Differential GABA<sub>B</sub> Receptor Modulation of Ethanol Effects on GABA<sub>A</sub> Synaptic Activity in Hippocampal CA1 Neurons

Peter H. Wu, Wolfgang Poelchen, and William R. Proctor

VA Eastern Colorado Health Care System, Medical Research Service, Denver, Colorado (P.H.W., W.R.P.); Department of Psychiatry, University of Colorado Health Sciences Center, Denver, Colorado (P.H.W., W.R.P.); and the Institut fur Neurophysiologie, Heinrich-Heine-Universitat, Dusseldorf, Germany (W.P.)

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ABSTRACT

We tested the hypothesis that differential sensitivity to ethanol of synaptic GABA<sub>A</sub> somatic and dendritic inhibitory postsynaptic currents (IPSCs) in hippocampal CA1 pyramidal neurons could be due to differences in the extent of GABA<sub>B</sub> receptor activity at GABAergic synapses in these two hippocampal subfields. Our present results show that dendritic (distally evoked) GABA IPSCs contain a larger GABA<sub>B</sub> IPSC component of the total GABA IPSC than the somatic (proximally evoked) subfield. The inhibition of GABA<sub>B</sub> receptors by pretreatment of hippocampal slices with CGP-52432 changes the basal ethanol-insensitive, distally evoked GABA<sub>B</sub> IPSCs to become more sensitive to ethanol. In addition, paired-pulse stimulation of the proximal and distal subfields of hippocampal pyramidal neurons shows that ethanol alone increases the probability of GABA release at proximal but not distal regions. Changes by ethanol on the probability of GABA release are only seen at distal locations during GABA<sub>B</sub> blockade. Finally, when the modulation of pre-synaptic GABA<sub>B</sub> receptors is minimized by the local application of 10 mM GABA directly onto somatic or dendritic GABAergic synaptic regions, postsynaptic GABA<sub>B</sub> receptors seem to exert significant negative (inhibiting) influence on the effects of ethanol on GABA<sub>B</sub> IPSCs in the distal subfields of CA1 pyramidal neurons. Together, our data suggest that differences in both presynaptic and postsynaptic GABA<sub>B</sub> receptor activity at these GABAergic synapses may modulate the differential ethanol sensitivity of proximal and distal GABA<sub>A</sub> IPSCs in hippocampal CA1 pyramidal neurons.
Activation of GABA<sub>B</sub> receptors in the septohippocampal pathway has also been shown to potentiate behavioral effects of several anesthetics (Ma et al., 2002) as well as ethanol (Mihic et al., 1997). Electrophysiological studies have shown that GABA<sub>B</sub> IPSCs evoked in the CA1 hippocampal stratum pyramidalis (proximal) subfield are enhanced to a greater extent by ethanol than GABA<sub>A</sub> IPSCs evoked in the stratum lacunosum-moleculare (distal) subfield (Weiner et al., 1997); however, the mechanisms that mediate the differential effect of ethanol on GABA currents evoked at these two pyramidal cell subfields are not well understood. The fact that zolpidem produced a similar enhancement of GABA<sub>B</sub> IPSCs at both proximally evoked (proximal GABA<sub>B</sub> IPSCs) and distally evoked (distal GABA<sub>B</sub> IPSCs) locations (Weiner et al., 1997) would seem to preclude the possibility that differences in GABA<sub>B</sub> receptor composition may underlie the differential ethanol sensitivity of GABA<sub>B</sub> IPSCs evoked at these two subfields in hippocampal CA1 pyramidal neurons. Other studies (Wan et al., 1996; Roberto et al., 2003; Ariwodola and Weiner, 2004) showed that a GABA<sub>B</sub> receptor antagonist can augment the ethanol enhancement of GABA<sub>B</sub> IPSCs, implying the involvement of GABA<sub>B</sub> receptors in modulating ethanolic effects of GABA<sub>A</sub> responses in the hippocampus, but the mechanisms are not well understood.

GABA<sub>B</sub> receptors mediate the slow synaptic inhibitory response of GABA transmission (Solis and Nicoll, 1992) and are found in both the presynaptic and postsynaptic elements of GABAergic terminals. The GABA<sub>B</sub> receptor is a typical seven transmembrane G protein-coupled receptor and is known to exert its action through the interaction with G<sub>protein</sub> and G<sub>protein<sub>gamma</sub></sub> subunits that modulate presynaptic calcium channels (Dittman and Regehr, 1996; Kubota et al., 2003) to regulate GABA release. The GABA<sub>B</sub> receptor has also been shown to modify postsynaptic potassium conductance (Newberry and Nicoll, 1984; Gahwiler and Brown, 1985; Osmanovic and Shefner, 1988), thereby altering cell activity. Although ethanol had little direct effect on the GABA<sub>B</sub> receptor response (Frye and Fincher, 1996; Roberto et al., 2003), blockade of the GABA<sub>B</sub> receptor in CA1 neurons was shown to increase ethanol potentiation of the GABA<sub>A</sub> response (Wan et al., 1996).

In the current study, we test the hypothesis that differences in GABA<sub>B</sub> receptor function may underlie the differential ethanol sensitivity of GABA<sub>B</sub> IPSCs observed in proximal and distal locations of CA1 pyramidal neurons. The results of this study support this concept and implicate a significant modulatory role of postsynaptic GABA<sub>B</sub> receptor activity in the differential ethanol sensitivity between proximal and distal subfields in hippocampal CA1 pyramidal neurons.

**Materials and Methods**

**Slice Preparation.** As described previously (Poelchen et al., 2000), transverse hippocampal slices (400 μm) were prepared from 6- to 8-week-old male Sprague-Dawley rats with a Sorvall tissue chopper (Sorvall, Newtown, CT). Slices were made in ice-cold artificial cerebrospinal fluid (aCSF) and were stored in a submersion chamber at 32°C in aCSF containing 126 mM NaCl, 3 mM KCl, 1.5 mM MgCl<sub>2</sub>, 2.4 mM CaCl<sub>2</sub>, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 11 mM glucose, and 26 mM NaHCO<sub>3</sub> saturated with 95% O<sub>2</sub>/<sub>5</sub>% CO<sub>2</sub>. The GABA<sub>B</sub> Activity Modulates Ethanol Action on GABA<sub>A</sub> Currents 1083

**Electrophysiological Recordings and Drug Application.** Hippocampal slices were transferred to a submersion recording chamber (H and H Custom Machining, Denver, CO) and superfused with gassed (95% O<sub>2</sub>/5% CO<sub>2</sub>) aCSF at a flow rate of 2 ml/min. Whole-cell patch-clamp recordings were made from CA1 pyramidal neurons at 32°C using an Axoclamp-2A amplifier (Axon Instruments Inc., Union City, CA) operating in voltage-clamp mode. Cells were not included in this study if their resting membrane potential was depolarized more than −65 mV (corrected for the liquid junction potential), if their access resistance was greater than 30 MΩ, or if this value changed by >15% over the course of the recording. Cells were voltage-clamped to −70 mV. To increase the chloride driving force to obtain GABA<sub>A</sub> IPSC responses of 50 to 100 pA (approximately 10–30% of the maximal response). Recording electrodes were constructed from fiber-containing borosilicate glass (1.5 mm o.d., 0.86 mm i.d.; Sutter Instrument Company, Navato, CA) and had resistances of 6 to 9 MΩ when filled with the following internal K<sup>+</sup>-glucuronate solution: 125 mM potassium gluconate, 1.5 mM KCl, 10 mM HEPES, 0.1 mM CaCl<sub>2</sub>, 2.0 mM Mg<sup>2+</sup>-ATP, and 0.2 mM Mg<sup>2+</sup> GTP. pH adjusted to 7.25 with KOH, 290 ± 5 mOsm. This solution was kept on ice until immediately before use. All drugs used to make up the aCSF and internal recording solution were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol and the receptor antagonists listed below were added to the artificial cerebrospinal fluid in known concentrations immediately before flowing into the slice chamber by direct infusion via calibrated syringe pumps (Razel Scientific Instruments, Stamford, CT). Glutamatergic N-methyl-d-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonists D-2-amino-5-phosphonovanoic acid (APV) and 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt (CNQX) and the GABA<sub>B</sub> receptor antagonist bicuculline methiodide (BMI) were also purchased —from Sigma-Aldrich. The GABA<sub>B</sub> receptor antagonist CGP-52432 was purchased from Tocris Cookson Inc. (Ellisville, MO). An 8.0 M ethanol solution (in deionized water) was prepared immediately before each experiment from a 95% stock solution (Aaper Alcohol and Chemical, Shelbyville, KY) and kept in a glass storage bottle at 4°C.

**Proximal and Distal GABA<sub>A</sub> and GABA<sub>B</sub> Receptor-Mediated IPSCs.** Evoked GABA<sub>B</sub> IPSCs were pharmacologically isolated by superfusion with the glutamatergic receptor antagonists APV (50 μM) and CNQX (20 μM) to block NMDA and AMPA receptors, respectively. Synaptic stimulation was delivered with the use of two bipolar twisted tungsten wire electrodes (0.2-μm pulses of 3–8 V), one placed in the stratum lacunosum-moleculare layer (for distal stimulation) and the other within 250 μm of the recorded cell soma in the CA1 stratum pyramidalis (for proximal stimulation), as illustrated in Fig. 1A. An interstimulation interval of 20 s was used, and the stimulus intensities were adjusted to evoke proximal and distal IPSCs of similar amplitude. When GABA<sub>A</sub> responses were isolated by superfusion with the GABA<sub>B</sub> receptor antagonist BMI, the remaining GABA<sub>A</sub> component was completely blocked by CGP-52432. For determinations of the extent of the GABA<sub>B</sub> fraction in proximal and distal responses, the percentage of the total GABA IPSC remaining following BMI treatment was calculated. Paired-pulse depression in these CA1 pyramidal neurons was investigated by delivering the stimuli to the electrodes located at both the proximal and distal subfields of the neuron. The paired-pulse stimuli were determined at an interpulse interval of 30 or 50 ms. Both intervals gave similar results, so data from both conditions were combined for the data analysis. The amplitudes of the evoked responses were measured, and the paired-pulse index (PPI) was calculated as the ratio of the two responses (second/first). Curve fitting was determined from responses to single pulses obtained throughout each experiment. In some experiments, differential interference contrast microscopy was used to measure the effect of ethanol from brief, local, direct pressure application of GABA to the soma or dendrites of CA1 pyramidal neurons. Micropipettes containing 10 mM GABA were placed 3 to 5 μm from the cell body or major dendritic processes under visual control. GABA was delivered through the micropipette (5–10 ms at 10–20 psi) via a Picosprizer II (General Valve, Fairfield, NJ).
Control responses were recorded, and then ethanol (80 mM) and/or CGP-52432 was applied by bath superfusion, and the GABA-evoked responses were measured before, during, and after washout of ethanol.

Statistical Analysis. Drug effects were quantified as the percentage of change in amplitude under the curve of the GABAA IPSCs following drug application relative to the mean control and washout values. Statistical analyses were carried out with the use of SigmaStat (SPSS Inc., Chicago, IL). The minimal significance level was set at \( p < 0.05 \).

Results

Effects of Ethanol on Proximally and Distally Evoked GABAA IPSCs in the Hippocampus. GABA IPSCs were evoked by electrical stimulation (3–8 V) by a bipolar stimulating electrode placed in the stratum pyramidale (proximal) or stratum radiatum (distal) subfields of the hippocampus (Fig. 1A) and isolated by blocking AMPA and NMDA receptors with 20 \( \mu \)M CNQX and 50 \( \mu \)M APV, respectively. This pharmacologically isolated GABA current usually contained two components: a fast GABAA response (peak amplitude latency of 5–10 ms) and a late GABAB component (peak amplitude latency of 150–200 ms) that was completely blocked by CGP-52432, a potent GABAB receptor antagonist. Ethanol (at concentrations of 40 mM and above) produced enhancements of proximally evoked GABAA IPSCs (proximal GABAA IPSCs) but much smaller increases by distally evoked GABAA IPSCs (distal GABAA IPSCs; Fig. 1, B and C), exhibiting time and ethanol concentration dependence (Fig. 1, D and E, respectively). A partial dose-response relationship for ethanol indicates that both proximal and distal GABAA IPSCs were enhanced by ethanol (40, 80, 100, and 120 mM) \([F(4,74) = 11.646, p < 0.001, \text{two-way ANOVA}]\) and that proximal GABAA IPSCs were potentiated to a significantly greater extent than distal GABAA IPSCs \([F(1,74) = 6.151, p < 0.015, \text{two-way ANOVA}]\). These results are consistent with those reported by Weiner et al. (1997). Statistical analysis of the present data shows that there is a significant ethanol concentration versus hippocampal subfield interaction \([F(4,74) = 3.447, p < 0.012, \text{two-way ANOVA}]\). Thus, ethanol exhibited differential potentiation of GABAA IPSCs in the proximal and distal subfields of hippocampus over this ethanol concentration range tested (40–120 mM).

Effect of GABAB Receptor Activity on Ethanol Potentiation of GABAA IPSCs. GABA IPSC traces typically
show that, under most recording conditions, the proximal GABA IPSC responses have a smaller late GABAB IPSC component than those evoked distally (Fig. 2, A1 and B1). Regardless of the proportion or size of the GABAB component, after pharmacological isolation with a GABA_A antagonist, BMI (10 μM), ethanol (80 mM) treatment did not significantly modify these GABA_A responses by either proximal (103 ± 9.8% of control, n = 7) or distal (102 ± 7.2% of control, n = 7) stimulation (Fig. 2, A2 and B2). Systematic subfield stimulation studies in the same CA1 pyramidal neurons in which GABA IPSCs were alternately evoked from proximal and distal locations show that the mean distally evoked GABA IPSC contains a significantly larger late GABAB IPSC component than the mean proximally evoked GABA IPSC (Fig. 2C) (Student’s t test, t = 3.209, p < 0.006). Furthermore, a significant correlation is seen between the ethanol potentiation of GABA_A IPSC and GABAB content; neurons having a smaller GABAB component have greater responses to ethanol \( [F(1,24) = 14.878, p < 0.001, \text{linear regression analysis}] \) (Fig. 2D).

To determine the effect of the possible GABA_B receptor activity on the ethanol enhancement of the GABA_A responses, we repeated the ethanol experiments in the presence of CGP-52432, a selective GABA_B receptor antagonist, to block the GABA_B IPSC component (Fig. 3, A1 and B1). Pretreatment of hippocampal slices with CGP-52432 shows that 80 mM ethanol now potentiates both the proximal and distal GABA_A IPSCs (Fig. 3, A2 and B2, respectively), and the enhancement is reversed upon washout of ethanol; however, the CGP-52432 block of the GABA_B component was not completely reversed after 30 min of washout (Fig. 3, A3 and B3). Composite results show that, in the presence of the GABA_B antagonist, 80 mM ethanol potentiates GABA_A IPSCs from the distally evoked location similarly to the ethanol enhancement via proximal stimulation (Fig. 3C) and that the proximal GABA_A IPSCs were not further enhanced by the pretreatment of slices with CGP-52432.

**Ethanol Enhancement of GABA_A IPSCs: Presynaptic and/or Postsynaptic?** A paired-pulse stimulation paradigm was used to examine a possible presynaptic role in the differential ethanol enhancement of proximal and distal GABA_A IPSC responses. An increase in the second of two

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**Fig. 2.** GABA_A activity modulates ethanol enhancement of distally evoked GABA_A responses. Synaptic GABA receptor-mediated responses usually consist of two components: a faster GABA_A and slower GABA_B component. Blockade of the GABA_A responses by 10 μM BMI reveals a small GABA_A response from proximal stimulation (A1), whereas the distally evoked IPSC response from the same cell (B1) typically has a much larger GABA_B component. Neither proximally (A2) nor distally (B2) evoked GABA_A responses are significantly modified by 80 mM ethanol (mean proximal GABA_B IPSCs: 103 ± 9.8% of control, n = 7; mean distal GABA_B IPSCs: 102 ± 7.2, n = 7). C, bar graph showing the paired mean and S.E.M. values for the proximal and distal GABA_B component (16.8 ± 5.24, n = 13; 49.1 ± 8.61, n = 13, respectively) of the total GABA IPSC response in these cells. **p < 0.01. The relationship of ethanol enhancement of the GABA_A response and the proportion of the GABA_B component is shown in D. These results are from proximally and distally evoked GABA_A responses measured in 13 cells treated with 80 mM ethanol. There is an inverse relationship between the sensitivity to ethanol and the proportion of the GABA_B component in the response, determined by blocking the GABA_B component following ethanol treatment and washout. Proximally evoked GABA_A responses (closed circles) showed greater ethanol enhancement and had the least GABA_B influence, whereas ethanol had a smaller effect on the distal responses (open circles) usually comprising a greater GABA_B component. A linear least-squares fit is shown (solid line) that has an r^2 value of 0.38, which is statistically significant (p < 0.001, n = 26). Calibration bars in A and B: 10 pA, 40 ms.
closely paired stimulations (30–50 ms apart) is thought to mainly be due to a presynaptic mechanism involving the phenomena of a higher probability of neurotransmitter vesicular release. Single and double (paired-pulse) GABA<sub>A</sub> IPSC responses were alternatively evoked in both proximal and distal locations on CA1 hippocampal pyramidal neurons (Fig. 4). The PPI was measured before and during ethanol (80 mM) treatment (Fig. 4, A<sub>1</sub> and B<sub>1</sub>). The PPI was determined as the ratio of the second peak amplitude response (P<sub>2</sub>, above the decay phase of the first peak) divided by the peak amplitude of the first peak (P<sub>1</sub>) as shown in Fig. 4B<sub>1</sub>. Ethanol treatment resulted in a significant increase in the proximally stimulated PPI (Fig. 4A<sub>1</sub>) (control: 0.63 ± 0.039, n = 11; ethanol: 0.90 ± 0.085, n = 10, p = 0.005), but no significant effect was measured by distal stimulation (Fig. 4B<sub>1</sub>) (control: 0.64 ± 0.028, n = 13; ethanol: 0.75 ± 0.070, n = 10, p = 0.186). However, in the presence of the GABA<sub>B</sub> antagonist CGP-52432, ethanol at both proximally and distally evoked sites show increased PPI (proximal, Fig. 4A<sub>2</sub>: 0.86 ± 0.051, n = 11, p = 0.013; distal, Fig. 4B<sub>2</sub>: 0.87 ± 0.052, n = 12, p = 0.003). The composite data from all cells tested are shown in Fig. 4C. Interestingly, CGP-52432 treatment alone significantly increased the PPI at both proximal (0.85 ± 0.073, n = 11, p = 0.018) and distal (0.86 ± 0.064, n = 13, p = 0.004) stimulation sites.

Finally, local application of GABA directly onto the somatic or dendritic subfields of CA1 neurons was used to test for the effects of ethanol on postsynaptic GABA responses. Brief pressure application (10–20 psi, 5–15 ms) of GABA onto the proximal subfield produces a GABA response that is enhanced by ethanol in the presence or absence of the GABA<sub>B</sub> antagonist CGP-52432 (Fig. 5, A<sub>1</sub> and A<sub>2</sub>). In contrast, although the application of GABA onto the dendritic subfield of CA1 neurons produces a robust GABA response, ethanol in the absence of the GABA<sub>B</sub> receptor antagonist does not significantly enhance the GABA response (Fig. 5B<sub>1</sub>). However, in the slices pretreated with CGP-52432, ethanol application produces a significant increase in the GABA response (Fig. 5B<sub>2</sub>). A comparison of the mean values during ethanol and/or CGP-52432 treatment (Fig. 5C) shows that, in the presence of CGP-52432, ethanol enhances responses to local GABA application to a similar extent in both proximal and distal subfields.

**Discussion**

Although it has been suggested that the differential sensitivity to ethanol in various brain preparations could be attributed to the composition of GABA<sub>A</sub> receptor subtypes (Harris, 1999; Mihic, 1999; Blednov et al., 2003), recent studies have shown that ethanol increased spontaneous GABA IPSC frequency in rat CA1 pyramidal neurons (Ariwodola and Weiner, 2004) and rat amygdala neurons (Roberto et al., 2004; Nie et al., 2004). These studies support the concept that ethanol may also increase the GABA<sub>A</sub> IPSC response via enhancement of presynaptic GABA release. Since pretreatment of hippocampal slices with a threshold concentration of 500 nM baclofen prevented ethanol (80 mM) from enhancing the proximally evoked GABA<sub>A</sub> IPSCs and can even cause IPSC inhibition by ethanol at a higher baclofen concentration (1.25 μM), Ariwodola and Weiner (2004) concluded that presynaptic GABA<sub>B</sub> receptors may modulate the actions of ethanol in the proximal subfield of CA1 pyramidal neurons in the hippocampus. On the other hand, our data show that...
Fig. 4. Paired-pulse response ratios of GABA<sub>A</sub> IPSCs are modulated by ethanol. Paired-pulse stimulation (30–50-ms interval) in both the proximal (A<sub>1</sub>, Control) and distal (B<sub>1</sub>, Control) regions of CA1 pyramidal neurons show paired-pulse depression (ratio, <1.0) of GABA<sub>A</sub> IPSCs determined by measuring the ratio of the amplitude of the second pulse, P<sub>2</sub>, and the amplitude of the first pulse response, P<sub>1</sub>, as depicted in B<sub>1</sub>. The second pulse amplitude values were obtained by subtracting the influence of the decay phase of the first, unconditioned pulse (shown as the dashed sweep for the mean single-pulse condition) from the absolute peak amplitude of the second pulse. These GABA IPSC responses were acquired using an alternating single-then double-pulse stimulation paradigm with a 20-s interval between to allow for full recovery of the previous response. Ethanol (80 mM) enhances both peaks by proximal stimulation (A<sub>1</sub>) but has little effect on either peak by distal stimulation (B<sub>1</sub>). In other pyramidal cells, the effects of the GABAB antagonist CGP-52432 alone and in the presence of 80 mM ethanol were tested (proximal, A<sub>2</sub>; distal, B<sub>2</sub>). Both conditions increased the PPI by proximal and distal stimulation, giving support to presynaptic modulation by these drugs. C, bar graph showing the summary data for these PPI determinations measured from 8 to 13 cells in each condition. *, p < 0.05 and **, p < 0.01. Calibration bars in A and B: 30 pA, 50 ms.

Fig. 5. GABA<sub>B</sub> antagonist modulates the GABA<sub>A</sub> response to ethanol in distal, but not proximal, responses to direct application of GABA. The local application of 10 mM GABA to the soma of CA1 pyramidal neurons (A<sub>1</sub>) produces a GABA<sub>A</sub> response that is enhanced by the bath perfusion of 80 mM ethanol and blocked by BMI (not shown). Local application of GABA to the distal dendrites (B<sub>1</sub>) also produces a GABA<sub>A</sub> response; however, this response is not enhanced by the bath perfusion of ethanol. In the presence of a GABA<sub>B</sub> antagonist (CGP-52432), however, responses by distal GABA application are now enhanced by ethanol (B<sub>2</sub>). The enhancement of ethanol on proximal GABA responses is not changed by the presence of CGP-52432 (A<sub>2</sub>). The mean values for all the local GABA application conditions are shown in C. Direct application of GABA enhances the GABA<sub>A</sub> response approximately 15 to 20% at distal sites only in the presence of the GABA<sub>B</sub> antagonist. In addition, ethanol enhances the GABA<sub>A</sub> response by direct proximal GABA application only about 15 to 20% compared with the 30 to 50% enhancement of the synaptic GABA<sub>A</sub> IPSC response. Calibration bars: A<sub>1</sub> and A<sub>2</sub>, 20 pA, 30 ms; B<sub>1</sub> and B<sub>2</sub>, 10 pA, 30 ms. The mean percentage of control values, S.E.M., and number of cells tested for each condition in C are as follows: proximal EtOH, 116 ± 6.6, n = 27; CGP + EtOH, 114 ± 7.47, n = 6; distal EtOH, 104 ± 3.7, n = 20; CGP + EtOH, 120 ± 3.0, n = 6. *, p < 0.05; NS, not significant.
proximally evoked GABA<sub>A</sub> IPSCs are enhanced to a similar extent by ethanol with or without pretreatment of a GABA<sub>B</sub> receptor antagonist, suggesting either very low endogenous GABA<sub>B</sub> receptor activity (our present results) or that ethanol enhancement of the proximal GABA<sub>A</sub> IPSC is not significantly modulated by presynaptic GABA<sub>B</sub> receptor activity. A low presynaptic GABA<sub>B</sub> activity at proximal GABAergic synapses would imply that inhibition of GABA<sub>B</sub> receptors would have little or no effect on the increase in probability of GABA release measured by paired-pulse stimulation. However, our experiments show that CGP-52432 enhances the paired-pulse ratio to a similar extent in both the proximally and distally evoked GABA<sub>A</sub> IPSCs, suggesting similar endogenous presynaptic GABA<sub>B</sub> receptor activity in both subfields of CA1 pyramidal neurons. Therefore, the differential sensitivity to ethanol in proximally and distally evoked GABA<sub>A</sub> IPSCs cannot be explained readily by differences in presynaptic GABA<sub>B</sub> receptor activity alone. In addition, presynaptic GABA<sub>B</sub> receptors have been shown to be inoperative under control or basal conditions in the adult CNS (Otis and Mody, 1992; McLean et al., 1996) unless tested under conditions causing large GABA release, such as during intense electrical stimulation (Deisz and Prince, 1989; Davies et al., 1993; Isaacs et al., 1993).

Evidence for a postsynaptic GABA<sub>B</sub> receptor-mediated IPSC has been shown in hippocampal neurons (Thalmann, 1988). This late inhibitory postsynaptic potential was mediated by pertussis toxin-sensitive, G protein-mediated activation of G protein-coupled inwardly rectifying potassium channels and inhibition of N-type Ca<sup>2+</sup> channels (Bertrand et al., 2003). The activation of this postsynaptic GABA<sub>B</sub> IPSC was thought to enhance neuronal inhibition at GABAergic synapses (Pham et al., 1998). To circumvent the effect of a postsynaptic GABA<sub>B</sub> receptor regulation of GABA release at the GABAergic synapses, we pressure-applied 10 mM GABA locally onto proximal or distal subfields of CA1 pyramidal neurons. Under these experimental conditions, ethanol enhanced proximally evoked GABA<sub>A</sub> IPSCs similarly in the absence or presence of a GABA<sub>B</sub> receptor antagonist, suggesting a minimal role for GABA<sub>B</sub> regulation of ethanol effects on these proximal GABA<sub>A</sub> IPSCs. In contrast, ethanol alone did not enhance the distally evoked GABA<sub>A</sub> IPSCs, but it did enhance the distal GABA<sub>A</sub> IPSCs in the presence of a GABA<sub>B</sub> receptor antagonist. Thus, our data suggest that the differences in ethanol sensitivity at proximally and distally evoked GABA<sub>A</sub> IPSCs may be due to differences in the levels of postsynaptic GABA<sub>B</sub> receptor number and/or activity between these two CA1 subfields. Since ethanol alone failed to increase the probability of GABA release in the distal GABAergic synapses, we conclude that differences in both presynaptic and postsynaptic GABA<sub>B</sub> receptor activity may modulate the differential ethanol effects on GABA<sub>A</sub> IPSCs in these two subfields in CA1 pyramidal neurons. Future studies are designed to use receptor antagonists and manipulations that are selective to presynaptic or postsynaptic GABA<sub>B</sub> subtypes to elucidate the precise mechanisms of ethanol action in these hippocampal neurons.

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Address correspondence to: Dr. William R. Proctor, Dept. of Psychiatry (C-261), University of Colorado Health Sciences Center, 4200 E. 9th Avenue, Denver, CO 80262. E-mail: bill.proctor@uchsc.edu