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Basis of the Gabamimetic Profile of Ethanol

G. R. Breese, **H. E. Criswell**, **M. Carta**, **P. D. Dodson**, **H. J. Hanchar**, **R. T. Khisti**, **M. Mameli**, **Z. Ming**, **A. L. Morrow**, **R. W. Olsen**, **T. S. Otis**, **L. H. Parsons**, **S. N. Penland**, **M. Roberto**, **G. R. Siggins**, **C. F. Valenzuela**, and **M. Wallner**

Bowles Center for Alcohol Studies, UNC School of Medicine, Chapel Hill, North Carolina (GRB, HEC, RTK ALM, SNP); the Department of Neurosciences, University of New Mexico HSC, Albuquerque, New Mexico (MC, MM, CFV); the Department of Molecular and Medical Pharmacology, Department of Neurobiology, University of California, Los Angeles, California (PDD, HJH, RWO, TSO, MW); and the Molecular and Integrative Neuroscience Department, The Scripps Research Institute, La Jolla, California (LHP, SNP, MR, GRS)

Abstract

This article summarizes the proceedings of a symposium held at the 2005 Research Society on Alcoholism meeting. The initial presentation by Dr. Wallner provided evidence that selected $GABA_A$ receptors containing the δ subunit display sensitivity to low intoxicating ethanol concentrations and this sensitivity is further increased by a mutation in the cerebellar $\alpha 6$ subunit, found in alcohol-hypersensitive rats. Dr. Mameli reported that ethanol affects γ-aminobutyric acid (GABA) function by affecting neural circuits that influence GABA release. Dr. Parsons presented data from electrophysiological and microdialysis investigations that ethanol is capable of releasing GABA from presynaptic terminals. Dr. Morrow demonstrated that systemic ethanol increases neuroactive steroids in brain, the absence of which alters various functional responses to ethanol. Dr. Criswell presented evidence that the ability of ethanol to increase GABA was apparent in some, but not all, brain regions indicative of regional specificity. Further, Dr. Criswell demonstrated that neurosteroids alone and when synthesized locally by ethanol act postsynaptically to enhance the effect of GABA released by ethanol in a region specific manner. Collectively, this series of reports support the GABAmimetic profile of acutely administered ethanol being dependent on several specific mechanisms distinct from a direct effect on the major synaptic isoforms of $GABA_A$ receptors.

Keywords

GABA Release; Neurosteroids; $GABA_A$ Receptor Subtypes; Neural Circuits; Integration; Regional Specificity

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For decades, the central nervous system (CNS) depression caused by ethanol has been associated with γ-aminobutyric acid (GABA) mechanisms (Allan and Harris, 1987; Frye and Breese, 1982; Harris, 1990; Liljequist and Engel 1982). Convincing evidence indicated that the behavioral consequences of benzodiazepines (BZDs) and barbiturates were similar to those induced by ethanol (Breese et al., 1983; Frye et al., 1979; White et al., 1997), Additionally, BZDs and barbiturates enhanced ethanol-induced impairment of motor function (Martz et al., 1983) and substituted for ethanol in discrimination investigations (Grant et al., 2000; Shannon et al., 2004). Although BZDs and barbiturates were known to rely on $GABA_A$-receptor function (Harris, 1990; Ticku, 1989), these findings suggested that ethanol influenced the function of GABA on $GABA_A$ receptors. In accord with this conclusion, $GABA_A$ receptor antagonists

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decreased the antipunishment action of ethanol (Koob et al., 1986, 1988; Liljequist and Engel, 1984) and decreased the sedative action of ethanol (Givens and Breese, 1990a; Harris, 1990; Ticku, 1989), and a BZD-inverse agonist and a GABA$_A$ receptor antagonist decreased operant responding for ethanol (Petry, 1995; Rassnick et al., 1993).

Nestoros (1980) reported that ethanol enhanced GABA-induced inhibition of cerebrocortical neurons. In vivo electrophysiological studies reported regionally specific effects of ethanol on cell firing (Bloom and Siggins, 1987; Givens and Breese, 1990a, 1990b). Particularly critical was the demonstration that ethanol enhanced the inhibitory action of GABA on single unit activity of medial septal neurons, but not from lateral septal neurons (Criswell et al., 1995; Givens and Breese, 1990b)—a finding consistent with the regionally specific action of ethanol on GABA effects on sleep time (Breese et al., 1984; McCown et al., 1986). Single-unit in vivo recording from neurons at other brain sites showing differing effects of ethanol on GABA responsiveness provided additional support for this view of regional specificity of ethanol on GABA function (Criswell et al., 1995, 1993). Additionally, Wafford et al. (1991) reported that transfection of the $\alpha_1\beta_1\gamma_2$ GABA$_A$ receptor subunit combination, which forms a type-1 BZD receptor, allowed ethanol enhancement of GABA responsives, whereas other GABA$_A$ receptor subunit combinations did not even at high ethanol concentrations—a finding suggestive that the GABA$_A$ receptor subtype dictated the regional specificity to ethanol to enhance GABA function. Other in vivo electrophysiological investigations demonstrated that the effect of zolpidem, a type-1 BZD agonist, predicted the ability of locally applied ethanol to enhance the effect of GABA on single-unit activity within specific regions of brain (Criswell et al., 1993, 1995, 1997).

However, in spite of the view that selected GABA$_A$ receptors with differing sensitivity to ethanol supported the regional specificity of ethanol on GABA function in brain (Wafford et al., 1991; Criswell et al., 1995, 1993), in vitro data began to accumulate that ethanol was not directly influencing GABA$_A$ receptors on neurons from brain regions where ethanol influenced GABA function in vivo (Palmer and Hoffer, 1990; Siggins et al., 1987b; White et al., 1990; Givens and Breese, 1990a, 1990b). Frye et al. (1994) were unable to demonstrate an effect of ethanol on GABA-gated currents from neurons isolated from medial septum, a brain site previously shown to be sensitive to ethanol (Criswell et al., 1993, 1995; Givens and Breese, 1990a, 1990b). Peoples and Weight (1999) found an ethanol enhancement of GABA function only at lethal concentrations. Later, Mori et al. (2000) and Criswell et al. (2003) reported that ethanol did not have a reliable action to enhance GABA function from neurons isolated from several regions of brain. These latter studies clearly discounted the view that ethanol acts directly on type-1 BZD or the majority of other GABA$_A$ receptors to influence GABA function. Consequently, given the lack of viability in vitro for a direct effect of ethanol affecting the majority of GABA$_A$ receptors to enhance GABA function (Criswell et al., 2003; Frye et al., 1994; Mori et al., 2000), it was concluded that the neural mechanism by which ethanol-enhanced GABA function was not available to individual enzymatically isolated neurons—a conclusion consistent with ethanol affecting neural mechanisms distinct from a direct action on the majority of postsynaptic receptors (see Criswell et al., 2004, 2005a, 2005b). Thus, the mechanism by which ethanol influences GABA function to enhance responsiveness in vivo in a regionally specific manner required resolution.

The focus of presentations from this symposium is to introduce various actions of ethanol that likely contribute to its GABAmimetic profile. These include data that implicate: (1) a direct effect of ethanol on specific δ-containing GABA$_A$ receptor subtypes, (2) ethanol modulating circuits which influence GABAergic transmission, (3) the ability of ethanol to induce presynaptic GABA release, (4) an increase in neurosteroid presence in brain that can influence GABA$_A$ receptor function, and finally (5) a regionally specific integration of ethanol's induction of brain neurosteroids enhancing the effect of GABA released by ethanol.

ROLE OF *δ* SUBUNIT-CONTAINING GABA$_A$ RECEPTORS IN ACUTE ETHANOL ACTIONS

Martin Wallner, H. Jake Hanchar, Paul Dodson, Thomas S. Otis, and Richard W. Olsen

GABA$_A$ Receptors as Alcohol Targets

GABA$_A$ receptors (GABA$_A$R) have long been implicated in the intoxicating actions of ethanol because GABA$_A$R agonistic barbiturates and ethanol show striking similarities in their effects on human behavior (Isbell et al., 1950). In addition, the GABA$_A$R agonist muscimol potentiates the sedative properties of alcohol, while the opposite effect, a reduction of ethanol-produced sedation, is observed after administration of GABA$_A$R blocking agents picrotoxin and bicuculline (Liljequist and Engel, 1982). Furthermore, GABA$_A$R agonistic BZDs share similarities with ethanol action and have additive, possibly even synergistic (super-additive), effects when taken together with ethanol. Using Cl$^-$ flux assays, a number of groups showed that alcohol at low concentrations increased GABA$_A$R-mediated Cl$^-$ flux in synaptoneurosomes (Suzdak et al., 1986).

However, the identification of recombinant GABA$_A$R subunit combinations sensitive to relevant (3–30 mM) intoxicating concentrations of ethanol has been accomplished only fairly recently (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003) and we showed that the *δ* as well as the *β*3 subunit is necessary for highly ethanol-sensitive receptors (Wallner et al., 2003). These receptors have not received much attention because the focus of alcohol research on GABA$_A$R have been the more abundant synaptic (*γ*2 subunit-containing) GABA$_A$R, that are increased in their activity by alcohol, but only at anesthetic concentrations of >30 mM ethanol (Mihic et al., 1997). In vivo GABA$_A$R *δ* subunits are thought to be virtually exclusively associated with *α*4 and cerebellar *α*6 subunits (Farrant and Nusser, 2005).

Why Are Extrasynaptic GABA$_A$ Receptors Appealing Targets for Alcohol and Anesthetics?

Delta subunit-containing GABA$_A$Rs have been shown to be excluded from GABAergic synapses, show slow desensitization, and are activated by ambient extracellular GABA concentrations thought to be on the order of 0.1 to 1 *μ*M (Farrant and Nusser, 2005). In contrast to synaptic receptors that open only sporadically and briefly in response to presynaptic GABA release, GABA$_A$Rs containing the *δ* subunit give rise to a continuously active mode of inhibition. Because of this continuous (tonic) activity, which more than compensates for their low abundance and low GABA efficacy, these extrasynaptic receptors are critical for setting overall neuronal excitability (Farrant and Nusser, 2005; Nusser and Mody, 2002). In addition, and in marked contrast to *γ*2-containing receptors, *δ* subunit-containing GABAR show low efficacy to GABA, that can be dramatically increased by anesthetics like etomidate, propofol, and anesthetic concentrations of neuroactive steroids. These features make extrasynaptic receptors excellent candidates for mediating not only the effects of some general anesthetics but also attractive targets for ethanol actions (Fig 1A).

A Point Mutation in the BZD Site of the Cerebellar *α*6 GABA$_A$R Subunit Leads to Increased Alcohol-Induced Motor-Impairment

It was reported that alcohol nontolerant (ANT) rats, selected based on their high susceptibility to the impairment of postural reflexes by ethanol (EtOH) and BZDs, have a point mutation in the "BZD site" *α*6(R100Q) of the cerebellar GABA receptor *α*6 subunit (Korpi et al., 1993). The α6R100Q mutation makes *α*6-containing receptors, when expressed with *β*2 and *γ*2 subunits, diazepam-sensitive (wild-type *α*6*β*2*γ*2-containing receptors are insensitive to classical BZ agonists), and this likely explains the higher BZD sensitivity of ANT animals. However, the reason(s) for the increased EtOH sensitivity of ANT rats remained unclear. The

same $\alpha6(R100Q)$ point mutation has also been found enriched in the independently derived "Sardinian alcohol nonpreferring" rats, (Saba et al., 2001). We reasoned that the identification of the same "mutation" occurring in 2 independently derived rat lines selected for an alcohol phenotype must be because of the selection of a fairly frequent naturally occurring $\alpha6(100Q)$ allele. Supporting this view is the fact that the published rat genomic sequence contains the $\alpha6$-100Q allele and our finding that in commercial Sprague–Dawley rats (Charles River Laboratories, Hollister, CA) the $\alpha6$-100Q variant is a frequently occurring allele. We showed that the $\alpha6$-R100Q variant (in animals that were not selectively bred) is sufficient to cause behavioral alcohol hypersensitivity at low alcohol doses in $\alpha6100^{QQ}$ homozygous animals (Hanchar et al., 2005). Most importantly we can show that the $\alpha6R100Q$ (ANT) mutation leads to a further increase in the already high alcohol sensitivity of $\alpha6\beta3\delta$ receptors (Fig. 1B), not only in recombinant systems, but also in tonic currents from cerebellar granule cells from animals homozygous for the $\alpha6R100Q$ allele (Hanchar et al., 2005). From our findings it would be expected that selectively bred mutant $\alpha6$-100QQ ANT rats should show alcohol-supersensitive tonic currents in cerebellar granule cells. The reason(s) why this has not been found remain unclear (Valenzuela et al., 2005).

The increase in alcohol sensitivity in the $\alpha4/6R100Q$ mutants (Fig. 1b) is unexpected because alcohol sensitivity in GABA$_A$R had been attributed to sites defined by mutations in GABA$_A$R transmembrane regions (Mihic et al., 1997) and it remains to be determined why a mutation at a site in the protein important for BZD sensitivity has such dramatic effects on alcohol sensitivity in these receptors.

We conclude that the direct activation of a subset of GABA$_A$R (in particular those containing the δ subunit) could be important for intoxicating ethanol effects experienced during social alcohol consumption. This is consistent with the observation that δ subunit knockout animals show multiple defects in behavioral responses to ethanol (Mihalek et al., 2001). Future challenges will be to determine how important these receptors are when compared with other potential alcohol targets and to determine the site and mechanism of alcohol action on these uniquely alcohol-sensitive GABA$_A$ receptors. However, results have recently been reported differing from those presented concerning the ethanol potentiation of GABA from δ-containing receptors combined with $\alpha4$ and $\beta3$ subunits (see Borghese et al., 2005). We are puzzled by this report as low-dose alcohol has had a consistent effect on this GABA$_A$ receptor subtype in our laboratory. The reason for the inability to reproduce low dose alcohol augmentation with the human recombinant $\alpha4\beta3\delta$ GABA$_A$ receptor expressed in oocytes and in a stable cell line remains to be clarified.

MODULATION OF GABAergic INTERNEURONS BY ETHANOL

Manuel Mameli, Mario Carta, and C. Fernando Valenzuela

In recent years, brain slices and patch-clamp electrophysiological techniques have been used to characterize the acute effect of ethanol on the function of GABAergic interneurons in the hippocampus and cerebellum. In the hippocampus, kainate receptors are important modulators of interneuronal excitability in the CA1 region. These receptors belong to the superfamily of glutamate-gated ion channels, which also includes NMDA and AMPA receptors. Crowder et al (2002) reported that 20 to 80 mM ethanol inhibits kainate receptor-mediated inhibition of evoked inhibitory postsynaptic currents (IPSCs) in CA1 pyramidal neurons, indicating that interneuronal kainate receptors are sensitive targets of ethanol. Consequently, a more detailed characterization of this effect was performed. In addition to modulating evoked IPSCs, activation of kainate receptors increases interneuronal firing, which robustly increases spontaneous IPSC (sIPSC) frequency in pyramidal neurons. Carta et al (2003) found that ethanol potently inhibits (IC$_{50}$ = 4.4 mM) this kainate receptor–mediated effect at

concentrations that can be achieved in blood after the ingestion of low amounts of ethanol. This effect was secondary to an ethanol-induced inhibition of interneuronal firing in response to kainate receptor activation by either exogenous agonist or synaptically released glutamate. Interneuronal firing in response to activation of AMPA receptors or a voltage step was not affected by ethanol. In the absence of kainate, ethanol (2–25 mM) did not significantly affect the frequency or amplitude of sIPSCs in CA1 pyramidal neurons; however, a small (~20%) but significant increase in sIPSC frequency was detected with 50 mM ethanol. Thus, ethanol increases excitability of CA1 pyramidal neurons indirectly by inhibiting the kainate receptor–dependent drive of GABAergic interneurons. We postulate that this effect of ethanol may explain some of the paradoxical excitatory actions of this widely abused central nervous system depressant.

We have also characterized modulation of cerebellar interneurons by ethanol. Recordings from cerebellar granule cells revealed that ethanol increases GABAergic transmission to these cells (Carta et al., 2004). These neurons receive GABAergic input in the form of phasic and tonic currents from Golgi cells, an interneuronal subtype that is present in the granule cell layer of the cerebellar cortex. Phasic currents are mediated by synaptic receptors containing $\alpha_1\beta_x\gamma$ subunits, whereas tonic currents are mediated by extrasynaptic receptors containing $\alpha_6\beta_x\delta$ subunits. It was found that ethanol increases the frequency but not the amplitude of action potential-dependent sIPSCs at a concentration as low as 20 mM. A similar finding was recently reported by Hanchar et al (2005). Tonic currents were also increased at this concentration of ethanol. However, in the presence of tetrodotoxin, ethanol failed to induce a significant change in the tonic current amplitude, noise variance, or miniature IPSC (mIPSC) amplitude. The peak, early phase, and spillover phase of inhibitory postsynaptic currents evoked by electrical stimulation of the Golgi cells were also unaffected by ethanol. Importantly, loose-patch cell-attached recordings from Golgi interneurons demonstrated that ethanol increases the excitability of these neurons. Taken together, these findings indicate that ethanol does not directly affect extrasynaptic GABA$_A$ receptors under our recording conditions (i.e., whole-cell patch clamping at 31°C with a high chloride internal solution, in the absence of exogenous GABA and GABA transporter inhibitors). Hanchar et al. (2005) reported that extrasynaptic receptors display high sensitivity to ethanol under different experimental conditions and a single amino acid difference (R100Q) in the sequence of the α_6 GABA$_A$ receptor subunit dramatically increases the ethanol sensitivity of these receptors (but see Valenzuela et al., 2005). Thus, future experiments will be required to fully understand modulation by ethanol of GABAergic transmission in cerebellar granule neurons.

Recently, we have begun to characterize modulation of molecular layer interneuronal function by acute ethanol exposure. Recordings from Purkinje neurons indicate that ethanol increases quantal GABA release from these inter-neurons without affecting postsynaptic GABA$_A$ receptor function. These findings are in agreement with a recent report (Criswell and Breese, 2005a, 2005b). Two classes of interneurons are present in the molecular layer: basket cells and stellate cells. We are currently investigating whether ethanol selectively affects 1 of these interneuronal populations. These experiments are being carried out using glass stimulation electrodes to selectively evoke IPSCs in response to GABA release from either basket cells or stellate cells. Specifically, we are looking at the effect of ethanol on paired-pulse plasticity of these events, as changes in this type of plasticity are inversely correlated to changes in the probability of transmitter release. We are also studying the impact of the ethanol-induced increase in quantal GABA release on the excitability of Purkinje neurons.

Collectively, the studies discussed above indicate that ethanol has diverse effects on interneurons, even within the same region of the brain. In the granule cell layer of the cerebellum, it increases GABAergic input in an action potential–dependent manner by increasing spontaneous firing in Golgi cells (Carta et al., 2004; Hanchar et al., 2005). In the

Purkinje cell layer of the cerebellum, it increases GABA release in an action potential–independent manner (Criswell and Breese, 2005a, 2005b). In CA1 and CA3 pyramidal hippocampal neurons from juvenile rats, subanesthetic concentrations of ethanol have little or no effect on sIPSC frequency or amplitude (Carta et al., 2003; Galindo et al., 2005). However, ethanol potently decreases the excitatory drive to CA1 interneurons, leading to disinhibition of pyramidal neurons. Thus, the effects of ethanol on the function of GABAergic interneurons are heterogeneous both in terms of mechanism and impact.

ACUTE AND CHRONIC ETHANOL ADMINISTRATION INCREASES GABA RELEASE IN THE CENTRAL AMYGDALA: IN VITRO AND IN VIVO STUDIES

Loren H. Parsons, Marisa Roberto, and George R. Siggins

GABAergic transmission is sensitive to ETOH in distinct brain regions and is clearly involved in the acute actions of ethanol and the development of ethanol tolerance and dependence. Ethanol allosterically modulates the $GABA_A$ receptor complex and is believed to potentiate the effects of GABA in some preparations, though inconsistent findings have contributed to a continuing controversy over this hypothesis. For example, in several brain regions the acute effects of ethanol on $GABA_A$ synaptic responses have been reported to be negligible (Nie et al., 2000; Proctor et al., 1992), contingent on additional manipulations such as $GABA_B$ receptor blockade (Nie et al., 2000; Wan et al., 1996) or stimulation in discrete segments of neuronal afferents (Weiner et al., 1997a). In addition, the differential effects of acute and chronic ethanol exposure on GABAergic function (Davis and Wu, 2001; Mihic, 1999) have further confounded consensus on the effects of ethanol on GABAergic neurotransmission in the CNS.

In an effort to gain more insight into these issues we have conducted a series of experiments that examined the effects of both acute and chronic ethanol on $GABA_A$-mediated neurotransmission in the central nucleus of the amygdala (CeA). The CeA, together with its connections to the bed nucleus of the stria terminalis and nucleus accumbens is considered a major component of the "extended amygdala," a limbic forebrain complex hypothesized to be a common neural substrate for the motivational effects of abused drugs and ethanol in particular. The GABAergic system in the CeA has been implicated in the expression of emotionality including behavioral states of fear and anxiety, as well as states associated with consummatory responses. Moreover, the CeA is considered to be integrally involved in mediating the behavioral effects of acute and chronic ethanol (Hyytia and Koob, 1995; Roberts et al., 1996). Many of the findings discussed below have recently been published (Roberto et al., 2003, 2004a, 2004b) and reviewed in a broader context (Siggins et al., 2005).

Using an intracellular recording procedure in CeA neurons of brain slices prepared from ethanol-naive male Sprague–Dawley rats we found that acute superfusion with a medium containing 44 mM ethanol clearly enhanced the amplitude of evoked GABA-mediated inhibitory postsynaptic currents (IPSCs). The magnitude of this effect was responsive to ethanol concentration (11–66 mM) with an apparent EC_{50} of 20 mM and was rapidly reversible by removal of ethanol from the super-fusion medium. Ethanol was also found to decrease the paired-pulse facilitation ratio of $GABA_A$ inhibitory IPSCs relative to the control condition. Changes in paired-pulse facilitation ratio are inversely related to transmitter release such that a reduction in paired-pulse facilitation is associated with an increased probability of transmitter release. Thus, these data suggest that acute ethanol exposure increases GABA release in the CeA of ethanol-naive rats. Using whole-cell patch-clamp measurements, ethanol was found to increase the frequency of spontaneously occurring mIPSCs in a bicuculline-sensitive manner, suggestive of an ethanol-induced enhancement of presynaptic GABA release. In addition, ethanol increased the amplitude of mIPSCs in approximately half the neurons evaluated, suggesting an influence of ethanol on postsynaptic mechanisms of some, but not all neurons.

In subsequent experiments, we sought to evaluate the effect of ethanol on GABA release in the CeA using an in vivo preparation. In these experiments ethanol was locally delivered to the CeA of ethanol-naïve rats by retrodialysis, and concurrent changes in dialysate GABA levels were determined using capillary electrophoresis coupled with laser-induced fluorescence. Based on previous work by others (Robinson et al., 2000), a 10% retrodialysis efficiency was estimated in this experiment, and thus the ethanol concentrations delivered to the tissue immediately surrounding the dialysis probe were approximately 10 to 100 mM, with the concentration declining sharply as a function of distance from the probe membrane because of dilution in the interstitial space. With this protocol, we observed that local ethanol administration induced a significant and dose-dependent (0.1–1.0 M) increase in dialysate GABA levels to maximum of approximately 200% of baseline (see example in Fig. 2). Interestingly, there was no effect of ethanol on glutamate, glycine, or taurine levels in these same dialysate samples indicating a selective effect of ethanol on GABA release under these conditions. Consequently, these findings provide important in vivo support for the electrophysiological observations made in the slice preparation, which collectively indicate that acutely administered ethanol increases GABA release in the CeA of ethanol-naïve rats.

Chronic ethanol exposure is known to induce many long-term adaptations in CNS function and some data suggest that chronic ethanol consumption reduces GABA ergic function thereby producing tolerance to many of the presumed GABA-mediated behavioral effects of ethanol. Based on these observations, we evaluated alterations in CeA GABA function in animals given chronic ethanol treatment (CET) through long-term ethanol vapor inhalation. Ethanol vapor concentrations were titrated to maintain blood alcohol levels of 150 to 200 mg% for more than 14 days in male Sprague–Dawley rats. Subsequent electrophysiological and microdialysis measures were made in the CeA 2 to 8 hours after removal from the ethanol vapor, a time frame during which significant signs of ethanol withdrawal have been observed using this paradigm (Macy et al., 1996). We found that the amplitude of evoked $GABA_A$-mediated IPSP/Cs was significantly enhanced in the CeA of rats previously exposed to CET relative to ethanol-naïve controls. Moreover, both the frequency and amplitude of spontaneously occurring mIPSCs were robustly enhanced in CET rats relative to controls and collectively these observations suggest that basal GABAergic transmission is enhanced following CET. This was supported by the observation of significantly higher baseline dialysate GABA levels collected from the CeA of CET rats compared with ethanol naïve rats. Baseline dialysate levels of glycine and taurine were unaltered by CET, though baseline glutamate levels were significantly increased in CeA microdialysates collected from CET rats. Interestingly, acute ethanol exposure enhanced the amplitude of evoked $GABA_A$-mediated IPSCs to a similar extent in CeA slices from CET and ethanol-naïve rats, and this similarity was observed across a range of stimulus intensities. In fact, estimated EC_{50}s for the ethanol-induced enhancement of evoked IPSCs amplitude was 18 and 20 mM for the CET and naïve groups, respectively. Ethanol was also found to produce a similar decrease in paired-pulse facilitation ratios and a similar increase in the frequency of spontaneous mIPSCs in the CeA of CET and naïve rats. These observations suggest that CET does not induce tolerance to ethanol-induced increases in GABA release in the CeA. This was supported by in vivo microdialysis measures in which local ethanol administration significantly enhanced GABA levels in CeA dialysates collected from CET rats, despite the nearly 5-fold increase in baseline dialysate GABA levels in these animals. As in ethanol-naïve rats, local ethanol administration did not alter glycine or taurine levels in CeA dialysates from CET rats, though a significant and ethanol concentration–dependent increase in dialysate glutamate levels was observed.

Collectively these findings indicate that ethanol increases GABA transmission in CeA neurons. The consistent observations of ethanol-induced decreases in paired-pulse facilitation ratios, increases in mIPSC frequencies and increased GABA levels in CeA dialysates indicate that acute ethanol increases presynaptic GABA release. In addition, ethanol increased the amplitude

of mIPSCs in approximately half the neurons evaluated, suggesting an influence of ethanol on postsynaptic mechanisms of some, but not all, neurons. The effects of acute ethanol on GABA transmission were preserved in CET rats, indicating a lack of tolerance to the stimulatory effects of ethanol. However, the robust increase in basal IPSCs, mIPSC frequency and baseline dialysate GABA levels following CET suggests a novel neuroadaptive mechanism that may be involved in the development of ethanol dependence.

GABAERGIC NEUROACTIVE STEROIDS MODULATE SELECTIVE ETHANOL ACTIONS: MECHANISMS AND SIGNIFICANCE

A. Leslie Morrow, Shannon N. Penland, and Rahul T. Khisti

Ethanol is anxiolytic, sedative/hypnotic, and anticonvulsant and causes impairments in cognitive processing (for a review, see Morrow et al., 2001). These effects of acute ethanol administration are remarkably similar to the effects of $3\alpha,5\alpha$-reduced neurosteroids as well as BZDs and barbiturates—all modulators of $GABA_A$ receptors. The neuroactive steroids 3α-hydroxy-5α-pregnan-20-one ($3\alpha,5\alpha$-THP) and $3\alpha,21$-dihydroxy-5α-pregnan-20-one ($3\alpha,5\alpha$-THDOC) are potent modulators of $GABA_A$ receptor inhibition (Majewska et al., 1986; Morrow et al., 1987). We have made a series of discoveries that led us to propose that endogenous neuroactive steroids play an important role in the complex and controversial interactions between ethanol and $GABA_A$ receptors.

Systemic ethanol administration dramatically elevates both plasma and brain $3\alpha,5\alpha$-THP and $3\alpha,5\alpha$-THDOC levels in male and female rats (Barbaccia et al., 1999; Morrow et al., 1999; O'Dell et al., 2004). In male rats, circulating $3\alpha,5\alpha$-THP levels increased 600% above handling-habituated saline-injected control rats (Morrow et al., 1999). Cerebral cortical $3\alpha,5\alpha$-THP levels were elevated approximately 700% above controls. Female rats were tested during the estrus phase of the estrous cycle when both circulating and cerebral cortical $3\alpha,5\alpha$-THP levels are substantially higher than male rats. Ethanol increased circulating $3\alpha,5\alpha$-THP levels by 157% and cerebral cortical $3\alpha,5\alpha$-THP levels by 234% in female rats. $3\alpha,21$-Dihydroxy-5α-pregnan-20-one and its precursor deoxycorticosterone are also elevated dramatically in plasma and brain following systemic ethanol administration (Barbaccia et al., 1999; Khisti et al., 2005). These increases in brain $3\alpha,5\alpha$-THP and $3\alpha,5\alpha$-THDOC levels are sufficient to enhance GABA-mediated neurotransmission and produce anxiolytic and anticonvulsant effects (VanDoren et al., 2000). We have explored whether increased brain concentrations of neuroactive steroids modulate the behavioral effects of ethanol discussed above.

Behavioral and Functional Effects of Ethanol Modulated by Endogenous Neuroactive Steroids

Systemic administration of moderate doses of ethanol increases both plasma and brain levels of $3\alpha,5\alpha$-THP and $3\alpha,5\alpha$-THDOC and their precursors progesterone and DOC in rodents, in a time-dependent and concentration-dependent manner (Barbaccia et al., 1999; Khisti et al., 2005; Morrow et al., 1999; O'Dell et al., 2004; VanDoren et al., 2000). Ethanol-induced elevations of GABAergic neuroactive steroids clearly contribute to many behavioral effects of ethanol in rodents. Neuroactive steroids modulate anticonvulsant effects (VanDoren et al., 2000), sedation (Khisti et al., 2003), impairment of spatial memory (Matthews et al., 2002; Morrow et al., 2001), anxiolytic-like (Hirani et al., 2005), antidepressant-like (Hirani et al., 2002), and reinforcing properties of ethanol in rodents (Janak et al., 1998; Morrow et al., 2001). Each of these behavioral responses is inhibited by pretreatment with the biosynthesis inhibitor finasteride and/or by previous adrenalectomy. Importantly, administration of the immediate precursor of $3\alpha,5\alpha$-THP restores effects of ethanol in adrenalectomized animals, showing that brain synthesis of neuroactive steroids modulates effects of ethanol (Khisti et al.,

2003). Additionally, VanDoren et al. (2000) were able to block the effects of moderate concentrations of ethanol on medial septal neurons and Tokunaga et al. (2003) demonstrated a reduction of ethanol activity in the hippocampus by minimizing the production of neurosteroids. Taken together, these studies suggest that elevations in neuroactive steroids are responsible for many of the GABAergic effects of ethanol in vivo and the effects of neuroactive steroids may determine sensitivity to the behavioral effects of ethanol.

Neurosteroids Do Not Affect All Ethanol-Mediated Behaviors

In contrast to the role of neurosteroids in many ethanol-induced behaviors, ethanol-induced ataxia and motor incoordination are insensitive to the effects of finasteride and adrenalectomy in rats. Although finasteride (25–50 mg/kg) reduces (VanDoren et al., 2000) and adrenalectomy prevents (Khisti et al., 2003) ethanol-induced elevations in $3\alpha,5\alpha$-THP levels in the cerebral cortex, these procedures did not prevent ethanol-induced impairment of the severity of intoxication or the loss of aerial righting or ataxia in the rotarod task up to 60 minutes following ethanol administration (Khisti et al., 2003). Although ethanol-induced impairment of motor behavior is thought to involve GABAergic neurotransmission, such impairment is more prominent at time points (<20 minutes) when ethanol-induced increases in cortical $3\alpha,5\alpha$-THP levels are not yet present. As procedures that reduce $3\alpha,5\alpha$-THP levels did not prevent ethanol impairment of motor coordination using several measures of motor impairment, it is likely that elevated GABAergic neuroactive steroid biosynthesis does not contribute to the motor-incoordinating effects of ethanol.

Suppression of Neurosteroid Responses Following Chronic Ethanol Exposure

It is well known that chronic ethanol stress results in adaptation of the hypothalamus–pituitary–adrenal (HPA) axis resulting in decreased levels of corticosterone in rats (Spencer and McEwen, 1990). Repeated exposure to alcohol also blunts the response of the HPA axis to a second drug challenge with a reduction in corticotropin-releasing factor and adrenocorticotrophin levels (Lee et al., 2001). Similar alterations in the HPA axis are observed in actively drinking or alcohol-dependent human subjects (Adinoff et al., 2005; Wand and Dobs, 1991). In line with these observations, chronic ethanol consumption in rats results in blunted elevation of cerebral cortical $3\alpha,5\alpha$-THP (Morrow et al., 2001) and plasma and brain DOC levels (Khisti et al., 2005) following ethanol challenge. These findings suggest that there is tolerance to ethanol-induced increases in neuroactive steroid levels. Although decreases in brain neurosteroid levels were concomitant with decreases in plasma neurosteroid levels, it is likely that the observed decreases in $3\alpha,5\alpha$-THP and DOC levels were dependent on blunted HPA axis activity. Thus, adaptations of the HPA axis may underlie tolerance to effects of ethanol that are mediated by the GABAergic neuroactive steroids.

Mechanism of Ethanol-Induced Increases in Neurosteroids

We investigated the hypothesis that ethanol could alter steroidogenic enzyme activity in rat brain and adrenal minces. Basal neurosteroidogenic enzyme activity was different in rat brain and adrenal gland. Fresh brain or adrenal tissue minces were incubated with radiolabeled steroid precursor and conversion to radiolabeled steroid product was monitored. 5α-Reductase enzyme activity was assessed by measuring total conversion of [^{14}C]progesterone to [^{14}C] dihydroprogesterone (DHP) and [^{14}C]$3\alpha,5\alpha$-THP. 5α-Reductase activity peaked at 10% to 12% conversion after 60 minutes in the cortex, cerebellum, and adrenal gland. The OB+T had a higher basal activity compared with other tissues (24% conversion). The 5α-reductase enzyme inhibitor finasteride (0.1 μM) significantly reduced the activity of the 5α-reductase enzyme only in the OB+T and adrenal gland. Ethanol did not alter 5α-reductase enzyme activity in rat brain or adrenal gland minces. The effect of ethanol on 5α-reductase activity was investigated in minces of rat cerebral cortex, cerebellum, OB+T, and adrenal gland. Ethanol

(10–100 mM) did not alter total percent conversion of $[^{14}C]$progesterone to $[^{14}C]$DHP above basal levels in these tissues.

3α-Hydroxysteroid dehydrogenase (HSD) reductive enzyme activity was assessed by measuring total conversion of $[^{14}C]$DHP to $[^{14}C]$3α,5α-THP. 3α-Hydroxysteroid dehydrogenase reductive activity was highest in the OB+T ($44.5 \pm 4.4\%$) and adrenal gland ($29.9 \pm 5.1\%$). The 3α-HSD enzyme inhibitor indomethacin ($0.2\,\mu$M) significantly reduced the reductive activity of the 3α-HSD enzyme in all regions. Ethanol (10–100 mM) increased the conversion of $[^{14}C]$5α-DHP to $[^{14}C]$3α,5α-THP by $30.0 \pm 3.6\%$ in the OB+T, but had no effect in the adrenal gland. In the cerebral cortex and cerebellum, ethanol did not increase 3α-HSD activity to the extent observed in the OB+T. Although there was a trend toward increased activity in the cerebral cortex and cerebellum in the presence of ethanol, the data were not statistically significant except for 1 dose (25 mM) in the cerebellum because of the small size of the changes (10%–15% above basal). We tested the effect of fluoxetine as a positive control as previous studies showed that fluoxetine decreased the K_m of a recombinant 3α-HSD enzyme (Griffin and Mellon, 1999). Fluoxetine increased the activity of 3α-HSD enzyme in the OB+T and adrenal gland and this effect was blocked by the 3α-HSD inhibitor indomethacin.

The 3α-HSD enzyme possesses bidirectional activity so we examined the effect of ethanol on the oxidative activity of 3α-HSD. 3α-Hydroxysteroid dehydrogenase oxidative enzyme activity was assessed by measuring total conversion of $[^{3}H]$3α,5α-THP to $[^{3}H]$DHP. In the oxidative direction, basal activity rates were $17.0 \pm 2.3\%$ total conversion in the OB+T and $24.4 \pm 3.9\%$ in the adrenal gland and were sensitive to indomethacin. The cerebral cortex exhibited less 3α-HSD oxidative activity ($9.9 \pm 1.4\%$) and was insensitive to indomethacin. Ethanol did not alter the total percent conversion of $[^{3}H]$-ALLO to $[^{3}H]$DHP in rat OB+T or adrenal gland. An increase in the reductive activity of the 3α-HSD with no change in the oxidative direction would cause a greater conversion of 5α-DHP to ALLO. This effect could contribute to ethanol-induced increases in brain 3α,5α-THP levels. Indeed, the increased reductive activity of 3α-HSD would be predicted to increase brain levels of both 3α,5α-THP and 3α,5α-THDOC.

Summary

We propose that the data reviewed in this chapter represent compelling evidence that 3α,5α-THP produced in response to systemic ethanol administration contributes to some, but not all effects of ethanol associated with modulation of GABA$_A$ receptors. These studies demonstrate an essential correlation between the time course of ethanol-induced 3α,5α-THP production in the brain and specific effects of ethanol. Furthermore, the anxiolytic, anticonvulsant, cognitive impairing, and inhibitory effects of ethanol on spontaneous neural activity in 2 brain regions were completely prevented by a key inhibitor of steroid biosynthesis or adrenalectomy. In contrast, ethanol-induced motor incoordination is not affected by either steroid synthesis inhibition or adrenalectomy. These data are consistent with the hypothesis that 3α,5α-THP contributes to ethanol action and this interaction represents an important mechanism of ethanol action.

NEUROSTEROID SYNERGISM OF THE EFFECT OF GABA RELEASED BY ETHANOL: RELEVANCE TO THE GABAMIMETIC PROFILE OF ETHANOL

Hugh E. Criswell, Z. Ming, and George R. Breese

While selected BZD-insensitive GABA$_A$ receptors, which have a limited localization in brain (Peng et al., 2002), have been reported to support the action of ethanol on GABA responsiveness in specific regions of brain (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003), there is controversy concerning these findings (see Borghese et al., 2005). Additionally,

unlike BZDs and barbiturates, ethanol does not appear to act directly on most other GABA$_A$ receptor subtypes to enhance GABA function (Criswell et al., 2003; Mori et al. 2000; Peoples and Weight, 1999; Siggins et al., 1987b; see review by Criswell and Breese, 2005a, 2005b; Siggins et al., 2005). Therefore, for systemic ethanol to display its GABAmimetic profile, it is hypothesized that ethanol affects mechanisms associated with GABA function distinct from a direct effect on the majority of postsynaptic GABA$_A$ receptors (Criswell and Breese, 2005a, 2005b). These neural mechanisms must be capable of supporting ethanol depression of neural rate (Givens and Breese, 1990b) and the action of ethanol on evoked IPSCs (Roberto et al., 2003; Weiner et al., 1997a) from some, but not all, neurons within and across brain regions (Breese et al., 1984; Givens and Breese, 1990a, 1990b; McCown et al., 1986; Weiner et al., 1997a). Current evidence is conistent with 2 indirect actions of ethanol having a major influence on GABA function. One relates to the ability of ethanol to increase release of GABA from presynaptic terminals (Carta et al., 2003; Crowder et al., 2002; Roberto et al., 2003; Ziskind-Conhaim et al., 2003) and the second relates to the action of ethanol to increase synthesis of GABAmimetic neuroactive steroids (Morrow et al. 1999, 2004, 2001; O'Dell et al., 2004; Sanna et al., 2004).

Effect of Ethanol on GABA Release

In very early work (Bloom and Siggins, 1987; Siggins et al., 1987a, 1987b; see review by Siggins et al., 2005), a possible presynaptic action of ethanol was proposed. Consistent with this view, Marszalec et al. (1998) found that perfusion of 100 mM ethanol augmented GABA$_A$ receptor–mediated IPSCs from rat cortical neurons, even though the effect of GABA applied to the neuron was not altered. Further, Melis et al. (2002) found that ethanol increased the frequency of miniature mIPSPs from ventral tegmental neurons, a finding in accord with GABA release, but in apparent conflict with data collected with microdialysis where ethanol did not increase GABA in the perfusate (Cowen et al., 1998). Additionally, reports found that ethanol increased mIPSPs from hippocampal neurons (Carta et al., 2003; Roberto et al., 2003; Sanna et al., 2004), from brain stem motor neurons (Sebe et al., 2003), and from neurons in slices of rat spinal cord (Ziskind-Conhaim et al., 2003). Most importantly, Roberto et al. (2003, 2004a) provided evidence that ethanol not only enhanced the effect of stimulus-induced GABA-mediated IPSPs and mIPSCs from neurons in slices from the central nucleus of the amygdala, but that ethanol caused release of GABA in vivo from this brain site into microdialysates (Roberto et al. 2004b)—clear evidence that ethanol can facilitate release of GABA.

In addition to the work on mIPSCs performed in slices (see Carta et al., 2004; Nie et al., 2004; Roberto et al., 2003, 2004a), the use of mechanically dissociated neurons that have terminals attached (see Akaike and Moorhouse, 2003) has allowed confirmation of the conclusion that ethanol indeed has the ability to release GABA directly from presynaptic terminals. Utilizing this approach, Criswell et al. (2004) provided evidence that ethanol increased the frequency of mIPSCs from cerebellar Purkinje neurons. Zhu and Lovinger (2004) reported similar ethanol enhancement of the frequency of mIPSCs from neurons mechanically dissociated from the basolateral amygdala. As these mIPSCs were blocked by bicuculline, postsynaptic GABA$_A$ receptors were being activated by the ethanol-induced release of GABA.

The demonstrated presynaptic action of ethanol to increase frequency of GABA-induced mIPSCs (Carta et al., 2003; Criswell et al., 2004; Roberto et al., 2003, 2004a; Zhu and Lovinger, 2004) supports the view that ethanol can affect a mechanism related to GABA function distinct from a postsynaptic action on GABA$_A$ receptors. These findings can explain the positive interaction of ethanol with modulators of GABA$_A$ receptor function, such as barbiturates and BZDs (Akaike et al., 1990; Puia et al., 1991), by these latter drugs enhancing the effect of the

increased GABA release by ethanol. For the present, the mechanism by which ethanol induces release of GABA from some, but not all, terminals is uncertain, but is a finding that should receive attention. The next question that arose was whether another mechanism or action of ethanol could contribute to the GABAmimetic profile of ethanol by affecting the degree of responsiveness to GABA released by ethanol.

Involvement of Neurosteroids

Progesterone released from the adrenal serves as a precursor for central neurosteroids (see Barbaccia et al., 2001; Mellon et al., 2001). Acute administration of ethanol activates the HPA axis and increases plasma precursors for neurosteroids resulting in their elevation in brain (Barbaccia et al., 1999; Morrow et al., 2001)—a change reduced by adrenalectomy (Khisti et al., 2002; 2003) and by combining adrenalectomy and gonadectomy (Follesa et al., 2005; O'Dell et al., 2004). However, evidence has been provided that neurosteroids can be produced in brain by de novo synthesis (Follesa et al., 2005), distinct from precursors produced by the adrenals (Khisti et al., 2003). Based on the findings that reducing neurosteroid content in brain minimized sedation and other actions of ethanol, Morrow et al. (1999, 2001, 2004) suggested that the increase in neurosteroids following acute ethanol administration contributes to its actions.

While adrenalectomy reduces neurosteroids (Khisti et al., 2002, 2003; O'Dell et al., 2004), this treatment reduces, but does not eliminate, the ethanol's ability to influence CNS function (Bowers et al., 1991; Khisti et al., 2003). The physiological significance of neurosteroids being produced in brain by de novo synthesis (Follesa et al., 2005; Sanna et al., 2004), distinct from precursors produced by the adrenals (Khisti et al., 2003), has yet to be resolved. Important to this discussion is that the neurosteroids, allopregnanalone and tetrahydro deoxycorticosterone, are potent allosteric modulators of GABA responsiveness from $GABA_A$ receptors, having this action at nanomolar concentrations (Criswell et al., 2003; Schwabe et al., 2005) on some, but not all, $GABA_A$ receptor subtypes (Belelli et al., 2002; Bianchi and Macdonald, 2003; Lambert et al., 2001; 2003; Stell et al., 2003).

A clue to the mechanism by which the ethanol-induced increase in neurosteroids influences GABA function was a study by Kang et al. (1998) who noted that the presence of neurosteroid-enhanced evoked IPSCs from hippocampal neurons. Further, alphaxalone and other neurosteroids have been shown to increase the decay time of mIPSCs associated with the GABA released by ethanol (Criswell and Breese, 2005a; Sanna et al., 2004; Vicini et al., 2002). These observations indicate that the neuosteroid is acting postsynaptically to enhance GABA responsiveness. The endogenous level of neurosteroids increased by pharmacologically relevant doses of ethanol is consistent with these compounds contributing to the pharmacological activity of ethanol by enhancing the action of GABA released by ethanol.

Regional Specificity of Ethanol on Presynaptic Release of GABA and Neurosteroids Involvement

Several studies have demonstrated that ethanol does not enhance GABA function from all brain regions in vivo (Criswell et al., 1993; Givens and Breese, 1990b). Studies from neurons from differing brain regions in vitro have demonstrated that ethanol does not have equivalent effects to increase mIPSCs from neurons (Criswell et al., 2004). For example, unlike data from cerebellar Purkinje neurons (Criswell et al., 2004, 2005a; Ming et al., 2006) and basolateral amygdala neurons (Zhu and Lovinger, 2004), ethanol did not increase the frequency of mIPSCs from mechanically dissociated cerebrocortical neurons (Criswell et al., 2004; Criswell and Breese, 2005a). Likewise, ethanol did not enhance IPSCs from some hippocampal neurons (Ariwodola and Weiner, 2004; Weiner et al., 1997a). Consequently, the means by which ethanol has this regional selectivity to release GABA from some, but not all, terminals is

uncertain, but must be resolved to provide a clear understanding of the GABAmimetic profile of ethanol.

Previous work has demonstrated the specificity of neurosteroids to enhance GABA function from specific $GABA_A$ receptor subtypes (see Belelli et al., 2002; Bianchi and Macdonald, 2003; Lambert et al., 2001, 2003; Spigelman et al., 2003; Stell et al, 2003; Wallner et al., 2003). Thus, this differing responsiveness of $GABA_A$ receptors to neurosteroid enhancement of GABA could conceivably contribute to the regional specificity of ethanol on GABA function. Furthermore, a differing presence of neurosteroids in specific regions of brain could be another means by which regional selectivity of ethanol could be realized. In this respect, the enzymes responsible for synthesizing neurosteroids have a differential distribution in brain (Dupont et al., 1994; Khanna et al., 1995; Korneyev et al., 1993; Pelletier et al., 1994). Thus, in addition to the regional effect of ethanol to release GABA, the differential synthesis of neurosteroids in differing brain regions and the differing response of $GABA_A$ receptor subtypes to neurosteroids can contribute to the regional specificity of ethanol to affect GABA function.

Integration of Ethanol Facilitation of GABA Release With the Postsynaptic Action of Neurosteroids Increased by Ethanol

The previous observations indicated that ethanol enhances GABA release and that neurosteroids increased by ethanol enhance GABA responsiveness in a regionally specific manner. The integration of these actions of ethanol presumably contributes to regional differences in ethanol's actions on GABA function within brain to contribute to its GABAmimetic profile. Brain areas where ethanol-induced enhancement of GABA release is prominent and where neurosteroids synthesized locally enhance GABA responsiveness most likely provide a major contribution to ethanol's GABAmimetic profile. In contrast, brain regions, where these processes do not occur, would not be likely to contribute to the GABAmimetic action of ethanol. Thus, the integration across brain regions of the magnitude of GABA release, with the increasing influence of neurosteroids over time on the responsiveness of the released GABA, provide a logical basis for the regional specificity of ethanol to affect GABA function.

The observation by Sanna et al (2004) that ethanol can increase synthesis of neurosteroids in a slice has provided a means to measure the integration of neurosteroid involvement on the effect of GABA released by ethanol. Using this approach, the overall contribution of the integration of these actions of ethanol will become apparent across brain regions. An example of this approach demonstrating the action of neurosteroid synthesized during incubation on the degree of enhancement of GABA responsiveness is provided in Fig. 3.

OTHER CONSIDERATIONS

Collectively, this symposium summarizes the involvement of several factors which contribute to the GABAmimetic profile of ethanol. Integration of these factors is likely to promote the regionally specific action of ethanol to influence GABA function. Nonetheless, clarification of the involvement of other mechanisms that influence GABA function will need to be integrated with GABA release and neurosteroid involvement before the means by which ethanol induces modification of GABA function in differing brain regions will be fully understood. Particularly, the involvement of ethanol on phosphorylation mechanisms (Weiner et al., 1997b; 1994a,b) and the modification of GABA release by $GABA_B$ receptors (Wan et al., 1996; Weiner et al., 1997a; Wu et al., 2004) or neurotransmitters other than GABA that influence release of GABA needs further clarification (Nie et al., 2004; see Siggins et al., 2005). A particularly important issue is whether involvement of these various mechanisms that influence GABA function differ across brain regions. Finally, as recently reported results (see Borghese et al., 2005) concerning ethanol potentiation of GABA from δ-containing receptors

combined with the α4 subunit differs from those presented in this symposium, the basis of this disparity will need future clarification so the role of this GABA$_A$ receptor subtype in the GABAmimetic profile of ethanol can be clearly delineated.

Acknowledgments

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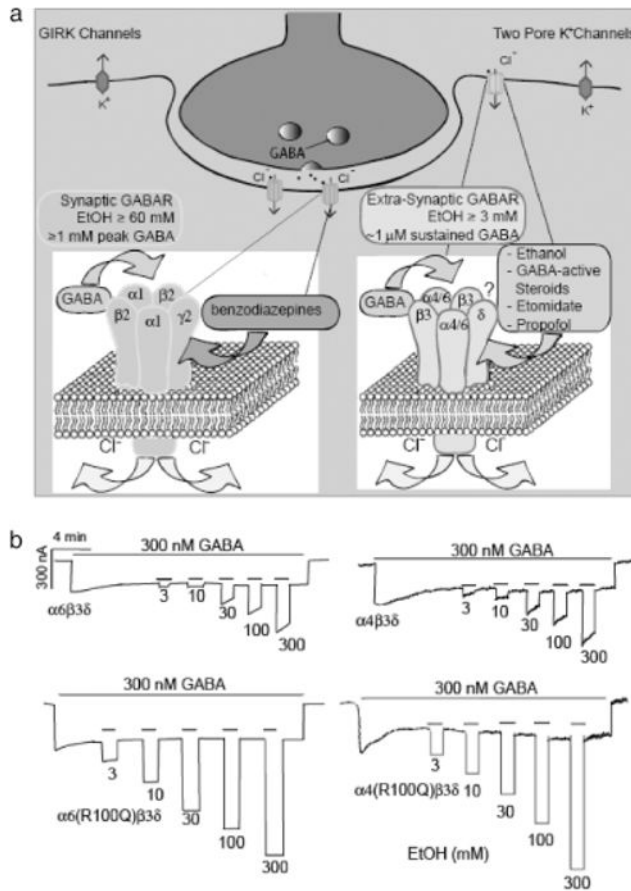**Fig. 1.**
(**a**) Proposed role of synaptic and extrasynaptic receptors. Synaptic receptors have low potency and high efficacy, whereas extrasynaptic δ subunit-containing receptors show high potency and low efficacy. GABA$_A$ receptor–specific anesthetics (etomidate, propofol, neurosteroids) can dramatically increase the low efficacy of extrasynaptic receptors (modified from Wallner et al., 2003). (**b**) The α4/6-R100Q mutation leads to a further increase in alcohol sensitivity of α6β3δ receptors. Original traces that illustrates the dramatic increase in ethanol sensitivity (concentrations indicated are in mM) of recombinant α4/6R100Qβ3δ receptors expressed in *Xenopus* oocytes (*lower panel*), particularly at low intoxicating (3–30 mM) alcohol concentrations. The threshold for activation of α4/6β3δ receptors is around 3 mM in α4β3δ receptors, a concentration almost 6 times lower than 17 mM, the legal driving limit in California, and even lower in mutant a4/6R100Qβ3δ receptors. Black bar indicates the application of 300 nM γ-aminobutyric acid. (Modified from Hanchar et al., 2005.)
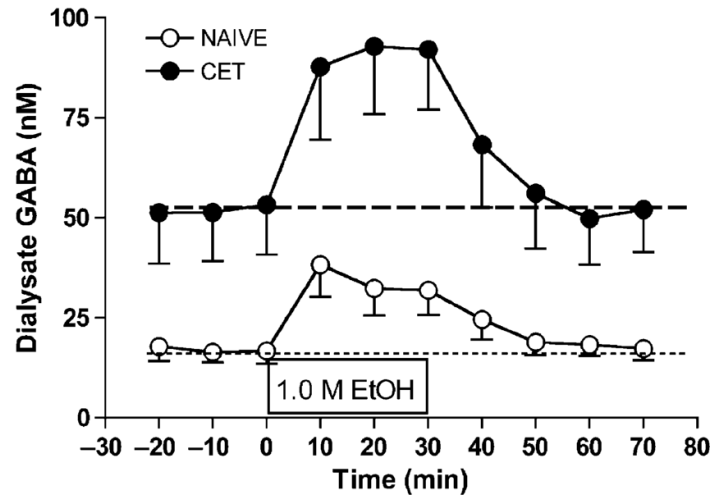
Fig. 2.
Effect of local ethanol (EtOH) administration on dialysate γ-aminobutyric acid (GABA) levels collected from the central nucleus of the amygdala (CeA) of EtOH-naive and chronic EtOH treatment (CET) rats. Stimulatory effect of acute and chronic EtOH administration on interstitial GABA levels in the CeA of the rat as determined by in vivo microdialysis. Chronic EtOH treatment resulted in a significant increase in baseline dialysate GABA levels collected from the CeA of CET rats ($n = 7$) compared with EtOH-naïve rats ($n = 11$). Subsequent local EtOH administration by retrodialysis (1.0 M perfusate EtOH concentration) significantly increased dialysate GABA levels in both EtOH-naive and CET animals (time 0–30 minutes). Dialysate GABA levels returned to baseline levels upon removal of perfusate EtOH (time 30–70 minutes). See text and Roberto et al. (2004a) for further details.
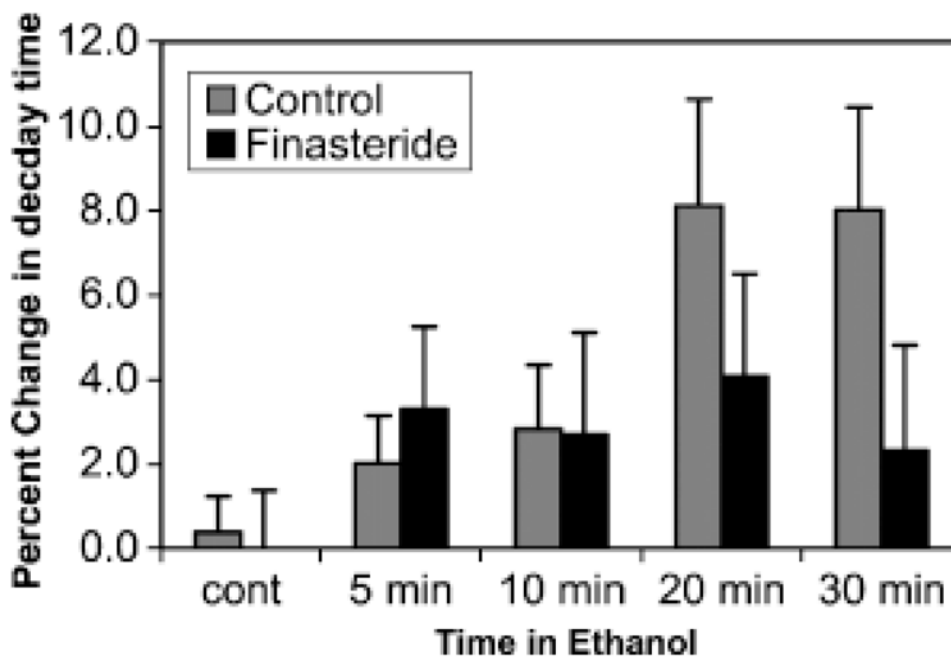
Fig. 3.
Increased decay time of mIPSCs of cerebellar Purkinje neurons with extended ethanol incubation. Cerebellar slices were incubated with ethanol (50 mM) for 30 minutes and decay times [τ_{slow}] of miniature inhibitory postsynaptic current (mIPSCs) from Purkinje neurons determined. Decay time of mIPSCs was significantly increased ($p<0.05$) at 20 and 30 minutes of ethanol incubation—a change minimized by finasteride indicative that the decay time of the mIPSCs related to steroidogenesis by ethanol. N =10 to 12 neurons.