Brief Communication

Low Ethanol Concentrations Selectively Augment the Tonic Inhibition Mediated by δ Subunit-Containing $GABA_A$ Receptors in Hippocampal Neurons

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In central neurons, a tonic conductance is activated by ambient levels of the inhibitory transmitter GABA. Here, we show that in dentate gyrus granule cells, where tonic inhibition is mediated by δ subunit-containing $GABA_A$ receptors, this conductance is augmented by low concentrations (30 mM) of ethanol. In contrast, the tonic inhibition mediated by α5 subunit-containing receptors of CA1 pyramidal cells is not affected. The effect of ethanol on tonic inhibition specifically reduces the excitability of the dentate gyrus and identifies the δ subunit-dependent tonic inhibition as a likely site of ethanol action in the brain.

Key words: **alcohol; CA1; dentate; GABA; hippocampus; inhibition**

Introduction

Over years of intensive research, it has been difficult to identify molecular targets for actions of ethanol at concentrations relevant for anxiolytic and social intoxicating effects in humans (10–30 mM; 17 mM or 0.08% blood alcohol level is the legal driving limit in California). Several ion channels, GABA and NMDA in particular, have been implicated in the actions of ethanol (Harris, 1999), but compelling effects were generally found at high concentrations, more applicable to the sedative–anesthetic actions of the drug rather than to its effects on anxiety and sobriety. Recently, low ethanol concentrations have been shown to affect central neurons through various mechanisms. The kainate receptor-dependent excitatory drive onto hippocampal interneurons was reduced by 10 mM ethanol (Carta et al., 2003), and ethanol (11–44 mM) was shown to augment GABA receptor (GABAR)-mediated IPSCs through corticotropin-releasing factor type 1 receptors (Nie et al., 2004). Recent reports have also identified the high-ethanol sensitivity of δ subunit-containing GABARs (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). Interestingly, these same receptors underlie a tonic form of inhibition in cerebellar and dentate gyrus granule cells (DGGCs) (Stell et al., 2003; Semyanov et al., 2004). Therefore, we investigated whether tonic inhibition in general is a target for ethanol action in the hippocampal formation.

Tonic inhibition is mediated primarily by specialized extrasynaptic GABARs and is activated by the ambient micromolar levels of GABA found in the CSF (Stell et al., 2003; Semyanov et al., 2004). The average charge carried by the tonically active GABARs is threefold to fivefold larger than the charge carried by all of the IPSCs (phasic inhibition) even when IPSCs occur at high frequencies (Nusser and Mody, 2002; Rossi et al., 2003). Consequently, tonic inhibition is considered to be an important regulator of neuronal gain control (Mitchell and Silver, 2003) and signal-to-noise ratio (Chadderton et al., 2004).

Materials and Methods

Tonic and phasic inhibitions were recorded as described previously (Stell et al., 2003) in slices prepared from C57BL/6J mice (40–60 d of age). The artificial CSF (aCSF) contained the following (in mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10–25 D-glucose, 0.005 GABA, and 3 kynurenic acid (for whole-cell recordings only), pH 7.3–7.4, when bubbled with 95% O_2/5% CO_2. Whole-cell recordings were made from DGGCs, and CA1 pyramidal cells (PCs) were identified by infrared videomicroscopy (Versascope; E. Marton Electronics, Cacoga Park, CA) using pipettes filled with the following (in mM): 125 CsCl, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 0.1 EGTA, 2 Na-ATP, 0.5 Na-GTP, and 5 *N*-ethyl bromide quaternary salt, pH 7.25 (280–290 mosm). The amount of tonic current was measured as described previously (Stell et al., 2003) by subtracting the current in the presence of saturating concentrations of SR95531. The IPSCs were detected and analyzed using a LabView-based software (Thotec). Extracellular field EPSPs (fEPSPs) and stimulus-response curves were recorded as described previously (Stell et al., 2003) in the molecular layer of the dentate gyrus and in the stratum radiatum of the CA1 under control conditions and during perfusion of 30 mM ethanol. The slopes of fEPSPs were fitted by a Boltzman equation of the form $f(W) = \mathrm{MAX}/[1 + \exp(-(W - W_{50})/k)]$, where W is stimulus duration (width in μsec), MAX is the maximum fEPSP slope, k is a steepness factor, and W_{50} is the stimulus width that elicits 50% of MAX.

Results

The effects of 30 mM ethanol on the tonic and phasic currents recorded in a DGGC are shown in Figure 1. In a total of seven

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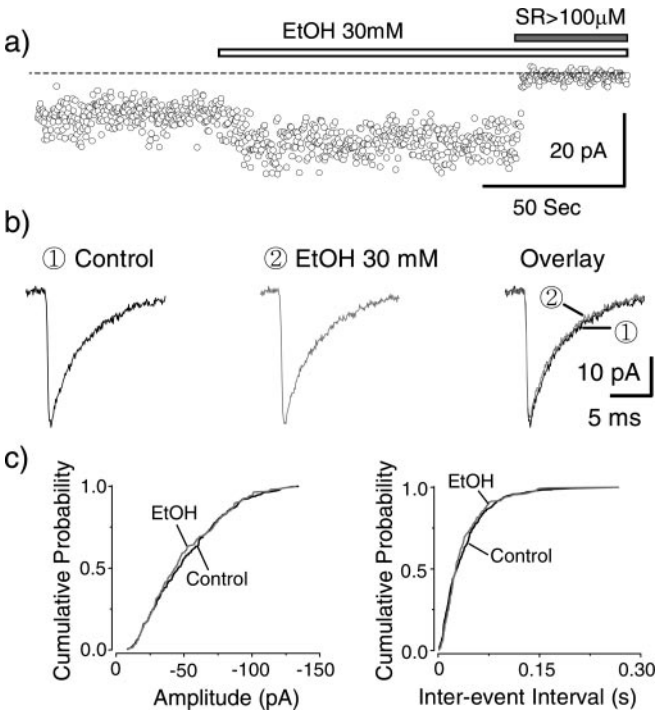

Figure 1. Effects of 30 mM ethanol on tonic and phasic inhibitions recorded in a dentate gyrus granule cell. *a*, Tonic inhibition was enhanced in the presence of 30 mM ethanol (open bar). The absolute change in tonic inhibition was measured after the application of the $GABA_AR$ antagonist SR95531 (100 μM). The dashed line represents the mean current after complete block of all $GABA_AR$-mediated currents used to calculate the magnitude of the tonic $GABA_AR$-mediated conductance (V_h, −70 mV). In this cell, the tonic current was potentiated by 94% from 8.1 to 15.7 pA. *b*, Averaged sIPSCs recorded in control aCSF did not change after perfusion of 30 mM ethanol. *c*, Cumulative probability plots of sIPSC amplitudes and inter-event intervals show no effect of 30 mM ethanol on sIPSCs in this DGGC.

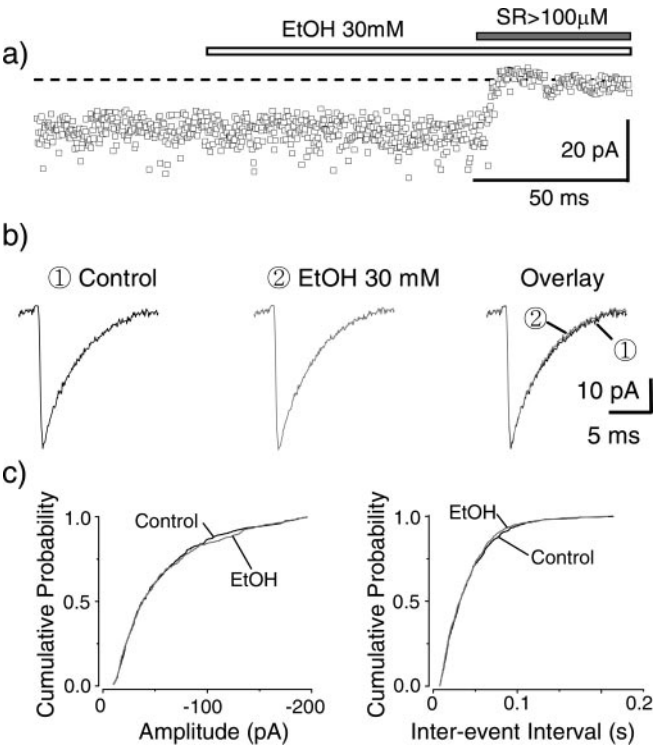

Figure 2. Perfusion of 30 mM ethanol has no effect on tonic and phasic inhibitions recorded in a CA1 pyramidal cell. *a*, Tonic inhibition was unaltered in the presence of 30 mM ethanol (open bar). The absolute value of tonic inhibition was measured after the application of the $GABA_AR$ antagonist SR95531 (100 μM). The dashed line is the mean current after the complete block of all $GABA_AR$-mediated currents used to calculate the magnitude of tonic inhibition (V_h, −70 mV). *b*, Averaged sIPSCs recorded in control aCSF did not change after perfusion of 30 mM ethanol. *c*, Cumulative probability plots of sIPSC amplitudes and inter-event intervals show no effect of 30 mM ethanol on sIPSCs in this neuron.

DGGCs, the average increase in tonic current produced by 30 mM ethanol was 81.4 ± 14.6% (from 13.7 ± 3.6 to 22.6 ± 4.5 pA; mean ± SEM; $p < 0.05$; paired two-tailed t test). It is clear that concentration-response curves would be desirable from a pharmacological point of view, but such experiments were outside the scope of the present study. Moreover, the resolution of tonic current measurements precluded us from testing lower ethanol concentrations, but the 81% increase found in DGGCs corresponds well to the 75% augmentation of GABA EC_{20} peak currents induced by 30 mM ethanol obtained in oocytes expressing $\alpha4\beta3\delta$ subunits (Wallner et al., 2003). Figure 1 also shows the lack of 30 mM ethanol effect on spontaneous IPSCs (sIPSCs) recorded in the same neuron. Table 1 summarizes the insensitivity to 30 mM ethanol of sIPSCs recorded in DGGC.

We wanted to know whether tonic inhibition in general was sensitive to relevant ethanol concentrations or whether the presence of δ subunits was necessary for the high-ethanol sensitivity. We performed additional experiments in DGGCs of δ subunit knock-out mice (Stell et al., 2003) and in CA1 pyramidal cells where tonic inhibition is not mediated by δ subunit-containing GABARs (Stell et al., 2003) but most likely by extrasynaptically located α5 subunit-containing GABARs (Brunig et al., 2002; Caraiscos et al., 2004). The tonic GABAR-mediated currents recorded in cells without δ subunits were insensitive to ethanol. The residual tonic currents recorded in δ subunit knock-out DGGCs were 104.1 ± 5% of predrug controls in the presence of 30 mM ethanol ($n = 8$). Similarly, our recordings of tonic inhibition in CA1 pyramidal cells indicated no sensitivity of this current to 30 mM ethanol. The change from 18.6 ± 3.6 to 19.8 ± 3.9 pA produced by 30 mM ethanol was not significant ($n = 6$). As expected from the results obtained in DGGCs, the properties of CA1 pyramidal cell sIPSCs were also insensitive to 30 mM ethanol (Fig. 2*b*,*c*; Table 1).

The fractional increase in tonic conductance produced in DGGCs by 30 mM ethanol was comparable with that induced by physiological concentrations (10 nM) of the neurosteroid al-

Table 1. Effects of 30 mM ethanol on sIPSCs in DGGCs and CA1 PCs in mouse hippocampal slices

	DGGCs		CA1 PCs	
	Control	Ethanol	Control	Ethanol
Amplitude (pA)	−43.0 ± 2.3	−42.2 ± 1.4	−56.7 ± 5.8	−57.0 ± 5.4
Frequency (Hz)	17.2 ± 2.8	16.8 ± 2.8	22.8 ± 4.2	23.4 ± 4.1
$RT_{10-90\%}$ (μsec)	373 ± 12	376 ± 11	333 ± 28	333 ± 10
τ_w (msec)	5.16 ± 0.20	5.13 ± 0.20	4.08 ± 0.56	4.07 ± 0.48
Number of cells	7	7	6	6

Values are mean ± SE. There were no significant differences between the control and ethanol-treated groups.

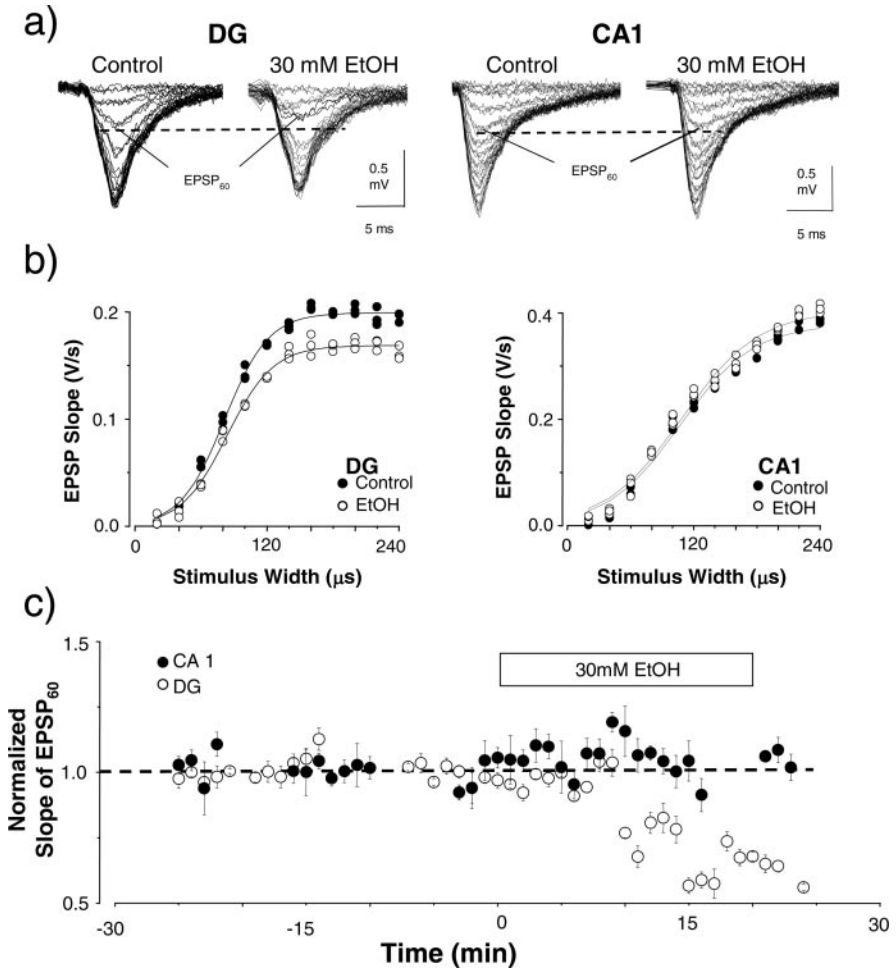

Figure 3. Ethanol (30 mM) reduces the fEPSP slope in the dentate gyrus (DG) where δ subunits are present. *a*, Raw traces of fEPSPs evoked during stimulus response curves before (left) and after (right) 30 mM ethanol (10 min) recorded in the molecular layer of the dentate gyrus and stratum radiatum of the CA1. The dashed line compares the two responses evoked by a stimulus width (*W*) of 60 μsec ($EPSP_{60}$). *b*, Representative stimulus-response curves recorded in the molecular layer of the DG or in the stratum radiatum of CA1 in control aCSF (●) and at the end of a 20 min 30 mM ethanol perfusion (○). Note the shift to the right in the DG, whereas the stimulus-response curve in the CA1 is unaltered. Lines represent the Boltzman functions fitted to the data points (three values each at each *W*). *c*, Plots of fEPSP slopes evoked by $W = 60$ μsec ($EPSP_{60}$) in the dentate gyrus and CA1 in two slices. Ethanol was perfused for 20 min as indicated by the box. Data points represent average slopes (±SEM) recorded during 1 min periods when fEPSPs were evoked every 15 sec. The values are normalized to the average of all responses evoked during the first 15 min. Gaps in the records represent the times when stimulus-response curves were evoked in the slices.

lotetrahydro-deoxy-corticosterone (Stell et al., 2003). Such an augmentation of the tonic current by the neurosteroid was sufficient to reduce the excitability of the dentate gyrus. We next addressed whether the effect of ethanol on tonic inhibition selectively reduced excitability in the dentate gyrus compared with CA1. Perfusion of 30 mM ethanol shifted the stimulus-response curves of fEPSPs to the right in the dentate gyrus but not in the CA1 (Fig. 3*a*,*b*). This was reflected by an increase of the W_{50} values in the dentate gyrus from 81.0 ± 3.4 to 89.5 ± 5.8 μsec ($n = 10$; $p < 0.05$ paired *t* test). Although in individual experiments there was a variable effect of ethanol on the values of MAX, on average, MAX and *k* were unaffected by ethanol. Figure 3*c* illustrates the pronounced dentate gyrus-selective effects of 30 mM ethanol on the slopes of fEPSPs evoked by less than half-maximal stimulus duration ($W = 60$ μsec). The most likely reason for the slower onset of ethanol action in the extracellular field recording experiments compared with the whole-cell recordings is the different recording chambers used for the two preparations (interfaced vs submerged).

Discussion

Our findings pinpoint the tonic inhibition mediated by δ subunit-containing GABARs as an important target for the action of ethanol concentrations relevant to intoxication in humans. Our results might have been predicted by the recent reports of an enhanced ethanol sensitivity of δ subunit-containing GABARs (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). However, it was not known whether tonic inhibition in general would be sensitive to ethanol. Based on the lack of ethanol effect in CA1 PCs, where tonic inhibition is likely mediated by GABARs composed of α5β3γ2/3 subunit combinations, apparently, not every type of GABAR-mediated tonic inhibition is a target for ethanol. If the δ subunit-containing GABARs are indeed a principal site of ethanol action, the distribution of δ subunit-containing GABARs should give some clues about the major *in vivo* effects of ethanol. The high concentration of δ subunit-containing GABARs in cerebellar granule cells and the existence of a strong δ subunit-mediated tonic current in these neurons (Stell et al., 2003) are compatible with the cerebellar motor effects of ethanol. An ethanol-induced change in tonic inhibition of cerebellar granule cells should change their input–output relationship to excitatory synaptic input in a predictable manner (Chadderton et al., 2004). In light of the partnership of δ and α6 subunits in these cells (Jones et al., 1997), cerebellar motor effects of ethanol should be altered in α6 knock-out mice, but only ethanol-induced sleep time, acute tolerance, and withdrawal were examined in these animals and were reported to be little affected (Homanics et al., 1998). The effects of ethanol still present in α6 knock-out mice (Homanics et al., 1998) might be mediated by the enhancement of tonic inhibition in areas of the brain, including the dentate gyrus, thalamus, and various regions of the cortex known to be rich in δ subunit-containing GABARs (Pirker et al., 2000; Peng et al., 2002). Our findings may have put gin and tonic into a new context, but it still remains to be determined whether any of the brain regions with δ subunit-mediated tonic GABA conductance are critical for the variety of ethanol actions, including reward, craving, tolerance, and dependence. The reduced ethanol consumption, attenuated withdrawal from chronic ethanol exposure, and reduced anticonvulsant effects of ethanol in δ subunit knock-out mice (Mihalek et al., 2001) are all consistent with a δ subunit-mediated tonic GABA conductance as an important factor in mediating the effects of ethanol on the CNS.

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