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Ethanol Potentiation of GABAergic Synaptic Transmission May Be Self-Limiting: Role of Presynaptic GABA$_B$ Receptors

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Ethanol enhances GABAergic synaptic inhibition, and this interaction contributes to many of the behavioral and cognitive effects of this drug. Most studies suggest that ethanol enhances GABAergic neurotransmission via an allosteric potentiation of the postsynaptic GABA$_A$ receptors that mediate fast synaptic inhibition in the mammalian CNS. Despite widespread acceptance of this hypothesis, direct support for such a mechanism has been difficult to obtain. Ethanol does not enhance GABA$_A$ receptor function in all brain regions or under all experimental conditions, and factors responsible for this variability remain mostly unknown. Notably, blockade of GABA$_B$ receptors dramatically enhances ethanol potentiation of hippocampal GABA$_A$ IPSPs and IPSCs, suggesting that some unknown GABA$_B$ receptor mechanism limits the overall potentiating effect of ethanol on GABAergic synapses. In this study, we demonstrate that, at perisomatic synapses in the rat hippocampus, ethanol enhances presynaptic GABA$_B$ autoreceptor function and that this interaction reduces the overall potentiating effect of ethanol at these synapses. We further show that ethanol significantly elevates basal presynaptic GABA$_B$ receptor tone, possibly via an increase in spontaneous GABA release, and that pretreatment with a subthreshold concentration of the GABA$_B$ receptor agonist baclofen blocks ethanol but not flunitrazepam or pentobarbital potentiation of GABA$_A$ IPSCs. These data suggest that an interaction between ethanol and presynaptic GABA$_B$ autoreceptor activity regulates the ethanol sensitivity of GABAergic synapses. Given that the *in vitro* ethanol sensitivity of these synapses correlates with *in vivo* ethanol responsiveness in a number of rodent lines, our data further suggest that presynaptic GABA$_B$ receptor activity may play a role in regulating behavioral sensitivity to ethanol.

Key words: alcohol; GABA; hippocampus; IPSP; patch clamp; slice

Introduction

Alcohol addiction remains an imposing medical and socioeconomic concern for many nations (Volpicelli, 2001). For example, in the United States, alcoholism and alcohol abuse rank among the top three psychiatric illnesses (Kessler et al., 1994). In addition, alcohol-related disorders are responsible for >105,000 deaths annually in the United States (McGinnis and Foege, 1999) at a cost of >150 billion dollars. Despite these staggering statistics, the neurophysiological mechanisms that mediate alcohol intoxication, reinforcement, and dependence are not fully understood. A hypothesis that has received increasing support over the past 20 years is that alcohol interacts with a subset of neuronal proteins that control excitatory and inhibitory synaptic communication in the CNS (Deitrich et al., 1989; Faingold et al., 1998). In particular, much attention has focused on the acute potentiating effects of alcohol on inhibitory synaptic transmission mediated by GABA$_A$ receptors.

GABA$_A$ receptors mediate the majority of fast inhibitory synaptic transmission in the mammalian CNS (Krnjevic, 1991;

Thompson, 1994). These receptors serve as the primary target for a variety of sedative and hypnotic drugs, such as barbiturates and benzodiazepines, which allosterically enhance GABA$_A$ receptor function (Macdonald and Olsen, 1994). There is also considerable evidence in support of the hypothesis that the behavioral and cognitive effects of ethanol are mediated, at least in part, via a potentiation of GABA$_A$ receptor-mediated synaptic inhibition (Grobin et al., 1998; Mihic, 1999). Despite the popularity of this hypothesis, considerable controversy remains. For example, studies that have examined the direct effects of ethanol on GABAergic synaptic transmission have reported potentiation (Proctor et al., 1992b; Weiner et al., 1994, 1997; Poelchen et al., 2000; Roberto et al., 2003), inhibition (Siggins et al., 1987), or no effect (Siggins et al., 1987; Soldo et al., 1994), and the reasons for these disparate results remain poorly understood. Interestingly, several studies have noted that blockade of GABA$_B$ receptors can dramatically enhance the acute potentiating effect of ethanol on GABA$_A$ receptor-mediated IPSCs in the rat hippocampus (Wan et al., 1996; Kang et al., 1998). These studies suggest that some unknown GABA$_B$ receptor-dependent process may play an important role in regulating the ethanol sensitivity of GABAergic synapses. Most GABAergic synapses contain both presynaptic and postsynaptic GABA$_B$ receptors. Presynaptic GABA$_B$ receptors function as autoreceptors, and their activation inhibits GABA$_A$ IPSCs, whereas postsynaptic GABA$_B$ receptors are primarily coupled to the activation of a potassium conductance (Misgeld et al., 1995). In this study, we demonstrate that ethanol

exerts a novel facilitatory effect on presynaptic GABA$_B$ receptor function at GABAergic synapses in the rat CA1 region and that this interaction serves to actively limit the overall potentiating effect of ethanol at these synapses.

Materials and Methods

Slice preparation. Transverse hippocampal slices (400 μm) were prepared from 4- to 6-week-old male Sprague Dawley rats. Slices were maintained at ambient temperature for at least 2 hr in oxygenated artificial CSF (aCSF) containing (in mM): 124 NaCl, 3.3 KCl, 2.4 MgCl$_2$, 2.5 CaCl$_2$, 1.2 KH$_2$PO$_4$, 10 D-glucose, and 25 NaHCO$_3$, saturated with 95% O$_2$ and 5% CO$_2$.

Electrophysiological recordings. Slices were transferred to a recording chamber maintained at ambient temperature and superfused with aerated aCSF at 2 ml/min. Recording electrodes were prepared from filamented borosilicate glass capillary tubes (inner diameter, 0.86 mm) using a horizontal micropipette puller (P-97; Sutter Instruments, Novato, CA). Patch-clamp recordings were made using a filling solution containing 130 mM K-gluconate, 10 mM KCl, 1 mM EGTA, 100 μM CaCl$_2$, 2 mM Mg-ATP, 200 μM Tris-guanosine 5′-triphosphate, and 10 mM HEPES, pH adjusted with KOH, 275–280 mOsm. In most experiments, 5 mM *N*-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314) was included in the recording solution to block voltage-gated sodium currents and GABA$_B$ IPSCs in the CA1 neurons being recorded (Horn et al., 1980; Nathan et al., 1990). QX-314 was omitted from the filling solution in experiments measuring postsynaptic GABA$_B$ receptor function (see Fig. 3). Whole-cell patch-clamp recordings were made from CA1 pyramidal neurons voltage-clamped at −45 to −60 mV. Only cells with a stable access resistance of 5–20 MΩ were used in these experiments. Whole-cell currents were acquired using an Axoclamp 2B or Axopatch 200B amplifier, digitized (Digidata1200 or Digidata 1321A; Axon Instruments, Union City, CA), and analyzed on- and off-line using an IBM-compatible personal computer and pClamp 8.0 or 9.0 software (Axon Instruments). In one set of experiments, we recorded spontaneous GABA$_A$ IPSCs (sIPSCs) using a filling solution identical to that described above, except that 140 mM CsCl$_2$ was substituted for K-gluconate and KCl. sIPSCs were digitized at 5–10 kHz in continuous 3 min epochs. Spontaneous events in each epoch were first identified using Clampfit event detection software (pClamp 9.0), and then all events were visually inspected to avoid inclusion of spurious responses in the data analysis (<2% of detected events were rejected). sIPSCs in each epoch were then averaged, and the amplitude and area of averaged traces were calculated using the Statistics function included in the Clampfit program (pClamp 9.0).

Pharmacological isolation of synaptic currents. GABA$_A$ IPSCs were evoked every 20 sec by electrical stimulation (0.2 msec duration) using a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) placed near the CA1 somatic layer ["proximal" stimulation (Weiner et al., 1997)]. Stimulation intensity was adjusted to evoke responses that were 10–20% of maximal currents (typically 50–200 pA). GABA$_A$ IPSCs were pharmacologically isolated using a mixture of 50 μM APV and 20 μM DNQX to block NMDA and AMPA/kainate receptors, respectively. GABA$_B$ IPSCs were recorded in the presence of a similar mixture that also included 20 μM bicuculline methiode to block GABA$_A$ receptors. Unless otherwise stated, all drugs used were purchased from Sigma (St. Louis, MO). Drugs were made up as 100- to 400-fold concentrates and applied to slices via calibrated syringe pumps (Razel Scientific Instruments, Stamford, CT). A 4 M ethanol solution was prepared immediately before each experiment from a 95% stock solution (Aaper Alcohol and Chemical, Shelbyville, KY) kept in a glass storage bottle.

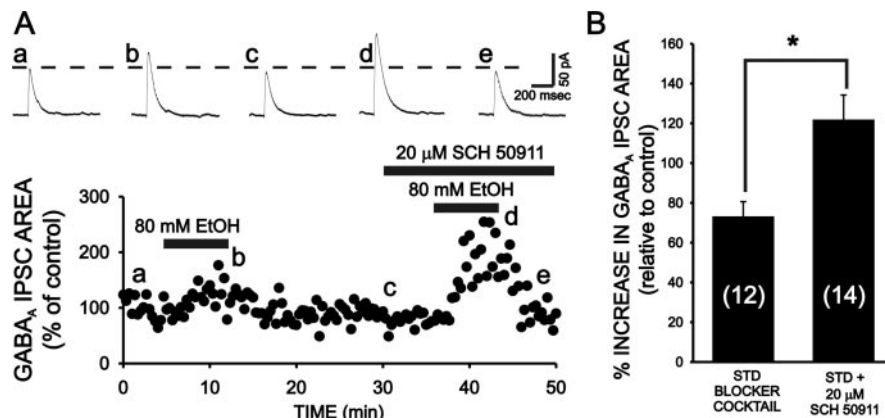*Statistics.* Drug effects were quantified as the percentage change in the

Figure 1. Pretreatment with a GABA$_B$ receptor antagonist enhances ethanol potentiation of proximal GABA$_A$ IPSCs. *A*, Representative time course of the effect of 80 mM ethanol (EtOH) on the area of proximal GABA$_A$ IPSCs recorded in the absence and presence of the GABA$_B$ receptor antagonist SCH 50911. Responses were pharmacologically isolated using the competitive NMDA and AMPA/kainate receptor antagonists APV (50 μM) and DNQX (20 μM). Traces above the graph are averages of five to seven IPSCs evoked at the times indicated by the letters. *B*, Bar graph summarizing the mean effect of 80 mM ethanol on the area of proximal GABA$_A$ IPSCs recorded in the absence and presence of 20 μM SCH 50911. STD, Standard. *$p < 0.05$, unpaired Student's *t* test. Numbers in parentheses represent the number of cells tested under each experimental condition.

area under the curve of synaptic currents relative to the mean of control and washout values. Statistical analyses of drug effects were performed using the two-tailed Student's paired or unpaired *t* tests or a one-way ANOVA followed by the Newman–Keuls *post hoc* test with a minimal level of significance of $p < 0.05$.

Results

A GABA$_B$ receptor antagonist enhances ethanol potentiation of proximal GABA$_A$ IPSCs

Several studies have shown that GABA$_A$ IPSCs evoked by stimulation of perisomatic GABAergic synapses proximal to the CA1 pyramidal layer are reliably potentiated by pharmacologically relevant concentrations of ethanol, even in the absence of a GABA$_B$ receptor antagonist (Weiner et al., 1997; Poelchen et al., 2000; Proctor et al., 2003). Therefore, we first sought to determine whether a GABA$_B$ receptor antagonist enhanced ethanol potentiation of proximal hippocampal GABA$_A$ IPSCs, as had been shown for ethanol potentiation of IPSCs evoked by stimulation of the stratum radiatum in this brain region (Wan et al., 1996). Under our standard recording conditions, bath application of 80 mM ethanol significantly potentiated the area of proximal GABA$_A$ IPSCs (72.8 ± 7.8%; $n = 14$; $p < 0.01$) (Fig. 1). This enhancement was stable for the duration of the ethanol application and reversed on ethanol washout. Pretreating slices with the GABA$_B$ receptor antagonist SCH 50911 ((+)-5,5-dimethyl-2-morpholineacetic acid hydrochloride) had no effect on GABA$_A$ IPSCs (2.4 ± 5.9% potentiation; $n = 14$; $p > 0.05$). However, in the presence of SCH 50911, ethanol potentiation of proximal GABA$_A$ IPSCs was significantly enhanced (121.5 ± 12.7%; $n = 14$; $p < 0.05$) (Fig. 1). Because of the long duration required for these and many of the experiments in this study, recordings were performed at ambient temperature, which we found to increase the stability of patch clamp recordings in brain slices. However, we did examine the effect of SCH 50911 on ethanol potentiation of GABA$_A$ IPSCs in nine slices maintained at 32°C. In these slices, we observed no difference in the magnitude of the acute effect of 80 mM ethanol (62.5 ± 19.0% potentiation; $n = 9$; $p < 0.05$) or 20 μM SCH 50911 alone (3.8 ± 6.8% inhibition; $n = 9$; $p > 0.05$) or the effect of ethanol in the presence of SCH 50911 (127.7 ± 15.4; $n = 9$; $p < 0.05$).
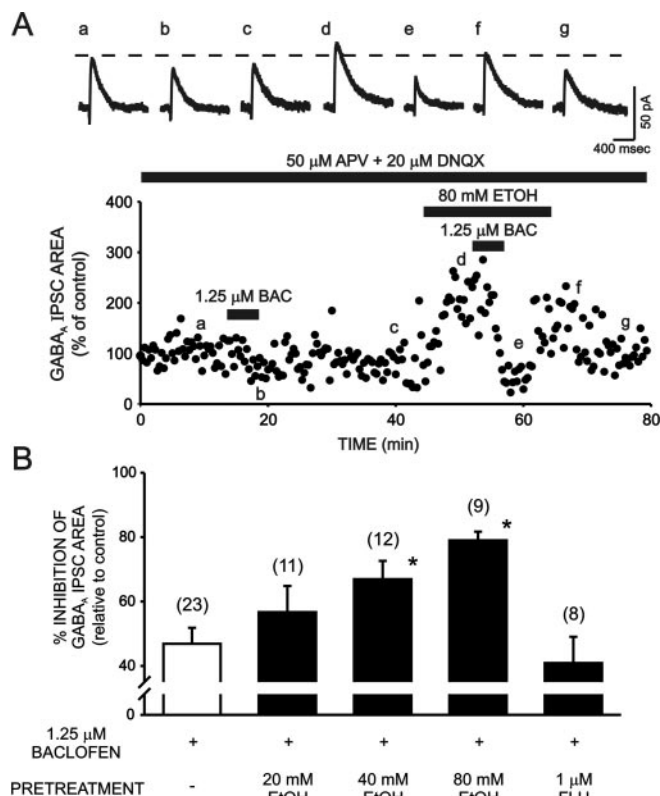
Figure 2. Ethanol increases presynaptic GABA_B receptor function. *A*, Representative time course illustrating the effect of 1.25 μM baclofen (BAC) in the absence and presence of 80 mM ethanol (EtOH) on the area of proximal GABA_A IPSCs. Responses were pharmacologically isolated as in Figure 1. Traces above the graph are averages of six to eight IPSCs evoked at the times indicated by the corresponding letters. *B*, Bar graph summarizing the effect of 1.25 μM BAC on proximal GABA_A IPSCs when applied alone or in the presence of 20, 40, or 80 mM EtOH or 1 μM FLU. *$p < 0.05$, relative to BAC alone, *post hoc* Neuman–Keuls test. Numbers in parentheses represent the number of cells tested under each experimental condition.

Ethanol increases presynaptic GABA_B receptor activity

The preceding experiments suggest that some unknown GABA_B receptor-mediated process may actively limit ethanol potentiation of proximal GABA_A IPSCs in the rat hippocampal CA1 region. Activation of presynaptic GABA_B receptors at these and many other inhibitory synapses in the mammalian CNS produces a well characterized inhibition of GABA release along with an associated decrease in the size of evoked GABA_A IPSCs (Bowery and Enna, 2000; Couve et al., 2000). We hypothesized that ethanol may enhance the activity of these presynaptic GABA_B autoreceptors and that such an interaction could serve to reduce the overall potentiating effect of ethanol at these synapses. To test this hypothesis, we assayed the effect of ethanol on presynaptic GABA_B receptor function at proximal GABAergic synapses. We determined the effect of a submaximal concentration of the GABA_B receptor agonist baclofen on the area of proximal GABA_A IPSCs in the absence and presence of 80 mM ethanol. These recordings were performed with 5 mM QX-314 in the patch pipette to block postsynaptic GABA_B IPSCs (Nathan et al., 1990). Under these recording conditions, bath application of 1.25 μM baclofen reduced the area of GABA_A IPSCs by $46.9 \pm 5.0\%$ ($n = 23$) (Fig. 2). Pretreating slices with 80 mM ethanol resulted in a significant potentiation of proximal GABA_A IPSCs (Fig. 2), as seen in Figure 1 and previous studies (Weiner et al., 1997; Poelchen et al., 2000). However, once a new baseline was established in the presence of 80 mM ethanol, 1.25 μM baclofen had a significantly greater in-

hibitory effect on GABA_A IPSCs, reducing their area by an average of $79.0 \pm 2.7\%$ ($n = 9$) (Fig. 2). In five cells in which ethanol was washed out for at least 20 min, baclofen inhibition of GABA_A IPSCs returned to baseline levels ($37.4 \pm 9.5\%$ inhibition).

We next examined the effect of a range of ethanol concentrations on baclofen inhibition of GABA_A IPSCs. Ethanol significantly enhanced the inhibitory effect of baclofen at 40 and 80 but not 20 mM (Fig. 2B), and the potency of this interaction was identical to that of the overall potentiating effect of ethanol at these synapses.

One possible confound of the above experiment is that, because ethanol potentiates proximal GABA_A IPSCs, it is possible that baclofen inhibition of proximal IPSCs is dependent on the initial size of these responses. To address this concern, we tested the effect of flunitrazepam (FLU), a well characterized benzodiazepine that potentiates GABA_A receptor activity (Sieghart, 1995), on presynaptic GABA_B receptor function. Bath application of 1 μM FLU increased the proximal GABA_A IPSC area by $80.1 \pm 13.5\%$ ($n = 8$; $p < 0.01$). After a new baseline was established, we tested the effect of 1.25 μM baclofen on proximal IPSCs. Unlike ethanol, FLU pretreatment had no significant effect on baclofen inhibition of GABA_A IPSCs (Fig. 2B).

Ethanol has no effect on postsynaptic GABA_B receptor function

The preceding experiments provide evidence that ethanol potentiates presynaptic GABA_B receptor function at proximal synapses in the rat hippocampus. Although this specific interaction has not been previously examined, several studies have characterized the acute effects of ethanol on postsynaptic GABA_B receptor activity in the hippocampus and have reported no effect of ethanol at concentrations similar to those used in our studies (Frye et al., 1991; Frye and Fincher, 1996; Wan et al., 1996). We performed two separate experiments to evaluate possible effects of ethanol on postsynaptic GABA_B receptor function under our recording conditions. First, we tested the effect of ethanol on GABA_B IPSCs recorded from rat hippocampal CA1 pyramidal neurons. GABA_B IPSCs were evoked by electrical stimulation of the stratum lacunosum in the presence of 50 μM APV and 20 μM DNQX, to block glutamatergic synaptic responses, and 20 μM bicuculline methiodide, to block GABA_A IPSCs. The remaining synaptic current was completely blocked by 20 μM SCH 50911, indicating that it was mediated by the activation of GABA_B receptors (Fig. 3A). Under these recording conditions, 80 mM ethanol had no effect on the area of GABA_B IPSCs ($4.3 \pm 8.3\%$ potentiation; $n = 7$; $p > 0.05$) (Fig. 3A).

In the second experiment, we tested the effect of ethanol on outward currents elicited by bath application of the GABA_B receptor agonist baclofen in cells voltage-clamped at −50 mV. This protocol has been used in other studies to activate the G-protein coupled inwardly rectifying potassium channels that underlie the slow GABA_B IPSCs recorded in Figure 3A (Newberry and Nicoll, 1984; Gahwiler and Brown, 1985; Sodickson and Bean, 1996; Liu and Leung, 2003). Under our recording conditions, bath application of 20 μM baclofen induced an outward current of 76.1 ± 14.8 pA ($n = 5$) (Fig 3B). Ethanol pretreatment (80 mM) had no effect on currents evoked by 20 μM baclofen (68.9 ± 8.6 pA; $n = 5$; $p > 0.05$). Although ethanol alone appeared to induce a small outward current in the example illustrated, this effect was not significant and has not been consistently observed in other studies (Frye and Fincher, 1996; Wan et al., 1996).
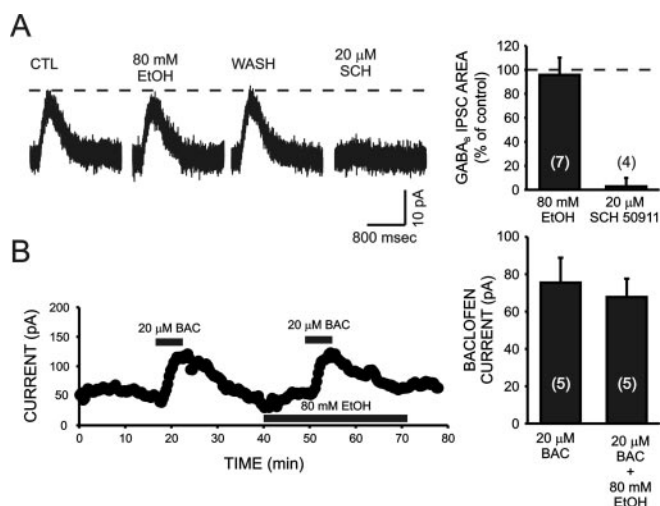
Figure 3. Ethanol does not potentiate postsynaptic GABA_B receptor function in rat CA1 pyramidal neurons. *A*, Representative traces illustrating the effect of 80 mM ethanol (EtOH) and 20 μM SCH 50911 on the area of GABA_B IPSCs evoked by stimulation of the stratum lacunosum-molecular in the presence of APV, DNQX, and 20 μM bicuculline methiodide. Traces are averages of five GABA_B IPSCs recorded under the conditions indicated. The bar graph summarizes the effect of EtOH and SCH 50911 on the area of GABA_B IPSCs. CTL, Control; WASH, washout. *B*, Representative time course of the outward current generated by bath application of 20 μM baclofen (BAC) in the absence and presence of 80 mM EtOH. The bar graph summarizes the maximal amplitude of BAC-evoked currents recorded in the absence and presence of EtOH. Numbers in parentheses represent the number of cells tested under each experimental condition.

Ethanol enhances presynaptic GABA_B receptor tone

The results of these studies suggest that ethanol selectively enhances presynaptic but not postsynaptic GABA_B receptor activity at GABAergic synapses in the rat hippocampal CA1 region. Because this interaction occurs across the same ethanol concentration range that results in an overall potentiation of proximal GABA_A IPSCs, this presynaptic interaction likely serves to reduce the overall potentiating effect of ethanol at these synapses. This interaction may thus account for the observation that a GABA_B receptor antagonist can facilitate ethanol potentiation of GABA_A IPSCs in this brain region. However, this interpretation implies that there must be some tonic presynaptic GABA_B receptor activity in the presence of ethanol. To address this issue, we examined the effect of the GABA_B receptor antagonist SCH 50911 on the area of proximal GABA_A IPSCs in the absence and presence of 80 mM ethanol. As shown in Figure 1, bath application of 20 μM SCH 50911 had no effect on proximal GABA_A IPSCs evoked every 20 sec ($4.0 \pm 8.0\%$ inhibition; $n = 9$; $p > 0.05$). This concentration of SCH 50911 was, however, sufficient to completely block the inhibitory effect of baclofen on these responses (compare Figs. 2A, 4A). Notably, in the presence of 80 mM ethanol, SCH 50911 significantly potentiated proximal GABA_A IPSCs ($35.9 \pm 4.1\%$ potentiation; $n = 11$; $p < 0.05$) (Fig. 4). The results of this experiment demonstrate that, in the presence but not the absence of ethanol, presynaptic GABA_B receptor activity tonically inhibits proximal GABAergic synapses in the rat hippocampal CA1 region.

Pretreatment with a GABA_B receptor antagonist selectively enhances ethanol potentiation of sIPSC frequency

We performed one additional series of experiments to further address both the mechanism through which ethanol enhances GABA_A receptor-mediated synaptic transmission in the CA1 re-
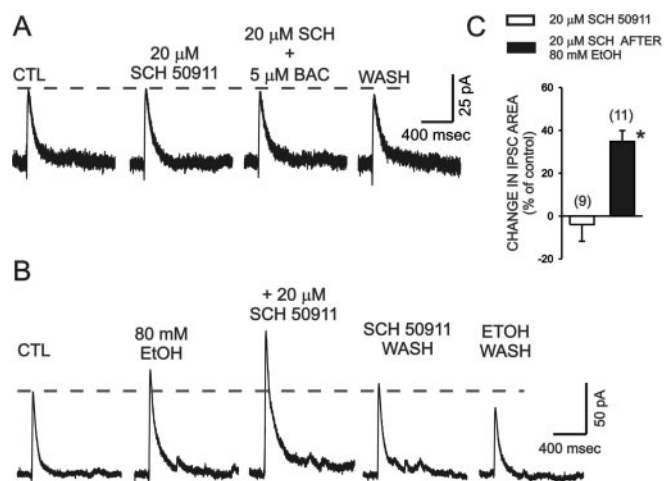

Figure 4. Ethanol increases presynaptic GABA_B receptor tone. Traces from representative experiments illustrate that 20 μM SCH 50911 potentiates the area of proximal GABA_A IPSCs in the presence (*B*) but not the absence (*A*) of ethanol (EtOH). Note that this concentration of SCH 50911 is sufficient to completely block the inhibitory effect of 5 μM BAC on GABA_A IPSCs. CTL, Control; WASH, washout. *C*, Summary of the effect of 20 μM SCH 50911 on the area of GABA_A IPSCs recorded in the absence and presence of EtOH. *$p < 0.05$, relative to control. Numbers in parentheses represent the number of cells tested under each experimental condition.

gion and the facilitation of this effect by blockade of GABA_B receptors. In these experiments, CA1 pyramidal neurons were voltage-clamped at −70 mV, and GABA_A sIPSCs were recorded in the presence of the glutamate receptor antagonist mixture used in the evoked GABA_A IPSC experiments. Under these recording conditions, the majority of neurons exhibited spontaneous synaptic responses that reversed near 0 mV and were completely blocked by the GABA_A receptor antagonist bicuculline methiodide (20 μM) (data not shown). In the first experiment, we tested the effect of 80 mM ethanol alone on the frequency, amplitude, and area of sIPSCs. Bath application of ethanol significantly and reversibly increased all three parameters (Fig. 5A,C) (frequency, $39.2 \pm 6.6\%$ potentiation; $p < 0.001$; amplitude, $16.7 \pm 5.6\%$ potentiation; $p < 0.05$; area, $21.9 \pm 6.7\%$ potentiation; $p < 0.05$; $n = 10$), with the increase in sIPSC frequency appearing to be the most robust effect (Fig. 5C). In the second experiment, we tested the effect of the GABA_B receptor antagonist SCH 50911 (20 μM) and SCH 50911 plus 80 mM ethanol on sIPSCs. Pretreating slices with SCH 50911 alone had no effect on any of the sIPSC parameters measured (frequency, $1.4 \pm 8.2\%$ potentiation; $p > 0.05$; amplitude, $2.1 \pm 5.2\%$ potentiation; $p > 0.05$; area, $1.0 \pm 4.9\%$ inhibition; $p > 0.05$; $n = 11$) (Fig. 5B,C). However, in the presence of SCH 50911, ethanol enhancement of sIPSC frequency was significantly greater than that observed when ethanol was applied alone ($99.8 \pm 8.6\%$ potentiation; $p < 0.001$) (Fig. 5B,C). In contrast, ethanol potentiation of sIPSC amplitude and area were not significantly altered by pretreatment with the GABA_B receptor antagonist (amplitude, $18.7 \pm 7.3\%$ potentiation; area, 30.6 ± 8.5 potentiation; $n = 11$) (Fig. 5B,C).

A subthreshold concentration of baclofen selectively blocks ethanol potentiation of proximal GABA_A IPSCs

Because ethanol appears to enhance tonic presynaptic GABA_B receptor activity, it might be possible to antagonize ethanol potentiation of proximal GABA_A IPSCs by pretreating slices with a low, subthreshold concentration of a GABA_B receptor agonist. To test this hypothesis, we examined the effect of 80 mM ethanol on proximal GABA_A IPSCs in the absence and presence of a
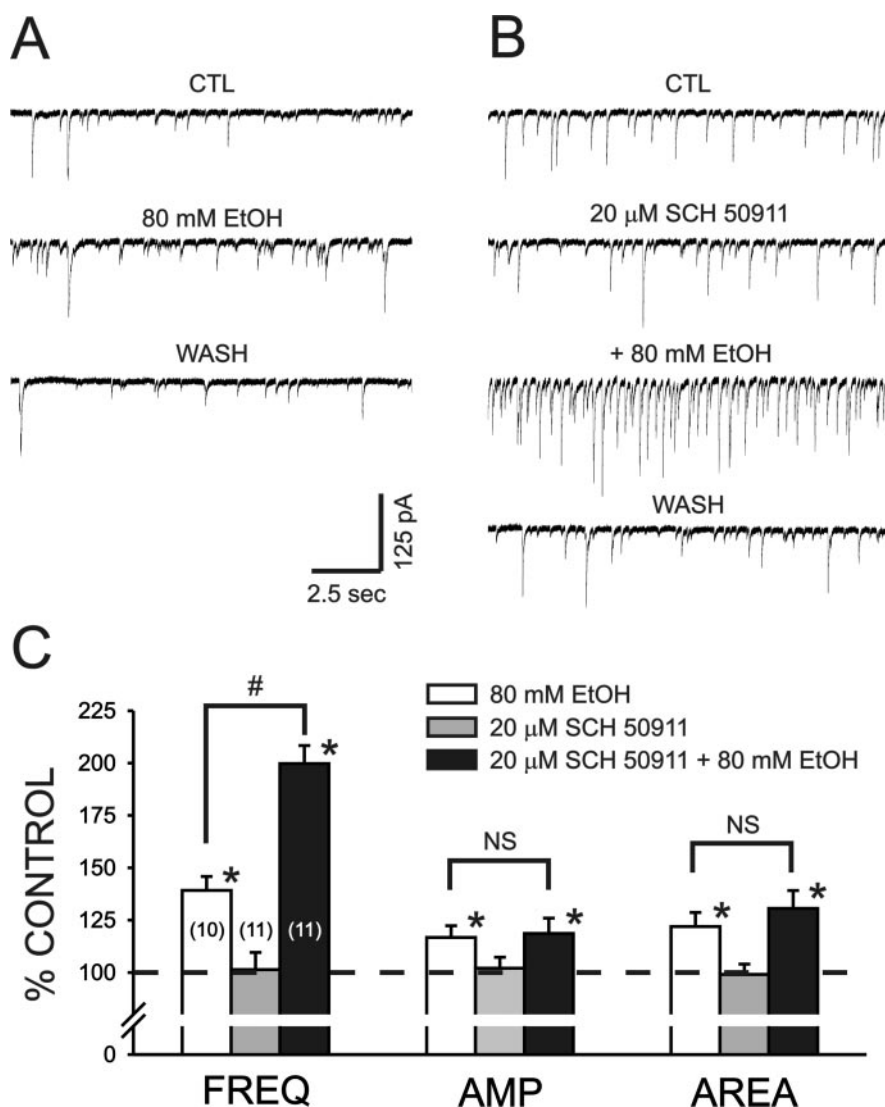
Figure 5. Pretreatment with a GABA$_B$ receptor antagonist selectively enhances ethanol potentiation of sIPSC frequency. *A*, sIPSCs recorded from a representative CA1 neuron voltage clamped at −70 mV before (CTL), during, and after bath application of 80 mM ethanol (EtOH, WASH). *B*, sIPSCs recorded from another representative neuron under control conditions, during bath application of 20 μM SCH 50911 alone and with 80 mM EtOH and after ethanol washout (WASH). *C*, Summary of the effect of ethanol, SCH 50911, and SCH 50911 plus EtOH on sIPSC frequency (FREQ), amplitude (AMP), and area. *$p < 0.05$, paired Student's *t* test; #significant difference between two conditions, one-way ANOVA followed by *post hoc* Neuman–Keuls test; NS, no significant difference. Numbers in parentheses represent the number of cells tested under each experimental condition. Note that SCH 50911 pretreatment significantly increases ethanol potentiation of sIPSC frequency but not their amplitude or area.

subthreshold concentration of baclofen. Under our recording conditions, a concentration of 500 nM baclofen was determined to be just below the threshold to elicit a significant inhibition of proximal GABA$_A$ IPSCs (to 90.2 ± 5.1% of control; $n = 21$; $p > 0.05$). We therefore pretreated slices with 500 nM baclofen and tested the effect of 80 mM ethanol on proximal IPSCs (Figs. 6, 7). Although 80 mM ethanol produced a robust potentiation of proximal GABA$_A$ IPSCs under control conditions (Fig. 1), in the presence of 500 nM baclofen, 80 mM ethanol caused a modest but significant inhibition of proximal GABA$_A$ IPSCs (to 78.5 ± 6.1% of control; $p < 0.05$; $n = 11$). In a few cells, we were able to wash out the baclofen for at least 30 min and challenge slices again with the same concentration of ethanol. After baclofen washout, 80 mM ethanol enhanced proximal GABA$_A$ IPSCs (Fig. 6).

We next examined the effect of 500 nM baclofen pretreatment on flunitrazepam and pentobarbital potentiation of GABA$_A$ IP-

SCs. In contrast to the inhibitory effect of baclofen pretreatment on ethanol potentiation of proximal IPSCs, baclofen had no effect on flunitrazepam or pentobarbital potentiation of these responses. Bath application of 1 μM flunitrazepam potentiated GABA$_A$ IPSCs to 198.0 ± 19.2% under control conditions ($n = 8$) and to 209.7 ± 19.2% of control in the presence of 500 nM baclofen ($n = 5$). Similar results were observed with 50 μM pentobarbital (254 ± 31.3% of control in the absence of baclofen; $n = 5$; 228.1 ± 27.8% of control in the presence of baclofen; $n = 5$) (Fig. 7).

Discussion

The results of this study suggest that acute ethanol exposure enhances presynaptic GABA$_B$ receptor function at proximal GABAergic synapses in the rat CA1 region. This interaction occurs across the same concentration range over which ethanol potentiates GABA$_A$ IPSCs at these synapses. Because presynaptic GABA$_B$ receptor activity reduces evoked GABA release (Misgeld et al., 1995), this interaction appears to actively limit the overall potentiating effect of ethanol at these synapses.

Although there is now compelling behavioral evidence that ethanol acts, in part, by potentiating GABA$_A$ receptor function (Grobin et al., 1998), direct evidence that ethanol actually enhances GABA$_A$ receptor-mediated synaptic inhibition has been somewhat difficult to demonstrate, particularly in the hippocampus. Our results suggest that variability in the level of presynaptic GABA$_B$ receptor activity, attributable to differences in the stimulation protocols used to evoke IPSCs, may have contributed to much of the disparity in the reported effects of ethanol on hippocampal GABAergic synapses. For example, within the CA1 region, ethanol has been reported to have little or no effect on compound IPSCs mediated by both GABA$_A$ and GABA$_B$ receptors (Siggins et al., 1987; Proctor et al., 1992a; Wan et al., 1996). Synaptic activation of GABA$_B$ receptors typically requires relatively intense stimulation or trains of stimuli (Thomson and Destexhe, 1999; Liu and Leung, 2003), possibly reflecting an extrasynaptic localization of these receptors (Fritschy et al., 1999). Synaptic GABA$_B$ IPSCs are also more readily evoked by stimulation of dendritic fields of the CA1 region (Williams and Lacaille, 1992; Thompson, 1994). In contrast, data presented in this study, as well as those of several other reports (Weiner et al., 1997; Poelchen et al., 2000; Crowder et al., 2002; Proctor et al., 2003), demonstrate that GABA$_A$ IPSCs evoked by minimal stimulation of perisomatic synapses in the CA1 region are reliably potentiated by intoxicating concentrations of ethanol. Our data suggest that the ethanol insensitivity of compound GABAergic synaptic responses may reflect a greater degree of presynaptic GABA$_B$ receptor modulation of such re-

sponses. In fact, presynaptic GABA$_B$ receptor activity inhibits dendritic IPSCs to a greater extent than those evoked by perisomatic stimulation (Lambert and Wilson, 1993b; Pearce et al., 1995). This hypothesis is further supported by the observation that blockade of GABA$_B$ receptors converts ethanol-insensitive compound IPSCs into ones that are reliably potentiated by ethanol (Wan et al., 1996) and by data presented in this study that a minimal elevation of presynaptic GABA$_B$ receptor tone, by pretreating slices with a subthreshold concentration of baclofen, was sufficient to convert ethanol-sensitive proximal GABA$_A$ IPSCs into responses that were actually inhibited by ethanol. Taken together, these data are consistent with the hypothesis that variation in the degree of presynaptic GABA$_B$ receptor activity can profoundly influence ethanol modulation of GABA$_A$ IPSCs in the hippocampus and that such variability may have contributed significantly to the disparity noted in earlier studies on ethanol modulation of GABAergic neurotransmission in this brain region.

It should be noted that, although proximal GABA$_A$ IPSCs were potentiated by ethanol under our standard recording conditions (i.e., in the absence of a GABA$_B$ receptor antagonist), pretreatment with a GABA$_B$ receptor antagonist significantly increased the ethanol sensitivity of these responses. Therefore, presynaptic GABA$_B$ receptor function can regulate the ethanol sensitivity of GABAergic synapses that are not under tonic regulation by these receptors. Interestingly, recent reports have demonstrated a strong correlation between the *in vitro* ethanol sensitivity of proximal GABA$_A$ IPSCs and behavioral sensitivity to some of the intoxicating effects of ethanol. This relationship has been found in lines of rats and mice bred for

Figure 6. Time course from a representative cell illustrating the effect of 80 mM ethanol (EtOH), in the presence and absence of a subthreshold concentration of baclofen (BAC), on the area of proximal GABA$_A$ IPSCs. Note that EtOH inhibits GABA$_A$ IPSCs in the presence of 500 nM BAC but potentiates GABA$_A$ IPSCs under standard recording conditions. Traces are averages of five to eight IPSCs recorded at the times indicated by the letters above the graph.
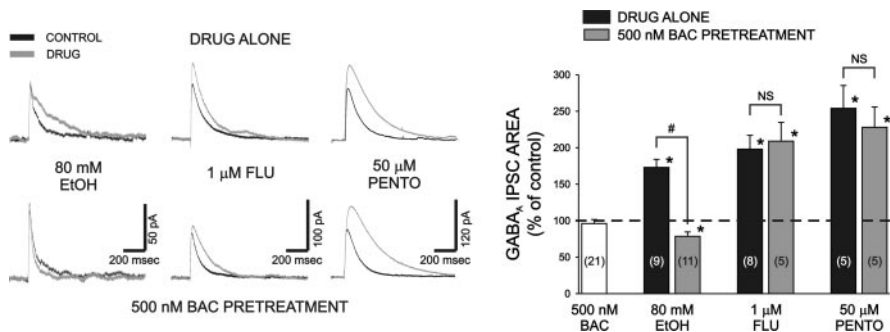
Figure 7. A subthreshold concentration of baclofen selectively blocks ethanol potentiation of proximal GABA$_A$ IPSCs. A summary of the effect of 500 nM baclofen (BAC) pretreatment on the effect of 80 mM ethanol (EtOH), 1 μM FLU, and 50 μM pentobarbital (PENTO) on the area of proximal GABA$_A$ IPSCs is shown. *Significant difference relative to control, paired *t* tests; #significant difference between two conditions, unpaired *t* tests; NS, no significant difference. Traces are averages of 6–10 GABA$_A$ IPSCs recorded from representative cells under the conditions indicated. Note that 500 nM BAC alone has no effect on proximal GABA$_A$ IPSCs and does not interact with FLU or PENTO potentiation of these responses. In contrast, 500 nM BAC pretreatment reverses the effect of ethanol on proximal IPSCs from potentiation to inhibition.

differences in ethanol-induced loss of the righting reflex (Poelchen et al., 2000) as well as in PKCγ or ε knock-out mice, which also exhibit alterations in a variety of ethanol-mediated behavioral effects (Harris et al., 1995; Hodge et al., 1999). In all of these rodent lines, proximal IPSCs from ethanol-sensitive strains were potentiated to a greater extent by ethanol than IPSCs recorded from slices prepared from the ethanol-resistant lines (Poelchen et al., 2000; Proctor et al., 2003). These findings suggest that factors that regulate the ethanol sensitivity of proximal GABA$_A$ IPSCs may also influence behavioral responsiveness to ethanol. Given that presynaptic GABA$_B$ receptor activity can profoundly influence the ethanol sensitivity of these synapses, it will be important in future studies to determine whether functional differences in presynaptic GABA$_B$ receptor activity contribute to these previously described *in vitro* and *in vivo* differences in ethanol sensitivity in these selected lines.

The importance of elucidating the physiological factor(s) that can influence behavioral sensitivity to ethanol is further under-
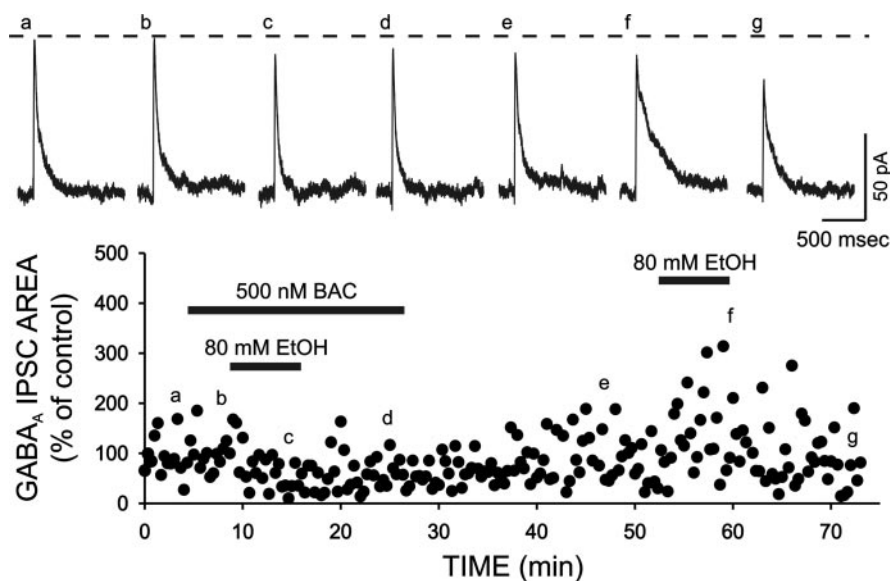
scored by epidemiological studies suggesting that lower initial sensitivity to ethanol may be an important risk factor associated with an elevated risk of developing ethanol-related problems later in life (Schuckit, 1994; Schuckit and Smith, 1996). Although much effort has been directed at identifying genetic differences that may contribute to the variance in behavioral responsiveness to ethanol consumption, the specific genes responsible for such differences remain mostly unknown. Our findings suggest that genes encoding for GABA$_B$ receptors, or any proteins that may modulate the activity of the cascade linking presynaptic GABA$_B$ receptor activation to a reduction in GABA release, represent genetic loci that could potentially be associated with an increased risk of alcoholism.

Another important question that remains to be fully addressed is the mechanism through which ethanol enhances presynaptic GABA$_B$ receptor function. One possibility is that ethanol interacts directly with presynaptic GABA$_B$ receptors. Our finding that ethanol potentiates baclofen inhibition of proximal GABA$_A$

IPSCs is consistent with such a mechanism. However, our data and those of others (Frye and Fincher, 1996; Wan et al., 1996) also suggest that ethanol does not enhance postsynaptic GABA_B receptor function in the CA1 region. All GABA_B receptors are heterodimers comprising GABA_B1 and GABA_B2 subunits (Bowery and Enna, 2000; Enna, 2001), and genetic deletion of the GABA_B1 isoform completely abolishes presynaptic and postsynaptic GABA_B receptor function (Prosser et al., 2001). These findings suggest a single, pharmacologically homogeneous distribution of GABA_B receptors in the CNS. It therefore seems unlikely that ethanol could directly interact with presynaptic GABA_B receptors but be devoid of activity at their postsynaptic counterparts. It should, however, be noted that ethanol has been shown to potentiate postsynaptic GABA_B receptor function in regions other than the hippocampus [e.g., cerebellar granule neurons (Lewohl et al., 1999)]. In addition, our data do not rule out the possibility that ethanol acts directly on some element of the downstream cascade linking presynaptic GABA_B receptor activation to the inhibition of GABA release because distinct coupling mechanisms are thought to mediate presynaptic and postsynaptic GABA_B receptor signaling in the hippocampus (Thompson and Gahwiler, 1992; Lambert and Wilson, 1993a; Pitler and Alger, 1994).

An alternative hypothesis that is consistent with much of our data is that ethanol may actually enhance GABAergic synaptic transmission, in part, via an increase in GABA release. Such an effect might raise ambient GABA levels to a level sufficient to enhance presynaptic GABA_B receptor function. In fact, GABA_B receptors have a much lower functional EC_{50} than do GABA_A receptors (Sodickson and Bean, 1996), and extracellular GABA levels have been estimated to be very near the threshold that we observed for activation of presynaptic GABA_B receptors at proximal GABAergic synapses ($\sim 0.5-1$ μM) (Lerma et al., 1986; Tossman et al., 1986).

Direct evidence in support of this hypothesis stems from our finding that bath application of ethanol significantly increased the frequency of sIPSCs onto CA1 neurons. This finding is consistent with other recent studies demonstrating ethanol-mediated presynaptic enhancement of action potential-dependent(Carta et al., 2003) and -independent (Sanna et al., 2004) GABA release in the hippocampus and amygdala (Roberto et al., 2003; Nie et al., 2004) as well as action potential-dependent GABA release onto cerebellar granule cells (Carta et al., 2004). In addition, although ethanol significantly increased the amplitude and area of sIPSCs in this study, possibly reflecting postsynaptic actions of this drug, pretreatment with a GABA_B receptor antagonist only facilitated the presynaptic ethanol enhancement of sIPSC frequency. An ethanol-mediated elevation of ambient GABA levels could also account for our observation that the GABA_B receptor antagonist SCH 50911 potentiated GABA_A IPSCs in the presence but not the absence of ethanol. This mechanism may also explain why the facilitatory effects of flunitrazepam and pentobarbital (allosteric potentiators of GABA_A receptor activity that do not enhance GABA release) on proximal GABA_A IPSCs are not subject to modulation by presynaptic GABA_B receptor activity.

Additional studies are clearly needed to fully elucidate the complex mechanisms through which ethanol modulates GABAergic synaptic inhibition in the rat hippocampus and other brain regions. Nevertheless, our data clearly demonstrate that ethanol can enhance presynaptic GABA_B receptor activity and that this interaction regulates the overall ethanol sensitivity of perisomatic hippocampal GABAergic synapses. These findings, coupled with the observation that ethanol can increase GABA release in the hippocampus and other brain regions, provide further evidence that ethanol modulation of GABAergic neurotransmission likely involves more than just a simple, allosteric interaction with the postsynaptic GABA_A receptor complex.

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