Published in final edited form as:
Pharmacol Biochem Behav. 2008 July ; 90(1): 90–94. doi:10.1016/j.pbb.2008.03.006.

$GABA_A$ receptors and alcohol

Ingrid A. Lobo and **R. Adron Harris***
Waggoner Center for Alcohol and Addiction Research, Section of Neurobiology and Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712, United States

Abstract

There is substantial evidence that GABAergic neurotransmission is important for many behavioral actions of ethanol and there are reports spanning more than 30 years of literature showing that low to moderate (3–30 mM) concentrations of ethanol enhance GABAergic neurotransmission. A key question is which GABA receptor subunits are sensitive to low concentrations of ethanol in vivo and in vitro. Recent evidence points to a role for extrasynaptic receptors. Another question is which behavioral actions of alcohol result from enhancement of GABAergic neurotransmission. Some clues are beginning to emerge from studies of knock-out and knock-in mice and from genetic analysis of human alcoholics. These approaches are converging on a role for GABAergic actions in regulating alcohol consumption and, perhaps, the development of alcoholism.

Keywords

Alcohol; GABA; Receptor

1. Introduction

Despite the fact that alcohol has been used and misused for hundreds of years, the mechanism of action of this simple molecule remains the subject of study. Alcohol use results in diverse behavioral effects, including intoxication, cognitive impairment, motor incoordination, tolerance and dependence, and these effects are likely due to its actions on multiple brain proteins (Davies, 2003; Follesa et al., 2006; Harris, 1999; Krystal et al., 2006). One of the most likely targets of ethanol in the central nervous system (CNS) is the $GABA_A$ receptor, a member of the ligand-gated ion channel superfamily of receptors. Gamma-aminobutyric acid (GABA), the neurotransmitter that activates $GABA_A$ receptors, is the major inhibitory neurotransmitter in the adult CNS (Barnard et al.,1998). In an adult neuron, activation of $GABA_A$ receptors by GABA results in an influx of chloride ions, which results in hyperpolarization of the cell. Along with alcohol, $GABA_A$ receptors are a target for benzodiazepines, barbiturates, neurosteroids and volatile and intravenous anesthetics. These drugs enhance $GABA_A$ receptor function to cause anesthesia, sedation, hypnosis and anxiolysis.

There are three types of GABA receptors. $GABA_A$ and $GABA_C$ receptors are ligand-gated, while $GABA_B$ receptors are G protein-coupled receptors. The ionotropic $GABA_A$ and $GABA_C$ receptors are composed of five subunits, which surround a central chloride pore. GABA receptors subunits are heterogeneous, allowing for tremendous receptor diversity. $GABA_A$ receptor subunits include α1–6, β1–3, γ1–3, δ, ε, θ, and π, and $GABA_C$ receptors are composed of the ρ1–3 subunits. The most common CNS $GABA_A$ receptor composition is α1β2γ2s, consisting of two α1, two β2 and one γ2s subunit. Detailed overviews of $GABA_A$

* Corresponding author. E-mail address: janacormack@mail.utexas.edu (R.A. Harris).

receptor structure, diversity, post-translational processing and modifications, associated diseases, pharmacology, distribution and biophysical properties have been reviewed previously (Ashcroft, 2000; Burt and Kamatchi, 1991; Macdonald and Olsen, 1994; Olsen and Tobin, 1990; Tyndale et al., 1995; Whiting et al., 1999).

$GABA_A$ receptors were first examined as a target of alcohol action in the 1980s. Drugs that increase GABAergic function, such as uptake inhibitors and GABA agonists, enhance the behavioral actions of ethanol. Meanwhile, drugs that decrease GABAergic function, such as receptor antagonists and synthesis inhibitors, reduce ethanol behaviors. Selectively bred long-sleep and short-sleep mice that differ in genetic sensitivity to ethanol were also found to differ in their behavioral sensitivities to GABAergic drugs (Martz et al., 1983). Intoxicating concentrations (5–50 mM) of ethanol were shown to enhance the function of $GABA_A$ receptors using chloride flux assays (Allan and Harris, 1986; Suzdak et al., 1986; Ticku et al., 1986). This literature is discussed in detail in reviews (Deitrich et al., 1989; Harris, 1999; Mihic and Harris, 1995).

Electrophysiological studies have measured ethanol potentiation in primary cultures including rat dorsal root ganglion neurons (Nakahiro et al., 1991; Nishio and Narahashi, 1990) and chick embryo cerebral cortical neurons (Reynolds and Prasad, 1991). Ethanol, at concentrations ranging from 1 to 50 mM, potentiated $GABA_A$ responses of acutely dissociated neurons from rat neocortical slices and primary neuronal cultures from chick, mouse and rat brain (Reynolds et al., 1992). $GABA_A$-activated chloride currents were also shown to be potentiated by ethanol in cultured mouse hippocampal and cortical neurons (Aguayo, 1990; Reynolds and Prasad, 1991). At the single channel level, ethanol enhanced the frequency of GABA-mediated channel opening events, mean open time, open time percentage, frequency of opening bursts, and mean burst duration (Tatebayashi et al., 1998). Studies of ethanol modulation of GABAergic neurotransmission in slice recordings have been recently reviewed (Weiner and Valenzuela, 2006).

2. Alcohol binding sites

Like $GABA_A$ receptors, glycine receptor currents are enhanced by volatile anesthetics and alcohols (Mascia et al., 1996a,b). Glycine receptors are a simpler model for study, since the α1 subunit can be homomerically expressed in *Xenopus laevis* oocytes with properties similar to heteromeric receptors (Taleb and Betz, 1994). Although GABA ρ1 receptors are evolutionarily related to glycine and $GABA_A$ receptors, homomeric ρ 1 receptors are inhibited by ethanol (Mihic and Harris, 1996). Taking advantage of this difference in pharmacology and the significant sequence homology between the two receptors, chimeric receptor constructs were created that combined sections of the GABA ρ 1 and glycine receptors. These chimeras were used to identify a small region of amino acids required for enhancement of $GABA_A$ and glycine receptor function (Mihic et al., 1997). Two amino acids in the $GABA_A$ receptor transmembrane segments (TM) 2 (S270) and 3 (A291) were shown to be critical for allosteric modulation of GABA and glycine receptors by alcohols and volatile anesthetics (Mihic et al., 1997). Replacing either of these two amino acids with the aligned amino acid from the GABA ρ 1 receptor resulted in loss of ethanol potentiation (Mihic et al., 1997). This study provided critical evidence that alcohols and volatile anesthetics specifically acted upon ion channels to alter channel function. Mutation of the aligned TM2 site in the β1 subunit also diminished the ethanol potentiation of $GABA_A$ receptors (Ueno et al., 1999).

The potency of alcohols increases with carbon chain length, up to a “cut-off” point when drug potency no longer increases. The amino acid volumes at the TM2 and TM3 positions are able to affect the alcohol cutoff in glycine receptors and GABA ρ 1 receptors, which indicates that the binding pocket has a finite size (Wick et al.,1998). While substitutions of smaller amino

acids increases the cut-off point to include longer chain alcohols, substitutions of these critical residues with larger amino acids lowers the cut-off size, indicating that the binding cavity is smaller (Wick et al., 1998). Mutation of TM2 and TM3 amino acids to larger amino acids in α2 and β1 subunits resulted in constitutive $GABA_A$ receptor activity (Findlay et al., 2000; Ueno et al., 2000, 1999). Using volatile anesthetics of different shapes and molecular sizes together with mutagenesis to manipulate amino acids side chain length, the volume of the alcohol and volatile anesthetic binding site in the $GABA_A$ receptor was estimated to be between 250 and 370 $Å^3$ (Jenkins et al.,2001). Additionally, a third amino acid in TM1, L232, was shown to contribute to the boundary of the amphipathic drug binding cavity in the $GABA_A$ receptor (Jenkins et al., 2001).

The substituted cysteine accessibility method (SCAM) couples site-directed mutagenesis and biochemical probing with methanethiosulfonate (MTS) reagents in a recombinant expression system (Karlin and Akabas, 1998). Specific positions in a receptor can be mutated to cysteine and probed with MTS reagents. Reaction with a substituted cysteine occurs in the presence of water when the cysteine is ionized. MTS reagents can be used as tools to explore changes in the local environment of specific positions in receptors under different conditions. For example, the SCAM can be used to identify movements of amino acids in the presence and absence of agonist or drug molecules. In electrophysiological experiments, reaction is measured by a change in current after exposure to MTS reagents. The key question of whether S270 was important to the action of alcohols and anesthetics and part of a binding pocket in the $GABA_A$ receptor was addressed using MTS reagents. The S270C mutant was covalently labeled by either an alkane thiol anesthetic or varieties of methanethiosulfonate compounds, and receptor function was irreversibly enhanced (Mascia et al., 2000). The usual ability of octanol, enflurane and isoflurane to enhance the receptor function was lost following upon occupation of the site, indicating that the action of alcohols and anesthetics stems from binding at a single binding pocket (Mascia et al., 2000). Using the SCAM, the $GABA_A$ receptor amino acid, A291, was shown to be surrounded by a water-filled cavity, which expanded in the presence of alcohol (Jung et al., 2005), shown in Fig. 1. This position was demonstrated to be a critical site for alcohol binding and alcohol-induced conformational changes, since mutation of the site prevented alcohol-induced conformational changes from occurring (Jung and Harris, 2006).

Further discussion of the effects of alcohol action on GABA receptors composed of defined subunits can be found in the following review articles (Mihic, 1999; Yamakura et al., 2001). While the $GABA_A$ receptor α1, α2, ρ1 and β1 subunits have been the subject of study, many other subunit combinations have only recently been tested for their ethanol responses. For example, it was reported that α4β2δ $GABA_A$ receptors expressed in *Xenopus* oocytes are very sensitive to alcohol, with a concentration of 1 mM ethanol significantly enhancing GABAergic currents (Sundstrom-Poromaa et al., 2002). Both α6 and α4, when combined with β3 and δ-containing subunits were associated with ethanol enhancement of function, which showed a threshold sensitivity at 3 mM and a progressive increase up to 300 mM (Wallner et al., 2003, 2006a). The α4β2δ $GABA_A$ receptors shown to be sensitive to 1 mM ethanol by Sundstrom-Poromaa et al. (2002) were not sensitive to concentrations of ethanol below 30 mM in the Wallner et al. (2003) study. In addition, other investigators were not able to obtain effects of low (1–30 mM) concentrations of ethanol on these receptors (Borghese et al., 2006a; Yamashita et al., 2006). Because GABA receptors containing the δ subunit are found in the extrasynaptic regions and are responsible for tonic inhibition, a model has emerged with ethanol selectively enhancing tonic, rather than synaptic, GABAergic transmission. However, as noted above, this model is controversial and a detailed discussion of the issues is beyond the scope of this review, but differences in results among laboratories was addressed by eight research groups in a special issue of *Alcohol* in 2007 (Volume 41).

The studies of putative alcohol binding sites between transmembrane regions discussed above used large concentrations of ethanol (50–200 mM). A key question is whether GABA receptors have other sites of action for low concentrations (e.g., 3–20 mM) of ethanol. Recent studies proposed that ethanol competes with a benzodiazepine, RO 15-4513, for binding to GABA receptors containing the δ subunits and that this represents a novel, high affinity ethanol binding site (Wallner et al., 2006b; Hanchar et al., 2006). However, Korpi et al. (2007) z and Mehta et al (2007) were not able to confirm this finding. Thus, the mechanism for actions of low concentrations of ethanol on GABA receptors remains controversial.

$GABA_A$ receptor subunit combinations have not been exhausted, and there may be more to learn about ethanol action on the GABAergic system by testing additional subunit combinations, including theπ, ε, γ1, γ3, θ, or ρ1–3 subunits. For instance, the ρ subunits, which are expressed in the brain and spinal cord, may coassemble with other subunits (Pan and Qian, 2005); however, to date the ρ 1 subunit has only been studied when expressed homomerically. Although, the untested receptor subtypes may be rare, or at least restricted to specific brain regions, experiments on new subtypes could provide new insight into ethanol action on $GABA_A$ receptors.

3. Transgenic, knock-out and knock-in mouse models

Transgenic and knock-out (null mutant) mice have been developed and used to study how $GABA_A$ receptor subunit composition influences the pharmacological and behavioral effects of ethanol and other drugs. Thus far, mice have been developed that individually knock-out the α1, α2, α5 α6, β2, β3, γ2S+L, γ2L and δ $GABA_A$ receptor subunits. These null mutant mice have been examined to identify subunit-specific changes in the behavioral effects of alcohol and understand the role of specific proteins in drug action. Additionally, transgenic lines of mice that overexpress either the γ2L or the γ2s subunits have been developed. These actions of ethanol of $GABA_A$ receptor subunit knock-out and transgenic mice were recently reviewed (Boehm et al., 2004) and Crabbe et al. (2006) recently published an extensive review on the genetic modifications of 93 alcohol-related genes in mice, including gene overexpression, gene knock-outs and gene knock-ins. It is difficult to draw generalized conclusions from all the GABA knock-out mice because the same behaviors are not measured in all mutants, but the most pervasive finding is that knock-out of GABA receptor subunits decreases alcohol consumption in the continuous two-bottle choice test (see Crabbe et al., 2006). However, it is important to note that genetic deletion of key neuronal proteins, such as GABA receptors, may lead to compensatory changes in gene expression and brain function. The adaptive changes have been studied for the GABA α1 subunit knockout mice and found to be quite extensive (Ponomarev et al., 2006).

Knock-in mice are potentially more powerful than knock-out mice because they allow for examination of specific point mutations that alter onlya single aspectof receptor function. Meanwhile, the mutated receptor functions normally in all other aspects, and compensation effects of other receptors and subunits are less likely to occur. Since $GABA_A$ receptors have multiple subunits and subtypes, this technique is particularly useful in studying the effects of a drug on a specific receptor subunit.

As an example, the use of knock-in mice has led to exciting developments in the understanding of benzodiazepine action on $GABA_A$ receptors in the CNS. Using mutagenesis and a recombinant expression system, Wieland et al. (1992) identified a single histidine residue in the $GABA_A$ receptor α1 subunit at position 101 was critical for high affinity binding of benzodiazepines. While the α1 subunit conferred high affinity benzodiazepine binding when co-expressed with β2 and γ2 subunits, α6β2γ2 receptors showed negligible benzodiazepine binding affinity (Wieland et al., 1992). When α1(H101) was replaced with arginine, the residue

present at the aligned site in α6 subunit, the α1 (H101R) decreased the binding affinity for benzodiazepines. Stemming from this study, knock-in mice were created that mutated the histidine to arginine at position 101 in the α1 subunit, which abolished the amnestic and sedative properties of diazepam (Rudolph et al., 1999; Valenzuela et al., 1995) and partially removed the anticonvulsant properties of the drug (Rudolph et al.,1999). Meanwhile, the α1(H101R) mice had normal GABA responses and showed no change in the anxiolytic, myorelaxant, motor-impairing and ethanol-potentiating effects when compared to the wild-type (Rudolph et al., 1999). Ethanol potentiation of the $GABA_A$ receptor is therefore due to action at a different site on the subunit, or a non-mutated receptor subunit. Following these studies, the α2 subunit was shown to be responsible for the anxiolytic properties of benzodiazepines, since the anxiolytic action of diazepam was absent in α2(H101R) knock-in mice (Vicini et al., 2001). There is also evidence that the α3 subunit is sufficient to be responsible for the anxiolytic properties of benzodiazepines (Atack et al., 2005; Dias et al., 2005). The α2(H101R) knock-in mouse was also used to show that the α2 subunit is mainly responsible for the myorelaxant activity of diazepam, with the α3 subunit possibly playing a role in response to high doses of diazepam (Jurd et al., 2003). Furthermore, α5(H105R) mice failed to display any sedative tolerance to diazepam (van Rijnsoever et al., 2004). This use of mutagenesis and expression studies culminated in the design of knock-in mice and has led new understandings of pharmacology based upon specificity for $GABA_A$ receptor subunits and subtypes.

A similar approach is being used to study and understand the effects of alcohol on $GABA_A$ receptors, as well as other ligand-gated ion channels. Thus far, two viable knock-in $GABA_A$ receptor mutant mouse lines have been produced. Following identification of S270 as a critical mediator of anesthetic and alcohol action (Mihic et al.,1997), a number of other mutations in the $GABA_A$ receptor α2 subunit at S270 were shown to decreased alcohol potentiation and increase sensitivity to GABA (Findlay et al., 2001; Ueno et al.,1999). A gain-of-function $GABA_A$ receptor α1(S270H) mutant mouse line was developed, and while they were resistant to the anesthetic isoflurane, the mice displayed synaptic and behavioral abnormalities (Homanics et al., 2005).

In order to create a mouse with GABA sensitivity like the wild-type receptor, a second mutation was introduced in the α1 subunit at L277A. The α1(S270H/L277A) double mutant had near-normal GABA sensitivity when expressed in HEK cells and in *Xenopus laevis* oocytes, but showed a faster deactivation in HEK293 cells (Borghese et al., 2006b). Potentiation by isoflurane and ethanol was greatly decreased in the S270H/L277A receptors. A viable, homozygous α1(S270H/L277A) knock-in mouse line was manufactured, which showed no overt abnormalities, except hyperactivity (Borghese et al., 2006b). The mutant receptors were less sensitive to ethanol potentiation in hippocampal slice recordings (Werner et al., 2006). Mutant α1(S270H/L277A) mice showed more rapid recovery from the motor-impairing effects of ethanol and displayed increased anxiolytic effects of ethanol in comparison to wild-type mice (Werner et al., 2006). Meanwhile, there were no differences in ethanol-induced hypnosis, locomotor stimulation or cognitive impairment. Ethanol preference and consumption were comparable to the wild-type mice (Werner et al., 2006).

This study demonstrates that GABAergic synapses containing the a 1 subunit are important for specific ethanol-induced behavioral effects. Since there are multiple $GABA_A$ receptor subtypes, different subunits may account for distinct ethanol-induced behavioral effects in the same manner as for benzodiazepine action. Individual $GABA_A$ receptor subunits have not yet been definitively linked with specific behavioral actions. Coupled with the knowledge derived from knock-out studies of different $GABA_A$ receptor subunits, future studies on knock-in mice may provide additional understanding of ethanol action on these receptors.

4. Genetic linkage

A number of research groups, including the Collaborative Study on the Genetics of Alcoholism (COGA), have studied human allelic variation and have collected detailed phenotypic data on individuals in families with multiple alcoholics in order to identify genes that increase the risk for alcoholism. Several GABA receptor clusters have emerged from these studies. The COGA, and other groups, identified a region of chromosome 4p that was associated with alcoholism, which includes a cluster of four $GABA_A$ receptor subunit-encoding genes for the γ1, α2, α4 and β1 subunits (Reich, 1996; Reich et al., 1998). A second cluster of $GABA_A$ receptor genes on chromosome 5 have also been associated with alcoholism. This $GABA_A$ receptor cluster encodes genes for the β2, α6, α1, and γ2 subunits. The third $GABA_A$ receptor gene cluster on chromosome 15q is also associated with alcoholism. This cluster contains the genes for the $GABA_A$ receptor α5, β3 and γ3 subunits (Dick et al., 2004). These results are reviewed in detail in an accompanying article.

5. Conclusions

There is substantial evidence that GABAergic neurotransmission is important for many behavioral actions of ethanol, but several key questions remain. As noted above, there are reports spanning more than 30 years of literature showing that moderate (3–30 mM) concentrations of ethanol enhance GABAergic neurotransmission. However, there are also many reports showing no effect of moderate or even large concentrations of ethanol in vivo and in vitro. Thus, a key question is whether there are specific GABA receptor subunits that are sensitive to low concentrations of ethanol in vivo and in vitro. Another possibility (for which there is also evidence — Weiner and Valenzuela, 2006) is that ethanol acts presynaptically to increase the release of GABA, and certain receptors (e.g., extrasynaptic) are particularly sensitive to this spillover of GABA resulting in enhanced GABAergic function. Another key question is which behavioral actions of alcohol require enhanced GABAergic neurotransmission? Some clues are beginning to emerge from studies of knock-out and knock-in mice and, intriguingly, from genetic analysis of human alcoholics. Both of these approaches point to a role for GABAergic actions of alcohol in regulating consumption and, perhaps, the development of alcoholism. Given these results, is it feasible to target this system to treat alcoholism? Medications that block the $GABA_A$ receptor are riddled with unwanted and severe side effects, such as convulsions. In order to therapeutically target the $GABA_A$ receptor safely and effectively, it is necessary to have a better understanding of receptor function, presynaptic and postsynaptic ethanol effects, the alcohol binding site and the subunit-specific effects of alcohol.

References

Aguayo LG. Ethanol potentiates the GABAA-activated Cl^- current in mouse hippocampal and cortical neurons. Eur J Pharmacol 1990;187(1):127–30. [PubMed: 1703076]

Allan AM, Harris RA. Gamma-aminobutyric acid and alcohol actions: neurochemical studies of long sleep and short sleep mice. Life Sci 1986;39(21):2005–15. [PubMed: 3784767]

Ashcroft, FM. Ion channels and disease. San Diego: Academic Press; 2000. GABAA receptors.

Atack JR, Hutson PH, Collinson N, Marshall G, Bentley G, Moyes C, et al. Anxiogenic properties of an inverse agonist selective for alpha3 subunit-containing GABA A receptors. Br J Pharmacol 2005;144 (3):357–66. [PubMed: 15655523]

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, et al. International Union of Pharmacology. XV Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. Pharmacol Rev 1998;50(2):291–313. [PubMed: 9647870]

Boehm SL II, Ponomarev I, Jennings AW, Whiting PJ, Rosahl TW, Garrett EM, et al. gamma-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions. Biochem Pharmacol 2004;68(8):1581–602. [PubMed: 15451402]

Borghese CM, Storustovu S, Ebert B, Herd MB, Belelli D, Lambert JJ, et al. The delta subunit of gamma-aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. J Pharmacol Exp Ther 2006a;316(3):1360–8. [PubMed: 16272217]

Borghese CM, Werner DF, Topf N, Baron NV, Henderson LA, Boehm SL II, et al. An isoflurane- and alcohol-insensitive mutant GABA(A) receptor alpha(1) subunit with near-normal apparent affinity for GABA: characterization in heterologous systems and production of knockin mice. J Pharmacol Exp Ther 2006b;319(1):208–18. [PubMed: 16807363]

Burt DR, Kamatchi GL. GABAA receptor subtypes: from pharmacology to molecular biology. Faseb J 1991;5(14):2916–23. [PubMed: 1661244]

Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF. Alcohol-related genes: contributions from studies with genetically engineered mice. Addict Biol 2006;11(3–4):195–269. [PubMed: 16961758]

Davies M. The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. J Psychiatry Neurosci 2003;28(4):263–74. [PubMed: 12921221]

Deitrich RA, Dunwiddie TV, Harris RA, Erwin VG. Mechanism of action of ethanol: initial central nervous system actions. Pharmacol Rev 1989;41(4):489–537. [PubMed: 2700603]

Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ, et al. Evidence for a significant role of alpha 3-containing GABAA receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci 2005;25(46):10682–8. [PubMed: 16291941]

Dick DM, Edenberg HJ, Xuei X, Goate A, Kuperman S, Schuckit M, et al. Association of GABRG3 with alcohol dependence. Alcohol Clin Exp Res 2004;28(1):4–9. [PubMed: 14745296]

Findlay GS, Ueno S, Harrison NL, Harris RA. Allosteric modulation in spontaneously active mutant gamma-aminobutyric acid(A) receptors [corrected]. Neurosci Lett 2000;293(3):155–8. [PubMed: 11036184]

Findlay GS, Ueno S, Harrison NL, Harris RA. Allosteric modulation in spontaneously active mutant gamma-aminobutyric acidA receptors. Neurosci Lett 2001;305(1):77–80. [PubMed: 11356312]

Follesa P, Biggio F, Talani G, Murru L, Serra M, Sanna E, et al. Neurosteroids, GABAA receptors, and ethanol dependence. Psychopharmacology (Berl) 2006;186(3):267–80. [PubMed: 16163526]

Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M, et al. Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to alpha4/6beta3delta GABAA receptors. Proc Natl Acad Sci U S A 2006;103(22):8546–51. [PubMed: 16581914]

Harris RA. Ethanol actions on multiple ion channels: which are important? Alcohol Clin Exp Res 1999;23 (10):1563–70. [PubMed: 10549986]

Homanics GE, Elsen FP, Ying SW, Jenkins A, Ferguson C, Sloat B, et al. A gain-of-function mutation in the GABA receptor produces synaptic and behavioral abnormalities in the mouse. Genes Brain Behav 2005;4(1):10–9. [PubMed: 15660664]

Jenkins A, Greenblatt EP, Faulkner HJ, Bertaccini E, Light A, Lin A, et al. Evidence for a common binding cavity for three general anesthetics within the GABAA receptor. J Neurosci 2001;21 (6):RC136. [PubMed: 11245705]

Jung, S. Conformational changes in the GABAA receptor during channel gating and alcohol modulation. University of Texas at Austin; Austin, TX: University Microfilms International; Austin: 2005.

Jung S, Harris RA. Sites in TM2 and 3 are critical for alcohol-induced conformational changes in GABA receptors. J Neurochem 2006;96(3):885–92. [PubMed: 16405501]

Jung S, Akabas MH, Harris RA. Functional and structural analysis of the GABAA receptor alpha 1 subunit during channel gating and alcohol modulation. J Biol Chem 2005;280(1):308–16. [PubMed: 15522868]

Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, et al. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. Faseb J 2003;17(2): 250–2. [PubMed: 12475885]

Karlin A, Akabas MH. Substituted-cysteine accessibility method. Methods Enzymol 1998;293:123–45. [PubMed: 9711606]

Korpi ER, Debus F, Linden AM, Malecot C, Leppa E, Vekovischeva O, et al. Does ethanol act preferentially via selected brain GABAA receptor subtypes? The current evidence is ambiguous. Alcohol 2007;41(3):163–76. [PubMed: 17591542]

Krystal JH, Staley J, Mason G, Petrakis IL, Kaufman J, Harris RA, et al. Gamma-aminobutyric acid type A receptors and alcoholism: intoxication, dependence, vulnerability, and treatment. Arch Gen Psychiatry 2006;63(9):957–68. [PubMed: 16952998]

Macdonald RL, Olsen RW. GABAA receptor channels. Annu Rev Neurosci 1994;17:569–602. [PubMed: 7516126]

Martz A, Deitrich RA, Harris RA. Behavioral evidence for the involvement of gamma-aminobutyric acid in the actions of ethanol. Eur J Pharmacol 1983;89(1–2):53–62. [PubMed: 6861890]

Mascia MP, Machu TK, Harris RA. Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics. Br J Pharmacol 1996a;119(7):1331–6. [PubMed: 8968539]

Mascia MP, Mihic SJ, Valenzuela CF, Schofield PR, Harris RA. A single amino acid determines differences in ethanol actions on strychnine-sensitive glycine receptors. Mol Pharmacol 1996b;50 (2):402–6. [PubMed: 8700149]

Mascia MP, Trudell JR, Harris RA. Specific binding sites for alcohols and anesthetics on ligand-gated ion channels. Proc Natl Acad Sci U S A 2000;97(16):9305–10. [PubMed: 10908659]

Mehta AK, Marutha Ravindran CR, Ticku MK. Low concentrations of ethanol do not affect radioligand binding to the delta-subunit-containing GABAA receptors in the rat brain. Brain Res 2007;1165:15–20. [PubMed: 17662260]

Mihic SJ. Acute effects of ethanol on GABAA and glycine receptor function. Neurochem Int 1999;35 (2):115–23. [PubMed: 10405995]

Mihic, JS.; Harris, RA. Alcohol actions at the GABAA receptor/choloride complex. In: Deitrich, R.; Erwin, VG., editors. Pharmacological effects of ethanol on the nervous system. Boca Raton: CRC Press, Inc; 1995. p. 51-72.

Mihic SJ, Harris RA. Inhibition of rho1 receptor GABAergic currents by alcohols and volatile anesthetics. J Pharmacol Exp Ther 1996;277(1):411–6. [PubMed: 8613949]

Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, et al. Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. Nature 1997;389(6649):385–9. [PubMed: 9311780]

Nakahiro M, Arakawa O, Narahashi T. Modulation of gamma-aminobutyric acid receptor-channel complex by alcohols. J Pharmacol Exp Ther 1991;259(1):235–40. [PubMed: 1656020]

Nishio M, Narahashi T. Ethanol enhancement of GABA-activated chloride current in rat dorsal root ganglion neurons. Brain Res 1990;518(1–2):283–6. [PubMed: 1697210]

Olsen RW, Tobin AJ. Molecular biology of GABAA receptors. Faseb J 1990;4(5):1469–80. [PubMed: 2155149]

Pan Y, Qian H. Interactions between rho and gamma2 subunits of the GABA receptor. J Neurochem 2005;94(2):482–90. [PubMed: 15998298]

Ponomarev I, Maiya R, Harnett MT, Schafer GL, Ryabinin AE, Blednov YA, et al. Transcriptional signatures of cellular plasticity in mice lacking the alpha1 subunit of GABAA receptors. J Neurosci 2006;26(21):5673–83. [PubMed: 16723524]

Reich T. A genomic survey of alcohol dependence and related phenotypes: results from the Collaborative Study on the Genetics of Alcoholism (COGA). Alcohol Clin Exp Res 1996;20(8 Suppl):133A–7A.

Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, et al. Genome-wide search for genes affecting the risk for alcohol dependence. Am J Med Genet 1998;81(3):207–15. [PubMed: 9603606]

Reynolds JN, Prasad A. Ethanol enhances GABAA receptor-activated chloride currents in chick cerebral cortical neurons. Brain Res 1991;564(1):138–42. [PubMed: 1723337]

Reynolds JN, Prasad A, MacDonald JF. Ethanol modulation of GABA receptor-activated Cl^- currents in neurons of the chick, rat and mouse central nervous system. Eur J Pharmacol 1992;224(2–3):173–81. [PubMed: 1281777]

Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, et al. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 1999;401(6755):796–800. [PubMed: 10548105]

Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, et al. Hormonally regulated alpha(4)beta(2)delta GABA(A) receptors are a target for alcohol. Nat Neurosci 2002;5(8):721–2. [PubMed: 12118257]

Suzdak PD, Schwartz RD, Skolnick P, Paul SM. Ethanol stimulates gamma-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. Proc Natl Acad Sci U S A 1986;83(11): 4071–5. [PubMed: 2424017]

Taleb O, Betz H. Expression of the human glycine receptor alpha 1 subunit in *Xenopus* oocytes: apparent affinities of agonists increase at high receptor density. EMBO J 1994;13(6):1318–24. [PubMed: 8137816]

Tatebayashi H, Motomura H, Narahashi T. Alcohol modulation of single GABA(A) receptor-channel kinetics. Neuroreport 1998;9(8):1769–75. [PubMed: 9665598]

Ticku MK, Lowrimore P, Lehoullier P. Ethanol enhances GABA-induced $36Cl^-$ influx in primary spinal cord cultured neurons. Brain Res Bull 1986;17(1):123–6. [PubMed: 3756540]

Tyndale, RF.; Olsen, RW.; Tobin, AJ. Handbook of receptors and channels. CRC Press, Inc; 1995. GABAA receptors; p. 265-89.

Ueno S, Wick MJ, Ye Q, Harrison NL, Harris RA. Subunit mutations affect ethanol actions on GABA (A) receptors expressed in *Xenopus* oocytes. Br J Pharmacol 1999;127(2):377–82. [PubMed: 10385236]

Ueno S, Lin A, Nikolaeva N, Trudell JR, Mihic SJ, Harris RA, et al. Tryptophan scanning mutagenesis in TM2 of the GABA(A) receptor alpha subunit: effects on channel gating and regulation by ethanol. Br J Pharmacol 2000;131(2):296–302. [PubMed: 10991923]

Valenzuela CF, Machu TK, McKernan RM, Whiting P, VanRenterghem BB, McManaman JL, et al. Tyrosine kinase phosphorylation of GABAA receptors. Brain Res Mol Brain Res 1995;31(1–2):165–72. [PubMed: 7476025]

van Rijnsoever C, Tauber M, Choulli MK, Keist R, Rudolph U, Mohler H, et al. Requirement of alpha5-GABAA receptors for the development of tolerance to the sedative action of diazepam in mice. J Neurosci 2004;24(30):6785–90. [PubMed: 15282283]

Vicini S, Ferguson C, Prybylowski K, Kralic J, Morrow AL, Homanics GE. GABA(A) receptor alpha1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. J Neurosci 2001;21(9):3009–16. [PubMed: 11312285]

Wallner M, Hanchar HJ, Olsen RW. Ethanol enhances alpha 4 beta 3 delta and alpha 6 beta 3 delta gamma-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc Natl Acad Sci U S A 2003;100(25):15218–23. [PubMed: 14625373]

Wallner M, Hanchar HJ, Olsen RW. Low dose acute alcohol effects on GABA A receptor subtypes. Pharmacol Ther 2006a;112(2):513–28. [PubMed: 16814864]

Wallner M, Hanchar HJ, Olsen RW. Low dose alcohol actions on alpha4beta3delta GABAA receptors are reversed by the behavioral alcohol antagonist Ro15-4513. Proc Natl Acad Sci U S A 2006b;103 (22):8540–5. [PubMed: 16698930]

Weiner JL, Valenzuela CF. Ethanol modulation of GABAergic transmission: the view from the slice. Pharmacol Ther 2006;111(3):533–54. [PubMed: 16427127]

Werner DF, Blednov YA, Ariwodola OJ, Silberman Y, Logan E, Berry RB, et al. Knockin mice with ethanol-insensitive alpha1-containing gamma-aminobutyric acid type A receptors display selective alterations in behavioral responses to ethanol. J Pharmacol Exp Ther 2006;319(1):219–27. [PubMed: 16785315]

Whiting PJ, Bonnert TP, McKernan RM, Farrar S, Le Bourdelles B, Heavens RP, et al. Molecular and functional diversity of the expanding GABA-A receptor gene family. Ann N Y Acad Sci 1999;868:645–53. [PubMed: 10414349]

Wick MJ, Mihic SJ, Ueno S, Mascia MP, Trudell JR, Brozowski SJ, et al. Mutations of gamma-aminobutyric acid and glycine receptors change alcohol cutoff: evidence for an alcohol receptor? Proc Natl Acad Sci U S A 1998;95(11):6504–9. [PubMed: 9600996]

Wieland HA, Luddens H, Seeburg PH. A single histidine in GABAA receptors is essential for benzodiazepine agonist binding. J Biol Chem 1992;267(3):1426–9. [PubMed: 1346133]

Yamakura T, Bertaccini E, Trudell JR, Harris RA. Anesthetics and ion channels: molecular models and sites of action. Annu Rev Pharmacol Toxicol 2001;41:23–51. [PubMed: 11264449]

Yamashita M, Marszalec W, Yeh JZ, Narahashi T. Effects of ethanol on tonic GABA currents in cerebellar granule cells and mammalian cells recombinantly expressing GABA(A) receptors. J Pharmacol Exp Ther 2006;319(1):431–8. [PubMed: 16844844]

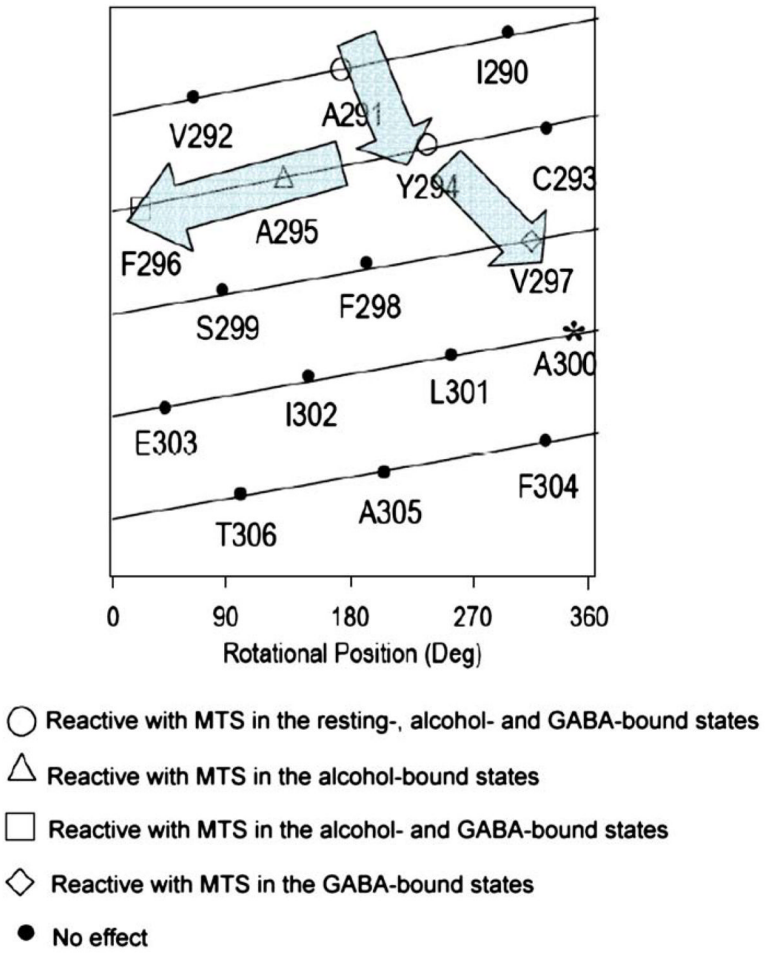

Fig. 1.

A helical net representation illustrating the change in GABA-induced currents before and after treatment of MTS reagents on TM3 single Cys substitution mutants in the absence and presence of GABA or alcohols. I290 is positioned at the extracellular end, and T306 is the most intracellular cysteine mutant that was tested in TM3. Each symbol indicates MTS reactivity for the different functional states. The arrows illustrate the expansion of the water-filled cavity. This figure was previously published (Jung, 2005).