were also sent to a data acquisition system (DigiPack 1200B and pClamp 7.0, Axon Instruments, Inc.) for continuous recording. To study the effects of ethanol on excitatory postsynaptic potential (EPSP), a bipolar stimulating electrode was placed on the dorsal root entry zone for activation of primary afferents; $GABA_A$ receptor antagonist bicuculline (10 μM) and glycine receptor blocker strychnine (1 μM) were included in the Krebs solution to minimize the interference from inhibitory synaptic responses. SG neurons with a short-latency (0.8–2 ms) EPSP that responded to electric stimulation at 0.1 Hz were selected for further study. For examining the effects of ethanol on excitatory amino acid-mediated responses, glutamate (400 μM), NMDA (100 μM), and AMPA (10 μM) dissolved in Krebs solution were applied every 5 min by superfusion for 5–10 s to induce a 10–20 mV depolarization [25]. Tetrodotoxin (0.3 μM) was added in the superfusion solution throughout to inhibit the spontaneous firing of neurons. Ethanol was applied by superfusion when the amplitudes of excitatory amino acid-induced depolarizations were constant over two consecutive tests. To evaluate the effects of mGluR modulators and kinase inhibitors on ethanol actions, these agents at known concentrations were given in combination with ethanol by superfusion. The reagents were applied by changing superfusion solution using a valve controller (VC-6, Warner Instrument Corp.).

2.3. Chemicals and statistical data analysis

Ethanol was purchased from Riedel-de Haen (Deisenhofen, Germany). Glutamate, NMDA, AMPA, and chemicals for electrophysiology were purchased from Sigma Co. (St. Louis, Missouri, USA). Tetrodotoxin citrate was from Alomone Laboratories (Jerusalem, Israel). The mGluR modulators, MPEP hydrochloride (MPEP), *(RS)*-APICA, *trans*-ACPD, and PKC inhibitor, GF109203X, were bought from Tocris (Brisol, UK).

The statistical evaluation of ethanol effects on EPSP and excitatory amino acid-induced depolarization were analyzed using repeated-measures ANOVA followed by Dunnett's post-test. The effects of mGluR modulators and kinase inhibitors on ethanol actions at different times were analyzed using two-way ANOVA followed by Bonferroni post-test. $P < 0.05$ is considered to be statistically significant. Data were expressed as means ± SEM, and were plotted and analyzed statistically with GraphPad Prism version 6.03 for Windows (GraphPad Software, La Jolla, CA).

3. Results

3.1. Effects of ethanol on excitatory postsynaptic potentials in SG neurons

The SG neurons were held at resting membrane potentials, being 52.5 ± 0.5 mV ($n = 77$, range − 42.8 ~ −66.2 mV), without a correction of liquid junction potential. Superfusion of ethanol (100 mM) for 5 min had no effects on the threshold for action potential firing and the amplitude of action potential evoked by injection of positive current pulses sufficient to reach threshold ($n = 4$). Superfusion of ethanol (50 and 100 mM) for 5 min significantly decreased the peak amplitude of EPSPs in SG neurons examined. The inhibition of EPSPs by ethanol was reversible upon washout (Fig. 1). Repeated-measures one-way ANOVA shows a significant time effect with $F_{(1,4)} = 7.768$, $p = 0.0495$ in ethanol 50 mM group and $F_{(1,10)} = 19.23$, $p = 0.0012$ in ethanol 100 mM group.

3.2. Effects of ethanol on excitatory amino acid-induced depolarizations in SG neurons

Superfusion of ethanol (100 mM) for 15 min, which caused no significant changes in membrane potentials, decreased NMDA- or AMPA-induced depolarizations in SG neurons tested. Ethanol consistently inhibited NMDA- and AMPA-induced depolarization during continuous superfusion. The inhibitory effect of ethanol was reversed within 5 min upon washout (Fig. 2A, B, $F_{(5,20)} = 19.22$, $p < 0.0001$ in NMDA-treated group and $F_{(5,20)} = 27.26$, $p < 0.0001$ in AMPA-treated group, repeated-measures one-way ANOVA). Superfusion of ethanol also inhibited glutamate-induced depolarizations. However, ethanol inhibition of glutamate-induced depolarization decreased over time. Ethanol inhibited glutamate-induced depolarizations initially, but the inhibition

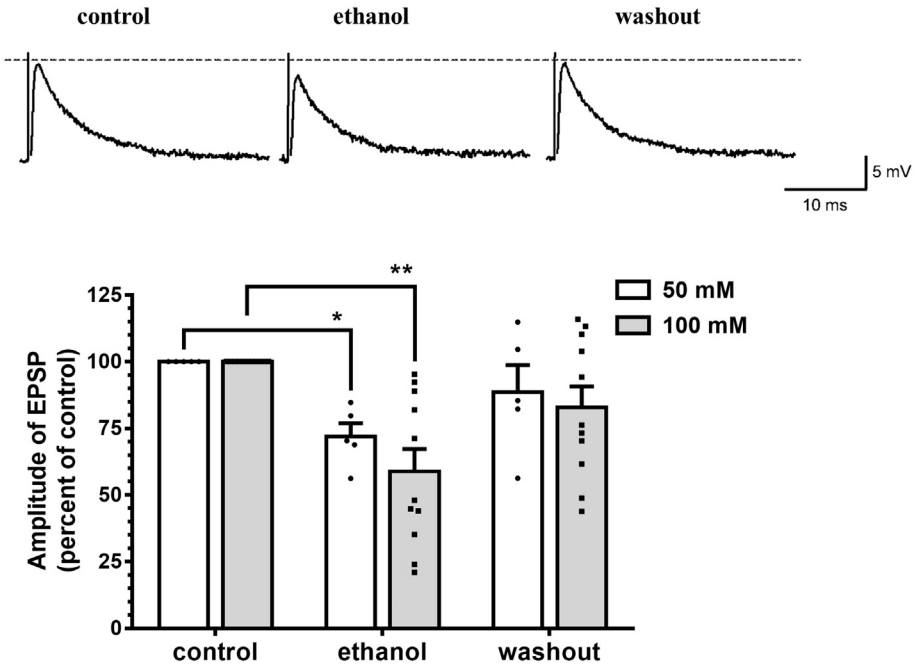

Fig. 1. Effects of ethanol on EPSPs in SG neurons. Top panel: Representative traces show averaged EPSPs (eight consecutive signals) taken before the application of ethanol (left), superfusion of 100 mM ethanol for 5 min (middle), and 5 min after removal of ethanol (right) in an SG neuron. The EPSPs were elicited by electrical stimulation of dorsal root entry zone in the presence of bicuculline (10 μM) and strychnine (1 μM) in Kreb's solution. Bottom panel: Bar graph shows percentage changes in amplitude of EPSPs following superfusion of 50 mM and 100 mM ethanol for 5 min. The peak amplitude of EPSPs prior to applications of ethanol is taken as control (100%). Values are mean ± S.E.M. from 5 and 11 SG neurons treated with 50 mM ethanol and 100 mM ethanol, respectively. *$p < 0.05$ and **$p < 0.01$ compared with control using repeated-measures one-way ANOVA followed by Dunnett's post-test.

was reduced at 15 min during superfusion of ethanol, suggesting the development of acute tolerance to ethanol inhibition during prolonged exposure (Fig. 2C, $F_{(5,20)} = 82.98$, $p < 0.0001$, repeated-measures one-way ANOVA).

3.3. Effects of mGluR modulators and PKC inhibitors on tolerance to ethanol inhibition of glutamate-induced responses

Because the application of glutamate would activate both ionotropic and metabotropic glutamate receptors, we hypothesize that metabotropic glutamate receptors play a role in the development of the tolerance. Superfusion with MPEP (50 nM), a mGluR5 antagonist, or *(RS)*-APICA (50 μM), a group II mGluR antagonist for 20 min had no noticeable effects on glutamate-induced depolarizations in SG neurons ($n = 3$, for each group). Ethanol consistently inhibited glutamate-induced depolarizations during the exposure of ethanol when MPEP (50 nM) was applied together with ethanol, suggesting that inhibition of mGluR5 pathways blocked the development of the tolerance (Fig. 3A, $F_{(5,20)} = 82.98$, $p < 0.0001$ in ethanol alone group, $F_{(5,10)} = 110.90$, $p < 0.0001$ in MPEP 10 nM group and $F_{(5,20)} = 53.84$, $p < 0.0001$ in MPEP 50 nM group, repeated-measures one-way ANOVA). Two-way ANOVA indicates a significant dose effect between three tested groups with $F_{(2,60)} = 9.205$, $p = 0.003$. Treatment with *(RS)*-APICA had no effects on the development of tolerance; ethanol inhibition of glutamate-induced depolarizations was reduced at 15 min (Fig. 3B, $F_{(5,20)} = 32.67$, $p < 0.0001$, in *(RS)*-APICA 50 μM group, repeated-measures one-way ANOVA). Given that activation of mGluR5 will enhance the NMDA receptor-mediated response *via* PKC, we evaluated a specific PKC inhibitor, GF109206X, on ethanol inhibition of glutamate-induced depolarization. Superfusion with GF109206X (500 nM, $n = 3$) for 20 min had no significant changes in glutamate-induced depolarizations in SG neurons. However, ethanol still retained its inhibitory effect on glutamate-induced depolarizations during the exposure of ethanol with GF109206X (Fig. 4), suggesting that inhibition of PKC pathways blocked the development of the tolerance. Repeated-measures one-way ANOVA shows a significant time effect with $F_{(5,15)} = 24.60$, $p < 0.0001$ in GF 109203 × 100 nM group and F (5,20) = 43.40, p < 0.0001 in GF 109203 × 500 nM group. Two-way ANOVA indicates a significant dose effect between three tested groups with $F_{(2,66)} = 3.999$, $p = 0.0229$.

3.4. Effects of mGluR agonists on ethanol inhibition of NMDA- and AMPA-induced responses

Ethanol inhibition of NMDA-induced depolarizations was significantly reduced at 15 min during the superfusion of ethanol in combination with trans-ACPD (15 μM), a mGluR agonist, suggesting the development of tolerance to ethanol inhibition of NMDA-induced depolarizations (Fig. 5A). Treatment with trans-ACPD (15 μM) had no significant effects on ethanol inhibition of AMPA-induced depolarizations (Fig. 5B). Repeated-measures one-way ANOVA shows a significant time effect with $F_{(5,20)} = 57.72$, $p < 0.0001$ and $F_{(5,20)} = 45.26$ $p < 0.0001$ in NMDA-treated group and AMPA-treated group, respectively.

4. Discussion

By using pharmacological and electrophysiological approaches, the present study showed the first evidence that ethanol inhibited the evoked excitatory synaptic transmission and the function of NMDA receptors and AMPA receptors in SG neurons. Our results also showed that prolonged exposure of ethanol might potentiate glutamate acting at mGluR5 resulting in the changes in NMDA receptor function and subsequent reduction of ethanol inhibition of NMDA receptor function. The regulation of NMDA receptors function by mGluR5 can explain the reduced inhibitory sensitivity of ethanol to the depolarizations induced by glutamate during prolonged exposure of ethanol.

Ethanol has effects on the function of neurotransmitter receptors and channels involved in excitatory and inhibitory synaptic transmission in many brain areas, which may contribute to ethanol-induced functional and behavioral changes [26]. Ethanol affects nociceptive transmission. Ethanol intake acutely produces analgesia dose-dependently in humans and animals. However, ethanol induces hyperalgesia in ethanol dependence and withdrawal [27]. An electrophysiological study in the SG neurons of spinal slices showed that ethanol augmented spontaneous inhibitory (GABAergic and glycinergic) synaptic transmission in the SG, which might be responsible for acute ethanol-induced analgesic action in the spinal dorsal horn [28]. Also in that study, ethanol had no effects on spontaneous excitatory synaptic transmission. However, in dentate granule neurons, ethanol modulated both excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic

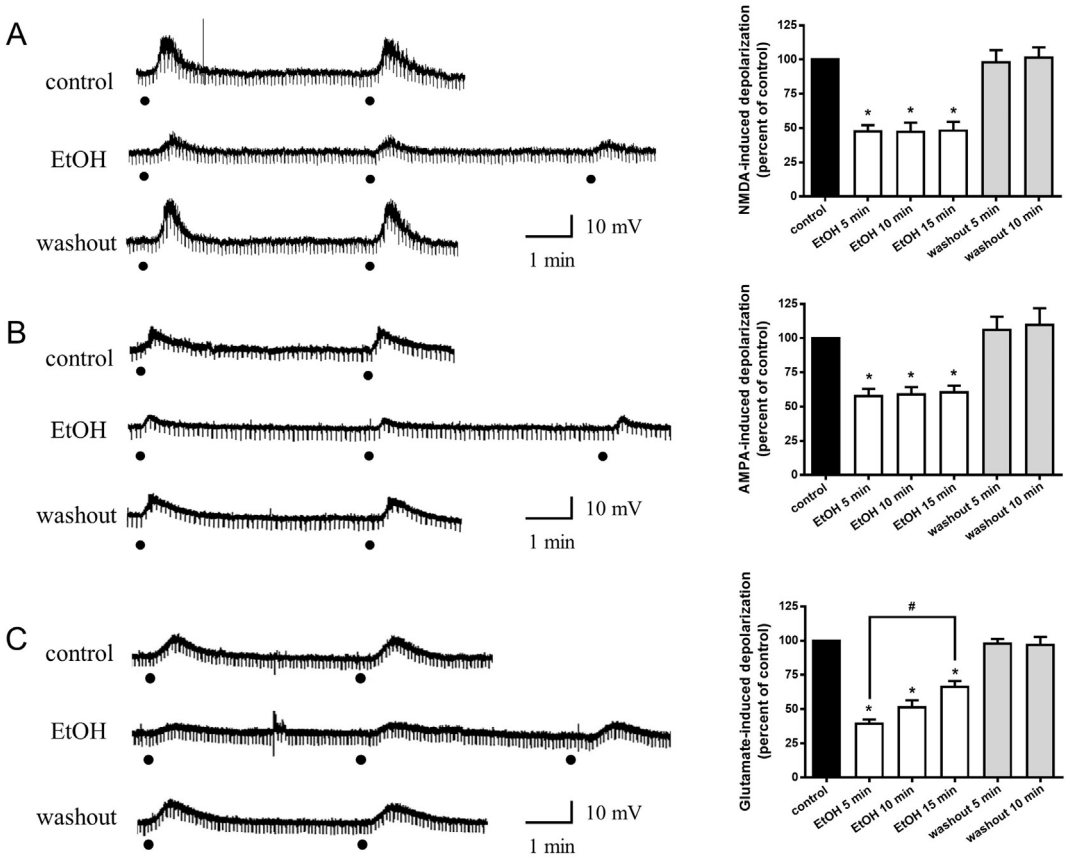

Fig. 2. Effects of ethanol on excitatory amino acid-induced depolarizations in SG neurons. (A) Left panel: a representative recording shows an NMDA-induced depolarization before (control) and during superfusion of spinal cord slice with ethanol for 15 min, and after removal (washout) of ethanol in an SG neuron. The membrane depolarizations were induced by consecutive superfusions of NMDA (100 μM, indicated by ●) for 8 s at intervals of 5 min in the presence of tetrodotoxin (0.3 μM). Downward deflections are hyperpolarizing electrotonic potentials induced by constant hyperpolarizing current pulses (not shown). Right panel: bar graph shows the time course of percentage changes in NMDA-induced depolarizations following superfusion of ethanol (100 mM), and after removal of ethanol (washout). The magnitude of NMDA-induced depolarization (15.3 ± 2.3 mV, $n = 5$) immediately prior to application of ethanol is taken as control of 100%. (B) and (C) Similar to (A) except that membrane depolarizations in representative traces was induced by superfusion of AMPA (10 μM for 8 s) and glutamate (400 μM for 10 s), respectively. The magnitude of AMPA-induced depolarization (13.8 ± 2.0 mV, n = 5) or glutamate-induced depolarization (11.5 ± 0.8 mV, n = 5) immediately prior to application of ethanol is taken as control of 100%. *$p < 0.0001$ compared with control; #$p < 0.001$ compared with 5 min following superfusion of ethanol analyzed using repeated-measures one-way ANOVA followed by Dunnett's post-test.

currents (IPSCs); ethanol inhibited NMDA receptor-mediated EPSCs but potentiated $GABA_A$ receptor-mediated IPSCs [29]. Besides, ethanol selectively inhibited NMDA-mediated EPSCs with little effects on IPSCs in prefrontal cortex neurons and agranular insular cortex neurons [30,31]. Based on these previous findings, ethanol may have different effects on excitatory and inhibitory synaptic transmission in different neurons. The present study showed that ethanol inhibited excitatory postsynaptic potential evoked from the dorsal root. Ethanol also inhibited the depolarizations induced by exogenous application of NMDA and AMPA. The results suggest that ethanol has inhibitory effects on iGluR-mediated neurotransmission in the SG neurons, which might contribute, at least in part, to acute ethanol-induced analgesia effects.

Some studies reported that ethanol inhibition of the responses mediated by NMDA receptors was much more sensitive to that by AMAP receptors in several neuronal preparations examined *in vitro* and *in vivo* [5,6,11,30–32]. However, several studies showed a similar inhibitory potency of ethanol on responses of both NMDA and AMPA receptors such as in locus coeruleus neurons, cultured rat cortical neurons, and spinal cord motor neurons [18,33,34]. In the present study, the degree of inhibition by ethanol of the depolarizations induced by NMDA and AMPA was similar in SG neurons, suggesting that both NMDA and AMPA receptors were responsible for the inhibitory effects of ethanol on evoked EPSPs.

Evidence has demonstrated a pivotal role of glutamate receptors in pain sensation and transmission [35]. While iGluRs mediate fast excitatory neurotransmission, mGluRs coupled to G-protein modulate nociceptive transmission. mGluR5 belongs to the group I family of mGluRs, a G-protein coupled receptors linked to phospholipase C and PKC signaling cascades. In the dorsal horn of the spinal cord, group I mGluRs are typically localized postsynaptically and are involved in the nociceptive transmission [36,37]. However, several reports also show that presynaptic mGluR5 plays an important role in the regulation of pain transmission in spinal dorsal horn neurons [38]. In the spinal dorsal horn, pharmacological activation of group I mGluRs modulates excitatory synaptic transmission [39]. Besides, mGluR5 is involved in the relay of nociceptive information in the spinal cord dorsal horn under inflammation conditions [40]. Studies have documented that activation of mGluR5 potentiated NMDA receptor function in several brain regions [17,41,42]. Group I mGluRs potentiation of NMDA receptor-mediated responses in the spinal cord dorsal horn might be involved in inflammatory hyperalgesia [43]. These studies suggest that regulation of NMDA receptor activation by group I mGluRs (mGluR5) play a critical role in nociceptive transmission. In the present study, both mGluR5 antagonists and PKC inhibitors blocked ethanol tolerance to glutamate-induced responses, suggesting that mGluR5/PKC signals play a functional role in regulating ethanol effects. Treatment with mGluR agonists counteracted ethanol inhibition of NMDA-induced depolarizations, but not AMPA-induced depolarizations, suggesting that

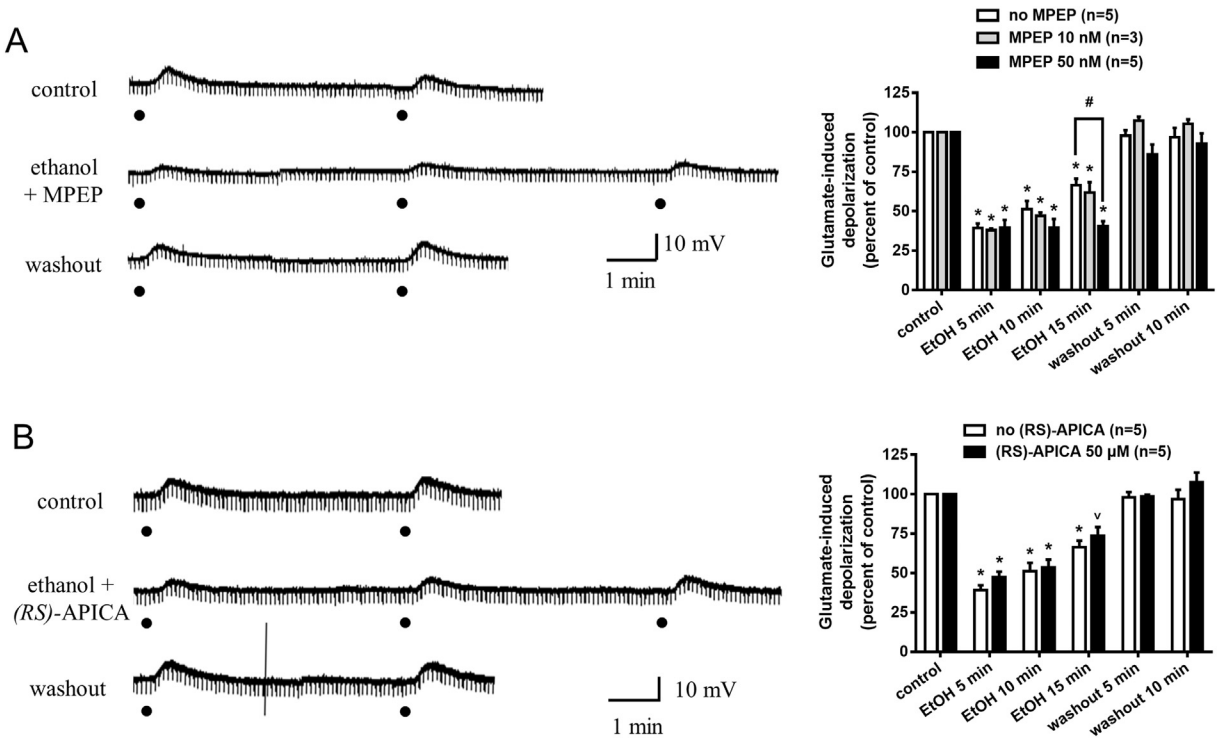

Fig. 3. Effects of mGluR modulators on tolerance to ethanol inhibition of glutamate-induced responses. (A) Left panel: A representative continuous recording shows glutamate-induced depolarizations before (control), during the superfusion of ethanol (100 mM) in combination with MPEP hydrochloride (MPEP, 50 nM), a mGluR5 antagonist, and after the removal (washout) of ethanol from the bath. The membrane depolarizations were induced by continuous superfusions of glutamate (400 μM, indicated by ●) for 10 s at intervals of 5 min in the presence of tetrodotoxin (0.3 μM). Right panel: statistical bar graph shows the time course of percentage changes in glutamate-induced depolarizations following superfusion of ethanol (100 mM) without (control) or with the addition of two concentrations of MPEP. The magnitude of glutamate-induced depolarization immediately before application of ethanol is taken as control of 100%. (B) Similar to the legend of (A) except that (*RS*)-APICA (50 μM), a group I metabotropic glutamate receptor antagonist, was added with ethanol in the superfusion medium. $^{v}p < 0.01$ and $^{*}p < 0.0001$ compared with control analyzed using repeated-measures one-way ANOVA followed by Dunnett's post-test.; $\# p < 0.001$ compared with ethanol alone group analyzed by two-way ANOVA followed by Bonferroni post-test.

the development of tolerance to ethanol inhibition of glutamate-induced responses is likely due to the regulation of the function of NMDA receptors. Previous studies have shown that ethanol-induced increases in NMDA receptor function might result from increases in NMDA receptor subunits expression, changes in phosphorylation state of the receptor and influences of co-operation of NMDA receptors with other neurotransmitter receptors [7,8].

Many studies have indicated that ethanol regulation of the activity of NMDA receptors in brain areas plays an important role in mediating ethanol-induced neuronal disorders. Ethanol inhibition of NMDA receptor-mediated responses and long-term potentiation in the hippocampus might contribute to alcohol-induced memory loss [44]. Ethanol inhibition of NMDA receptor function in the cerebellum might play a critical role in cerebellar-dependent behaviors affected by ethanol [45].

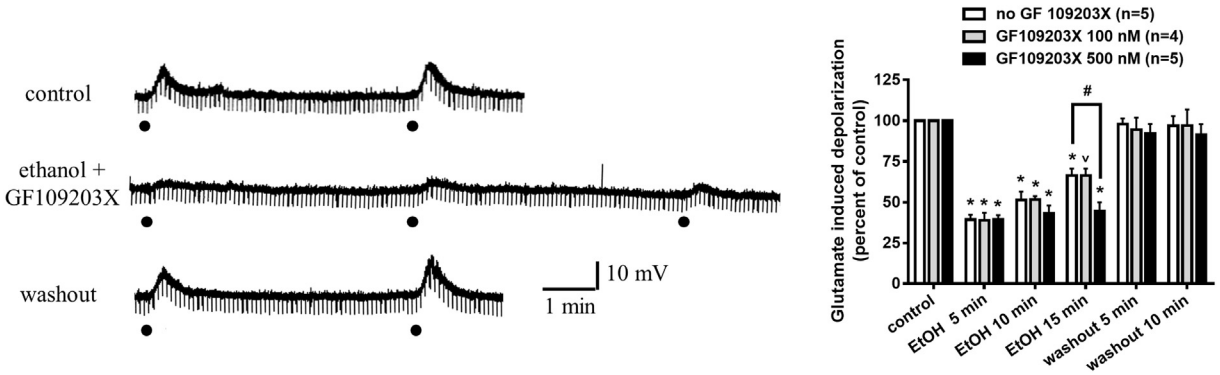

Fig. 4. Effects of PKC inhibitors on tolerance to ethanol inhibition of glutamate-induced responses. Left panel: A representative continuous recording shows glutamate-induced depolarizations before (control), during the superfusion of ethanol (100 mM) in combination with GF109203X (500 nM), a protein kinase C (PKC) inhibitor, and after the removal (washout) of ethanol from the bath. The membrane depolarizations were induced by continuous superfusions of glutamate (400 μM, indicated by ●) for 10 s at intervals of 5 min in the presence of tetrodotoxin (0.3 μM). Right panel: bar graph shows the time course of percentage changes in glutamate-induced depolarizations following superfusion of ethanol without (control) or with two concentrations of GF109203X. The magnitude of glutamate-induced depolarization immediately before application of ethanol is taken as control of 100%. $^{v}p < 0.01$ and $^{*}p < 0.0001$ compared with control analyzed using repeated-measures one-way ANOVA followed by Dunnett's post-test.; $\# p < 0.05$ compared with ethanol alone group analyzed by two-way ANOVA followed by Bonferroni post-test.

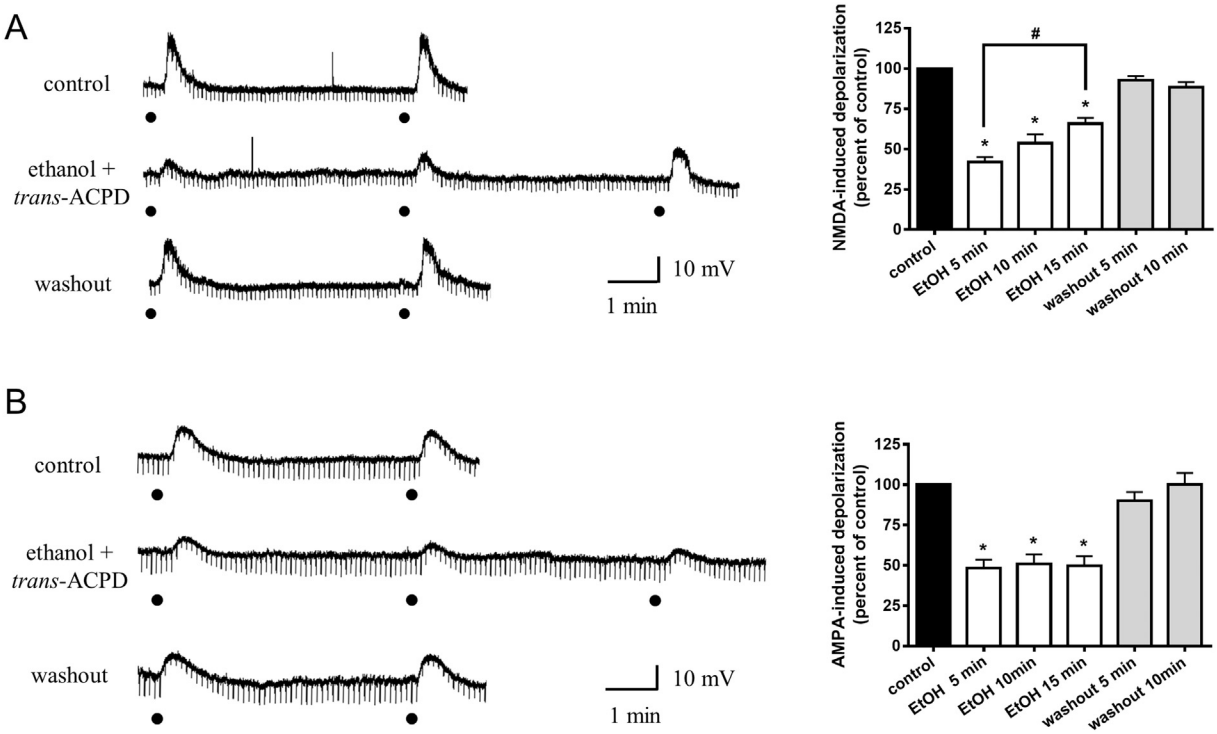

Fig. 5. Effects of mGluR agonists on ethanol inhibition of NMDA- and AMPA-induced responses. (A) Left panel: A representative continuous recording shows NMDA-induced depolarizations before (control), during the superfusion of ethanol (100 mM) in combination with trans-ACPD (ACPD, 15 nM), a metabotropic glutamate receptor agonist, and after the removal (washout) of ethanol from the bath. The membrane depolarizations were induced by continuous superfusions of NMDA (100 μM, indicated by ●) for 5 s at intervals of 5 min in the presence of tetrodotoxin (0.3 μM). Right panel: bar graph shows the time course of percentage changes in NMDA-induced depolarizations following superfusion of ethanol without (control) or with the addition of ACPD. The magnitude of NMDA-induced depolarization (13.4 ± 2.4 mV, $n = 5$) immediately before application of ethanol is taken as control of 100%. (B) Similar to (A) except that membrane depolarizations in representative traces was induced by superfusion of AMPA (10 μM for 7 s). The magnitude of AMPA-induced depolarization (15.5 ± 4.0 mV, $n = 3$) before application of ethanol is taken as control of 100%. *$p < 0.0001$compared with control; # $p < 0.001$ compared with 5 min following superfusion of ethanol analyzed using repeated-measures one-way ANOVA followed by Dunnett's post-test.

The selective inhibition by ethanol of neuronal activity in thalamocortical circuits through potentiation of GABAergic transmission and inhibition of NMDA receptors might be an important pathophysiological mechanism of massive neuroapoptosis induced by ethanol in developing brain and deleteriously neurological consequences of fetal ethanol exposure [46]. Our results showed that mGluR5 modulated the inhibitory effects of ethanol on NMDA- or glutamate-induced responses during prolonged ethanol exposure. This finding suggests that mGluR5 might participate in the regulation of several neuronal disorders induced by ethanol. In the present study, the mGluR5 regulation of ethanol inhibition of glutamate or NMDA-induced depolarizations occurred by 15 min following continuous superfusion of ethanol. The level of mGluR5 is unlikely to be changed during the short period of exposure. More studies are required to clarify the molecular mechanisms underlying ethanol modification of mGluR5 activity in SG neurons.

5. Conclusion

The present study showed inhibition by ethanol of iGluR-mediated neurotransmission in SG neurons. The degree of the inhibition varied depending on the duration of exposure; ethanol potentiation of mGluR5/PKC signals and subsequent modification of the inhibitory effects of ethanol on NMDA receptor function led to reduced sensitivity of ethanol to iGluR-mediated responses during prolonged exposure. The modulation by ethanol of the function of iGluRs and mGluR5, given their critical role in synaptic transmission, in the substantia gelatinosa neurons may participate in the mechanism underlying ethanol regulation of nociceptive transmission.

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Declaration of competing interest

The authors declare that there are no conflicts of interests.

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