Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 5049–5054, May 1996
Neurobiology

Low ethanol concentrations enhance GABAergic inhibitory postsynaptic potentials in hippocampal pyramidal neurons only after block of $GABA_B$ receptors

(alcohol/$GABA_B$ receptor antagonist/monosynaptic/hippocampal slices/$GABA_A$ receptors)

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Communicated by Floyd E. Bloom, The Scripps Research Institute, La Jolla, CA, December 29, 1995 (received for review November 22, 1995)

ABSTRACT **Despite considerable evidence that ethanol can enhance chloride flux through the γ-aminobutyric acid type A ($GABA_A$) receptor–channel complex in several central neuron types, the effect of ethanol on hippocampal GABAergic systems is still controversial. Therefore, we have reevaluated this interaction in hippocampal pyramidal neurons subjected to local monosynaptic activation combined with pharmacological isolation of the various components of excitatory and inhibitory synaptic potentials, using intracellular current- and voltage-clamp recording methods in the hippocampal slice. In accord with our previous findings, we found that ethanol had little effect on compound inhibitory postsynaptic potentials/currents (IPSP/Cs) containing both $GABA_A$ and $GABA_B$ components. However, after selective pharmacological blockade of the $GABA_B$ component of the IPSP ($GABA_B$-IPSP/C) by CGP-35348, low concentrations of ethanol (22–66 mM) markedly enhanced the peak amplitude, and especially the area, of the $GABA_A$ component ($GABA_A$-IPSP/C) in most CA1 pyramidal neurons. Ethanol had no significant effect on the peak amplitude or area of the pharmacologically isolated $GABA_B$-inhibitory postsynaptic current (IPSC). These results provide new data showing that activation of $GABA_B$ receptors can obscure ethanol enhancement of $GABA_A$ receptor function in hippocampus and suggest that similar methods of pharmacological isolation might be applied to other brain regions showing negative or mixed ethanol–GABA interactions.**

It is common knowledge that alcohol intoxication and the resulting loss of motor and cognitive control in humans have led to untold trauma and suffering. Despite the likelihood that such problems arise from the action of ethanol on the central nervous system (CNS) and several decades of alcohol research suggesting a general depressant effect of intoxicating doses of ethanol on CNS neurons, until recently little has been known about the mechanisms behind this depression. Studies over the past decade have shown that the most sensitive site for ethanol action is the synapse (1–5), and more recently it has been suggested that ethanol-evoked neuronal depression might arise from either a blunting of excitatory glutamatergic synaptic transmission (see, e.g., refs. 6–8) and/or an enhancement of inhibitory γ-aminobutyric acid (GABA)ergic transmission (see refs. 4 and 9).

With regard to inhibitory neurotransmission, ethanol has often been reported to enhance $GABA_A$ receptor activation, and the resulting Cl^- currents or fluxes, in neurons or isolated preparations of several brain regions (4, 9). However, the action of ethanol on GABAergic responses [e.g., $GABA_A$-mediated inhibitory postsynaptic potentials (IPSPs) or responses to exogenous GABA] in hippocampus has been controversial. Although there are a few recent studies suggesting that ethanol can enhance GABA responses in mouse hippocampal cultures (10) or inhibitory postsynaptic currents (IPSCs) in rat hippocampal slices (11) under certain conditions in a percentage of neurons, most *in vivo* (12) and *in vitro* studies (13–15) have found little evidence for such an effect in rat hippocampus. For example, our laboratory (13) reported that ethanol had little effect, or even an inhibitory action, on evoked IPSPs or GABA-induced hyperpolarizations in CA1 and CA3 pyramidal neurons of rat hippocampal slices. To some degree, such negative findings with physiological concentrations of ethanol in hippocampus and other brain regions (6, 16, 17) and preparations (18, 19) have weakened hypotheses on the role of GABAergic systems in alcohol intoxication.

However, most of these negative findings, including those from our laboratory, involved ethanol tests of stratum radiatum (SR)-evoked polysynaptic IPSPs or GABA responses potentially confounded by responses of multiple receptor types, including various glutamate [(*R*,*S*)-α-amino-3-hydroxyisoxazole-4-propionic acid, kainate and *N*-methyl-D-aspartate (NMDA)] and GABA ($GABA_A$ and $GABA_B$) receptors. This could be an important confound for ethanol studies, especially in the light of recent data showing that (*i*) polysynaptic IPSPs can be driven by glutamatergic transmission (see, e.g., ref. 20), and (*ii*) ethanol can antagonize kainate and NMDA glutamate receptor subtypes (6, 21–24), known to mediate portions of SR-evoked excitatory postsynaptic potentials. Recently, several new and selective antagonists have become available that allow complete blockade or pharmacological isolation of these amino acid receptor subtypes (see refs. 24–26). In addition, the development of local or focal stimulation techniques (27, 28), combined with these selective antagonists, now allows study of pharmacologically isolated synaptic components. Therefore, we have repeated earlier studies of ethanol effects on GABAergic monosynaptic IPSPs with two different slice preparation methods, including the one used in previous studies from our laboratory (13), but now with pharmacologically isolated IPSP components. We now report that, under these conditions, low ethanol concentrations reproducibly enhance $GABA_A$ergic IPSPs of hippocampal pyramidal neurons (HPNs), but only when $GABA_B$ receptors are blocked.

MATERIALS AND METHODS

Preparation. The two hippocampal slice preparations used were as described (13, 29, 30). In brief, male Sprague–Dawley

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Abbreviations: IPSP/C, inhibitory postsynaptic potential/current; IPSC, inhibitory postsynaptic current; HPN, hippocampal pyramidal neuron; GABA, γ-aminobutyric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; d-APV, DL-2-amino-5-phosphonovaleric acid; ACSF, artificial cerebrospinal fluid; RMP, resting membrane potential; PKA, protein kinase A; PKC, protein kinase C.

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rats (100–200 g) were anesthetized with halothane (3%) and decapitated, and their hippocampal formations were rapidly removed. We cut transverse slices of 350–400 μm thickness on either a McIlwain brain slicer or a Vibroslice (Campden Instruments) and placed them in ice-cold (6–10°C) artificial cerebrospinal fluid (ACSF), gassed with carbogen (95% O_2/5% CO_2), of the following composition: 130 mM NaCl, 3.5 mM KCl, 1.25 mM NaH_2PO_4, 1.5 mM $MgSO_4$, 2 mM $CaCl_2$, 24 mM $NaHCO_3$, 10 mM glucose. Most slices were incubated at room temperature for up to several hours before being placed in the recording chamber. Once in the chamber, and after 15–30 min of incubation with their upper surfaces exposed to warmed, humidified carbogen, the slices were completely submerged and continuously superfused with ACSF at a constant rate (2–4 ml/min) for the remainder of the experiment. The inner chamber had a total volume of ≈0.5 ml; at the superfusion rates used, 90% replacement of the chamber solution could be obtained within 1–1.5 min (13). We maintained the bath temperature constant during testing at 31–35°C.

Electrophysiology. We used sharp glass micropipettes filled with KCl (3 M; tip resistances, 50–80 MΩ) or KOAc (3 M; tip resistances, 80–95 MΩ) to penetrate CA1 pyramidal neurons. Methods of superfusion, current- and voltage-clamp recording, cell identification, drug administration, and data analysis were as described (29, 31). We performed current- and voltage-clamp studies with an Axon Instruments Axoclamp 2A or 2B headstage (Burlingame, CA). In voltage-clamp mode (discontinuous single electrode voltage-clamp), the switching frequency between current injection and voltage sampling was 3–4 kHz. We continuously monitored electrode settling time and input capacitance neutralization at the headstage on an oscilloscope (32). Current and voltage records were filtered at 0.3 kHz and stored on polygraph paper and also were acquired and stored by A/D sampling and acquisition software (pClamp; Axon Instruments). For A/D sampling via computer, we used two to five sweeps of each evoked IPSP/C for subsequent averaging. The various problems (for example, space-clamping) associated with voltage-clamping of neurons with extended processes are discussed elsewhere (32–34). However, these problems may be less acute when dealing with relative changes after drug application (see, e.g., ref. 35), as in the present study.

Drug Treatment and Pharmacological Isolation of Synaptic Components. Drugs and receptor channel blockers were added, from a concentrated stock solution, to the ACSF in known concentrations immediately before administration to the slice chamber. The usual ethanol-testing protocol was as follows: recording of IPSPs or IPSCs for 10–15 min during superfusion of ACSF alone (control), followed by switching to ACSF with ethanol and repeating these measures after 5–15 min of drug, followed by switching again to ACSF alone for 30–35 min with subsequent current measures (wash or washout).

For pharmacological isolation of synaptic components, we first continuously superfused slices with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (20 μM) and DL-2-amino-5-phosphonovaleric acid (d-APV) (60 μM) to block excitatory glutamatergic transmission and then recorded monosynaptic compound IPSPs in response to local stimulation (within 0.5 mm of, and temporal to, the recording electrode) of Schaffer collateral/commissural fibers. The stimulating electrodes consisted of two bipolar tungsten wires each 130 μm in diameter and with a tip separation of 200 μm; the two wires were placed perpendicularly to the pyramidal cell layer. Stimulation parameters were 50 μs duration, 2–9 V, and 0.05 Hz. To avoid adding ethanol twice to the same neuron, we examined the effects of ethanol superfusion in two paradigms on two separate populations of CA1 HPNs: (*i*) those with isolated compound IPSP/Cs (that is, containing both the early $GABA_A$ and the late $GABA_B$ components), with CNQX and d-APV in the bath; (*ii*) those with isolated $GABA_A$ components of the evoked IPSP/C, in the presence of CNQX, d-APV, and 0.5 mM CGP-35348, the $GABA_B$ antagonist. At the end of the latter experiments, we added 30 μM bicuculline to the superfusate to verify that the early IPSP/C tested was mediated by $GABA_A$ receptors. In some cases we examined ethanol effects on the $GABA_B$ IPSP/C component by including d-APV, CNQX, and bicuculline in the superfusate.

We purchased ethanol from Remet (La Mirada, CA), CNQX from Research Biochemicals, and bicuculline and d-APV from Sigma. CGP-35348 was a gift to W.F. and G.R.S. from CIBA–Geigy.

Data Analysis. We analyzed IPSP/C amplitudes (peaks) and areas (time integrals) with CLAMPFIT 6.0 and AXOGRAPH software (Axon Instruments) for subsequent numerical and graphic evaluation in Microsoft Excel and Microcal Origin. Data are expressed as means ± SEM. For statistical analysis we used either: (*i*) ANOVA for repeated measures with the Newman–Keuls post hoc test, for comparison of control, ethanol-treated and washout conditions; or (*ii*) Student's paired *t* test, for cases where only control and ethanol comparisons were needed. We considered $P = 0.05$ as statistically significant.

RESULTS

Neuronal Sample. We studied a total of 61 neurons identified as pyramidal cells on the basis of electrophysiological criteria (13) and their presence in the CA1 pyramidal cell layer. The mean value of the resting membrane potential (RMP) for this sample was −68 ± 9.6 mV.

Ethanol Effects on GABA-Mediated IPSPs. We recorded monosynaptic IPSPs intracellularly from CA1 pyramidal neurons in response to local Schaffer collateral/commissural fiber stimulation, using current-clamp mode in slices exposed to a mixture of CNQX (20 μM) and d-APV (60 μM) to block excitatory transmission. These compound IPSPs (Fig. 1) consisted of an early and a late component as described (13, 36), and both components were particularly evident at membrane potentials slightly negative to the firing threshold. As shown in Fig. 1*B1*, superfusion of 0.5 mM CGP-35348, a $GABA_B$ receptor antagonist, completely abolished the late component of the IPSP, suggesting that this component was mediated by $GABA_B$ receptors. Subsequent superfusion of 30 μM bicuculline totally blocked the remaining early component of the IPSP (Fig. 1*B3*), suggesting mediation of this component by $GABA_A$ receptor activation.

We studied the effects of ethanol on monosynaptic compound IPSPs and on $GABA_A$-mediated IPSPs in 14 pyramidal cells. As shown in Fig. 1*A*, bath application of 66 mM ethanol did not measurably affect the peak amplitude of the early component of the compound IPSP (102% ± 4% of control) and had only a slight enhancing effect on the compound IPSP duration. Statistical analysis of the mean peak amplitude of the early component of the IPSPs in nine cells revealed no significant change (ANOVA, $F_{(2,16)} = 1.354$; P = not significant). Ethanol increased the peak amplitude of the early component of the compound IPSP (by >20% above the control) in only one of nine cells.

However, in slices superfused with 0.5 mM CGP-35348 to block $GABA_B$ receptors, 66 mM ethanol markedly increased the early ($GABA_A$) IPSP (Fig. 1*B2*). The mean peak amplitude and area under the IPSP increased to 123% ± 6.8% and 148% ± 10.4% of control (ANOVA, $F_{(2,8)} = 7.01$; $P = 0.01$; $F_{(2,8)} = 17.61$; $P = 0.005$; $n = 5$), respectively. Subsequent superfusion of 30 μM bicuculline completely abolished the early IPSP, suggesting that this synaptic response was mediated by $GABA_A$ receptors. Washout of ethanol with ACSF readily reversed enhancement of the early $GABA_A$ IPSPs to control

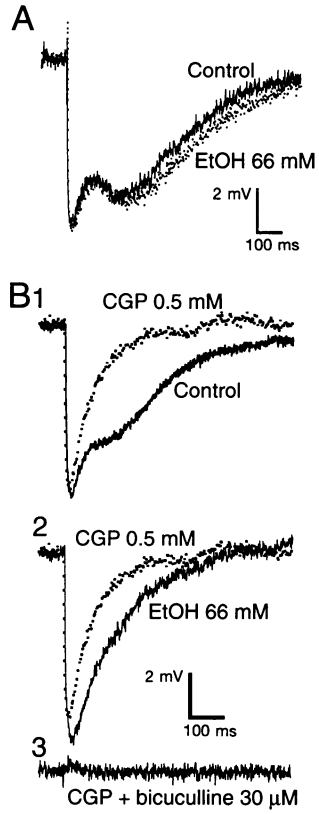

FIG. 1. Ethanol effects on compound IPSPs compared to pharmacologically isolated $GABA_A$-IPSPs: Superimposed current-clamp recordings of CA1 pyramidal neurons. (*A*) Ethanol (66 mM superfused for 8 min; dotted trace) has little effect on the compound monosynaptic IPSP (control; solid trace) evoked by local stimulation and recorded in the presence of 20 μM CNQX and 60 μM d-APV to block glutamatergic synaptic potentials. RMP, −68 mV. (*B*) A different CA1 neuron, also in the presence of 20 μM CNQX and 60 μM d-APV; 66 mM ethanol clearly enhances the amplitude and especially the duration (i.e., the area) of the pharmacologically isolated $GABA_A$-IPSP. (*B1*) 0.5 mM CGP-35348 superfused for 5 min eliminated the late $GABA_B$-IPSP (CGP: dotted trace) component of the compound IPSP (control). (*B2*) Subsequent superfusion of 66 mM ethanol (solid trace) together with CGP-35348 enlarges the IPSP area compared to that in CGP-35348 alone (CGP, 0.5 mM; dotted trace). (*B3*) Superfusion of CGP-35348 and 30 μM bicuculline (together with CNQX and d-APV) totally blocks all synaptic potentials evoked by local stimulation. Thus, the early IPSP was entirely due to activation of $GABA_A$ receptors. RMP, −66 mV.

levels. In fact, the peak ethanol effect at 5–8 min of ethanol superfusion was often followed by a tachyphylaxis (short-term tolerance) of the IPSP enhancement with longer superfusion times, so that the amplitudes or areas of the IPSPs returned to near control levels despite the continued superfusion of ethanol. We did not see ethanol inhibition of IPSPs.

Effects of Ethanol on GABA-Mediated IPSCs. The results obtained in current-clamp mode also were replicated in voltage-clamp mode in 36 neurons. As previously reported (14, 37), ethanol did not modify the compound evoked current (Fig. 2*A*). The mean peak amplitude of the compound IPSC increased to only 101% ± 4.7% of control 5–8 min after superfusion of 66 mM ethanol. Only one cell of eight showed an IPSC increase of >20% over control. Statistical analysis of these eight cells demonstrated no significant change in the mean peak amplitude of the early (peak) component or the area (Table 1) of the compound IPSC (respectively, $F_{(2,14)} = 0.45$; P = not significant; $F_{(2,14)} = 0.39$; P = not significant).

We recorded isolated $GABA_A$ currents from 12 neurons in slices superfused with 0.5 mM CGP-35348 (Fig. 2*B*). The decay

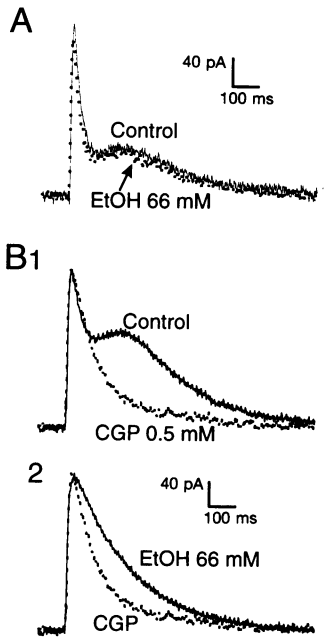

FIG. 2. Voltage-clamp recordings. Comparison of effects of ethanol on compound- and $GABA_A$-IPSCs. (*A*) Monosynaptic compound IPSC (control; solid trace) evoked by focal stimulation in the presence of 20 μM CNQX and 60 μM d-APV. Superfusion of 66 mM ethanol (dotted trace) has no effect on this IPSC. Holding potential, −65 mV. (*B*) Another CA1 HPN, also in the presence of 20 μM CNQX and 60 μM d-APV. (*B1*) 0.5 mM CGP-35348 superfused for 10 min eliminated the late $GABA_B$-IPSC (CGP; dotted trace) component of the compound IPSC (control). (*B2*) Subsequent superfusion of 66 mM ethanol for 5 min (solid trace) together with CGP-35348 enlarges the IPSC duration (area) compared to that in CGP-35348 alone (CGP, 0.5 mM; dotted trace). Holding potential, −65 mV.

phase (τ) of the $GABA_A$-mediated Cl^- current was best fitted with a single exponential with a mean τ of 60.8 ± 8.5 ms. Superfusion of 66 mM ethanol greatly increased the isolated $GABA_A$-IPSC (Fig. 2*B2*). Analysis of the average peak amplitude and the area (Table 2) under the IPSCs for all 12 cells revealed a significant increase of both measures 5–8 min after ethanol superfusion (respectively, $F_{(2,22)} = 9.429$; $P = 0.001$; $F_{(2,22)} = 15.69$; $P = 0.0001$). Ethanol (66 mM) increased the area under the IPSCs (to 162% ± 18.6% of control) much more than it did the IPSC amplitude (to 116% ± 4.2% of control), suggesting a greater effect on IPSC duration than on amplitude. Superfusion of 66 mM ethanol also increased the mean τ to 170% ± 26% of control. Statistical analysis demonstrated a significant change of the τ value (t test, $P = 0.0112$; $n = 12$), confirming that ethanol mainly affected the decay phase of the $GABA_A$-IPSC. Washout of ethanol with ACSF readily reversed these changes of the $GABA_A$ IPSCs and τs to control levels. As with the IPSPs, the effects of ethanol superfusion on the IPSCs was often followed by tachyphylaxis

Table 1. Comparison of effects of 66 mM ethanol on the $GABA_A$ IPSC component and the compound IPSC

IPSC component	% control ± SEM*	n cells with IPSC increase/total n†	ANOVA
Compound IPSC	102 ± 3.4	1/8	$P = 0.6824$
			$F_{(2,14)} = 0.393$
$GABA_A$-IPSC	162 ± 18.5	8/12	$P = 0.001$
			$F_{(2,22)} = 9.429$

Table does not include current-clamp recordings of ethanol effects on IPSPs.
*Mean area under the IPSC.
†No. of cells showing an IPSC increase of >20% of control.

Table 2. Ethanol concentration–response relationships for enhancement of the area of the $GABA_A$ IPSC

EtOH, mM	$GABA_A$-IPSC, % control ± SEM	*n* cells with $GABA_A$-IPSC increase/total *n**	ANOVA
1	93 ± 4.9	0/8	$P = 0.45$
			$F_{(2,14)} = 0.853$
22	140 ± 7.5	5/6	$P = 0.003$
			$F_{(2,10)} = 20.674$
44	139 ± 11.8	5/8	$P = 0.0109$
			$F_{(2,14)} = 6.341$
66	162 ± 18.5	8/12	$P = 0.0001$
			$F_{(2,22)} = 15.69$

*No. of cells showing a $GABA_A$-IPSC increase of >20% of control (current-clamp data not included).

(short-term tolerance) of the effect with superfusion times longer than 8 min; that is, the increase in IPSC size reversed to control levels despite continued ethanol superfusion.

Dose–Response Relationships. We analyzed concentration–response relationships of ethanol on the $GABA_A$ IPSCs in 14 neurons. As shown in Fig. 3 and Table 2, ethanol at all concentrations tested except 1 mM significantly increased the area under the isolated $GABA_A$ current (see Table 2 for statistical analyses). The mean increases of the IPSC amplitudes and the areas under the IPSCs for all ethanol concentrations tested are shown in Fig. 3. It is evident from these curves that the enhancement of $GABA_A$ IPSCs is nearly maximal at 22–44 mM ethanol.

Other Controls. Ethanol enhancement of $GABA_A$ IPSPs was unaffected by the type of slice preparation used (i.e., whether cut on a McIlwain type slicer or on the Vibroslice); thus, three of four cells showed 66 mM ethanol increasing the $GABA_A$ component in slices cut on the McIlwain slicer, and five of eight cells showed the $GABA_A$ component increase in slices cut on the Vibroslice.

We also sought to determine, in both current- and voltage-clamp modes, whether ethanol might alter the late IPSP/C, likely to be mediated by $GABA_B$ receptors. As shown in Fig. 4, 66 mM ethanol had no measurable effect on the monosynaptic $GABA_B$ component. The mean peak amplitude and the area under the mean isolated $GABA_B$ IPSC with ethanol was 99.4% ± 1.2% and 102.5% ± 3.5% of control, respectively. Statistical analysis demonstrated no significant change of these values (*t* test: peak, $P = 0.84$; area, $P = 0.43$; $n = 5$).

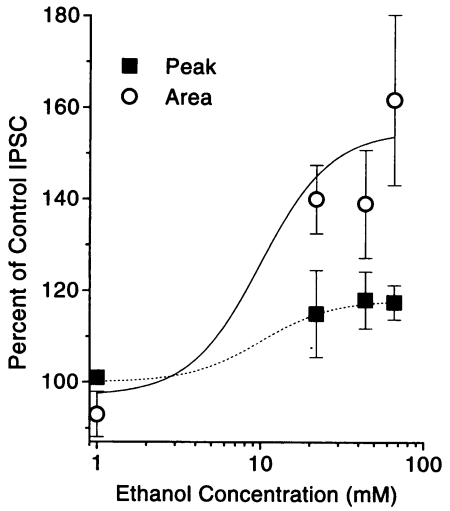

FIG. 3. Concentration–response relationships for ethanol effects on $GABA_A$-IPSCs, comparing effects on the peak current amplitudes versus the area under the IPSC. Ethanol concentration plotted on a logarithmic scale; dose–response curves fitted by software (ORIGIN; Microcal Software Inc.) to a logistic curve: $y = (A_1 - A_2)/\{1 + (x/x_0)^p\} + A_2$, where A_1 is the initial *y* value (97 or 100% of control), A_2 is the estimated final (maximum) *y* value, x_0 is the center *x* value estimated to be about 10 mM, and *p* is the power at 1.6–2. Note the much greater enhancement of $GABA_A$ IPSP/C area compared to peak amplitude values.

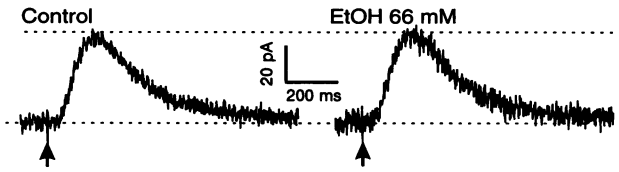

FIG. 4. Ethanol (66 mM) has no measurable effect on monosynaptic $GABA_B$ IPSCs isolated by superfusion of 20 μM CNQX, 60 μM d-APV, and 30 μM bicuculline. Here, 66 mM ethanol was superfused for 6 min with no effect on the outward current generated by local stimulation (arrows). Dotted lines are added for comparison of IPSC amplitudes. Holding potential, −65 mV.

DISCUSSION

In this study of possible ethanol interactions with CA1 hippocampal GABAergic systems, we have found that intoxicating concentrations of ethanol: (*i*) had no effect on monosynaptic compound IPSP/Cs evoked by local stimulation; (*ii*) significantly enhanced pharmacologically isolated $GABA_A$ IPSP/Cs in the presence of a $GABA_B$ receptor antagonist; and (*iii*) had no effect on pharmacologically isolated $GABA_B$ IPSPs in the presence of a $GABA_A$ receptor antagonist. We believe these data help resolve the controversy surrounding possible ethanol interactions with hippocampal GABAergic systems.

As stated in the Introduction, the recent literature on ethanol–GABA interactions in the hippocampus has been confusing, with many papers showing no interaction (e.g., refs. 12–14, 18, and 37–41) and a few others showing ethanol potentiation of GABAergic responses (10, 11, 42). Often, the positive findings depended on satisfaction of certain conditions, such as species (10) or activation of protein kinase C (PKC) (11). In the case of hippocampal cultures, rat neurons generally required much higher ethanol concentrations for augmentation of GABA responses than did cultured mouse neurons. Moreover, even with mouse hippocampal cultures, previously robust GABA interactions with reasonably low ethanol concentrations later became more difficult to observe over a period of years (18, 42), even within the same laboratory. Such inconsistent findings in hippocampus contrast with the positive data from several other brain regions, even when studied by the same group (14, 37, 40). This has led to hypotheses of region-specific differences in $GABA_A$ receptor subunit composition (e.g., the presence of the γ_{2L} subunit or the subunit responsible for zolpidem binding) postulated to be responsible for the brain region differences (4, 5, 9, 41, 43–45). However, such differences have been difficult to prove (41, 46–49). For example, whereas it was once postulated that the γ_{2L} subunit (rather than the γ_{2S} subunit) was required for ethanol–GABA interactions (50), it is now known that the hippocampus expresses abundant levels of this subunit (see, e.g., refs. 41, 51, and 52).

Recent electrophysiological data from the Carlen and Dunwiddie groups have helped to resolve the hippocampal GABA–ethanol controversy, at least for hippocampal slices. Thus, Weiner *et al.* (53) have preliminary data suggesting that the use of low initial temperatures, termed cold-shocked, during preparation and incubation of the slices [as used in previous studies by the Carlen group (11)] permits observation of ethanol enhancement of GABAergic IPSP/Cs. Such cold-shock treatment results in a significant reduction in the

baseline amplitude of evoked CA1 IPSPs (J. Weiner and T. Dunwiddie, personal communication), in contrast to the large IPSPs (with both an early and a pronounced later $GABA_B$ component) seen in both our standard slice preparations. Our data showing ethanol potentiation of IPSPs only during $GABA_B$ receptor blockade suggest that the reduced control IPSPs, and particularly reduced late $GABA_B$ergic components, in the cold-shocked slices could account for the ethanol enhancement of $GABA_A$ IPSP components seen in this preparation. This argument is consistent with the earlier studies of Weiner *et al.* (11), who used a stimulation protocol that did not evoke a visible late $GABA_B$-IPSC. It also is interesting to speculate that differences in $GABA_B$ receptor function between culture conditions and species used could account for the inconsistency of ethanol–GABA interactions in hippocampal cultures.

Our data with the $GABA_B$ receptor antagonist suggests that activation of this receptor somehow prevents the observation of ethanol enhancement of IPSPs. However, the mechanism or site of this $GABA_B$ receptor interference is not yet certain from our data. It is possible that $GABA_B$ receptor activation has some posttranscriptional effect on $GABA_A$ receptor function, perhaps via a second messenger system mediating phosphorylation of $GABA_A$ receptor subunits. Activation of $GABA_B$ receptors has been shown to inhibit adenylyl cyclase activity (54–56), which should lead to reduced protein kinase A (PKA) activity. Therefore, antagonism of constitutively activated $GABA_B$ receptors, as might occur in our study, should then lead to enhanced cyclic AMP levels and PKA activity. This idea is consistent with cerebellar data suggesting that adenylyl cyclase and PKA activation is necessary for ethanol potentiation of GABA responses (57, 58). $GABA_B$ receptor activation has also been shown to reduce PKC activation (59), postulated by Weiner *et al.* (11) to be required for ethanol potentiation of IPSPs in hippocampal slices.

However, it might be argued that the $GABA_B$ receptor influence was exerted presynaptically. $GABA_B$ receptors are known to be localized both pre- and postsynaptically in CA1 neurons; activation of presynaptic $GABA_B$ autoreceptors reduces GABA release (36, 60, 61). It is possible that removal of this influence by the $GABA_B$ antagonist could lead to an ethanol–IPSP interaction via enhanced GABA release. Our finding that ethanol had no effect on pharmacologically isolated late $GABA_B$ IPSPs [see also the dentate data of Morrisett and Swartzwelder (62)] weakens the possibility that ethanol acts generally to release GABA. In addition, most biochemical release studies have shown that ethanol reduces rather than enhances GABA release from isolated cortical regions (63–65). Reduction of GABA reuptake by ethanol is a related concern; the few biochemical studies on this question appear to show an enhancement (66–68), rather than reduction, of GABA uptake by low or intoxicating concentrations of ethanol.

However, the lack of ethanol effects on the isolated $GABA_B$ IPSC cannot be taken as proof of a postsynaptic site of ethanol action in enhancing the $GABA_A$ IPSP/C, since the $GABA_B$ antagonist could not be present when studying the $GABA_B$ IPSC. Indeed, preliminary studies in our laboratory have shown little effect of ethanol superfusion on responses of HPNs to exogenous GABA, despite the presence of tetrodotoxin, glutamate, and $GABA_B$ receptor antagonists in the superfusate (unpublished data). This suggests that presynaptic $GABA_B$ receptors may be involved in suppressing ethanol enhancement of IPSPs.

We cannot account for the finding of Allan *et al.* (69) that $GABA_B$ receptor activation was required for ethanol augmentation of $GABA_A$ receptor-activated Cl^- flux in cortical microsacs and *Xenopus* oocytes expressing mouse brain RNA. However, in another paper by the same group (70), baclofen activation of $GABA_B$ receptors inhibited the function of $GABA_A$ receptors in cerebellar (and to a lesser extent) cortical membrane vesicles; other data by these authors suggest that this action of baclofen results from activation of phospholipase C and phosphorylation of a subtype of $GABA_A$ receptor by PKC. Significantly, the only positive reports of ethanol enhancement of hippocampal slice IPSCs (11) were obtained under conditions where late IPSCs were not apparent and PKC activity was enhanced.

In our study, ethanol increased the area under the IPSCs much more (to 162% of control value with 66 mM) than the amplitude of the evoked IPSCs (to 116% of control at 66 mM), and the mean IPSP/C decay τ also increased significantly. In a study of cultured hippocampal neurons, the time constant of decay of miniature IPSCs was comparable to previously published values of the mean open time of GABA-activated Cl^- channels (71), supporting the hypothesis that the IPSC decay is determined solely by single-channel kinetics and that each receptor is bound only once by GABA during an IPSC (72, 73). Thus, in the presence of a $GABA_B$ receptor antagonist, ethanol could either increase mean channel open-time or allow activation of each $GABA_A$ receptor more than once during an IPSC.

In preliminary studies (unpublished data), 66 mM ethanol did not significantly alter the IPSP reversal potential (E_{IPSC}). Thus, the ethanol-induced potentiation of $GABA_A$ IPSP/Cs probably was not the result of changes in chloride distribution or equilibrium conditions, in agreement with the report by Weiner *et al.* (11) on hippocampal IPSCs. Therefore, the potentiation in hippocampus probably does not involve the mechanism suggested for cortical neurons (74), where E_{IPSC} was shifted in the hyperpolarized direction while IPSC conductance was unchanged. In addition, ethanol had no effect on monosynaptic compound IPSP/Cs evoked by local stimulation without CGP in the bath but in the presence of the specific glutamate receptor antagonists CNQX and d-APV. This important control suggests that ethanol effects on glutamate receptors appears not to have been the major confound in previous studies failing to observe ethanol enhancement of IPSP/Cs (13, 40).

Analysis of dose–response relationships for the ethanol–$GABA_A$ interaction in HPNs may also be informative. The rather sharp increase in IPSC enhancement between 1 and 22 mM ethanol, with a relatively flat curve from 22 to 66 mM, suggests the possibility of desensitization (acute tolerance) at the higher ethanol concentrations. This is also supported by noticable short-term tolerance of the ethanol effect with longer superfusion times and the relatively constant percentage of cells showing the IPSP/C interaction with 22–66 mM ethanol. Such short-term or rapid tolerance to ethanol has been reported in a number of electrophysiological (75, 76) and behavioral (see, e.g., refs. 77–79) studies.

As to the functional or behavioral significance of our findings, it might be argued that the concomitant activation of $GABA_B$ receptors along with $GABA_A$ receptors would occur normally with general activation of inhibitory GABAergic pathways. However, the standard methods of pathway stimulation in brain slices may be highly artificial compared to more discrete synaptic events occurring with ongoing neural net activity. We posit that low strengths of GABAergic pathway activation might lead to release of low GABA levels for stimulation of postsynaptic $GABA_A$ receptors alone, without activation of potentially presynaptic or extrasynaptic $GABA_B$ receptors, allowing an ethanol interaction in this state. In this case, the low effective concentrations of ethanol we have seen suggest a role for the hippocampal GABAergic system in the behavioral sequelae of alcohol ingestion. It is also interesting to speculate that our hippocampal findings apply to other brain regions. Such a possibility should be tested with $GABA_B$ antagonists in future studies of other brain regions previously showing little or mixed effects of ethanol on GABAergic

processes (16, 17, 41, 43). Finally, our data, combined with those from other studies, suggest that the sensitivity of $GABA_A$ receptors to ethanol may hinge more on posttranscriptional events such as receptor phosphorylation than on regional differences in $GABA_A$ receptor subunit composition.

We thank D. Gruol, S. J. Henriksen, P. Schweitzer (all from Scripps), and Simon Brailowsky (of Mexico City) for helpful criticisms and F. Bellinger for technical support. This work was supported by grants from the National Institutes of Health (AA06420) and Groupe LIPHA.

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