

γ -Aminobutyric Acid Type A Receptors and Alcoholism

Intoxication, Dependence, Vulnerability, and Treatment

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Context: Alcohol facilitates γ -aminobutyric acid (GABA) function, and GABA type A (GABA_A) receptor-facilitating agents suppress alcohol withdrawal symptoms. Advances in molecular neuroscience, genetics, and neuroimaging provide new insights into the role of brain GABA systems in short- and long-term alcohol effects.

Objective: To review the role of brain GABA systems in alcohol response, alcohol dependence, alcoholism vulnerability, and alcoholism pharmacotherapy.

Design: Literature review.

Results: Alcohol increases GABA release, raises neurosteroid levels, and may potently enhance the function of a GABA_A receptor subclass that shows high affinity for GABA and neurosteroids, relative insensitivity to benzodiazepines, low chloride conductance, and an extrasynaptic location. Variation in GABA_A receptor subunit genes may contribute to the vulnerability to alcoholism, particularly in the context of environmental risk factors. Alcohol dependence is associated with time-

dependent changes in brain GABA_A receptor density and subunit gene expression levels that contribute to a withdrawal-related deficit in GABA_A receptor function. However, cortical GABA levels are not reduced during acute withdrawal. Benzodiazepine-assisted detoxification enhances a phasic component of GABA function. However, novel treatments target the tonic component of GABA neurotransmission mediated by benzodiazepine-insensitive GABA_A receptors. Smoking attenuates withdrawal-related disturbances in brain GABA function, perhaps contributing to comorbid nicotine and alcohol dependence. The GABA systems show recovery with long-term sobriety.

Conclusions: Recent research deepens our understanding of the role of GABA systems in alcohol action, alcohol dependence, and the vulnerability to alcoholism. Also, GABA_A receptor subtype-selective treatments merit exploration for reducing withdrawal symptoms and drinking in alcohol-dependent individuals.

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STUDIES OF γ -AMINOBUTYRIC acid (GABA) systems are at the core of alcoholism research.¹⁻³ The facilitation of GABA type A (GABA_A) receptor function by ethanol may be among the potent consequences of ethanol intoxication (**Figure 1**). Deficits in GABA function associated with ethanol dependence may account for the ability of barbiturates, benzodiazepines, and GABA-modulating anticonvulsant agents to suppress alcohol withdrawal symptoms. However, it is still not clear whether ethanol acts directly on GABA_A receptors to produce effects associated with social drinking. Also, the GABAergic adaptations to long-term ethanol administration remain a focus of intensive study.

Groundbreaking research conducted during the past 10 years raises important questions about ethanol actions on GABA_A

receptors, the mechanisms underlying ethanol dependence, and the role of GABA systems in the vulnerability to alcoholism. This review summarizes and integrates this new generation of translational neuroscience research. In doing so, it highlights new directions for the pharmacotherapy of alcohol abuse and dependence.

DOES ALCOHOL PREFERENTIALLY FACILITATE EXTRASYNAPTIC BENZODIAZEPINE-INSENSITIVE GABA_A RECEPTORS?

The family of GABA receptors includes GABA_A receptors that contain a chloride channel,^{6,7} GABA_B receptors that are coupled by G-proteins to second messenger systems,⁸ and GABA_C receptors that are neither blocked by bicuculline nor stimulated by baclofen.⁹ The GABA_A receptors

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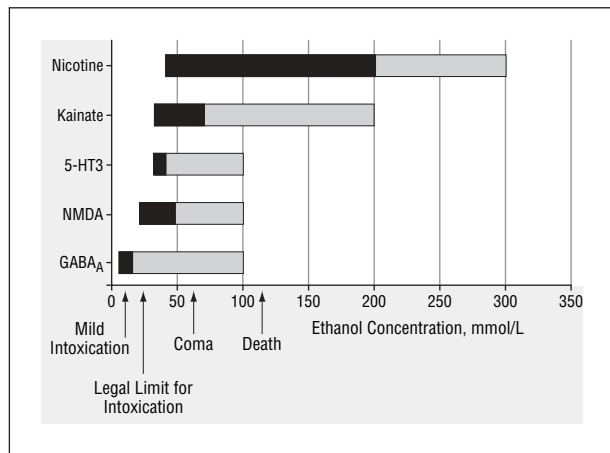


Figure 1. Range of ethanol concentrations with modulatory effects at selected ionotropic receptors. Each bar is black up to the concentration at which ethanol produces 50% of its maximum effect (EC_{50}) or the concentration at which ethanol produces a 50% inhibition of ligand binding to a receptor target (IC_{50}) (ie, the boundary of the black and gray sections of the bars). The ranges primarily reflect electrophysiologic data reviewed by Grant and Lovinger⁴ (where additional details related to the EC_{50} or IC_{50} values can be found). The figure has been modified to include studies reviewed in the text and to be consistent with a recent review.⁵ The clinical consequences of intoxication, also from Grant and Lovinger,⁴ vary across individuals depending on the initial level of alcohol sensitivity and extent of alcohol tolerance. 5-HT3 indicates serotonin type 3; $GABA_A$, γ -aminobutyric acid type A; NMDA, *N*-methyl-D-aspartate.

are pentamers that potentially contain combinations of 19 known subunits designated as α_{1-6} , β_{1-3} , γ_{1-3} , δ , ϵ , θ_{1-3} , and $\pi_{4,5}$.

Ethanol enhances $GABA_A$ receptor function in vivo. For example, benzodiazepine and barbiturate $GABA_A$ receptor-facilitating hypnotic drugs and ethanol produce similar behavioral effects,¹⁰⁻¹³ have additive or even synergistic interactive neurobehavioral effects,¹⁴⁻¹⁷ produce cross-tolerance with ethanol, and suppress ethanol withdrawal syndromes.^{1,2,11,18-24} Aspects of ethanol response are blocked by substances that have effects that oppose those of typical benzodiazepines, benzodiazepine inverse agonists, whereas benzodiazepine antagonists produce mixed effects.²⁵⁻³¹

Electrophysiologic and biochemical studies directly implicated $GABA_A$ receptors as targets for ethanol in the brain. The application of ethanol at doses associated with human ethanol intoxication increased the $GABA_A$ receptor-mediated chloride flux and inhibitory postsynaptic potentials.³²⁻³⁷ However, other studies found that ethanol facilitated $GABA_A$ receptor function to a variable extent, enhanced $GABA_A$ receptor function only at doses associated with very high levels of human intoxication, or did not alter $GABA_A$ receptor function.³⁸⁻⁴³

Differential ethanol actions on $GABA_A$ receptor subtypes may help explain conflicting evidence of direct ethanol effects on GABA receptors (**Table**). The $GABA_A$ receptors typically found in the GABA synapse, that is, synaptic receptors, mediate responses to benzodiazepine-like drugs.^{7,51} Synaptic $GABA_A$ receptors typically include α , β , and γ subunits in $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, or $\alpha_3\beta_3\gamma_2$ subunit combinations. Synaptic $GABA_A$ receptors have lower affinity for GABA than receptor subclasses located at the periphery of GABA synapses, that is, extrasynaptic receptors.^{52,53} However, synaptic receptors mediate a phasic, high-

Table. Differences Between $GABA_A$ Receptor Classes Distinguished by the Potency of Ethanol*

Feature	$GABA_A$ Receptor Type	
	High-Potency Ethanol	Low-Potency Ethanol
Ethanol EC_{50} , mmol/L	3-30	50-100
GABA potency	Higher	Lower
GABA action	Partial agonist	Full agonist
Location	Extrasynaptic	Synaptic
Chloride ion conductance	Low	High
Inhibition	Tonic	Phasic
α Subunit subtype	α_4 , α_6	α_{1-3} , α_5
γ/δ Subunit subtype	δ	γ
Frequency in brain, %†	≤ 5	> 95
Modulator	Neurosteroid Ro15-4513 (BZ inverse agonist) Tiagabine Topiramate	BZ agonist

Abbreviations: BZ, benzodiazepine; EC_{50} , concentration at which ethanol produces 50% of its maximum effect; GABA, γ -aminobutyric acid; $GABA_A$, γ -aminobutyric acid type A.

*This table highlights recent findings suggesting that ethanol may have a potent direct extrasynaptic effect on $GABA_A$ receptors. As noted in the text, caveats to this hypothesis include a recently published nonreplication and the possibility that ethanol may have potent indirect effects mediated by increased synaptic or extrasynaptic levels of GABA or neurosteroids.⁴⁴⁻⁵⁰ †Percentage of the total pool of $GABA_A$ receptors.

chloride conductance GABA response by virtue of their exposure to brief pulses (10 milliseconds) of near-saturating (≥ 1 mM) levels of GABA during GABA neuronal firing.⁵²⁻⁵⁵ In contrast, extrasynaptic receptors typically do not respond to benzodiazepine agonist stimulation, although they respond to neurosteroids and benzodiazepine inverse agonists.⁵⁵⁻⁵⁷ Instead of the $\alpha_{1-3,5}$ subunits typical of synaptic receptors, extrasynaptic receptors on cerebellar granule cells contain α_6 subunits, and extrasynaptic receptors on other cell types likely contain α_4 subunits. Extrasynaptic receptors also contain a δ subunit instead of a γ subunit. Although these receptors have a higher affinity for GABA than the synaptic receptors, they are exposed to relatively low GABA levels associated with GABA spillover from the synapse into extracellular spaces ($< 3\mu M$).⁵⁸⁻⁶⁰ These receptors constitute a small minority of $GABA_A$ receptors in the brain, but they play an important role in mediating the tonic component of GABAergic inhibition.⁵⁹

Studies^{54,61} suggest that ethanol potentially facilitates extrasynaptic "benzodiazepine-insensitive" $GABA_A$ receptors (concentration at which ethanol produces 50% of its maximum effect [EC_{50}], 3mM-30mM) and less potently acts at synaptic or "benzodiazepine-sensitive" $GABA_A$ receptors (EC_{50} , 50mM-100mM) (Table). Aspects of the physiologic, discriminative stimulus as well as behavioral effects of ethanol are blocked by drugs that act as inverse agonists on synaptic receptors and partial agonists or inverse agonists on extrasynaptic $GABA_A$ receptors.^{27,62-65} Ethanol also potently and competitively inhibits the binding of the benzodiazepine partial inverse agonist, Ro15-4513, to extrasynaptic $GABA_A$ recep-

tors.^{66,67} Ethanol may also preferentially facilitate tonic rather than phasic GABAergic neurotransmission.⁶⁸

The δ subunit may contribute to high-potency ethanol effects. Although knocking out this subunit in mice increases the expression of γ_2 subunits and increases benzodiazepine binding,⁶⁹ it eliminates some high-potency ethanol and neurosteroid actions on GABA_A receptors.⁶⁹⁻⁷² Knockouts of other GABA_A receptor subunits also produce changes in ethanol response that reflect the normal functions associated with each GABA_A receptor subunit, as has been reviewed elsewhere.³ For example, knocking out the α_6 subunit eliminates the cerebellar high-affinity binding of Ro15-4513 and ataxic, but not sedative, ethanol effects.^{73,74}

However, other data question the role of δ subunits in potent ethanol effects. The discriminative stimulus effects of ethanol are preserved in δ subunit knockout mice.^{71,75} Also, a recent multicenter study⁶⁰ did not find high-potency (<100mM) ethanol actions on rat and human $\alpha_1\beta_2\delta$ GABA_A receptors expressed in *Xenopus* oocytes or a fibroblast cell line. These new data question whether ethanol has a potent direct action on GABA_A receptors and raise the possibility that unknown factors missing from the in vitro systems may account for the lack of potent ethanol actions in this study. For example, it is possible that the high sensitivity of $\alpha_1\beta_2\delta$ GABA_A receptors depends on the phosphorylation status of GABA_A receptor subunits or other features of GABA-related signal transduction.^{76,77} The sensitivity of GABA_A receptors to ethanol is reduced by protein kinase C inhibition.⁷⁸ Also, protein kinase C γ -null mice show reduced ethanol but not benzodiazepine sensitivity of GABA_A receptors and increased ethanol preference compared with wild-type mice, whereas protein kinase C ϵ -null mice show increased ethanol sensitivity of GABA_A receptors, less ethanol preference, and lower ethanol consumption than wild-type mice.⁷⁹⁻⁸⁵

The low potency of ethanol at benzodiazepine-sensitive GABA_A receptors makes it unlikely that direct ethanol actions at these receptors are relevant to typical human alcohol consumption. Ethanol-induced coma develops in the 50mM to 100mM range, and the lethal level of ethanol for humans is approximately 100mM (Figure 1).^{6,44} Thus, ethanol actions on benzodiazepine-sensitive GABA_A receptors may contribute to the lethality of alcohol intoxication or alcohol-benzodiazepine/barbiturate combinations.⁴⁵

Even if ethanol has no potent direct actions on GABA_A receptors, it may have potent indirect effects on these receptors by its well-established ability to raise brain neurosteroid levels and to stimulate GABA release.^{46-48,86} The ability of ethanol to raise brain neurosteroid levels^{87,88} may distinctively contribute to ethanol effects on extrasynaptic GABA_A receptors.^{56,58,72} Ethanol might raise neurosteroid levels by increasing steroidogenesis in the adrenal glands and gonads.⁸⁹ However, a role for central neurosteroid synthesis is suggested by the absence of changes in human plasma neurosteroid levels during ethanol intoxication⁹⁰ and the presence of ethanol-induced elevations in neurosteroid levels in hippocampal slices.⁴⁹ Also, reductions in neurosteroid synthesis produced by the 5- α -reductase inhibitor finasteride reduce ethanol ef-

fects in animals⁵⁰ and humans.⁹¹ Ethanol also acts presynaptically to increase GABA release.^{47,92,93} These increases may reflect local presynaptic effects of ethanol and disinhibitory effects of ethanol on glutamate release.^{94,95} The GABA_B receptor function may modulate ethanol effects in part by affecting the ability of ethanol to stimulate GABA release.^{92,96} The GABA released by ethanol may stimulate synaptic and extrasynaptic GABA_A receptors. Thus, ethanol may indirectly modulate the function of GABA_A receptors where its direct effects are weak.

ALCOHOL DEPENDENCE ASSOCIATED WITH ADAPTATIONS IN GABA_A RECEPTORS: EFFECTS OF SUBUNIT SUBSTITUTION AND ALTERED SUBUNIT TRAFFICKING

Adaptations by GABA systems to long-term ethanol exposure contribute to ethanol tolerance, dependence, and withdrawal symptoms, including anxiety and seizures.¹ Although ethanol does not act potently on benzodiazepine-sensitive GABA_A receptors, tolerance is associated with reduced GABA synaptic function.⁹⁷ Also, ethanol dependence is associated with reduced responses to drugs acting on the GABA and benzodiazepine binding sites of GABA_A receptors.^{2,22,54,98}

The "subunit substitution hypothesis" posits that ethanol tolerance and dependence may be related to shifts in the expression levels of GABA_A receptor subunits.^{2,98-104} These studies find that ethanol dependence is associated with reduced messenger RNA levels for benzodiazepine-sensitive $\alpha_{1-3,5}$ and δ subunits and increased expression of benzodiazepine-insensitive α_4 , α_6 , and γ_2 subunits. Consistent with reductions in α_1 subunit levels, long-term intermittent ethanol exposure attenuates substantially the benzodiazepine-sensitive facilitation of synaptic and extrasynaptic GABA_A activity in hippocampal pyramidal neurons.¹⁰⁴ Consistent with the expectation that increases in γ_2 subunits and reductions in δ subunits should increase the presence of GABA_A receptor subtypes, such as $\alpha_4\beta_3\gamma_2$, that are not typical for synaptic or extrasynaptic sites,¹⁰⁵ long-term intermittent ethanol exposure increased the synaptic effects of a neurosteroid, benzodiazepine inverse agonist Ro15-4513, and ethanol on GABA_A receptor function.^{104,106} The emergence of "atypical" GABA_A receptors could impair GABAergic contributions to cortical network functions^{107,108} and could contribute to cognitive impairments in patients without neurotoxicity.^{109,110}

There are several limitations of the receptor subunit substitution hypothesis. Increases in subunit expression do not consistently match the levels of the replaced subunits, potentially altering receptor density and subunit composition.⁹⁸ Furthermore, subunit substitution could not account for all GABA_A receptor adaptations associated with long-term ethanol administration because altered GABA_A receptor responses to ethanol are observed in cell lines stably transfected with GABA_A receptor subunits.¹¹¹ Also, alcohol dependence-related changes in GABA_A receptor subunit gene expression may not be proportionately reflected in changes in the subunit composition of GABA_A receptors at the cell surface. For example, ethanol dependence may "uncouple" the benzo-

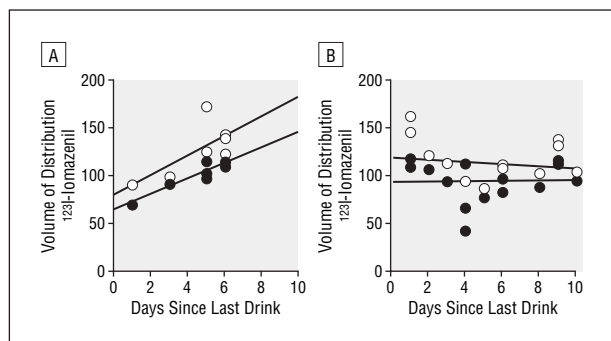


Figure 2. Relationship between the duration of sobriety and the volume of distribution of iodine 123-labeled iomazenil (^{123}I -iomazenil) in the medial frontal cortex (mFC) (open circles) and cerebellum (CB) (filled circles) in recovering alcohol-dependent nonsmoking (mFC: $r=0.72$, $P=.04$; and CB: $r=0.93$, $P=.001$) (A) and smoking (B) patients. The ^{123}I -iomazenil volume of distribution provides a measure related to the density of the benzodiazepine binding site of γ -aminobutyric acid type A receptors. Each dot represents data from an individual. Data are from Staley et al.¹⁴²

diazepine and muscimol binding sites in that there are reductions in the percentage of GABA_A receptors that contain α_2 , α_3 , and γ_2 subunits and bind benzodiazepines; no reductions in the percentage of GABA_A receptors that contain α_2 , α_3 , and γ_2 subunits and bind muscimol; and no change in the percentage of receptors that bear α_1 subunits.¹¹²⁻¹¹⁴

Mismatches between GABA_A receptor subunit gene transcription levels and receptor subunit composition may reflect alterations in receptor trafficking, that is, changes in a growing array of mechanisms that regulate the movement of receptor subunits across cellular compartments.^{115,116} For example, long-term ethanol exposure increases the internalization of α_1 subunits.¹¹⁷ This step might give rise to situations in which subunit protein levels at the cell surface might be reduced but total cellular subunit protein levels might be unchanged or even increased. Recent research also raises the possibility that long-term ethanol administration alters the distribution of GABA_A receptor subtypes across the synaptic-extrasynaptic boundary, a change that might contribute to shifts in the balance of tonic and phasic inhibitory neurotransmission.¹¹⁸ Benzodiazepine-sensitive GABA_A receptors may cluster in the synapse in part because the γ_2 subunit essential for benzodiazepine actions is also important for interactions with proteins, including gephyrin, that promote the clustering of these receptors.¹¹⁹ However, in the context of use-dependent changes in the localization of GABA_A receptor subunits, subunits normally associated with extrasynaptic receptors may diffuse into the synapse¹²⁰ or, presumably, from the synapse to perisynaptic regions.

Several studies¹²¹⁻¹²³ did not find changes in ligand binding to GABA_A receptors in ethanol-dependent animals. However, other ligand-binding studies found data consistent with changes in receptor subunit composition. For example, many brain regions showed decreases in the binding density of tritiated zolpidem, a ligand selective for GABA_A receptors containing the α_1 subunit,¹²⁴ and increases in inverse agonist ligand binding to GABA_A receptors¹²⁵ associated with repeated ethanol exposure.

Postmortem studies of animals and humans yielded different findings that may reflect distinct distributions of benzodiazepine receptors in rodents and humans.¹²⁶ Human studies reported increased messenger RNA levels of α_1 and α_3 subunits¹²⁷⁻¹²⁹ and increases in ligand binding to the muscimol or benzodiazepine sites in the frontal cortex, particularly in patients who did not show significant medical complications of alcoholism (cirrhosis or Wernicke-Korsakoff dementia),¹³⁰⁻¹³³ but reductions in benzodiazepine ligand binding were reported in postmortem studies as well.¹³⁴⁻¹³⁶ Early in vivo human neuroreceptor imaging studies comparing ethanol-dependent patients and controls using positron emission tomography and single-photon emission computed tomography also generated conflicting results. These studies identified cortical and cerebellar regions with increased variability in receptor density¹³⁷ and significant or near-significant reductions in outcomes reflecting receptor density, even when adjusting for tissue atrophy.¹³⁸⁻¹⁴¹

The GABAergic findings in humans changed across time during the first month of sobriety, and these changes were strongly affected by cigarette smoking.^{142,143} Benzodiazepine receptor density (total volume of distribution of the single-photon emission computed tomography tracer iomazenil labeled with iodine 123 [^{123}I -iomazenil]) increased across the group of nonsmoking, alcohol-dependent patients in cortical, limbic, and cerebellar regions during the first week of sobriety, reaching a level that was significantly greater than that in healthy individuals and alcohol-dependent smokers (**Figure 2**). Within individuals, receptor density decreased between the first and fourth weeks of sobriety and then returned to normal levels. Although smoking had no effect on ^{123}I -iomazenil uptake in healthy individuals, alcohol-dependent patients who smoked cigarettes did not exhibit any withdrawal-related alterations in benzodiazepine receptor density. At longer levels of sobriety (1-6 months), decreased benzodiazepine receptor density was reported in a separate sample of patients.¹⁴¹ Thus, confusion in the cross-sectional neuroimaging and postmortem literature is understandable. Depending on whether patients smoked, on whether analyses were adjusted for tissue atrophy, and on the duration of sobriety of individuals, these studies might be expected to produce increased, normal, or decreased density of ligand binding to GABA_A receptors.

A WORKING MODEL FOR HUMAN GABAergic ADAPTATIONS DURING DEPENDENCE AND WITHDRAWAL

We hypothesize that the shifting patterns in ^{123}I -iomazenil uptake across time in nonsmoking, alcohol-dependent patients are directly related to the alterations in transcription, translation, and receptor trafficking reviewed in the previous section and summarized in **Figure 3**. The preclinical studies suggest that alcohol dependence is associated with normal densities of ligand binding to GABA_A receptors, but these receptors comprise the less functional GABA_A receptor subtypes containing subunit compositions exemplified by the $\alpha_4\beta_3\gamma_2$ combination. During withdrawal, the functional abnormality in GABA_A receptors and the associated glutamatergic hyperactivity^{144,145} may evoke the

reemergence of typical synaptic and extrasynaptic forms of GABA_A receptors. Although the new receptors eventually replace the alcohol dependence–related forms of GABA_A receptors, the elevated levels of regional ¹²³I-iomazenil uptake occurring at 1 week of sobriety suggest that in many brain regions there is a transitional phase of recovery associated with normal and alcohol dependence–related distribution patterns and densities of GABA_A receptor subtypes. Between 1 week and 1 month of sobriety, the GABA_A receptor population returns to baseline conditions.

There are several limitations to these models. As a partial inverse agonist, ¹²³I-iomazenil would be expected to bind to synaptic and extrasynaptic GABA_A receptors. Thus, the attribution of shifts to binding across time to particular receptor populations is somewhat speculative. However, the proposed model is consistent with time-dependent changes in GABA_A receptor subunit gene expression across time in animals.⁹⁷ Also, it is possible that ¹²³I-iomazenil binds to receptors in multiple cellular compartments. Therefore, it is premature to assume that all of the changes reported in the clinical study reflect alterations in receptors at the cell surface.

This model is supported by proton magnetic resonance spectroscopy data¹⁴³ collected in the same patients and at similar time points as studied using single-photon emission computed tomography.¹⁴² A previous study¹⁴⁶ showed that occipital cortex GABA levels are reduced in healthy individuals by the administration of a benzodiazepine agonist. These data predict that reductions in benzodiazepine-sensitive GABA_A receptors associated with alcohol dependence would correlate with elevations in cortical GABA levels during acute withdrawal. Also, cortical GABA levels would be expected to decline with extended sobriety as benzodiazepine-sensitive GABA_A receptors reemerged. Consistent with this view,¹⁴³ occipital cortex GABA levels were normal or elevated in alcohol-dependent patients experiencing acute withdrawal, and cortical GABA levels declined during the first month of sobriety. Thus, acute alcohol withdrawal is not associated with a GABA deficit, although normal GABA levels may not compensate adequately for reductions in GABA_A receptor function related to alcohol dependence. Smoking prevented withdrawal-related changes in ¹²³I-iomazenil uptake and prevented the withdrawal-related changes in cortical GABA levels. An earlier pilot magnetic resonance spectroscopy study¹⁴⁷ that included patients with sobriety sustained longer than 1 month found reductions in occipital cortex GABA levels in alcohol-dependent patients compared with healthy individuals. These data raise the possibility that the “normal” state for recovered patients is normal GABA_A receptor density but reduced cortical GABA levels. This stable pattern of abnormality may result from the combined impact of genetic factors and persisting neurotoxicity.

GABA AND VULNERABILITY TO ALCOHOL DEPENDENCE

Familial vulnerability to alcohol dependence is associated with reduced sedative or dysphoric responses and in-

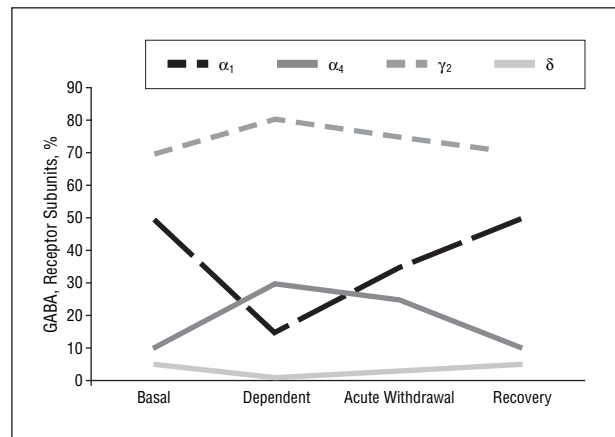


Figure 3. The general trajectory of selected γ -aminobutyric acid type A (GABA_A) receptor subunits during alcohol dependence and the initiation of sobriety. The results of several studies considered in this review are summarized. The gross estimate of the percentage of GABA_A receptors bearing a given subunit is based on the reported percentage of receptor subtypes in the brain from Whiting⁷ and on the quantitative time-dependent description of GABA_A receptor subunit gene expression levels during ethanol withdrawal from Sanna et al.⁹⁷ In the basal state, the synaptic GABA_A receptors bearing the α_{1-3} , β_{2-3} , and γ_2 subunits dominate. With the development of alcohol dependence, synaptic and extrasynaptic receptor subunit composition changes, with $\alpha_{4,6}$ subunits replacing α_{1-3} subunits and γ_2 subunits replacing δ subunits. The GABA_A receptors associated with alcohol dependence are less functional than the array associated with the basal state. In response, synaptic forms of GABA_A receptors are recruited during acute withdrawal. The recent single-photon emission computed tomography data suggest that there is a transitional period during the late phase of acute withdrawal in which receptors associated with alcohol dependence are still present but the synaptic receptors are reemerging. This transitional period is associated with increased GABA_A receptor density. As the transition to the basal array of GABA_A receptor is completed (1 week to 1 month of sobriety), the GABA_A receptor contribution to acute and protracted alcohol withdrawal is presumed to be completed. It is possible, however, that intracellular disturbances downstream of GABA_A receptors may have a more protracted time course than that presented in this figure.

creased stimulatory or euphoric responses to ethanol.¹⁴⁸⁻¹⁵² To test the hypothesis that alteration in ethanol response related to alcoholism vulnerability is conveyed by heritable differences in GABA_A receptors, several groups have studied the response to benzodiazepines¹⁵³⁻¹⁵⁶ and barbiturates^{12,157,158} in healthy individuals with and without a family history of alcohol dependence. Family history–positive individuals, compared with family history–negative individuals, show reduced depression of orbitofrontal cortex glucose metabolism,¹⁵⁹ decreased shifts in electroencephalographic power spectra,¹⁵⁵ and less disruption of smooth-pursuit eye tracking but increased euphoric responses to benzodiazepine agonists.¹⁵³⁻¹⁵⁶ However, no differences in sensitivity to benzodiazepine effects on body sway¹⁶⁰ or in diazepam self-administration were observed in family history–positive or family history–negative groups.¹¹ Genetic variation in several GABA_A receptor subunit genes may contribute to vulnerability to alcohol abuse and dependence with an interaction with environmental factors. To date, 3 published studies¹⁶¹⁻¹⁶³ link single-nucleotide polymorphisms or haplotypes in the gene coding for the α_2 subunit of the GABA_A receptor (*GABRA2*) to alcohol dependence.

Variation in *GABRA2* might contribute to alcoholism risk in several ways. The *GABRA2* variation seems to affect human responses to low doses of ethanol⁹¹ despite the

low potency of ethanol at these receptors. This impact of polymorphic variation in *GABRA2* could be explained by mutations that alter the responses of GABA_A receptors containing α_2 subunits to ethanol as well as neurosteroids or GABA released by ethanol¹⁶⁴ and alterations in the competition among GABA_A receptor subunits for inclusion in GABA_A receptors expressed on the cell surface.¹⁶⁵ The *GABRA2* variation might also convey risk for alcoholism by altering cortical and limbic function in ways that have broader functional significance. This GABA_A receptor subclass plays important regulatory roles in circuitry central to anxiety, judgment, and impulse control.¹⁶⁶⁻¹⁶⁸ Thus, the *GABRA2* contributions to alcoholism risk could reflect the self-medication of emotional distress and the expression of behavioral impulsivity. This hypothesis is supported by the following observations: (1) *GABRA2* haplotypes are associated with an alcohol dependence-related endophenotype, electroencephalographic β frequency power^{161,169}; (2) the *GABRA2* variation may be associated with increased alcoholism risk in anxious but not nonanxious alcohol-dependent patients¹⁷⁰; and (3) preliminary data suggest that the risk of early drinking is associated with an allele of a *GABRA2* single-nucleotide polymorphism in abused or neglected children but not in sociodemographically matched children who were not exposed to extreme stressors (J.H.K., B.-Z. Yang, PhD, H. Douglas-Palumberi, BA, J.K., and J.G., unpublished data, 2006).

It is also possible that variation in *GABRA2* alters the response to other components of ethanol action in the brain. For example, preliminary data suggest that *GABRA2* single-nucleotide polymorphisms associated with alcohol dependence and early drinking may alter the subjective response to an *N*-methyl-D-aspartate glutamate receptor antagonist (I.L.P., J.G., and J.H.K., unpublished data, 2006). As noted in Figure 1, ethanol blocks *N*-methyl-D-aspartate glutamate receptors at levels associated with human ethanol consumption.^{5,171}

A cluster of GABA_A receptor subunit genes located on chromosome arm 5q34, including the genes coding for the β_2 (*GABRB2*), α_6 (*GABRA6*), α_1 (*GABRA1*), and γ_2 (*GABRG2*) subunits, has also been implicated in alcohol dependence.¹⁷² The *GABRB2* is of interest for alcohol dependence in part because shifting $\alpha_4\beta\delta$ and $\alpha_6\beta\delta$ GABA_A receptor subunit composition from the β_3 to the β_2 subunit reduces the potency of ethanol 10-fold.⁵⁴ Two^{172,173} of the 3¹⁷⁴ published studies support an association between *GABRB2* alleles and alcohol dependence. The *GABRA6* single-nucleotide polymorphisms and haplotypes were associated with alcohol dependence in some^{172,173,175} but not all^{176,177} studies. The Pro385Ser [1236C→T] amino acid substitution in this gene was also associated with reduced diazepam response in healthy, family history-positive individuals.¹⁷⁸ The association of variation in *GABRA6* and alcohol dependence is consistent with the potency of ethanol at $\alpha_6\beta\delta$ receptors.^{54,61} However, the broad impact of *GABRA6* polymorphisms on alcoholism risk is surprising because this subunit is expressed only in cerebellar granule cells.^{73,74} In addition, deletion of *GABRA6* in animals does not seem to affect ethanol intoxication, ethanol tolerance, or ethanol dependence.^{73,179} As with *GABRA2*, it is possible that

the *GABRA6* markers are in linkage disequilibrium with a neighboring gene that affects alcohol response or that genetic variation in *GABRA6* affects other dimensions of circuit function that might be relevant to alcoholism. A polymorphism in *GABRG2* was associated with alcoholism in some of the populations in which this relationship was studied.^{172,176,180} Variation in this gene was also associated with altered alcohol response.¹⁸¹ Reductions in the γ_2 subunit might increase alcoholism risk by increasing anxiety¹⁸² or by increasing the relative levels of ethanol-sensitive δ subunits. The latter response might be the converse of the impact of deletions of the gene coding for δ subunits.¹⁰⁵ Deletions of the α_1 subunit increase the locomotor response to ethanol but leave most other aspects of ethanol response unchanged,¹⁸³ although tritiated zolpidem binding sites are completely lost and ligand binding for other benzodiazepine agonists are decreased by 50%.¹⁸⁴ However, there are no compelling data supporting a link between *GABRAA1* and risk of alcohol dependence.^{172,185} A recent study¹⁸⁶ implicated *GABRG3* in risk of alcohol dependence, although the role of the γ_3 subunit in ethanol action and alcoholism vulnerability is not yet clear.

COMORBIDITY OF ALCOHOL DEPENDENCE AND SMOKING

Alcohol-dependent patients may smoke to attenuate the severity of alcohol withdrawal, contributing to the comorbidity of drinking and smoking.¹⁸⁷ As noted earlier in this article, smoking prevents alcohol withdrawal-related alterations in occipital cortex GABA levels and benzodiazepine receptors.^{142,143} Alcohol-dependent patients show elevated rates of smoking,¹⁸⁸ and smoking is associated with heavier drinking.¹⁸⁹⁻¹⁹¹ People who smoke moderately report increasing their cigarette smoking during ethanol detoxification to suppress ethanol withdrawal symptoms.¹⁹² Also, heavy smokers reduce their smoking after they complete ethanol detoxification.¹⁹⁰ Nicotine withdrawal may increase alcohol craving and alcohol consumption in nicotine-dependent drinkers.^{193,194} In addition, cigarette smokers drink less after detoxification than nonsmokers as long as they continue to smoke.^{190,191}

Cigarette smoking might act in several ways to reduce the severity of alcohol dependence or withdrawal¹⁸⁷: (1) nicotine may maintain GABA neuronal function by stimulating GABA neurons and promoting GABA synthesis and release¹⁹⁵⁻¹⁹⁷; (2) nicotine may alter the levels of GABA receptor-modulating neurosteroids¹⁹⁸⁻²⁰²; and (3) other constituents of cigarette smoke modulate GABA adaptations associated with alcohol dependence, including carbon monoxide,²⁰³ harman, and norharman. The latter 2 substances inhibit monoamine oxidase type B and may act as benzodiazepine inverse agonists (harman: benzodiazepine receptor: $K_i = 1.6\mu\text{M}$; norharman: benzodiazepine receptor: $K_i = 12.4\mu\text{M}$).²⁰⁴⁻²⁰⁹

The emerging data on the interplay of smoking and alcohol dependence raise the possibility that pharmacotherapies targeting nicotine receptors might be explored as possible pharmacotherapies for alcoholism.

GABAergic PHARMACOTHERAPIES FOR ALCOHOLISM

Ethanol may act potently on extrasynaptic GABA_A receptors to facilitate the tonic component of GABA neurotransmission, but it does not seem to act directly to increase the benzodiazepine-sensitive synaptic component of phasic GABA neurotransmission. These findings suggest that it may be useful to explore whether extrasynaptic or benzodiazepine-insensitive GABA_A receptors might serve as a target for alcoholism medication development. Candidate agents to achieve this end include GABA_A receptor subtype-selective benzodiazepine partial inverse agonists and neurosteroid compounds.^{46,210-214} The development of partial inverse agonists for the benzodiazepine receptor as treatments for alcoholism may be challenging because high-activity benzodiazepine inverse agonists are anxiogenic and proconvulsant,²¹⁵⁻²¹⁷ and alcohol dependence increases the response to these drugs.²² The development of corticosteroid anesthetic agents, such as alphaxolone, ganaxolone, and the new sedative hypnotic drug gaboxadol, signal promise that new drugs that target extrasynaptic receptors may play a role in mechanistic and therapeutic alcoholism clinical research.^{104,218,219} Alternatively, signal transduction mechanisms might be targeted directly by novel medication development to influence signal transduction pathways that may affect the ethanol sensitivity of extrasynaptic GABA_A receptors, perhaps by affecting the phosphorylation state of receptor subunits.⁷⁹⁻⁸⁵

A growing number of anticonvulsant medications show efficacy for reducing alcohol withdrawal and relapse to drinking.²²⁰⁻²²⁴ Part of the attractiveness of the anticonvulsants is the possibility that one might avoid prescribing benzodiazepine agonists to alcohol-dependent patients. Benzodiazepine treatment may prolong some alcohol dependence-related alterations in GABA_A receptor subunit composition that are also seen in benzodiazepine dependence.²²⁵ Consistent with this view, one study²²³ found that patients who underwent benzodiazepine-assisted detoxification had higher levels of withdrawal symptoms and greater alcohol consumption after detoxification compared with patients treated with carbamazepine. As noted earlier in this article, alcohol dependence is associated with deficits in tonic and phasic GABAergic inhibition. The utility of benzodiazepines may also be limited by their preferential facilitation of the phasic component of GABA neurotransmission, whereas some anticonvulsants, such as the GABA transporter antagonist tiagabine, preferentially facilitate the tonic component of GABA neurotransmission.²²⁶ In addition, although shifts in GABA receptor subunit composition associated with alcohol dependence may reduce some aspects of the effectiveness of benzodiazepines, these shifts increase the efficacy of tiagabine.²²⁷ Tiagabine has yet to be proved effective in suppressing withdrawal or preventing relapse to drinking in humans.

Topiramate therapy suppressed alcohol withdrawal and reduced relapse to alcohol use in recovering alcohol-dependent patients.^{224,228} It has complex effects on GABA_A receptor subtypes. Taken at low doses (10 μmol/L), topiramate antagonized the function of synaptic GABA_A receptors (α₁β₂γ_{2S} and α₂β₂γ_{2S}). Taken at higher doses (100 μmol/L), topiramate had no effects on synaptic GABA_A receptors,

but it facilitated the function of cerebellar extrasynaptic GABA_A receptors, that is, α₆β₂γ_{2S} but not α₄β₂γ_{2S} receptors.²²⁹ In epileptic patients, topiramate therapy also increased occipital cortex GABA levels,^{230,231} raising the possibility that it indirectly facilitated GABA_A receptor function by enhancing GABA release.

IMPLICATIONS

The role of GABA systems in alcohol dependence and withdrawal is more complex than the simple notion that alcohol stimulates GABA receptors and GABA deficits contribute to withdrawal. This review highlights several mechanisms through which alcohol affects GABA_A receptors, the complex adaptations of GABA_A receptor-related mechanisms to long-term alcohol exposure, genetic variation in GABA_A receptor genes that contributes to the vulnerability to alcohol dependence, and novel targets for alcoholism pharmacotherapy that may lead to improved treatment of alcohol withdrawal and reduced relapse to heavy drinking in recovering patients.

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