ETHANOL DUAL MODULATORY ACTIONS ON SPONTANEOUS POSTSYNAPTIC CURRENTS IN SPINAL MOTONEURONS

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Abbreviated Title: Ethanol dual modulation of synaptic transmission

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ABSTRACT

Recently we have shown that acute ethanol (EtOH) exposure suppresses dorsal root-evoked synaptic potentials in spinal motoneurons (Cheng et al., 1999). To examine the synaptic mechanisms underlying the reduced excitatory activity, EtOH actions on properties of action potential-independent miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) were studied in spinal motoneurons of newborn rats. Properties of mEPSCs generated by activation of $N$-methyl-$D$-aspartate receptors (NMDARs) and non-NMDA receptors, and of mIPSCs mediated by glycine and $\gamma$-aminobutyric acid$_A$ receptors (GlyR and GABA$_A$R) were examined during acute exposure to 70 and 200 mM EtOH. In the presence of 70 mM EtOH, the frequency of NMDAR- and non-NMDAR-mediated mEPSCs decreased to 53 ± 5% (SE) and 45 ± 7% of control values, respectively. In contrast, the frequency of GlyR- and GABA$_A$R-mediated mIPSCs increased to 138 ± 15% and 167 ± 23% of control, respectively. Based on the quantal theory of transmitter release, changes in the frequency of miniature currents are correlated with changes in transmitter release, suggesting that EtOH decreased presynaptic glutamate release and increased the release of both glycine and GABA. EtOH did not change the amplitude, rise and decay times of either mEPSCs or mIPSCs, indicating that the presynaptic changes were not associated with changes in the properties of postsynaptic receptors/channels. Acute exposure to 200 mM EtOH increased mIPSC frequency 2-3-fold, significantly higher than the increase induced by 70 mM EtOH. However, the decrease in mEPSC frequency was similar to that observed in 70 mM EtOH. Those findings implied that the regulatory effect of EtOH on glycine and GABA release was dose-dependent. Exposure to the higher EtOH concentration had opposite actions on mEPSC and mIPSC amplitudes: it attenuated the amplitude of NMDAR- and non-NMDAR-mediated mEPSCs to approximately 80% of control, and increased GlyR- and GABA$_A$R-mediated mIPSC amplitude by about 20%. EtOH-induced changes in the amplitude of postsynaptic currents were not associated with
changes in their basic kinetic properties. Our data suggested that in spinal networks of newborn rats, EtOH was more effective in modulating the release of excitatory and inhibitory neurotransmitters than changing the properties of their receptors/channels.

INTRODUCTION

EtOH, a potent modulator of synaptic transmission, affects neural network function by interacting with a spectrum of specific membrane proteins that initiate cellular signal trasduction processes (reviewed by Weight, 1992; Faingold et al, 1998). In a variety of neuronal preparations it modulates both excitatory and inhibitory synaptic transmission, reducing glutamate-mediated excitation and increasing GABA-, glycine- and adenosine-mediated inhibition (reviewed by Crews et al., 1996; Peoples et al., 1996; Narahashi et al., 2001). Its dual modulatory action on nicotinic acetylcholine receptors resulted in depressing or facilitating acetylcholine-mediated currents depending on the composition of the receptor subunits (Aistrup et al., 1999).

Glutamate receptors, the major excitatory receptors in the CNS, consist of three ionotropic receptor subtypes: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors. Until recently the general consensus was that NMDA receptors are the primary targets of EtOH, because at relatively low concentrations it antagonizes NMDAR-mediated responses in a diverse populations of neurons (Lovingier et al., 1990; Lima-Lindman and Albuquerque, 1989; Morrisett and Swartzwelder, 1993; reviewed by Tabakoff and Hoffman, 1996; Faingold et al., 1998; Woodward, 2000; Li et al., 2002). NMDA receptor sensitivity to EtOH is regulated by its subunit composition, but factors such as phosphorylation also play important role in modulating EtOH sensitivity (reviewed by Woodward, 2000). The effect of EtOH on non-NMDA receptors is somewhat controversial
(Crews et al., 1996; Lovinger, 1997), but in spinal and cortical neurons, NMDAR- and non-NMDAR-
mediated synaptic events are similarly affected by EtOH inhibitory actions (Wang et al., 1999, Wirkner et
al., 2000).

Numerous studies have demonstrated that EtOH-induced intoxication is correlated with its
interaction with $\gamma$-aminobutyric acid$_A$ (GABA$_A$) receptors (reviewed by Crews et al., 1996). In isolated
brain preparations and cultured neurons, EtOH increases GABA$_A$ receptor-mediated Cl$^-\text{conductance}$
(Nestores, 1980; Celentano et al., 1988; Aguayo, 1990; Reynolds and Prasad, 1991), and similarly it
potentiates glycine-mediated Cl$^-\text{flux}$ in synaptoneurosomes (Engblom and Akerman, 1991), and
enhances glycine currents generated in hippocampal neurons (Aguayo and Pancetti, 1994). A
comparison between EtOH actions on glycine- and GABA-mediated Cl$^-\text{currents}$ in dissociated spinal
neurons indicated that glycine receptors are more sensitive to EtOH than GABA receptors (Celentano et
al., 1988). It has been proposed that GABA$_A$ and glycine receptor sensitivity to EtOH depends primarily
on the expression of the $\alpha1$ receptor subunit (Mascia et al., 1996; Eggers et al., 2000).

In addition to EtOH opposite actions on excitatory and inhibitory neurotransmitter receptors, its
dual effects on presynaptic release of excitatory and inhibitory neurotransmitters have also been
documented. EtOH inhibits NMDAR-mediated glutamate release in the rat striatum (Carboni et la.,
1993), and in the hippocampus it suppresses high-K$^+$-evoked release of endogenous glutamate, aspartate
and GABA (Martin and Swartzwelder, 1992). In contrast, EtOH increases the frequency of action
potential-independent glycinergic currents in hypoglossal motoneurons, implying that it increases
presynaptic glycine release (Eggers et al., 2000).

We have demonstrated that EtOH suppresses motoneuron electrical activity by reducing
motoneuron excitability, decreasing the amplitude of dorsal root-evoked excitatory postsynaptic
potentials and decreasing the frequency of spontaneous excitatory postsynaptic currents, while
increasing the frequency of inhibitory postsynaptic currents (Cheng et al., 1999). In that study, spontaneous excitatory and inhibitory currents were recorded concurrently, therefore it is conceivable that EtOH action partly resulted from modifying the synaptic interaction between those currents rather than directly influencing excitatory and inhibitory synaptic transmission (Marszalec et al., 1998). In this study, properties of pharmacologically isolated action potential-independent postsynaptic currents were examined to determine EtOH effects on action potential-independent presynaptic release of glutamate, glycine and GABA and its modulatory actions on postsynaptic NMDA, non-NMDA, glycine and GABA_A receptors/channels.

A preliminary report of this study was published in an abstract form (Gao and Ziskind-Conhaim, 2000).
METHODS

Spinal cord preparation

Lumbar spinal cords were isolated from 1-4-day old postnatal Sprague-Dawley rats (P1-4). The procedure for spinal cord dissection was similar to that described previously (Ziskind-Conhaim, 1990; Gao and Ziskind-Conhaim 1998). Postnatal rats were anesthetized by hypothermia. The lumbar region of the spinal cord was removed and placed in oxygenated cold dissection solution. The dissection solution contained (in mM): 113 NaCl, 3 KCl, 1 CaCl₂, 6 MgCl₂, 25 NaHCO₃, 1 NaH₂PO₄, and 11 glucose (pH 7.2-7.4). The isolated spinal cord was embedded in agar (2% in extracellular solution). Transverse slices, 350 µm thick were cut using a Vibratome (Technical Products International). Prior to whole-cell recordings, slices were incubated in extracellular solution at room temperature for 30-60 mins. The extracellular solution contained (in mM): 113 NaCl, 3 KCl, 2 CaCl₂, 1 MgCl₂, 25 NaHCO₃, 1 NaH₂PO₄, and 11 glucose. The solution was equilibrated with 95% O₂ and 5% CO₂ (pH 7.2 at 20-22 °C).

Whole-cell recording

Slices were transferred into a recording chamber, which was mounted on the stage of an upright microscope and were superfused with aerated extracellular solution at room temperature (20-22 °C). Large neurons in the medial and lateral ventral horn were visualized using infrared DIC optics, and those were assumed to be motoneurons. Whole-cell patch-clamp recordings in motoneurons were performed using patch electrodes pulled to tip resistances of 3-5 MΩ using a multi-stage puller (Sutter Instruments Co.). Electrodes were filled with solution containing (in mM): 149 CsCl, 10 HEPES, 0.2 EGTA, 1 Mg-ATP, and 0.1 GTP. The solution was adjusted to pH 7.2 using CsOH, and the osmolarity was 290 mOsm. Typically, the series resistance was 2-3-fold higher than the pipette resistance. Experiments were rejected if the series resistance changed > 15%.
Action potential-independent miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) were recorded in the presence of TTX (1 µM). At P1-4, mEPSC and mIPSC frequencies were 0.5 Hz and 0.3 Hz, respectively (Gao et al., 1999), and the frequencies of pharmacologically isolated NMDAR- and non-NMDAR-mediated mEPSCs and GlyR- and GABA_AR-mediated mIPSCs were approximately 50% lower. Therefore, to acquire a large sample of mEPSCs and mIPSCs for statistical analysis, experiments were performed in high extracellular K⁺ (18 mM, Gao et al., 1998 and 2001). Synaptic currents were recorded using an Axopatch 200A amplifier (Axon Instruments Co.). Currents were filtered at 1 kHz, digitized at 5 kHz and stored on a disk for later analysis. In most motoneurons, continuous recordings were carried out until > 100 events were recorded.

**Data Analysis**

The threshold for detection of miniature currents was set at 2 pA above the background noise. The average noise standard deviation was 1.7 pA at –60 mV (see also Gao et al., 2001), not significantly different from the 1.9 pA measured at +40 mV. mEPSCs and mIPSCs were measured using Mini Analysis software (Synaptosoft Inc.). Kinetic analysis was performed on averaged miniature currents, which were obtained by lining up the rising phase of single currents. In most motoneurons, more than 100 events were averaged. Kinetic analysis included: peak amplitude, rise time from 10 to 90% peak amplitude and decay time constant (decay τ). Typically, the time course of decay of mEPSCs and mIPSCs was best fitted with the sum of two exponentials. Data are presented as mean ± SE. Student’s *t*-test was used to determine the statistical significance (*p* < 0.05).
Figure 1: EtOH reversibly suppressed the frequency of mEPSCs in a P4 motoneuron. (A) Traces of continuously recorded mEPSCs before (control), during EtOH exposure (EtOH) and after the removal of EtOH from the extracellular solution (wash). mEPSC frequency decreased from 3.7 Hz before EtOH application to 3.0 Hz in its presence. mEPSC frequency of 3.2 Hz was recorded 20 min after EtOH removal. (B) mEPSC frequency and amplitude during EtOH exposure (% of control) and after its removal. EtOH significantly suppressed mEPSC frequency to 84 ± 6% of control (p < 0.04), and the frequency increased to 91 ± 6% of control 20 min after EtOH removal. The averaged amplitude did not significantly change, decreasing to 86 ± 4% of the control value (p < 0.07) in the presence of EtOH and increasing to 101 ± 8% after its removal. Values are means ± SE of 6 motoneurons.
RESULTS

EtOH modulation of mEPSC and mIPSC properties was examined in spinal motoneurons of P1-4 rats. Acute EtOH exposure does not significantly change the properties of dorsal root-evoked potentials at concentrations lower than 70 mM (Cheng et al., 1999). To determine whether lower EtOH concentration altered the properties of action potential-independent miniature synaptic currents, mEPSC and mIPSC frequencies and amplitudes were examined during a 10-15 min exposure to 30 mM EtOH (n = 4). At that concentration, EtOH did not alter mEPSC and mIPSC frequencies (not shown), confirming our previous findings that higher EtOH concentrations were required to significantly reduce excitatory synaptic transmission and motoneuron excitability in newly formed spinal networks (Cheng et al., 1999). Therefore, in this study, EtOH actions on pharmacologically isolated miniature synaptic currents were examined only at higher concentrations of 70 and 200 mM.
**Figure 2:** EtOH significantly reduced the frequency of fast-decaying non-NMDAR-mediated mEPSCs, without changing mEPSC amplitudes and basic kinetic properties. *Upper panels:* Traces of continuously recorded mEPSCs in a P4 motoneuron before and during a brief exposure to 70 mM EtOH. *Bottom left panel:* amplitude distributions of non-NMDAR-mediated mEPSCs recorded in control and EtOH-containing solution. mEPSC amplitudes larger than 45 pA are not shown. Mean peak amplitudes were 16.5 (control) and 15.0 pA (EtOH). mEPSC frequency decreased from 0.6 Hz before EtOH application to 0.2 Hz in its presence. *Bottom right panel:* averaged non-NMDAR-mediated mEPSCs before and during EtOH exposure (control mEPSCs, n = 373; EtOH mEPSCs, n = 134). Overlapping currents were not averaged.

**EtOH decreased mEPSC frequency and increased mIPSC frequency**

Glutamate-mediated mEPSCs were recorded in the presence of strychnine (0.5 μM) and bicuculline (5 μM), glycine and GABA_A receptor antagonists, respectively. mEPSCs were recorded at a holding potential (HP) of +40 mV to remove the voltage-dependent Mg2+ block of NMDA channels. Some experiments were initially carried out at a HP of −60 mV and Mg2+ was omitted from the extracellular solution (see below). However, because of the possibility that Mg2+ modulated presynaptic release, mEPSC properties were analyzed only when recorded at +40 mV.

At +40 mV, mEPSCs were recorded as outward currents (Fig. 1), and consisted of a mixed population of fast-rising, fast-decaying non-NMDAR-mediated mEPSCs and slow-rising, slow-decaying NMDAR-mediated mEPSCs. A third sub-population of dual-component mixed fast- and slow-decaying mEPSCs was also apparent, and it was assumed that those were generated by co-activation of NMDA and non-NMDA receptors. Pharmacologically isolated non-NMDAR-mediated mEPSCs were recorded in the presence of D-2-amino-5-phosphonovaleric acid (D-APV, 20 μM), a NMDA receptor antagonist (Fig 2). NMDAR-mediated mEPSCs were recorded in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 5-10 μM), an AMPA/kainate receptors antagonist (Fig. 3).
Figure 3: EtOH significantly reduced the frequency of slow-decaying NMDAR-mediated mEPSCs, but did not change mEPSC amplitudes and basic kinetic properties. *Upper panels*: Traces of continuously recorded mEPSCs in a P3 motoneuron before EtOH application and in its presence (70 mM). *Bottom left panel*: Amplitude distributions of NMDAR-mediated mEPSCs before and during EtOH exposure. mEPSC amplitudes larger than 25 pA are not shown. Mean peak amplitudes were 10.6 and 10.7 pA before and after EtOH exposure, respectively. mEPSC frequency was reduced from 0.4 Hz (control) to 0.2 Hz (EtOH). *Bottom right panel*: Averaged NMDAR-mediated mEPSCs before and during EtOH exposure (control mEPSCs, n = 192; EtOH mEPSCs, n = 79).

mIPSCs were recorded in the presence of D-APV (20 µM) and CNQX (5-10 µM), and at a HP of −60 mV they appeared as inward currents (Cl\textsuperscript{-} equilibrium potential was approximately -5 mV).

Based on their kinetic properties mIPSC population consisted of three groups: fast-decaying glycinergic mIPSCs, slow-decaying GABAergic mIPSCs and dual-component, fast-slow-decaying mixed glycine-GABA-mediated mIPSCs (Gao et al., 2001). Pharmacologically isolated glycineR- (GlyR-) mediated mIPSCs were recorded in the presence of bicuculline (5 µM, Fig. 4), and GABA\textsubscript{A}R-mediated mIPSCs were recorded in the presence of strychnine (0.5 µM, Fig. 5).
EtOH significantly increased the frequency of fast-decaying GlyR-mediated mIPSCs, without changing mIPSC basic kinetic properties. **Upper panels**: Traces of continuously recorded mIPSCs in a P4 motoneuron before and during exposure to 70 mM EtOH. **Bottom left panel**: amplitude distributions of GlyR-mediated mIPSCs in control and EtOH-containing solution. mIPSC frequency increased from 0.6 Hz (control) to 0.9 Hz (EtOH). mIPSC amplitudes larger than 65 pA are not shown. Mean peak amplitudes were 18.3 (control) and 25.1 pA (EtOH), the largest amplitude increase induced by 70 mM EtOH. **Bottom right panel**: averaged GlyR-mediated mIPSCs before and during EtOH exposure (control mIPSCs, n = 194; EtOH mIPSCs, n = 253). The large EtOH-induce increase in the amplitude of GlyR-mediated mIPSCs was not associated with changes in their rise time and decay time constants.
EtOH significantly increased the frequency of slow-decaying GABA_A-mediated mIPSCs, but did not change mIPSC amplitudes and basic kinetic properties. *Upper panels:* Traces of continuously recorded mIPSCs in a P3 motoneuron before EtOH application and in its presence (70 mM). *Bottom left panel:* amplitude distributions of GABA_A-mediated mIPSCs before and during EtOH exposure. mIPSC amplitudes larger than 130 pA are not shown. Mean peak amplitude increased from 35.5 (control) to 36.5 pA (EtOH). mIPSC frequency increased from 0.2 Hz (control) to 0.6 Hz (EtOH), with a significant number of overlapping currents. *Bottom right panel:* averaged GABA_A-mediated mIPSCs before and during EtOH exposure (control mIPSCs, n = 111; EtOH mIPSCs, n = 161).

A brief (10-15 min) exposure to 70 mM EtOH reversibly suppressed mEPSC frequency to approximately 50% of control (Figs. 1-3). The frequency of non-NMDAR-mediated mEPSCs was significantly reduced from 0.64 ± 0.16 Hz (SE, n = 8) before EtOH exposure to 0.29 ± 0.11 Hz in its presence, and that of NMDAR-mediated mEPSCs decreased from 0.51 ± 0.13 Hz (n = 11) to 0.27 ± 0.09 Hz (Fig. 6). Opposite modulatory action was apparent on mIPSC frequency, significantly increasing the frequency of GlyR- and GABA_A-mediated mIPSCs to 138 ± 15% (n = 8) and 167 ± 23% (n = 9) of control, respectively (Fig. 6). Although we cannot rule it out, it is unlikely that the apparent reduction in mEPSC frequency reflected a failure to detect currents that decreased in amplitude during
EtOH exposure. EtOH did not significantly change mEPSC amplitude distributions (Figs. 2-3), and small mEPSCs (2.5-to-6 pA) constituted only a small fraction of mEPSC population. Therefore, even if EtOH acted on postsynaptic glutamatergic receptor to reduce the amplitude of the small currents below the level of detection, it could not have accounted for the 50% reduction in mEPSC frequency.

![Graph showing frequency and amplitude changes during EtOH exposure.](image)

**Figure 6:** The frequency of non-NMDAR- and NMDAR-mediated mEPSCs significantly decreased and the frequency of GlyR- and GABA_A receptor-mediated mIPSCs significantly increased during EtOH exposure (70 mM). At this concentration, EtOH did not significantly alter mEPSC and mIPSC amplitudes. Averages ± SE of 8 and 11 motoneurons for non-NMDAR- and NMDAR-mediated mEPSCs, respectively and 8 and 9 motoneurons for GlyR- and GABA_A receptor-mediated mIPSCs.

At this concentration, EtOH did not significantly alter the amplitude, rise and decay times of either mEPSCs or mIPSCs. The mean amplitude of non-NMDAR-mediated mEPSCs was 18.3 ± 1.1 pA before EtOH application and 16.7 ± 1.2 pA in its presence, and that of NMDAR-mediated mEPSCs
changed from $11.2 \pm 1.8$ pA to $11.8 \pm 1.8$ pA (Fig 6). It is conceivable that small mEPSCs were not detected at a HP of $+40$ mV, because of a potential leak, which could have masked changes in mEPSC amplitudes during EtOH exposure. However, at a HP of $-60$ mV (extracellular Mg$^{2+}$ was omitted), the mean amplitude of non-NMDAR-mediated mEPSCs was $18.9 \pm 2.1$ pA ($n = 5$) and that of NMDAR-mediated mEPSCs was $11.6 \pm 1.6$ ($n = 4$), not significantly different from those recorded at $+40$ mV. Therefore, it is unlikely that the failure to detect changes in mEPSC amplitudes resulted from the inability to record small currents at a HP of $+40$ mV.

Figure 7: mIPSC frequency and amplitude significantly increased during acute exposure to 200 mM EtOH. (A) Continuously recorded mIPSCs in a P3 motoneuron before (control), in the presence of EtOH (EtOH) and after its removal (wash). mIPSC frequency was 1.4 Hz and the mean amplitude was 94.7 pA before EtOH application and 4.6 Hz and 136 pA in its presence. EtOH effects were partially reversible and the frequency and amplitude decreased to 0.9 Hz and 45.4 pA after its removal. (B) mIPSC frequency and amplitude during EtOH exposure (% of control) and after its removal. EtOH significantly increased mIPSC frequency to $295 \pm 73\%$ of control ($p < 0.01$), and the frequency decreased to $70 \pm 18\%$ of control 20 min after EtOH removal. The averaged amplitude increased to $142 \pm 29\%$ of control in the presence of EtOH and decreased to $80 \pm 20\%$ after its removal. Values are means $\pm$ SE of 4 motoneurons.
The amplitude of GlyR- and GABA\textsubscript{A}R-mediated mIPSCs increased approximately 10% during EtOH exposure. Our recent study has demonstrated that EtOH does not significantly change motoneuron membrane resistance (Cheng et al., 1999), therefore it is unlikely the inability to detect changes in mEPSC and mIPSC amplitudes resulted from a decrease in membrane resistance during EtOH exposure.

Our findings that EtOH changed the frequency but not amplitude of mEPSCs and mIPSCs implied that acute exposure to 70 mM EtOH modulated presynaptic release of glutamate, glycine and GABA without changing the properties of their postsynaptic receptors/channels.

**High EtOH concentration reduced mEPSC amplitude and increased mIPSC amplitude**

To determine whether the amplitude and basic kinetic properties of mEPSCs and mIPSCs were modulated by high EtOH concentration, current properties were examined in the presence of 200 mM EtOH. The approximately 30% decrease in mEPSC frequency (Table 1) was similar to the reduction induced by 70 mM EtOH, indicating that the lower concentration produced a maximal inhibitory effect on mEPSC frequency. At that concentration, the decrease in mEPSC frequency was associated with a significant decrease in mEPSC amplitude, with a reduction of approximately 20% in the amplitude of both NMDAR- and non-NMDAR-mediated mEPSCs (Table 1). EtOH-induced amplitude attenuation was not associated with changes in basic kinetic properties of glutamate-mediated mEPSCs.

mIPSC frequency increased 2-3-fold during the exposure to 200 mM EtOH (Table 2), significantly higher than the increase induced during the exposure to 70 mM EtOH. This effect was reversible upon removal of EtOH from the extracellular solution (Fig. 7). These data demonstrated that unlike its inhibitory action on mEPSC frequency, EtOH facilitatory action on mIPSC frequency was
dose-dependent. At the higher concentration EtOH induced an approximately 20% increase in the amplitude of both GlyR- and GABA<sub>A</sub>R-mediated mIPSCs, but it did not affect mIPSC basic kinetic properties (Table 2).

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<th>Frequency (Hz)</th>
<th>Amplitude (pA)</th>
<th>Rise Time (ms)</th>
<th>Decay t1 (ms)</th>
<th>Decay t2 (ms)</th>
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<td>Control (n = 6)</td>
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Table 1  Acute exposure to 200 mM EtOH significantly decreased the frequency and amplitude of non-NMDAR- and NMDAR-mediated mEPSCs. The frequencies of non-NMDAR- and NMDAR-mediated mEPSCs decreased to 67 and 68 % of control levels, respectively and their amplitudes decreased to 83 and 78 % of control. EtOH did not significantly change the rise time and decay time constants of the two populations of mEPSCs.

n = number of cells.

* Significantly different than control, p < 0.05.
Table 2: The frequency and amplitude of GlyR- and GABA<sub>A</sub>R-mediated mIPSCs significantly increased during exposure to 200 mM EtOH, but there was no change in their rise time and decay time constants. The frequencies of GlyR- and GABA<sub>A</sub>R-mediated mIPSCs increased 2-3-fold, but their amplitudes increased only by 19.9 and 24.5%, respectively.

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DISCUSSION

This study demonstrated that EtOH shifted the balance between excitation and inhibition toward inhibition by modulating both pre- and postsynaptic mechanisms underlying spontaneous synaptic transmission. EtOH dominant action was to increase high-K<sup>+</sup>-induced mIPSC frequency and depress mEPSC frequency, probably reflecting an increase in glycine and GABA release and a suppression of glutamate release. The dual presynaptic effects might contribute to EtOH-induced attenuation of dorsal root evoked potentials in spinal motoneurons (Cheng et al., 1999; Wang et al., 1999). At high concentrations, presynaptic modulation was associated with opposite actions on excitatory and inhibitory postsynaptic receptors/channels as evident by the decrease in mEPSC amplitude and the
increase in mIPSC amplitude. The concept of opposite EtOH actions on excitatory and inhibitory synaptic transmission is not new, but this is the first study to show that during the period of spinal network formation in the rat (Seebach and Ziskind-Conhaim, 1994), EtOH at the lower concentration (70 mM) was more effective in modulating presynaptic release of excitatory and inhibitory neurotransmitters than changing the properties of their postsynaptic receptors/channels. The finding that relatively high EtOH concentrations were required to affect miniature current properties might be related to the age-dependent sensitivity to EtOH in the rat (Fang et al., 1997). It is conceivable that lower EtOH concentrations would effectively modulate synaptic currents in more mature rats.

**Dual EtOH actions on neurotransmitter release**

Our observations that EtOH depressed mEPSC frequency supported previous reports showing that it inhibited NMDA-mediated glutamate release in the rat striatum (Carboni et al., 1993), and suppressed high-K⁺-evoked release of endogenous glutamate and aspartate in the hippocampal slice (Martin and Swartzwelder, 1992). The finding that EtOH increased mIPSC frequency was similar to the increased frequency of glycinergic mIPSCs in postnatal hypoglossal motoneurons (Eggers et al., 2000), but contradicted its inhibitory effect on high-K⁺-evoked GABA release in the hippocampus (Martin and Swartzwelder, 1992). It should be noted that the decrease in endogenous GABA release was measured in the whole tissue rather than its release at synaptic sites as reflected in our study.

The mechanisms underlying EtOH-induced opposite actions on vesicular release of excitatory and inhibitory neurotransmitters are unknown. It is conceivable that different presynaptic receptors that influence intracellular calcium concentrations are expressed on terminals releasing glutamate, glycine
and GABA. Dual EtOH actions on muscarine-stimulated release of norepinephrine (NE) have been demonstrated in PC12 cells (Rabe and Weight, 1988). At a concentration of 25 mM, EtOH inhibited both muscarine-induced NE release and the increase in intracellular free Ca\(^{2+}\). However, at a concentration of 100 mM, it increased NE release and elevated intracellular Ca\(^{2+}\). Those findings implied that the dose-dependent dual EtOH actions were associated with its opposite effects on intracellular Ca\(^{2+}\). Therefore, it is possible that suppression of Ca\(^{2+}\) release from internal stores in glutamate containing nerve terminals contributed to the reduced mEPSC frequency. It has been shown that spontaneous transmitter release can result from spontaneous Ca\(^{2+}\) release from internal stores in hippocampal synaptic boutons (Emptage et al., 2001), and in Purkinje cells, large mIPSCs are generated by multivesicular release mediated by Ca\(^{2+}\) release from presynaptic stores (Llano et al., 2000).

In our study, miniature postsynaptic currents were recorded in the presence of 18 mM extracellular K\(^+\) that produced ~ 20 mV depolarization, sufficient to reach the threshold potential for Ca\(^{2+}\) current activation (Gao and Ziskind-Conhaim, 1998). One of the most significant effects of EtOH on voltage-gated ion channels is its inhibitory action on Ca\(^{2+}\) channels, in particular the L-type channel (reviewed by Crews et al., 1996). It is feasible that the decrease in mEPSC frequency during EtOH exposure resulted from its suppression of voltage-gated Ca\(^{2+}\) current. Our recent findings have indicated that EtOH reduced the amplitude of Ca\(^{2+}\) current in spinal motoneurons of postnatal rats (Gao and Ziskind-Conhaim, unpublished data). That current was previously characterized as the N-type current (Gao and Ziskind-Conhaim, 1998), which appeared to regulate synaptic transmission in spinal cords of developing rodents (e.g., Gruner and Silva, 1994; Xie and Ziskind-Conhaim, 1995).

It is conceivable that nerve terminals releasing glutamate have different components in the vesicular release sequence than those releasing glycine and GABA (Varoqueaux et al., 2002), resulting in different EtOH actions on the release of excitatory and inhibitory neurotransmitters. The mechanisms
underlying the increase in mIPSC frequency are unknown, but it is possible that presynaptic facilitation via ligand- or voltage-gated channels is responsible for the increased mIPSC frequency.

**Opposite EtOH actions on postsynaptic receptors**

EtOH opposite actions on mEPSC and mIPSC amplitudes supported the general consensus that its interaction with glutamate receptors/channels depressed glutamatergic responses, while it increased glycine- and GABA-mediated Cl− currents (reviewed by Weight et al., 1992; Faingold et al., 1998). It is thought that NMDAR-mediated synaptic transmission is especially sensitive to acute EtOH exposure (Dildy-Mayfield et al., 1996; Lovinger et al., 1989; Morrisett and Swartzwelder, 1993). However, our findings indicated that there was no differential sensitivity to EtOH of NMDA and non-NMDA receptors (see also Wang et al., 1999; Wrikner et al., 2000). NMDA receptor sensitivity to EtOH depends, at least in part, on the receptor subunit compositions (Lovinger, 1995; Masood et al., 1994, Yang et al., 1996, reviewed by Crews et al., 1996), and studies have suggested that EtOH has a more prominent action on NMDAR-mediated currents in cells expressing the NR1/NR2B combination than any other receptor subunit composition (Masood et al., 1994; Woodward, 2000). Although we cannot rule it out, it is unlikely that the low sensitivity of the NMDA receptor to EtOH is related to its receptor subunit composition in developing spinal motoneurons, because NR1 and NR2B subunits are expressed in postnatal motoneurons (Stegenga and Kalb, 2001). Moreover, EtOH (100 mM) has similar inhibitory action on both AMPAR- and NMDAR-mediated synaptic currents in motoneurons of 14-23-day-old rats (Wang et al., 1999), close to the time when adult-like NMDA receptor subunit composition is expressed (Stegenga and Kalb, 2001). It is conceivable that other postsynaptic mechanisms, such as EtOH actions on pathways that regulate receptor phosphorylation (reviewed by Woodward, 2000), mediate at least some of its effects on glutamate-mediate synaptic transmission.
Our observation that EtOH increased the amplitudes of GlyR- and GABA\(_A\)R-mediated mIPSCs supported previous reports showing an increase in GABA- and glycine-induced Cl\(^{-}\) flux during EtOH exposure (Aguayo and Pancetti, 1994; Celentano et al., 1988; Nestores, 1980; Celentano et al., 1988; Aguayo, 1990; Reynolds and Prasad, 1991; Eggers et al., 2000). EtOH had no differential actions on GlyR- and GABA\(_A\)R-mediated mIPSCs in postnatal motoneurons as reported in dissociated spinal neurons, in which EtOH induced larger increase in glycine- than GABA-induced current (Celentano et al., 1988). It is possible that EtOH significantly increased mIPSC amplitude only at a concentration of 200 mM because of the immature composition of glycine and GABA receptor subunits. Expression of \(\alpha_1\), \(\beta_2\) and \(\gamma_2\) GABA\(_A\) receptor subunits (Wafford et al., 1991; Criswell et al., 1993; Duncan et al., 1995), and \(\alpha_1\) glycine receptor domain (Mascia et al., 1996; Eggers et al., 2000) are correlated with high sensitivity to EtOH. In spinal sensory neurons, the switch in glycine receptor subunits from the embryonic \(\alpha_2\) to the adult \(\alpha_1\) occurs between P8 to P16 (Takahashi et al., 1992), implying that motoneurons expressed primarily the \(\alpha_2\) subunit during the period examined in our study.

Our data demonstrated that EtOH dual modulatory actions on excitatory and inhibitory synaptic currents resulted from its interactions with both pre- and postsynaptic mechanisms that regulate synaptic transmission in the developing rat spinal cord. EtOH opposite effects on presynaptic release of excitatory and inhibitory neurotransmitters were predominant in newly established spinal networks.

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