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Ethanol Antagonizes Kainate Receptor-Mediated Inhibition of Evoked $GABA_A$ Inhibitory Postsynaptic Currents in the Rat Hippocampal CA1 Region

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ABSTRACT

Many studies have demonstrated that ethanol reduces glutamatergic synaptic transmission primarily by inhibiting the *N*-methyl-D-aspartate subtype of glutamate receptor. In contrast, the other two subtypes of ionotropic glutamate receptor (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate) have generally been shown to be insensitive to intoxicating concentrations of ethanol. However, we have previously identified a population of kainate receptors that mediate slow excitatory postsynaptic currents in the rat hippocampal CA3 pyramidal cell region that is potently inhibited by low concentrations of ethanol. In this study, we examined the effect of ethanol on kainate receptor-mediated inhibition of evoked $GABA_A$ inhibitory postsynaptic currents (IPSCs) in the rat hippocampal CA1 pyramidal cell region. Under our recording conditions, bath application of 1 μM kainate significantly inhibited $GABA_A$ IPSCs. This inhibition seemed to be mediated by the activation of somatodendritic kainate receptors on GABAergic interneurons and the subsequent activation of metabotropic $GABA_B$ receptors, because the kainate inhibition was largely blocked by pretreating slices with a $GABA_B$ receptor antagonist. Ethanol pretreatment significantly antagonized the inhibitory effect of kainate on $GABA_A$ IPSCs, at concentrations as low as 20 mM. In contrast, ethanol did not block the direct inhibitory effect of a $GABA_B$ receptor agonist on $GABA_A$ IPSCs. The results of this study suggest that modest concentrations of ethanol may antagonize presynaptic, as well as postsynaptic, kainate receptor function in the rat hippocampus.

Alcoholism represents an imposing medical and socioeconomic concern for our society (Volpicelli, 2001). Surprisingly, little is known about the physiological factors that predispose an individual to this disease or the molecular mechanisms that mediate the intoxicating actions of ethanol. Recent studies have suggested that ethanol acts primarily by modulating the activity of a select group of neurotransmitter systems that mediate excitatory and inhibitory synaptic transmission (Faingold et al., 1998; Tsai and Coyle, 1998). It is thought that the summation of these multiple synaptic effects of ethanol underlies the complex behavioral sequelae associated with the intoxicating and reinforcing actions of this drug, and ultimately, the addiction process.

The majority of excitatory synaptic communication in the mammalian central nervous system (CNS) is mediated by the neurotransmitter glutamate. Glutamate activates three major classes of ionotropic receptors, named for the ligands α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), kainite (KA), and *N*-methyl-D-aspartate (NMDA) (Mayer and Westbrook, 1987). Given the central role that glutamate receptors play in numerous aspects of normal brain function, many studies have examined ethanol effects on glutamatergic synaptic transmission. To that end, there is now compelling evidence, from behavioral, neurochemical, and electrophysiological studies, that ethanol potently inhibits the activity of the NMDA subtype of glutamate receptor and that this inhibition contributes, in part, to some of the behavioral and cognitive effects of this drug (Deitrich et al., 1989; Tsai and Coyle, 1998; Woodward, 2000). In contrast, most studies have reported little or no effect of ethanol on glutamatergic responses mediated by non-NMDA (AMPA and kainate) receptors (Lovinger et al., 1990;

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ABBREVIATIONS: CNS, central nervous system; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; KA, kainate; NMDA, *N*-methyl-D-aspartate; IPSC, inhibitory postsynaptic current; EPSC, excitatory postsynaptic current; eIPSC, evoked inhibitory postsynaptic current; aCSF, artificial cerebrospinal fluid; QX-314, *N*-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium chloride; LY 303070, (−)-1-(4-aminophenyl)-3-methylcarbamoyl-4-methyl-7,8-methylenedioxy-5*H*-2,3,-benzodiazepine; IPSC, inhibitory postsynaptic current; APV, DL-(−)-2-amino-5-phosphonovaleric acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline; DNQX, 6,7-dinitroquinoxaline-2,3-dione; SCH 50911, (−)-(*R*)-5,5-dimethylmorpholinyl-2-acetic acid ethyl ester HCl.

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Martin et al., 1991; but see Nie et al., 1994; Martin et al., 1995; Valenzuela et al., 1998a).

Interestingly, in many of these previous studies, it was not possible to distinguish between AMPA and kainate receptor-mediated responses. With the relatively recent development of selective AMPA receptor antagonists (Paternain et al., 1995), it is now apparent that AMPA receptors are the primary mediators of fast excitation at most non-NMDA receptor-gated synapses. Thus, in many previous reports of ethanol-insensitive non-NMDA receptors, responses were likely mediated predominantly by AMPA receptors.

The physiological role of the kainate subtype of glutamate receptor is only now beginning to emerge and little is known about the pharmacological properties of native kainate receptors. Although kainate receptors are widely expressed in the CNS, functional kainate receptor-gated synapses have only been identified in a limited number of brain regions (for reviews, see Chittajallu et al., 1999; Frerking and Nicoll, 2000; Lerma et al., 2001). However, in addition to their somewhat limited postsynaptic role, functional presynaptic kainate receptors have been identified in a variety of brain areas. Activation of presynaptic kainate receptors has been shown to potently modulate neurotransmitter release in several brain regions, for example, the hippocampus (Chittajallu et al., 1996; Cossart et al., 1998; Frerking et al., 1999) and the striatum (Chergui et al., 2000; Crowder and Weiner, 2002).

We recently demonstrated that at least one population of kainate receptors in the rat hippocampus is sensitive to low concentrations of ethanol (Weiner et al., 1999). Ethanol, at concentrations as low as 20 mM, significantly inhibited kainate EPSCs recorded from rat hippocampal CA3 pyramidal neurons. In contrast, AMPA EPSCs in this brain region were insensitive to ethanol, even at the highest concentration tested (80 mM). These findings suggest that kainate receptors may represent a novel neuronal target of ethanol action in the mammalian CNS.

In the present study, we sought to determine whether another kainate receptor-mediated response within the hippocampus might also be inhibited by intoxicating concentrations of ethanol. We evaluated the effect of ethanol on kainate receptor-mediated inhibition of evoked $GABA_A$ IPSCs (eIPSCs) in the rat hippocampal CA1 region. Recent evidence suggests that this effect is mediated by the activation of somatodendritic kainate receptors on presynaptic GABAergic interneurons (Cossart et al., 1998; Frerking et al., 1998) and that the subunit composition of these receptors may differ from that of the postsynaptic receptors underlying kainate EPSCs onto CA3 pyramidal neurons (Mulle et al., 2000). Our data suggest that ethanol, at concentrations similar to those that inhibit postsynaptic kainate receptors in the CA3 region, also inhibits kainate receptor-mediated inhibition of eIPSCs onto rat hippocampal CA1 pyramidal neurons. These results further support the hypothesis that native kainate receptors are significantly inhibited by relatively modest concentrations of ethanol and may potentially mediate some of the behavioral and cognitive effects of this drug.

Materials and Methods

Hippocampal Slice Preparation. Transverse hippocampal slices (400 μm) were prepared from 4- to 6-week-old male Sprague-Dawley rats as described previously (Weiner et al., 1997). Slices were incubated at ambient temperature (20–23°C) for ≥2 h before recording in artificial cerebrospinal fluid (aCSF) containing 126 mM NaCl, 3 mM KCl, 1.5 mM $MgCl_2$, 2.4 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 11 mM glucose, and 26 mM $NaHCO_3$, saturated with 95% O_2, 5% CO_2.

Electrophysiological Recordings. Slices were transferred to a recording chamber maintained at 20–23°C and superfused with aerated aCSF at 2 ml/min. Patch electrodes were prepared from filamented borosilicate glass capillary tubes (inner diameter 0.86 mm) using a horizontal micropipette puller (P-97; Sutter, Novato, CA). Electrodes were filled with a recording solution containing 130 mM KGlu, 15 mM KCl, 0.1 mM $CaCl_2$, 1.0 mM EGTA, and 2 mM Mg-ATP (Sigma-Aldrich, St. Louis, MO), 0.2 mM Tris-GTP (Sigma-Aldrich), 10 mM HEPES, and 5 mM QX-314 (pH adjusted with KOH; 275–285 mOsM). Reagents used in the preparation of the recording solution were purchased from Fluka (Buchs, Switzerland) unless otherwise indicated. Whole-cell patch-clamp recordings were made from individual CA1 pyramidal neurons voltage-clamped at −45 to −55 mV. Only cells with a stable access resistance of 5 to 20 MΩ were used in these experiments. Whole-cell currents were acquired using an Axoclamp 2B or Axopatch 200B amplifier, digitized (Digidata 1200B; Axon Instruments, Union City, CA), and analyzed on- and off-line using an IBM compatible PC computer and pClamp 8.0 software (Axon Instruments).

Pharmacological Isolation of IPSCs. Evoked $GABA_A$ receptor-mediated inhibitory postsynaptic currents were evoked every 20 s by electrical stimulation (0.2-ms duration) using a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) placed near the CA1 pyramidal cell body region ("proximal" stimulation; Weiner et al., 1997). Unless otherwise indicated, eIPSCs were pharmacologically isolated using a cocktail of 50 μM APV to block NMDA receptors and either 10 μM LY 303070 (generous gift from Eli Lilly & Co., Indianapolis, IN) or 1 μM NBQX to block AMPA receptor function. QX-314 (5 mM; Alamone Laboratories, Jerusalem, Israel) was included in the patch-pipette solution to block $GABA_B$ IPSCs. Unless otherwise stated, all drugs used were purchased from Sigma-Aldrich. A 4 M ethanol solution (Aaper Alcohol and Chemical, Shelbyville, KY), diluted in deionized water, was prepared immediately before each experiment from a 100% stock solution kept in a glass storage bottle. All drugs were applied directly to the aCSF via calibrated syringe pumps (Razel, Stanford, CT).

Statistics. All drug effects were quantified as the percentage of change in IPSC amplitude relative to the mean of control and washout values. Statistical analyses of drug effects were performed using the two-tailed Student's paired t test or a one-way analysis of variance followed by the Newman-Keuls post hoc test with a minimal level of significance of $P < 0.05$.

Results

Effect of Kainate on eIPSCs. We first examined the effects of exogenous kainate application on the amplitude of pharmacologically isolated eIPSCs recorded from rat hippocampal CA1 pyramidal cells. Neurons were voltage-clamped at depolarized potentials (−45 to −55 mV) and eIPSCs were evoked every 20 s in the presence of the NMDA receptor antagonist APV (50 μM) and the noncompetitive AMPA receptor antagonist LY303070 (10 μM). We have previously shown that these concentrations of APV and LY303070 completely block NMDA and AMPA EPSCs, but have no significant effect on kainate receptor function in rat hippocampal neurons (Weiner et al., 1999). Synaptic currents evoked under these recording conditions were mediated solely by the activation of $GABA_A$ receptors because they were completely antagonized by bath application of the selective $GABA_A$ receptor antagonist bicuculline methiodide (data not shown). A 5- to 7-min bath application of 1 μM kainate significantly inhibited

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the amplitude of eIPSCs in all cells tested (to 34.3 ± 3.3% of control, $n = 11$, $P < 0.01$) (Fig. 1, A and D). The onset of this inhibition was rapid and persisted for the duration of the kainate application. The effect was fully reversible upon washout with recovery taking between 20 to 45 min. Under these recording conditions, the inhibition of eIPSCs by 1 μM kainate was not accompanied by a significant change in holding current or input resistance.

It has been reported in murine studies that low concentrations of NBQX can be used to selectively antagonize AMPA receptor function (Bureau et al., 1999; Mulle et al., 2000). We therefore determined whether a low concentration of NBQX was selective for AMPA over kainate receptors in the rat hippocampal CA1 region. Bath application of 1 μM NBQX completely blocked AMPA EPSCs (by 97.8 ± 2.9%; $n = 4$; data not shown) and notably, 1 μM kainate had the same inhibitory effect on $GABA_A$ IPSCs regardless of whether 1 μM NBQX (38.5 ± 3.1% of control, $n = 10$, $P < 0.01$) or 10 μM LY303070 was used to antagonize AMPA receptor activity (Fig. 1, B and D).

We next sought to demonstrate that the inhibitory effect of kainate on eIPSCs required the activation of kainate receptors. Because selective kainate receptor antagonists are not commercially available, we used a protocol in which eIPSCs were first pharmacologically isolated using a blocker cocktail containing maximally effective concentrations of NMDA and AMPA receptor antagonists (either APV + LY303070 or APV + NBQX). Slices were then perfused with a high concentration of the mixed AMPA/kainate (KA) receptor antagonist DNQX and subsequently challenged with 1 μM kainate. In the presence of the blocker cocktail, bath application of 80 μM DNQX had no effect on the amplitude of eIPSCs (Fig. 1C), suggesting that, under our recording conditions, there was no tonic kainate receptor-dependent regulation of GABAergic synaptic transmission. However, DNQX pretreatment completely blocked the inhibitory effect of exogenous kainate application on the amplitude of eIPSCs (94.7 ± 4.5% of control, $n = 6$, $P > 0.05$) (Fig. 1, C and D).

Effect of Ethanol on Kainate Modulation of eIPSCs. We next examined the effect of ethanol on kainate inhibition of eIPSCs recorded in the presence of the AMPA and NMDA receptor antagonist blocker cocktail. Bath application of 80 mM ethanol significantly increased the amplitude and area of eIPSCs, as we have reported previously (Fig. 2A) (Weiner et al., 1997). After a 10-min pretreatment in 80 mM ethanol, slices were then challenged with 1 μM kainate in the contin-

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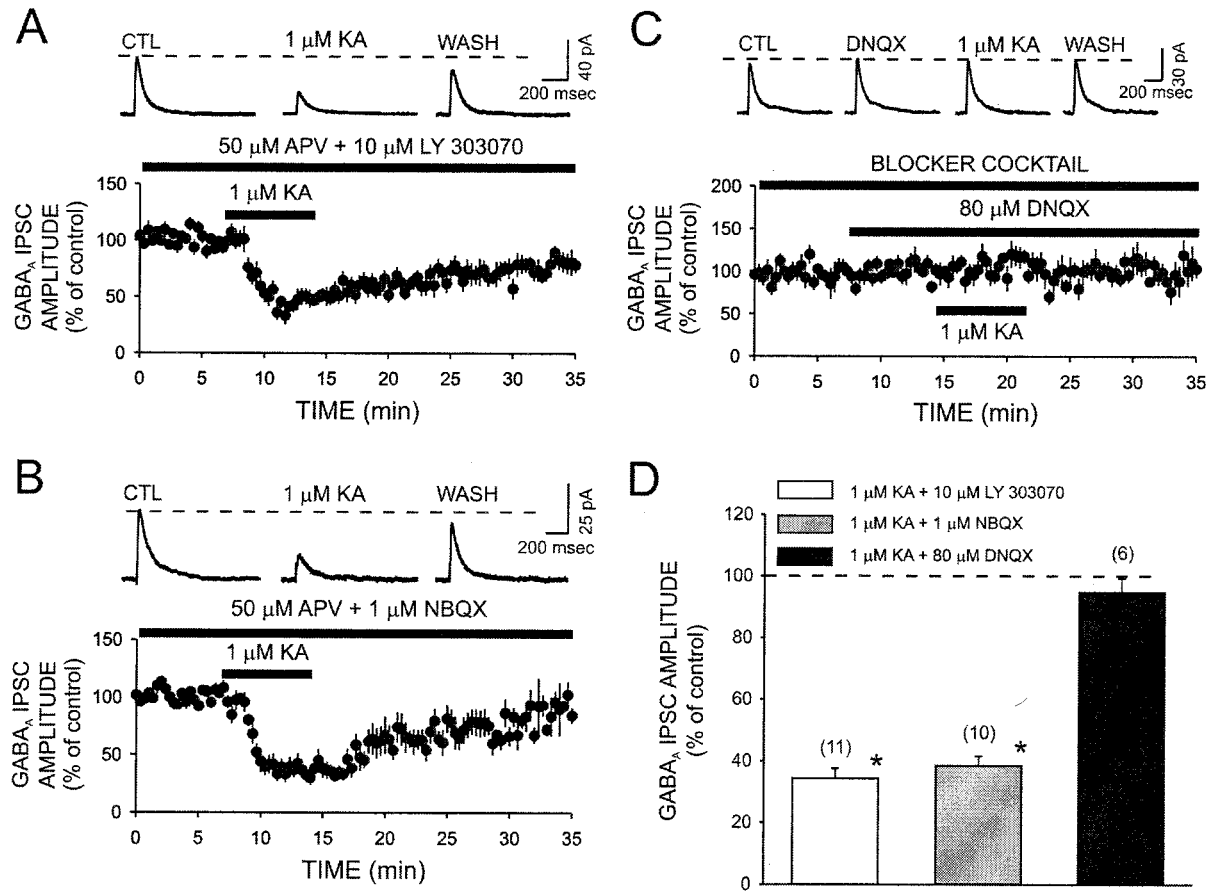

Fig. 1. Activation of kainate receptors inhibits eIPSCs in rat hippocampal CA1 pyramidal neurons. Summary time courses (6–11 cells) of the effect of a 5- to 7-min bath application of 1 μM KA on the amplitude of eIPSCs pharmacologically isolated with 50 μM APV and 10 μM LY303070 (A) or 50 μM APV and 1 μM NBQX (B). The summary time course in C illustrates that bath application of 1 μM kainate has no effect on eIPSCs in the presence of a maximal concentration of the mixed AMPA/kainate receptor antagonist DNQX. Traces above each graph are averages of five to eight eIPSCs recorded under the conditions indicated. D, bar graph summarizing the effect of bath application of 1 μM kainate on the amplitude of eIPSCs recorded in the presence of the selective AMPA receptor antagonists LY303070 and NBQX, and the mixed AMPA/kainate receptor antagonist DNQX. All recordings were carried out in the presence of 50 μM APV. ⋆, significant difference relative to control, $P < 0.05$. Numbers in parentheses indicate the number of cells tested under each experimental condition.

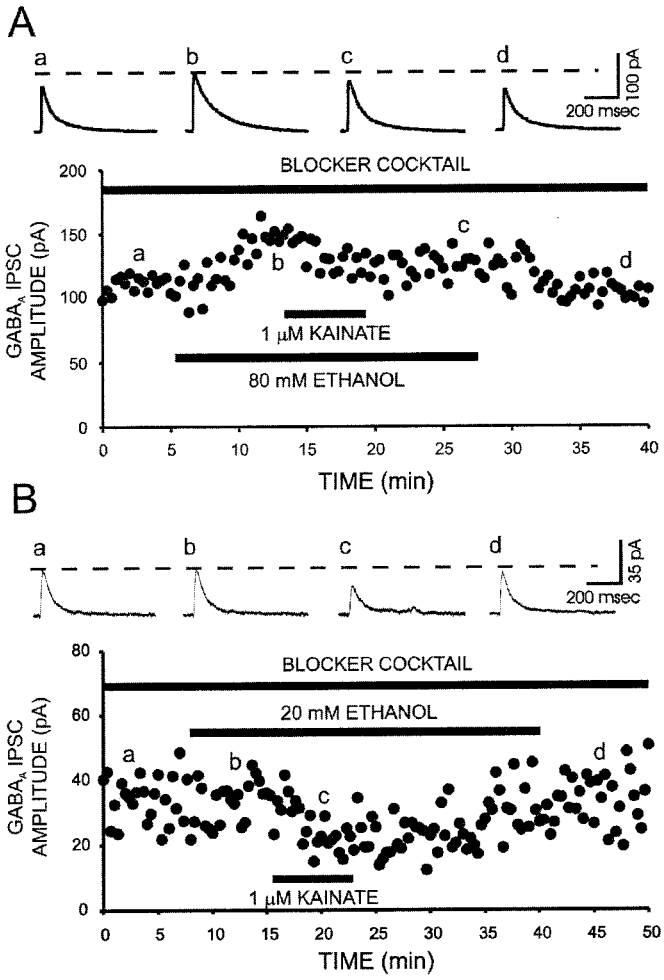

Fig. 2. Effect of ethanol on kainate inhibition of eIPSCs. Time course illustrating the effect of 80 mM (A) and 20 mM (B) ethanol on the inhibitory effect of 1 μM kainate on eIPSCs recorded from rat hippocampal CA1 pyramidal neurons. Traces above the graph are averages of five to eight eIPSCs recorded at the times indicated by the letters.

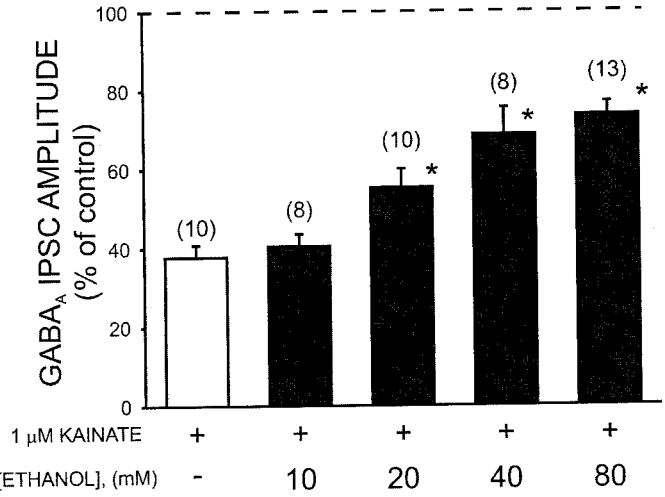

Fig. 3. Concentration dependence of ethanol antagonism of kainate inhibition of eIPSCs. ⋆, $P < 0.05$, relative to the effect of 1 μM kainate alone. Numbers in brackets indicate the number of cells recorded under each experimental condition.

ued presence of ethanol. Although kainate did significantly inhibit the amplitude of eIPSCs in the presence of 80 mM ethanol (to 73.6 ± 3.3% of control, $n = 13$, $P < 0.05$), the magnitude of this inhibition was significantly less than that observed in the absence of ethanol ($P < 0.01$) (Fig. 3). We next examined the concentration dependence of the ethanol antagonism of kainate-mediated inhibition of eIPSCs. Ethanol pretreatment produced a concentration dependent reduction of kainate-mediated inhibition of eIPSCs, with a significant effect being observed at 20 mM ethanol (to 55.3 ± 4.8% of control, $n = 10$, $P < 0.05$), a concentration that had no effect on the amplitude or area of GABA$_A$ IPSCs under these recording conditions (Figs. 2B and 3).

Mechanism of Kainate Inhibition of eIPSCs. A number of mechanisms have been described to account for the inhibitory effect of kainate on eIPSCs in the rat hippocampus (for reviews, see Chittajallu et al., 1999; Frerking and Nicoll, 2000; Ben-Ari and Cossart, 2000; Lerma et al., 2001). A recent study demonstrated that the inhibitory effect of a relatively high concentration of kainate (10 μM) on eIPSCs in the rat CA1 region could be blocked to a significant extent by pretreating slices with a GABA$_B$ receptor antagonist (Frerking et al., 1999). The authors concluded that kainate activates somatodendritic kainate receptors on presynaptic GABAergic interneurons, resulting in a large increase in spontaneous GABA release. This increased GABA release in turn activates presynaptic GABA$_B$ receptors that are known to produce a pronounced decrease in evoked GABA release (Davies et al., 1990), thereby contributing to the kainate-mediated decrease in eIPSCs. To determine whether a similar mechanism was responsible for the inhibitory effect of a lower concentration of KA, we tested the effect of 1 μM kainate on eIPSCs in the presence of the GABA$_B$ receptor antagonist SCH 50911. Under our recording conditions, bath application of 20 μM SCH 50911 dramatically reduced the inhibitory effect of 1 μM kainate on eIPSCs. In fact, kainate had no significant effect on eIPSCs in the presence of SCH 50911, reducing eIPSC amplitude to only 83.2 ± 6.7% of control ($n = 11$, $P < 0.08$). This experiment suggests that the majority of the kainate inhibition of eIPSCs observed under our recording conditions is likely due to presynaptic kainate receptor-dependent release of GABA and the subsequent activation of presynaptic GABA$_B$ receptors (Fig. 4).

Effect of Ethanol on GABA$_B$ Receptor-Mediated Inhibition of eIPSCs. The preceding experiment suggested that the inhibitory effect of 1 μM kainate on GABA$_A$ IPSCs was triggered by the activation of somatodendritic KA receptors on presynaptic GABAergic interneurons but also involved the secondary activation of presynaptic GABA$_B$ receptors. We therefore sought to determine whether ethanol was acting to inhibit the function of these interneuronal KA receptors or, perhaps, was acting downstream to antagonize presynaptic GABA$_B$ receptor function. To differentiate between these two possible mechanisms, we directly assessed the effect of ethanol on presynaptic GABA$_B$ receptor-mediated inhibition of GABA$_A$ IPSCs. Under our recording conditions, bath application of 2.5 μM baclofen, a selective GABA$_B$ receptor agonist, significantly inhibited the amplitude of GABA$_A$ IPSCs (to 42.2 ± 6.1% of control, $n = 8$, $P < 0.001$) (Fig. 5A). This inhibition was completely blocked by pretreating slices with the GABA$_B$ receptor antagonist SCH 50911 (Fig. 5, A and C), suggesting that baclofen inhibition of GABA$_A$ IPSCs was mediated by the activation of GABA$_B$

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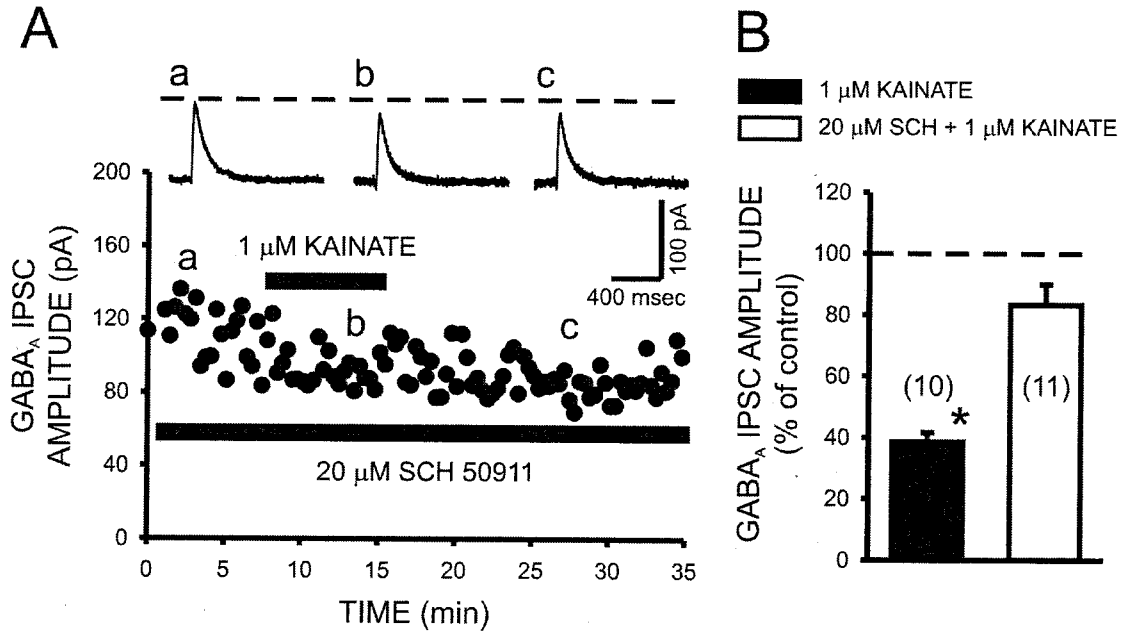

Fig. 4. Blockade of kainate inhibition of $GABA_A$ IPSCs by the selective $GABA_B$ receptor antagonist SCH50911. A, time course illustrating that 1 μM kainate has no effect on the amplitude of eIPSCs in the presence of 20 μM SCH50911. Traces above the graph are averages of five to six eIPSCs recorded at the times indicated by the letters. B, bar graph summarizing the effect 1 μM kainate on the amplitude of eIPSCs in the absence and presence of 20 μM SCH50911. $\star$, $P < 0.05$, relative to control. Numbers in parentheses indicate the number of cells recorded under each experimental condition.

receptors. We next tested the effect of 2.5 μM baclofen in the presence of ethanol. As observed above, pretreating slices with 80 mM ethanol significantly potentiated $GABA_A$ IPSCs. However, ethanol pretreatment did not block the inhibitory effect of baclofen on $GABA_A$ IPSCs (Fig. 5B). In fact, the inhibitory effect of 2.5 μM baclofen was modestly enhanced in the presence of 80 mM ethanol (to 27.0 $\pm$ 3.8% of control, $n = 9$, $P < 0.01$) (Fig. 5C).

Discussion

Previous work from our laboratory has demonstrated that relatively low concentrations of ethanol significantly inhibit postsynaptic kainate receptor function in rat hippocampal CA3 neurons (Weiner et al., 1999). The current study sought to evaluate the effect of ethanol on kainate receptor-mediated inhibition of eIPSCs in rat hippocampal CA1 pyramidal cells. Consistent with previous studies, we found that activation of kainate receptors by 1 μM kainate significantly inhibited eIPSCs recorded from rat hippocampal CA1 pyramidal neurons. This inhibition involved the indirect activation of presynaptic $GABA_B$ receptors, because pretreating slices with a $GABA_B$ receptor antagonist blocked the inhibitory effect of kainate on eIPSCs. Pretreating slices with ethanol, at concentrations as low as 20 mM, significantly reduced kainate inhibition of eIPSCs. In contrast, ethanol did not antagonize the depressant effect of a $GABA_B$ receptor agonist on eIPSCs. Taken together, these results demonstrate that, in addition to its inhibitory effect on postsynaptic kainate receptors in CA3 neurons, relatively modest concentrations of ethanol also significantly antagonize kainate receptor-mediated inhibition of GABAergic synaptic transmission in the CA1 region of the rat hippocampus.

Ethanol Inhibition of Interneuronal Kainate Receptor Function. In this study, bath application of ethanol significantly potentiated eIPSCs evoked by proximal stimulation, as we (Weiner et al., 1997) and others (Poelchen et al., 2000) have reported previously. This effect was primarily on the area of eIPSCs and was significant at 40 and 80 mM ethanol. Bath application of 1 μM kainate inhibited eIPSCs in the presence of ethanol; however, the magnitude of this inhibition was significantly reduced at all but the lowest ethanol concentration tested (10 mM). Thus, ethanol antagonism of kainate inhibition of eIPSCs seemed to be more potent than its direct potentiating effect on eIPSCs. Moreover, the potency of ethanol's depressant effect on kainate inhibition of eIPSCs was the same as that of ethanol antagonism of kainate EPSCs in CA3 pyramidal cells (Weiner et al., 1999). These data suggest that ethanol's overall facilitatory effect on proximal GABAergic synapses may be even more potent under physiological conditions in which presynaptic kainate receptors are active. Although we did not observe any regulatory effect of presynaptic kainate receptors on eIPSCs in the absence of exogenous kainate application in this study, synaptically released glutamate has been shown to modulate GABAergic synaptic transmission via activation of kainate receptors in other studies (Min et al., 1999; Jiang et al., 2001). In general, the synaptic activation of kainate receptors is most readily observed after intense or high-frequency stimulation of glutamatergic afferents (for review, see Frerking and Nicoll, 2000). Therefore, when glutamatergic synaptic transmission is increased, for example during chronic ethanol withdrawal (Tsai and Coyle, 1998), ethanol inhibition of presynaptic kainate receptor function at GABAergic synapses may serve to further enhance the depressant effects of this drug on hippocampal function. Interestingly, there is some evidence that ethanol potentiation of eIPSCs is enhanced after chronic intermittent ethanol exposure (Kang et al., 1998).

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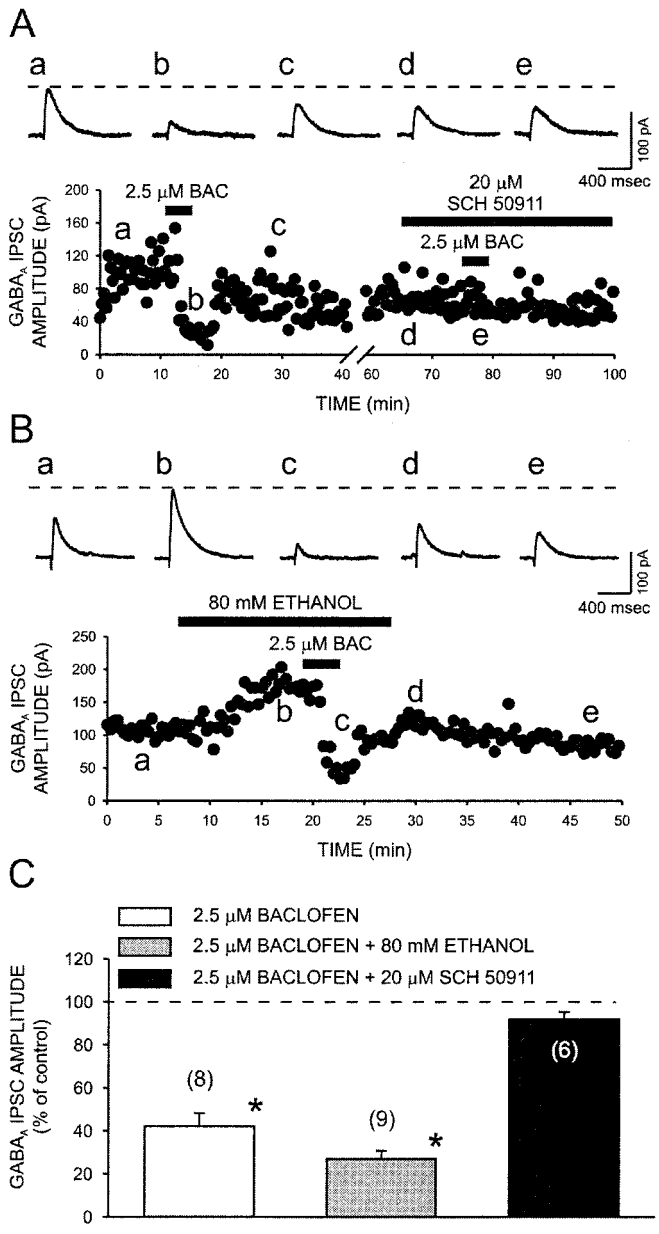

Fig. 5. Ethanol does not antagonize kainate inhibition of $GABA_A$ IPSCs via an indirect effect on presynaptic $GABA_B$ receptors. A, time course of the effect of the $GABA_B$ receptor agonist baclofen (BAC) on the amplitude of $GABA_A$ IPSCs in the absence and presence of SCH 50911. Note that BAC inhibits the amplitude of $GABA_A$ IPSCs and this effect is blocked by 20 μM SCH 50911. B, time course illustrating the effect of 80 mM ethanol pretreatment on BAC inhibition of $GABA_A$ IPSCs. Note that ethanol potentiates the amplitude and area of $GABA_A$ IPSCs but does not occlude the inhibitory effect of BAC. C, bar graph summarizing the effect of bath application of 2.5 μM BAC on the amplitude of $GABA_A$ IPSCs alone, or after pretreatment with 80 mM ethanol or 20 μM SCH50911. $\star$, $P < 0.05$, relative to control.

As previously shown (Frerking et al., 1999), kainate receptor-dependent inhibition of eIPSCs at interneuron-CA1 pyramidal cell synapses in the current study seemed to be largely due to the secondary activation of presynaptic $GABA_B$ receptors. It was therefore necessary to determine whether ethanol was interacting with interneuronal kainate receptor function or rather with the presynaptic $GABA_B$ receptors that are known to depress GABA release (Davies et al., 1990). We therefore tested whether ethanol had any effect on inhibition of eIPSCs mediated by the direct activation of presynaptic $GABA_B$ receptors. Under our recording conditions, 2.5 μM baclofen inhibited eIPSCs to a similar extent as 1 μM kainate. Pretreating slices with the highest concentration of ethanol tested in this study (80 mM) did not inhibit the effect of baclofen on eIPSCs. In fact, ethanol had a modest but significant facilitatory effect on baclofen inhibition of eIPSCs and this novel interaction is currently under further investigation in our laboratory. Therefore, the inhibitory effect of ethanol on kainate inhibition of eIPSCs could not be attributed to an interaction between ethanol and presynaptic $GABA_B$ receptors.

Postsynaptic shunting has also been shown to contribute significantly to the inhibition of eIPSCs by kainate receptor activation at interneuron-CA1 pyramidal cell synapses (Frerking et al., 1999). However, postsynaptic shunting did not seem to contribute to the inhibitory effect of kainate on eIPSCs in this study because bath application of 1 μM kainate was not associated with any changes in input resistance or holding current. It should be noted that postsynaptic shunting of eIPSCs observed in the previous study was demonstrated with a kainate concentration 10 times higher than that used in the present study. Lower concentrations of kainate have previously been reported to have only minimal effects on the passive membrane properties of CA1 pyramidal cells (Bureau et al., 1999).

Taken together, these results further suggest that ethanol may interact directly with interneuronal kainate receptors, in a manner similar to its inhibitory effect on postsynaptic kainate receptors on CA3 pyramidal cells.

Ethanol Sensitivity of Non-NMDA Receptors. Our data suggest that hippocampal kainate receptors may be particularly sensitive to low concentrations of ethanol. These findings are somewhat surprising because few studies have demonstrated ethanol sensitive non-NMDA receptors in neuronal preparations (Martin et al., 1991; Nie et al., 1994). In the current study, as well as in another recent study from our laboratory (Weiner et al., 1999), ethanol was shown to potently inhibit pre- and postsynaptic kainate receptor function, but not AMPA receptor function, in the rat hippocampus. It might then be hypothesized that the ethanol sensitivity of non-NMDA receptors is dependent on the receptor subtype (i.e., kainate versus AMPA) and/or on the subunit composition of these receptors. However, studies conducted with recombinant kainate receptors or native receptors in cultured cells suggest that this is unlikely. For example, Valenzuela and Cardoso (1999) demonstrated that the ethanol sensitivity of recombinant kainate receptors, unlike that of NMDA and $GABA_A$ receptors (Masood et al., 1994; Harris et al., 1997), does not vary with the particular subunits being expressed. Second, recombinant AMPA receptors expressed in either *Xenopus* oocytes (Dildy-Mayfield and Harris, 1992) or human embryonic kidney 293 cells (Lovinger 1993), as well as AMPA receptors in primary culture (Wirkner et al., 2000) are potently inhibited by ethanol. Finally, studies conducted in cultured neurons have reported that ethanol inhibits both kainate and AMPA receptors, with little difference in the potency of these effects (Valenzuela et al., 1998a).

The factors responsible for the differential ethanol sensitivity of native non-NMDA receptors in tissue slices, native receptors in cultured cells, and recombinant receptors in

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expression systems are not known. One hypothesis is that receptors in these different environments might undergo differences in post-translational modifications such as phosphorylation, glycosylation, or protein-protein interactions that might alter the ethanol sensitivity of these receptors. Post-translational modifications have been shown to underlie changes in the ethanol sensitivity of NMDA receptors (for review, see Chandler et al., 1998). For example, phosphorylation reduces the ethanol sensitivity of these receptors during acute tolerance (Miyakawa et al., 1997). Although the ethanol sensitivity of kainate receptors seems to be unaltered by phosphorylation (Valenzuela et al., 1998b), the effect of phosphorylation on the ethanol sensitivity of AMPA receptors has not been examined. Post-translational modifications have been shown to account for other differences in the physiological and pharmacological properties of native and recombinant glutamate receptors (Standley and Baudry, 2000). For example, modulation of glutamate receptor function by concanavalin A has been shown to require glycosylation (Everts et al., 1997). Differences in the post-translational regulation of glutamate receptors in brain slices, cultured cells, and expression systems could contribute to previously observed differences in the ethanol sensitivity of these receptors. Clearly, further studies are needed to resolve the physiological mechanisms underlying the differential ethanol sensitivity of native and recombinant glutamate receptors.

Possible Behavioral Significance of Ethanol Inhibition of Interneuronal Kainate Receptors. Our data suggest that, at concentrations relevant to the pharmacological effects of ethanol, this drug may inhibit the activity of at least two populations of kainate receptors in the rat hippocampus. Assessing the behavioral significance of these observations at present is difficult because the physiological role of kainate receptors in this brain region is complex and not fully defined. For example, although kainate clearly inhibits eIPSCs, it likely does so via a profound excitation of presynaptic GABAergic interneurons and an associated increase in spontaneous GABA release. Moreover, recent data suggests that kainate may actually increase unitary eIPSCs under some conditions (Jiang et al., 2001). A further complication is that presynaptic kainate receptors also regulate glutamate release in the CA3 and CA1 regions and the ethanol sensitivity of these receptors remains to be determined. Nevertheless, studies with systemic administration or local infusion of kainate into the hippocampus clearly indicate that the overall effect of kainate receptor activation on hippocampal physiology is profoundly excitatory in nature (Ben-Ari and Cossart, 2000). Therefore, it is likely that acute inhibitory effects of ethanol on hippocampal kainate receptor function will have a predominantly depressant effect on CNS activity, consistent with the known physiological sequelae associated with ethanol ingestion. Continued research into the physiological role of kainate receptors in the mammalian CNS will ultimately allow us to place the effects of ethanol on kainate receptor function into their proper context.

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