Differential Effects of Ethanol on GABA<sub>A</sub> and Glycine Receptor-Mediated Synaptic Currents in Brain Stem Motoneurons

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Sebe, Joy Y., Erika D. Eggers, and Albert J. Berger. Differential effects of ethanol on GABA<sub>A</sub> and glycine receptor-mediated synaptic currents in brain stem motoneurons. J Neurophysiol 90: 870–875, 2003. First published April 17, 2003; 10.1152/jn.00119.2003. Ethanol potentiates glycine receptor-mediated synaptic transmission to hypoglossal motoneurons (HMs). This effect on glycine receptor transmission changes with postnatal development in that juvenile HMs (P9–13) are more sensitive to ethanol than neonate HMs (P1–3). We have now extended our previous study to investigate ethanol modulation of synaptic GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs), because both GABA and glycine mediate inhibitory synaptic transmission to brain stem motoneurons. We tested the effects of ethanol on GABAergic and glycineric miniature inhibitory postsynaptic currents (mIPSCs) recorded from neonate and juvenile rat HMs in an in vitro slice preparation. Bath application of 30 mM ethanol had no significant effect on the GABAergic mIPSC amplitude or frequency recorded at either age. At 100 mM, ethanol significantly decreased the GABAergic mIPSC frequency recorded from neonate (6 ± 3%, P < 0.05) and juvenile (16 ± 3%, P < 0.01) HMs. The same concentration of ethanol increased the GABAergic mIPSC frequency recorded from neonate (64 ± 17%, P < 0.05) and juvenile (40 ± 15%, n.s.) HMs. In contrast, 100 mM ethanol robustly potentiated glycineric mIPSC amplitude in neonate (31 ± 3%, P < 0.0001) and juvenile (41 ± 7%, P < 0.001) HMs. These results suggest that glycine receptors are more sensitive to modulation by ethanol than GABA<sub>A</sub> receptors and that 100 mM ethanol has the opposite effect on GABA<sub>A</sub>R-mediated currents in juvenile HMs, that is, inhibition rather than enhancement. Further, comparing ethanol’s effects on GABAergic mIPSC amplitude and frequency, ethanol modulates GABAergic synaptic transmission to HMs differentially. Presynaptically, ethanol enhances mIPSC frequency while postsynaptically it decreases mIPSC amplitude.

Introduction

Ethanol acts as a general nervous system depressant (Draski and Deitrich 1996). This effect is at least partially mediated by ethanol’s actions on ligand-gated channels to depress overall synaptic activity in the nervous system. Ethanol is generally thought to inhibit excitatory transmission and increase inhibitory transmission via its modulation of neurotransmitter receptor-mediated currents (Crews et al. 1996). Inhibitory synaptic transmission is mediated by two neurotransmitters, GABA and glycine. Currents mediated by both GABA and glycine neurotransmitter receptors are modulated by ethanol. Ethanol typically potentiates glycine-activated currents in cultured cells as shown by studies in mouse hippocampal and cortical neurons (Aguayo and Pancetti 1994), mouse spinal cord motoneurons (Aguayo et al. 1996), and Xenopus oocytes (Mascia et al. 1996), although ethanol has also been shown to inhibit glycineric current in some cultured rat ventral tegmental neurons (Ye et al. 2001). Ethanol’s effects on GABAergic currents are characteristically variable and depend on many factors including cell type, dose of ethanol, and method of ethanol application. In cultured and dissociated rat neurons, extracted from a variety of brain regions, and rat brain slices, GABA-activated currents were potentiated by low concentrations of ethanol in a cell type specific manner (Nishio and Narahashi 1990; Proctor et al. 1992). Ethanol (1–50 mM) increased the current to a greater degree and in a larger percentage of sampled cells in cerebral cortex compared with the cerebellum (Reynolds et al. 1992). Ethanol-mediated potentiation of GABAergic currents recorded from chick spinal cord neurons was protocol dependent, while the potentiation of glycineric currents was more robust and consistent (Celentano et al. 1988). Studies from our laboratory have shown that ethanol potentiates currents mediated by native glycine receptors in hypoglossal motoneurons (HMs) in a developmentally dependent manner (Eggers et al. 2000). The effects of ethanol on GABA<sub>A</sub> receptor (GABA<sub>A</sub>R)-mediated synaptic currents are of interest as well, because GABA and glycine are co-released onto HMs (O’Brien and Berger 1999) and spinal cord motoneurons (Jonas et al. 1998) and therefore jointly determine the extent of inhibition to these neurons. Therefore we examined the effect of ethanol on miniature inhibitory postsynaptic currents (mIPSCs) mediated by native GABA<sub>A</sub>Rs and compared these data to the ethanol-induced potentiation of glycineric mIPSCs.

Methods

These experiments utilized the in vitro brain stem slice preparation to record from neonate (P1–3) and juvenile (P9–13) Sprague-Dawley rat HMs. All procedures were approved by the University of Washington’s Institutional Animal Care and Use Committee. Rats were anesthetized with halothane and decapitated. The brain stem was removed and 300-μm slices were prepared with a vibratome (Pelco). During slicing, incubation (1 h at 37°C), and recording, the slices were perfused with a carbogen-bubbled Ringer solution containing (in mM) 119 NaCl, 26.2 NaHCO<sub>3</sub>, 1 NaH<sub>2</sub>PO<sub>4</sub>, 2.5 KCl, 11 glucose, 2.5 CaCl<sub>2</sub>, and 1.3 MgSO<sub>4</sub>. Using near-infrared differential interference contrast optics, HMs were identified based on their characteristic location and morphology (Umemiya and Berger 1994).

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Whole cell patch-clamp recordings were performed at room temperature using 2–4 MΩ resistance patch electrodes. The electrodes were filled with (in mM) 140 CsCl, 2 MgCl₂, 10 HEPES, 10 EGTA, 2 ATP, and 0.2 GTP (pH 7.2). HMs were voltage clamped at −55 to −60 mV. The bath solution contained 6,7-dimino-quinoxaline (DNQX, RBI), n(-)-2-amino-5-phosphonopentanoic acid (APV, Tocris), TTX (Alomone Labs), and bicuculline methiodide (BMI, Sigma) or strychnine hydrochloride (Sigma). In some experiments in which glycinergic mIPSCs were recorded CdCl₂ (100 μM) was also added to the bath solution. The series resistance was measured after each recording period and the data were discarded if the resistance changed by more than 25% or if the series resistance was >10 MΩ. Data were acquired at 5 kHz using Clampex software (Axon Instruments) and filtered at 2 kHz. Representative raw traces were additionally filtered at 1 kHz in Clampfit (Axon Instruments). Spontaneous mIPSCs were analyzed using software developed in our laboratory and MiniAnalysis software (Synaptosoft). For all cells, the decay of the average mIPSC was fit to one or two exponentials. A weighted decay time constant, τ_weighted, was calculated for all traces fit by two exponentials using the time constants and the relative amplitudes: τ_weighted = (τ₁α₁ + τ₂α₂)/(α₁ + α₂). A paired t-test was used to compare differences in mean values for different conditions. Data are reported as mean ± SE. Kolmogorov-Smirnov (K-S) test was used to assess differences in mIPSC histogram distributions.

**RESULTS**

To examine the effects of ethanol on GABAergic currents, we made whole cell voltage-clamp recordings of GABAergic mIPSCs from HMs at two postnatal age groups. We were interested in determining whether the effects of ethanol changed during the postnatal period. Previously, we observed a developmentally dependent effect of ethanol on glycineric mIPSCs (Egger et al. 2000). Glycinergic currents from juvenile HMs are more sensitive to ethanol than glycineric currents from neonate HMs and this increase in ethanol sensitivity observed with postnatal development correlates with both a shortened decay and a subunit shift from the α₂ to α₁ glycine receptor subunit.

**GABAergic mIPSC decay duration decreases with postnatal development, but amplitude and frequency do not change**

We compared the amplitude, frequency, and decay of GABAergic mIPSCs recorded in control conditions for neonate and juvenile HMs (Table 1). We found that the amplitude and frequency of mIPSCs between the two age groups were not significantly different. In contrast, the weighted decay time constant significantly decreased from neonate to juvenile ages. In previous studies, our laboratory has reported similar mean GABAergic mIPSC amplitudes in HMs (O’Brien and Berger 1999, 2001). Also, the reduction in the GABAergic mIPSC decay with postnatal development is consistent with our laboratory’s previous results (O’Brien and Berger 1999, 2001).

**Table 1. GABAergic mIPSC properties**

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Amplitude (pA)</th>
<th>Frequency (Hz)</th>
<th>τ_weighted decay (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate (P1–3)</td>
<td>17</td>
<td>38 ± 2</td>
<td>1.7 ± 0.4</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>Juvenile (P9–13)</td>
<td>16</td>
<td>39 ± 2</td>
<td>1.1 ± 0.2</td>
<td>41 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. GABAergic miniature inhibitory postsynaptic currents (mIPSCs) recorded in control conditions from all cells were analyzed. n, number of hypoglossal motoneurons. * GABAergic mIPSC average weighted decay time constant decreases between neonate and juvenile stages of development (unpaired t-test, P < 0.05), but amplitude and frequency do not change.

**Bath application of 30 mM ethanol had no effect on GABAergic mIPSC frequency, amplitude, and decay**

Bath application of 30 mM ethanol did not change the frequency, amplitude, or decay of GABAergic mIPSCs recorded from neonate and juvenile HMs. Representative raw traces in Fig. 1. A1, A2 and B1, B2 show that the frequency of GABAergic mIPSCs did not change following ethanol application. For the overall population of HMs tested, the average frequency of mIPSCs did not significantly change following ethanol application at neonate (1.7 ± 1.1 to 2.2 ± 1.3 Hz, n = 5, P > 0.1, paired t-test) and juvenile (1.1 ± 0.7 to 1.1 ± 0.9 Hz, n = 5, P > 0.7, paired t-test) ages. The average τ_weighted measured from the same GABAergic mIPSCs did not change following ethanol application at neonate (47 ± 9 to 45 ± 7 ms, n = 5, P > 0.6, paired t-test) and juvenile (41 ± 8 to 40 ± 4 ms, n = 5, P > 0.9, paired t-test) ages. The average traces, each from more than 100 mIPSCs recorded from two HMs, are shown in Fig. 1, A3 and B3, and show no change in either peak amplitude or decay in the presence of ethanol. The average amplitudes of GABAergic mIPSCs recorded from HMs were unaffected by ethanol application at neonate (43 ± 4 to 39 ± 2 pA, n = 5, P > 0.3, paired t-test) and juvenile (38 ± 3 and 35 ± 1 pA, n = 5, P > 0.3, paired t-test) ages.

**Bath application of 100 mM ethanol increased the frequency, but decreased the amplitude of GABAergic mIPSCs in a developmentally dependent manner**

Representative raw traces in Fig. 2 reflect an increased mIPSC frequency for both age groups following 100 mM ethanol application. The frequency histogram distributions of GABAergic mIPSCs amplitudes recorded from the cells in Fig. 2, A1, A2 and B1, B2, are shown in Fig. 2, A3 and B3. The distribution of mIPSC amplitudes from juvenile HMs shows a leftward shift toward smaller peak amplitudes following ethanol application that is not apparent in the neonate recordings.
The mIPSC amplitude distributions for seven of eight cells tested are significantly different between control and ethanol for juvenile HMs (K-S, n = 7, P < 0.05), but the amplitude distributions for neonate HMs were not significantly different (K-S, n = 12, P > 0.9). The average GABAergic mIPSC amplitudes recorded from neonate HMs (n = 12) in the control condition slightly but significantly decreased following 100 mM ethanol application by 6 ± 3% (paired t-test, P < 0.05), from 36 ± 3 to 33 ± 2 pA. Ethanol application significantly decreased the average amplitudes recorded from juvenile HMs (n = 8) by 16 ± 3% (paired t-test, P < 0.01) from 42 ± 4 pA, in the control condition, to 33 ± 3 pA with ethanol. The average traces (insets) are consistent with this finding in that the mIPSC peak amplitude recorded from a neonate decreases only slightly in the presence of 100 mM ethanol, but the average mIPSC peak amplitude recorded from the older animal decreased to a larger extent when 100 mM ethanol was applied. The average mIPSC frequency recorded from neonate HMs (n = 12) increased by 64 ± 17% (1.7 ± 0.3 to 2.8 ± 0.6 Hz, paired t-test, P < 0.01). The frequency histogram distribution of inter-event intervals for the juvenile HM (Fig. 2B4) shows a decrease in interval following ethanol application. However, the frequency of GABAergic mIPSCs recorded from all juvenile HMs studied did not increase significantly (1.5 ± 0.3 to 2.0 ± 0.5 Hz, n = 8, paired t-test, P < 0.2). The average $\tau_{\text{weighted decay}}$ did not change following ethanol application at neonate (56 ± 5 to 58 ± 8 ms, n = 5, P > 0.7, paired t-test) and juvenile (41 ± 6 to 41 ± 6 ms, n = 5, P > 0.8, paired t-test) ages.

The effects of 30 and 100 mM ethanol on GABAergic mIPSC amplitude, frequency, and decay are summarized and presented as a percentage of change from control values in Fig. 3. We see that the amplitude and frequency were modulated by ethanol in a dose- and developmentally dependent manner, although the decay time was unaffected. The amplitude of GABAergic mIPSCs recorded from older rats decreased more following 100 mM ethanol bath application than were mIPSCs recorded from neonates. The frequency of GABAergic mIPSCs significantly increased only at the higher ethanol concentration in neonate HMs. The observation common among the data sets is the large variability of ethanol’s effects.

Ethanol at 100 mM differentially affected GABAergic and glycinergic mIPSCs

We next compared our results on GABAergic mIPSCs with the effects of 100 mM ethanol on glycinergic mIPSCs. Responses of glycinergic mIPSCs recorded in this study support our previously published data (Eggers et al. 2000) that demonstrated ethanol (100 mM) potentiated mIPSC amplitude and increased mIPSC frequency. We pooled our previously published data (n = 18, Eggers et al. 2000) with additional data from glycinergic mIPSCs (n = 8) and compared all these responses to those of GABAergic mIPSCs. Ethanol at 100 mM potentiated glycinergic mIPSC peak amplitude by 31 ± 3 and 41 ± 7%, in neonates and juveniles, respectively. In contrast, ethanol decreased the amplitude of GABAergic mIPSC peak amplitude by 6 ± 3 and 16 ± 3% in neonates and juveniles, respectively. Although ethanol modulated GABAergic and glycinergic mIPSC peak amplitudes in opposite directions, it increased the frequency of both types of mIPSCs.
Developmental dependence of ethanol’s actions

The glycine receptor (GlyR) undergoes a subunit switch during postnatal development, from the α2 to α1 subunit, that correlates with a shortened glycinergic mIPSC decay (Singer et al. 1998). This subunit switch is likely to explain the more robust ethanol-mediated potentiation of glycinergic currents recorded from juvenile HMs (Eggers et al. 2000; Mascia et al. 1996). The $\tau_{\text{weighted}}$ decay of GABAergic mIPSCs in the present study also shortened over postnatal development. The present study demonstrates an interesting parallel between glycinergic mIPSCs and GABAergic mIPSCs recorded in neonate and juvenile HMs. GABAergic mIPSC decay shortened during postnatal development and GABAergic mIPSCs recorded in juvenile HMs were more sensitive to ethanol modulation than those recorded in neonate HMs. The shortened decay accompanied by an increased sensitivity to ethanol suggest that a subunit shift in the GABA<sub>A</sub>R occurs during development. Immunohistochemical studies have shown that neonatal HMs express the GABA<sub>A</sub>R α<sub>2</sub> subunit but not the α<sub>1</sub> subunit (Donato and Nistri 2000). A recent study has shown that GABA<sub>A</sub>R α<sub>1</sub>, α<sub>2</sub>, and γ<sub>2</sub> subunits are the predominant GABA<sub>A</sub>R subunits expressed (Fritschy and Mohler 1995). It is therefore possible that α<sub>2</sub> subunit expression is favored in neonatal HMs followed by an increase in α<sub>1</sub> subunit expression during development. Ethanol’s brain region specific effects suggest that some subunits or subunit combinations render GABA<sub>A</sub>Rs more readily modulated by ethanol (Blednov et al. 2003; Reynolds et al. 1992; Ueno et al. 1999). A GABA<sub>A</sub>R subunit composition change in HMs during postnatal development might explain the greater degree of ethanol-mediated inhibition in older HMs.

Variability of ethanol’s effect on GABAergic currents

Most electrophysiological studies have shown that ethanol increases GABAergic currents (Aguayo 1990; Reynolds et al. 1992; Wafford et al. 1991; Weiner et al. 1994). However, this is not a wholly consistent result. One characteristic of ethanol’s action on GABAergic currents is that the effects vary depending on GABA<sub>A</sub>R subunit composition expressed, preparation used, concentration of ethanol, brain region, and age of animals. Wafford et al. reported that low concentrations of ethanol increased currents mediated by GABA<sub>A</sub>Rs expressed in Xenopus oocytes, and that the γ<sub>2L</sub> subunit was required for potentiation (Wafford et al. 1990, 1991). Subsequent studies investigated this proposal that the γ<sub>2L</sub> subunit is required for ethanol-mediated potentiation (reviewed by Mihic 1999). These studies showed that GABAergic currents recorded from Xenopus oocytes (Harris et al. 1997) and dorsal root ganglion (Zhai et al. 1998) cells expressing the γ<sub>2L</sub> subunit were not potentiated at low doses (<100 mM). In another study, GABAergic currents recorded from Xenopus oocytes expressing α<sub>1</sub>β<sub>1</sub>γ<sub>2S</sub>, α<sub>1</sub>β<sub>1</sub>γ<sub>2L</sub>, and α<sub>1</sub>β<sub>2</sub>γ<sub>2S</sub> GABA<sub>A</sub>Rs were potentiated only at higher ethanol concentrations (>60 mM) (Sigel et al. 1993). Ethanol had no differential effect on currents mediated by receptors containing the γ<sub>2S</sub> versus the γ<sub>2L</sub> subunit. Further, a lower dose of ethanol (50 mM) potentiated GABA-activated currents mediated by heteromeric GABA<sub>A</sub>Rs (α<sub>1</sub>β<sub>1</sub>γ<sub>2L</sub>) expressed in Xenopus oocytes. The potentiation was more depen-
dent on the expression of the $\alpha_2$ and $\beta_1$ than the $\gamma_{2L}$ subunit (Ueno et al. 1999). Thus in the *Xenopus* oocyte expression system, the sensitivity of GABA$_A$Rs to ethanol modulation depends not on the presence of the $\gamma_{2L}$ subunit but on the combination of subunits expressed. The effect of ethanol on GABAergic currents recorded from chick-, mouse-, and rat-cultured neurons is highly concentration dependent and cell type specific. Low concentrations of ethanol ($\leq 50$ mM) potentiate GABA-activated currents recorded from neurons in the mouse hippocampus, chick cortex, and spinal cord and rat cortex and cerebellum (Reynolds et al. 1992). GABAergic currents recorded from cultured rat dorsal root ganglion neurons (White et al. 1990), however, were insensitive to ethanol at concentrations $\leq 100$ mM, while GABAergic currents recorded from superior cervical ganglion neurons were inhibited by the same range of ethanol concentrations (Aguayo and Alarcon 1993). In the same study, Aguayo et al. also showed that ethanol at concentrations $\leq 100$ mM inhibited GABAergic currents recorded from adult SCG cells more than currents recorded from newborn SCG cells. The effect of ethanol on GABAergic currents recorded from slice preparations is no more consistent. GABAergic currents recorded from rat brain slices dissected from different regions have shown that ethanol potentiates evoked IPSCs in the cortex, and the intermediate and medial septal nuclei, but has no effect on the hippocampus (Soldo et al. 1994). In spinal cord slices, bath application of $70\mu M$ ethanol significantly increased the frequency of GABAergic spontaneous inhibitory postsynaptic currents (Cheng et al. 1993). In the same study, Aguayo et al. also showed that ethanol at concentrations $\leq 100$ mM inhibited GABAergic currents recorded from adult SCG cells more than currents recorded from adult SCG cells. The effect of ethanol on GABAergic currents recorded from rat brain slices dissected from different regions have shown that ethanol potentiates evoked IPSCs in the cortex, and the intermediate and medial septal nuclei, but has no effect on the hippocampus (Soldo et al. 1994). In spinal cord slices, bath application of $70\mu M$ ethanol significantly increased the frequency of GABAergic spontaneous inhibitory postsynaptic currents (Cheng et al. 1993) and mIPSCs (Ziskind-Conhaim et al. 2003) recorded from PC12 cells that correlate with changes in intracellular $\mathrm{Ca}^{2+}$ concentrations (Rabe and Weight 1988). At the physiological concentration of ethanol tested (100 mM), ethanol inhibits muscarine-stimulated NE release and the increase of intracellular-free $\mathrm{Ca}^{2+}$. Ethanol also inhibits voltage-gated channels, particularly L-type channels, in PC12 cells and intact brain (Crews et al. 1996). Releasing $\mathrm{Ca}^{2+}$ from intracellular stores stimulates spontaneous neurotransmitter release (Emptage et al. 2001). Ethanol may act on voltage-gated $\mathrm{Ca}^{2+}$ channels or intracellular $\mathrm{Ca}^{2+}$ stores to change the presynaptic $\mathrm{Ca}^{2+}$ concentration and modulate synaptic vesicle release probability.

In addition to its effects on GABAergic mIPSCs recorded from neonate HMs, 100 mM ethanol also induced an average increase in the frequency of GABAergic mIPSCs recorded from juvenile HMs. However, this effect was not statistically significant. It is possible that the release machinery in neurons innervating the HMs of the juvenile rat is not as sensitive to ethanol modulation as in the neonatal rat. Alternatively, a significant increase in average mIPSC frequency at juvenile ages may have gone undetected because ethanol decreased the mIPSC peak amplitudes to a value below the detection threshold.

Although both pre- and postsynaptic mechanisms can determine the peak mIPSC amplitude, conventionally mIPSC amplitude changes are thought to reflect changes in the response of postsynaptic receptors (Thompson et al. 1993). Ethanol is thought to potentiate the amplitude of glycinergic currents by acting on postsynaptic GlyRs. A single amino acid on the GlyR $\alpha_1$ subunit confers greater ethanol sensitivity when homomeric GlyR are expressed in *Xenopus* oocytes (Mascia et al. 1996). An analogous site for ethanol’s actions on the GABA$_A$R has not been found. Instead, as discussed above, it is thought that the combination of receptor subunits expressed determines ethanol sensitivity. The opposing effects ethanol has on frequency and amplitude suggest that ethanol modulates GABAergic synaptic transmission differently presynaptically versus postsynaptically.

**Physiological effects of ethanol modulation**

The reliability of ethanol’s potentiation of glycinergic currents and the variability of its effects on GABAergic currents suggest that ethanol modulates inhibitory synaptic transmission in the hypoglossal nucleus primarily by acting on glycine receptors. HMs innervate the tongue muscle, which plays a role in regulating airway patency. Inhibition of HMs partially contributes to obstructive sleep apnea (OSA) (Yamuy et al. 1999), which occurs when the tongue collapses into the airway (Remmers et al. 1980). During a pharmacologically induced model of REM sleep, a sleep state when OSA is generally most prevalent, HMs receive large glycinergic IPSPs. Administering ethanol to humans suffering from OSA intensifies the severity of sleep apnea by increasing the duration and frequency of apneic events and decreasing the arterial oxygen saturation (Issa and Sullivan 1982; Scrima et al. 1982; Taasan et al. 1981). Ethanol consumption not only intensifies sleep apnea in patients suffering from OSA, it also induces apneic events in

**Differential effects on presynaptic and postsynaptic transmission**

At 100 mM, ethanol significantly increased the GABAergic mIPSC frequency in neonate, but not in juvenile HMs. The frequency of mIPSCs is controlled by presynaptic mechanisms that determine the synaptic vesicle release probability. Although the mechanisms involved in ethanol’s actions on neurotransmitter release are unknown, it is possible that ethanol increases the release probability of synaptic vesicles containing inhibitory neurotransmitters by modulating the presynaptic $\mathrm{Ca}^{2+}$ concentration. Ethanol has a concentration-dependent effect on muscarine-stimulated norepinephrine (NE) release from PC12 cells that correlate with changes in intracellular $\mathrm{Ca}^{2+}$ concentrations (Rabe and Weight 1988). At the physiological concentration of ethanol tested (100 mM), ethanol inhibits muscarine-stimulated NE release and the increase of intracellular-free $\mathrm{Ca}^{2+}$. Ethanol also inhibits voltage-gated channels, particularly L-type channels, in PC12 cells and intact brain (Crews et al. 1996). Releasing $\mathrm{Ca}^{2+}$ from intracellular stores stimulates spontaneous neurotransmitter release (Emptage et al. 2001). Ethanol may act on voltage-gated $\mathrm{Ca}^{2+}$ channels or intracellular $\mathrm{Ca}^{2+}$ stores to change the presynaptic $\mathrm{Ca}^{2+}$ concentration and modulate synaptic vesicle release probability.
chronic snorers (Issa and Sullivan 1982). Ethanol may exacerbate OSA in part by increasing inhibition by potentiating glycinergic currents. Although ethanol had opposing effects on pre- and postsynaptic transmission by potentiating and inhibiting GABAergic currents, the effect was generally weaker than the response of glycinergic currents. Therefore GABAergic currents may not significantly contribute to the heightened inhibition of HMs during OSA, particularly following ethanol consumption.

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**Disclosures**

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**References**


