Review Paper
Examen critique

The role of $GABA_A$ receptors in mediating the effects of alcohol in the central nervous system

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Ethanol is a chemically simple compound that produces many well-known effects in humans. The prevailing idea for many years was that ethanol and other alcohols exerted their effects on the central nervous system (CNS) by non-selectively disrupting the lipid bilayers of neurons. However, in recent years, there has been an accumulation of evidence pointing to the importance of ligand-gated ion channels (LGICs) in mediating the effects of ethanol. Of these LGICs, γ-aminobutyric acid type A ($GABA_A$) receptors appear to occupy a central role in mediating the effects of ethanol in the CNS. GABA is the primary inhibitory neurotransmitter in the mammalian CNS, and activation of $GABA_A$ receptors by GABA tends to decrease neuronal excitability. This article reviews several aspects of $GABA_A$ receptor and ethanol interactions, including the evidence for short- and long-term modulation of $GABA_A$ receptors by ethanol and evidence for a $GABA_A$ receptor-related genetic component of alcoholism.

L'éthanol est un composé chimiquement simple qui produit de nombreux effets biens connus chez les humains. Le concept selon lequel l'éthanol et les autres alcools exercent leurs effets sur le système nerveux central (SNC) par une perturbation non sélective de la bicouche lipidique des neurones a prévalu pendant des années. En revanche, ces dernières années, une masse de données probantes ont souligné l'importance des canaux ioniques dont l'ouverture est contrôlée par un ligand (LGIC) dans la médiation des effets de l'éthanol. Au nombre des LGIC, les récepteurs de l'acide gamma-aminobutyrique de type A ($GABA_A$) semblent jouer un rôle pivot dans la médiation des effets de l'éthanol sur le SNC. Le GABA est le principal neurotransmetteur inhibiteur dans le SNC des mammifères et l'activation par le GABA des récepteurs $GABA_A$ a tendance à diminuer l'excitabilité neuronale. Le présent article examine divers aspects des interactions entre les récepteurs $GABA_A$ et l'éthanol, y compris les données probantes sur la modulation à court et à long terme des récepteurs $GABA_A$ par l'éthanol et les données probantes sur une dimension génétique de l'alcoolisme se rattachant aux récepteurs $GABA_A$.

Introduction

Alcohol is the most frequently abused drug in our society. The abuse of this substance has a societal impact not only in terms of the general health of the population, but also in economic output, where it is estimated that loss of productivity costs many billions of dollars each year.[1] Alcohol abuse has many long-term effects

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that result in premature death and an increased propensity for serious illness.[2] Additionally, fetal alcohol syndrome is a major health issue worldwide[3–7] and often leads to health problems throughout life.[8,9] Another alcohol-related health issue is ethanol–drug interactions which, in some cases, can be fatal.[10–12]

Alcohol, or more specifically, ethanol, produces several effects in humans. It is a central nervous system (CNS) depressant and shares many of the effects of other CNS depressants, such as sedatives, hypnotics and anesthetic agents. Although it has dramatic effects on the CNS, ethanol is not a particularly potent drug. For example, threshold effects generally do not occur until the concentration of ethanol in the blood is relatively high (5–10 mmol/L).[13] However, a single serving of a strong spirit can contain as much as 12 g of ethanol, and thus it is possible to consume large amounts of ethanol in a single sitting and quickly reach these blood concentrations.

The effects of ethanol in humans are well documented.[13] At low blood concentrations, there is a feeling of euphoria or disinhibition. As the concentration of ethanol in the blood increases, motor function is impaired and speech becomes slurred. With blood alcohol concentrations between 200 mg/dL and 300 mg/dL (i.e., between 43 mmol/L and 65 mmol/L), vomiting can occur and the subject can fall into a stupor. Blood concentrations higher than this can result in coma, and at high blood concentrations (500 mg/dL or 109 mmol/L), there is the potential for respiratory failure and death.[14]

For many years, it was believed that these effects were mediated through the non-specific disruption of neuronal lipid bilayers by ethanol. However, it is now generally accepted that ethanol acts by binding with and altering the function of specific proteins, particularly membrane-bound ligand-gated ion channels and voltage-dependent ion channels.[15–18] Additionally, there is mounting evidence that ethanol can alter the function of second-messenger proteins.[19,20]

Membrane-bound proteins and ethanol

The 2 major types of membrane-bound proteins that are directly affected by pharmacologically relevant concentrations of ethanol (i.e., concentrations up to 100 mmol/L or 460 mg/dL, at which point ethanol can be lethal in humans) are ligand-gated ion channels (LGICs) and voltage-dependent calcium channels.[21] LGICs are a family of neurotransmitter receptors that are widely distributed in the mammalian CNS and play a major role in synaptic transmission and the regulation of neuronal excitability. In particular, the gamma-aminobutyric acid type A ($GABA_A$), *N*-methyl-D-aspartate (NMDA), glycine,[22] neuronal nicotinic[23] and 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) receptors[24] are LGICs that have been shown to be directly modulated by ethanol. Ethanol does not modulate these receptors in a non-specific fashion; interestingly, it potentiates ligand-gated currents at some receptors but inhibits them at others. For example, it is well documented that acute ethanol exposure potentiates these currents at $GABA_A$ and glycine receptors[25] but inhibits them at NMDA receptors.[26] Voltage-gated calcium channels play key roles in neurotransmitter release, hormone secretion, gene regulation and differentiation. Ethanol, when administered acutely, has been shown to block voltage-gated calcium channels at pharmacologically relevant concentrations.[27,28]

Because of ethanol's capacity to change neuronal excitability by interacting with the proteins mentioned above, it is not surprising that after long-term exposure to ethanol, neurons adapt to its presence by altering the expression of their complement of these receptors and ion channels.

Current therapies to treat alcohol addiction

Current therapeutics for alcohol-related health problems include the drugs disulfiram, naltrexone and acamprosate.[29,30]

Disulfiram inhibits aldehyde dehydrogenase, one of the enzymes involved in alcohol metabolism, which converts acetaldehyde to acetic acid.[31,32] Administration of disulfiram in the absence of alcohol has little or no effect. However, if alcohol is consumed, there is a build up of acetaldehyde, and this results in several unpleasant effects such as tachycardia, nausea, vomiting and hyperventilation, often accompanied by feelings of anxiety or panic.[33] Compliance taking this drug is often low.

Evidence for a link between opioid receptors and alcohol has been shown, and it has been postulated that opioid receptors are involved in the reinforcing effects of chronic ethanol consumption.[34] With this idea in mind, naltrexone, an opioid receptor antagonist, was tested for its effects on ethanol consumption and proved to be effective in decreasing it.[35,36] Naltrexone has been described as an anti-craving medication

because clinical trials have shown that it helps relieve the urge that alcoholics have to consume ethanol.

Acamprosate is a relative newcomer as a therapy for alcohol abuse and is best used to maintain abstinence in patients who have stopped drinking.[37] Acamprosate (*N*-acetylhomotaurine) has a structure similar to GABA and has been shown to interact with presynaptic $GABA_B$ receptors, increasing the release of GABA from presynaptic terminals.[38] Additionally, it appears to inhibit calcium ion influx through voltage-dependent calcium channels and NMDA receptors.[39] The $5\text{-}HT_3$ receptor antagonist, ondansetron, has shown some efficacy in the treatment of early-onset alcoholism. This group of patients is considered to have a biological predisposition to alcohol abuse. It has been postulated that ondansetron acts by reducing the craving for alcohol.[40]

Benzodiazepines are often used to treat some of the symptoms of alcohol abuse, especially during withdrawal, but have yet to be shown as effective drugs in treating addiction itself.[41,42]

Ethanol and $GABA_A$ receptors

As described above, ethanol interacts with and modifies the function of a number of membrane-bound proteins. However, it is likely that some play more important roles than others in mediating the effects of ethanol in the CNS.[15] There is a large body of evidence showing that $GABA_A$ receptors play central roles in both the short- and long-term effects of ethanol in the CNS.[43] $GABA_A$ receptors belong to a family of transmembrane ligand-gated ion channels that includes the nicotinic acetylcholine, glycine and $5\text{-}HT_3$ receptors.[44] These receptors are responsible for rapid neuronal transmission in the mammalian CNS. $GABA_A$ receptors primarily occur in the postsynaptic membrane, although there is evidence that certain subtypes may occur extra-synaptically.[45] These receptors are the site of action of a number of drugs, including barbiturates, benzodiazepines and anesthetics. The $GABA_A$ receptors are pentameric receptors with a high degree of homology with nicotinic receptors.[46,47] $GABA_A$ receptors have a multitude of subunits, including 6α, 4β, 3γ, 2ρ, δ, ε, θ and π.[48] They have a rich pharmacology, and this is dependent upon the particular subunits that are present within the receptor pentamer.[49,50] Each subunit has an extracellular N-terminal domain that typically contains ligand-recognition sites and 4 membrane-spanning domains.[51] The second membrane-spanning domain forms the lining of the ion channel. The subunits are arranged in a radial fashion such that they surround a central ion pore that opens in the presence of ligand. Once the ion channel opens, ion transport follows the electrochemical gradient that is established across the neuronal membrane. In the case of $GABA_A$ receptors, the ion pore conducts chloride ions. GABA is classified as an inhibitory amino acid neurotransmitter because the influx of chloride ions into the postsynaptic cell after the activation of these receptors moves the postsynaptic membrane potential further away from its firing threshold. The discrete distribution of receptor subtypes suggests that each has a specific function within the CNS.[52,53] In mammalian tissue, the most common receptor subtype contains $\alpha1$, $\beta2$ and $\gamma2$ subunits.

Within each subunit is a large N-terminal domain, which includes clusters or "loops" of amino acids thought to form the neurotransmitter binding domain(s),[54,55] 4 transmembrane domains, of which the second (TM2) is thought to line the ion channel, and a large intracellular loop that contains consensus sites for phosphorylation by various kinases.[47,56] Interestingly, these receptors not only bind their neurotransmitter ligands, but can also interact with a number of compounds that bind at distant sites on the protein and allosterically modulate the actions of the neurotransmitter.[57,58] These modulatory agents include benzodiazepines, barbiturates, neurosteroids and anesthetics. With some exceptions, these modulators are not able to activate the receptors unless GABA is present and bound to the receptor. Indeed, the capacity of some of these compounds to modulate LGICs, and thus neuronal function, has been exploited in the development of therapeutic agents. Typically, those modulators that enhance the actions of GABA at the receptor have sedative or hypnotic effects. Benzodiazepines, one of the most commonly prescribed groups of psychoactive drugs, are positive allosteric modulators of $GABA_A$ receptors.[59] Clinically useful benzodiazepines and benzodiazepine-like compounds act by potentiating the effects of GABA at the receptor. This is manifested as an increased probability of channel opening, thus leading to a greater hyperpolarization of the postsynaptic membrane and a further decrease in neuronal excitability than would be seen with GABA activation alone. Benzodiazepines are used to treat clinical symptoms such as anxiety, convulsions, muscle tension and insomnia. Not all ligands for this site are positive

allosteric modulators, however. In fact, ligands for this particular binding site show a spectrum of efficacies.[60]

Apart from positive allosteric modulators, there are those that act as negative allosteric modulators by decreasing the probability of channel opening events in the presence of GABA. In animal models, these compounds lead to anxiety, seizures or both; other compounds have no intrinsic efficacy at this site and are therefore classified as antagonists.[44]

When $GABA_A$ receptors are acutely exposed to ethanol, there is a potentiation of GABA-gated current.[61] Some of the first experiments showing that the ionic flux generating this current is increased by ethanol were done using native receptors in rat brain microsacs and synaptoneurosome preparations. Exposure of these preparations to ethanol increased GABA-gated chloride uptake by as much as 260%.[62] Subsequently, the effects of ethanol on recombinant receptors expressed in *Xenopus* oocytes were examined. These effects generally occur in a pharmacologically relevant range of concentrations (below 100 mmol/L) and potentiate the actions of GABA at $GABA_A$ receptors. However, this issue is somewhat contentious; some laboratories report seeing no effect of alcohols at $GABA_A$ receptors, and the range of concentrations at which effects are seen also seems to vary.[63–65] A study using $\alpha1\beta2\gamma2$ receptors showed that ethanol potentiation occurred, but only in those receptors in which a particular splice variant of the $\gamma2$ subunit was present.[66] The "long" version of $\gamma2$ ($\gamma2L$), so-called because of the presence of an extra 8 amino acids in the intracellular loop, was deemed necessary for potentiation to occur. However, other laboratories have been unable to duplicate this finding and have shown that the effects of ethanol appear to be independent of the type of $\gamma2$ subunit that is contained within the oligomer.[67] So far, there appears to be no subunit dependence with respect to the effects of ethanol on $GABA_A$ receptors,[68] with the exception of the $\alpha4\beta1\delta$ subtype, which seems to be more sensitive to ethanol than other receptor subtypes tested to date.[69]

Originally, it was believed that alcohols and anesthetics acted by non-selectively disturbing the lipid bilayers of neurons, thus exerting a global disruption in neuronal membrane protein activity. This idea was based on evidence showing that the lipid solubility of anesthetic agents and their potency was highly correlated.[70–72] One aspect of anesthesia not easily explained by the lipid hypothesis was the cut-off effect seen with many series of anesthetic agents, in which the carbon length of the anesthetic agent incrementally increased.[73] Typically, the potencies of these agents increase until a certain backbone length is reached, at which point the anesthetic no longer shows any effect. It was originally proposed that this phenomenon was due to anesthetics over a particular size or carbon length having poor solubility in lipid bilayers.[71,73]

A turning point in the idea of alcohol and anesthetic targets was provided by experiments showing that the activity of a soluble enzyme, luciferase, could be modified by alcohols.[74] Further, this modification showed distinct "cut-offs" where, after a certain chain length was reached, the inhibitory effects were lost or did not increase further.[74] (See below.) As this protein was soluble and not associated with lipid, the best explanation for this phenomenon was that the protein contained a binding pocket of fixed dimensions that could only accommodate alcohols of a certain size. Additionally, it has been shown that long-chain anesthetics are able to partition into membranes long after they have lost their anesthetic effect,[75] thus negating the idea that the lipid cut-off was due to a partitioning effect. Other evidence for the protein theory comes from experiments showing that optical isomers of anesthetic agents, while having the same bilayer disrupting properties, nonetheless show very different effects on LGICs.[76,77] Additionally, agents that can disrupt the lipid bilayer do not necessarily induce anesthesia.[78]

With respect to $GABA_A$ receptors, a number of alcohols with different carbon numbers in their backbone (indicated in parenthesis), including methanol (3), butanol (4), hexanol (6), octanol (8), decanol (10) and dodecanol (12), potentiate GABA-gated current. The potencies of these alcohols tend to increase with chain length. However, once the length of the backbone exceeds 12 carbons, there is no longer an effect on GABA-gated current. Interestingly, dodecanol is the longest n-alcohol that is able to bring about a loss of the righting reflex in tadpoles.[79] This lack of effect with n-alcohols longer than dodecanol has been taken to show that there is a binding pocket with a defined size that is able to accommodate alcohols with carbon lengths up to 12, with those exceeding 12 being excluded on the basis of size.[80] Similar molecular cut-offs have been found for neuronal nicotinic, NMDA[81] and AMPA receptors,[82] although other studies suggest that this cut-off effect may be due to poor aqueous solubility.[83]

A study of the single-channel properties of $GABA_A$

receptors revealed that ethanol-induced potentiation of GABA-gated currents is due to an increase in the frequency and duration of channel opening and an increase in channel bursts and burst duration.[84] Additionally, the amount of time that the channel spends in the closed state is decreased. The summation of these effects leads to increased ion flux through the open channel in the presence of GABA and ethanol.[84]

Evidence for a putative ethanol binding site on GABA$_A$ receptors was provided by a study from Mihic et al.[85] Ethanol potentiates glycine-gated chloride current through glycine receptors composed of glycine α subunits, but it inhibits GABA-gated current through receptors composed entirely of the ρ subunit. By creating a series of chimeras between these 2 subunits, Ye and colleagues[86] identified regions of the subunits involved in mediating this differential ethanol response. They identified a region encompassing part of the TM2 and TM3 regions and the extracellular loop between as being important for the effects of ethanol. However, there was some question about whether this site was part of a transduction mechanism brought about by ethanol rather than the ethanol-binding site itself. Subsequent mutagenesis studies of this region identified an amino acid in TM2 as being an important component of ethanol sensitivity. It was shown that replacement of the serine residue in position 267 of the glycine receptor α1 subunit by those occupying a smaller volume can increase the alcohol cut-off effect described above, supporting the idea that this region does constitute a binding site for alcohol.[86] Conversely, replacing the residue at position 267 with amino acids of a larger size decreased the cut-off size of alcohols. Further manipulation of this region resulted in receptors at which glycine-gated currents were inhibited, rather than potentiated, by ethanol.

GABA$_A$ receptors in self-administration

There is strong evidence that GABAergic systems are involved in mediating self-administration of ethanol in animal models, likely by stimulating reward circuitry in the mesolimbic system.[87] For the most part, there is a correlation between compounds that activate or potentiate GABAergic systems and increased ethanol intake,[88,89] while the reverse is true for compounds that block or inhibit GABAergic systems.[90–92] Benzodiazepines, which display a spectrum of efficacies at GABA$_A$ receptors, likewise can increase or decrease self-administration depending on the efficacy of the particular drug being studied.[43] However, some studies have not seen this effect with benzodiazepine agents.[93,94] It is believed that the mesolimbic dopamine system is intimately involved in this behaviour and that GABA$_A$ receptors play a role in mediating dopamine levels in this region.[43] For example, infusion of muscimol directly into the ventral tegmental area (VTA), a component of the mesolimbic system, results in a dose-dependent increase in dopamine levels.[95] Also, long-term ethanol exposure decreases GABA$_A$ receptor α1 subunits in the VTA,[96] which would in turn result in an increase in dopamine release.[34] An inverse agonist selective for α5-containing receptors when infused into rat hippocampus was effective in reducing ethanol self-administration.[97] α5-Containing receptors are enriched in the CA1 and CA3 fields, both of which innervate the nucleus accumbens and amygdala, components of the mesolimbic system.

Long-term effects of ethanol on GABA$_A$ receptors

Long-term exposure to ethanol affects the baseline of neuronal excitability.[98,99] Animals chronically treated with ethanol and then withdrawn are typically more prone to seizure activity than naive controls. Several studies have shown that GABA$_A$ receptor subunit mRNA and protein levels change during repeated ethanol exposure. At the functional level, examination of receptors in brain preparations derived from chronically treated rats shows that ethanol no longer potentiates GABA-gated chloride flux to as great an extent as is seen in non-treated rats. Additionally, the response to allosteric modulators such as benzodiazepine-site ligands is altered.[100] Positive allosteric modulators such as flunitrazepam appear to be less effective in potentiating the GABA response, whereas negative allosteric modulators such as Ro 15-4513 appear to have an enhanced effect.[101] Additionally, the response to neurosteroids, a group of endogenous allosteric modulators, is altered.[102]

Some of the more consistent findings in studies of repeated alcohol exposure and changes in GABA$_A$ receptor subunit mRNA and protein involve changes in relative levels of α1 and α4 protein and mRNA. In animals exposed to ethanol, the mRNA and protein levels of α1 decrease, whereas those for α4 increase in certain brain regions such as cerebral cortex.[103,104] The reason for

this change is not understood, but it appears to be a common adaptive change. We have observed this pattern of change in the brains of rats repeatedly exposed to other allosteric modulators such as benzodiazepines,[105] and others have observed increases in α4 expression in response to progesterone exposure.[106]

Ethanol can pass freely through the lipid bilayer of cells and affects several intracellular proteins, including many involved in second-messenger pathways. In particular, many studies have indicated that protein kinase C (PKC) is affected by exposure to ethanol.[19] Ethanol-induced alteration of PKC function leads to changes in the expression or function of voltage dependent calcium channels[107] and ligand-gated ion channels.[108] In brain tissue from mice lacking PKCε, GABA receptors are highly sensitive to allosteric modulation by ethanol, with 20 mmol/L ethanol bringing about twice the potentiation of GABA agonist-induced chloride ion flux compared with controls.[109] Additionally, these mice displayed less ethanol self-administration. Similarly, in a line of mutant mice lacking PKC-γ, ethanol lost its potentiating effect on $GABA_A$ receptors.[110] These mice show a distinct increase in the consumption of ethanol and appear to have a higher level of impulsive behaviour than wild-type mice. Interestingly, decreased sensitivity to the effects of ethanol seems to be predictive of a high probability of alcoholism in humans (see below).

Protein kinase A (PKA) activity is affected by ethanol and, in turn, appears to alter the function of other proteins.[20] In some rat brain regions, modulation of $GABA_A$ receptors by ethanol will occur only if the region has been pre-exposed to β-adrenergic stimulating agents.[111] This shows that increased cyclic adenosine monophosphate (cAMP) levels, and therefore an increase in PKA activity, are needed for ethanol to potentiate GABA-gated currents. In support of this are findings that repeated ethanol administration results in increased PKA activity and cAMP levels and alters the subcellular location of PKA.[112–114] Knockout mice in which one of the regulatory subunits of PKA was disrupted were much less sensitive to the sedative effects of ethanol and consumed more ethanol than controls.[115]

$GABA_A$ receptor subunit knockouts and ethanol

Several $GABA_A$ receptor subunit knockout mice have been developed over the past several years. Because it was hypothesized that the long splice variant of the γ2 subunit was required for ethanol responses, this gene was disrupted to determine its role in ethanol sensitivity in mice. This line of mice showed no difference between wild-type mice in terms of the behavioural effects of ethanol.[116] A line of mice in which the α6 subunit had been knocked out also showed no difference in response to ethanol when compared with wild-type animals.[117] However, a different outcome was seen with δ subunit knockout mice; these animals consumed less ethanol, were less sensitive to withdrawal and displayed less ethanol-induced seizure protection than wild-type mice.[118] Interestingly, δ subunits have been found to co-localize with α4 subunits in various brain regions,[119] and, as mentioned previously, expression of this subunit increases in models of repeated ethanol exposure and appears to be a neuroadaptive response to the presence of ethanol.

$GABA_A$ receptor as a target for therapeutics

A large body of evidence indicates that pharmacological manipulation of $GABA_A$ receptors may be one way to treat alcohol-related diseases. Numerous behavioural studies have shown that allosteric modulators of $GABA_A$ receptors, specifically those that recognize the benzodiazepine binding site, can reverse the effects of ethanol. One compound in particular, the imidazobenzodiazepine Ro 15-4513, has been reported to be particularly effective in reversing the effects of ethanol intoxication in rats.[120] This compound is a negative allosteric modulator. At the molecular level, Ro 15-4513 has been shown to reverse the potentiating effects of ethanol at recombinant receptors expressed in *Xenopus* oocytes. However, because of its anxiogenic and proconvulsant activity, Ro 15-4513 has not been tested as an alcohol antagonist in humans. Interestingly, this drug has also been shown to protect against ethanol-induced damage of the gastric mucosa by interacting with $GABA_A$ receptors in the stomach.[121] Ro 15-4513 binds to subtypes that contain any α, β and γ subunits. It binds with particularly high affinity to α5β2γ2 subtypes, with an affinity approximately 10-fold higher than for α1-containing receptors.

Other compounds that act at the benzodiazepine binding site, for example, members of the β-carboline structural family, also show potential for being alcohol antagonists. FG 7142, a β-carboline inverse agonist, has been shown to block the effects of ethanol in various

$GABA_A$ receptor-containing models. However, like Ro 15-4513, its proconvulsant activity precludes its use as a therapeutic agent. Allosteric modulators with no intrinsic activity at the benzodiazepine site, which therefore have the potential to be used therapeutically, show some promise in ameliorating the effects of ethanol. Although these compounds have not yet been shown to antagonize the potentiating effects of ethanol at $GABA_A$ receptors, they do seem able to antagonize some of the complex behaviours associated with ethanol intake.[122,123] This has prompted some speculation that an endogenous compound produced during chronic ethanol ingestion is prevented from binding to $GABA_A$ receptors by these compounds. Flumazenil (also known as Ro 15-1788), an imidazobenzodiazepine antagonist, has been the subject of several trials. There is conflicting evidence with respect to its ability to reduce ethanol tolerance and dependence,[124-127] but it seems to be somewhat effective in lessening withdrawal-associated anxiety and convulsions.[128] Both flumazenil and ZK 93426 (a β-carboline antagonist) have been shown to reduce alcohol-induced aggression in rats and monkey.[129] Flumazenil is also reported to block ethanol-induced sleep when injected into the medial preoptic area of the hypothalamus of rats.[130] Other antagonists such as CGS 8216 have also been shown to modulate some of the behaviours associated with ethanol ingestion, and drugs that block the chloride channel (picrotoxin) or are GABA antagonists (bicuculline) have shown some potential in antagonizing the effects of ethanol.[131–133]

Genetic component to alcoholism

It has been long-debated whether alcohol dependence is a due to environment or genotype. A genetic component for alcohol-related disorders is supported by many many studies.[134] Early genetic studies revealed polymorphisms in the gene coding for aldehyde dehydrogenase, an enzyme that plays a major role in the metabolism of ethanol. There are 2 common polymorphisms in these genes. One causes an increase in acetaldehyde production, the other a decrease in the rate of its removal. A build-up of acetaldehyde results, producing symptoms such as headache, vasodilation and nausea. At high enough concentrations, acetaldehyde can produce symptoms similar to those of disulfiram.[134,135] Poor alcohol metabolism due to these polymorphisms is prevalent in Chinese and Japanese populations. The side-effects of poor metabolism seem to, in turn, reduce the probability of alcoholism.[136,137]

Several strains of rodents have been bred to be sensitive to ethanol, and there appears to be a GABAergic component underlying this trait. For example, long sleep (LS) and short sleep (SS) mice are strains that were selectively bred on the basis of the duration of sleep induced by acute exposure to ethanol. When the mRNA obtained from the brains of these mice was injected into *Xenopus* oocytes, the $GABA_A$ receptors that were expressed were differentially modulated by ethanol.[138] mRNA from SS mice produced receptors at which ethanol antagonized GABA-gated currents, and mRNA from LS mice produced receptors at which ethanol potentiated GABA-gated currents. In a line of alcohol non-tolerant (ANT) rats, $GABA_A$ receptors are highly sensitive to allosteric modulators of $GABA_A$ receptors such as benzodiazepines. When $GABA_A$ receptor subunits from this line were cloned and sequenced, it was discovered that the α6 subunits contained a mutation at position 100, where a glutamine residue replaced an arginine residue.[139] Interestingly, the amino acid occupying this position has been shown to be a major determinant of benzodiazepine affinity and efficacy,[140] but not of ethanol sensitivity, and so its significance in the ANT phenotype is uncertain.[141] $GABA_A$ receptor polymorphism appears to be the underlying cause in differences among mice strains with different reactions to alcohol withdrawal.

Quantitative trait loci (QTL) studies are based on statistical analysis of the association of a measurable trait and molecular markers that segregate with them. As the position of the markers is known, the relation between the trait(s) and regions of specific chromosomes can be determined. These studies are useful to determine the relation between complex traits that likely involve more than 1 gene and genotype. Application of this technique to understanding the genetic basis of alcohol-related behaviours has met with some success and has implicated the GABAergic system in these processes.[142] The C57BL/6J and DBA/2J mouse lines have high and low acute ethanol withdrawal sensitivity, respectively. QTL mapping shows that a cluster of genes on chromosome 11 affects the severity of acute alcohol withdrawal.[143] Included in this cluster are the genes for the $GABA_A$ receptor α1, α6, β2 and γ2 subunits. Comparison of the γ2 gene of the 2 mice strains shows that the γ2 subunit gene differs at position 11, with alanine present in the high withdrawal sensitivity

mice and a threonine present in the low withdrawal sensitivity mice.[143] This allelic variation is highly correlated with susceptibility to behaviours such as withdrawal sensitivity and ethanol-induced motor incoordination.[144] How this polymorphism affects receptor function is unknown, but it has been proposed that it might disrupt an α-helical region of the N-terminus.

Several studies have also implicated a region of mouse chromosome 1 in mediating alcohol-related behaviours.[145] This region also contains genes involved in diazepam withdrawal convulsions and pentobarbital withdrawal. The identity of these genes has yet to be resolved. QTL mapping has also revealed a cluster of genes on mouse chromosome 2 that include those coding for glutamic acid decarboxylase, the enzyme that synthesizes GABA from glutamate, as being a determinant of ethanol withdrawal behaviours.[142] Interestingly, these same regions overlap with those linked to pentobarbital withdrawal.[145] Other neurotransmitter systems, namely those for dopamine and serotonin, have also been shown in QTL studies to be linked to alcohol-related behaviours.[146,147]

Polymorphisms in $GABA_A$ receptor subunits have also been linked to alcohol response in humans. One study showed that the offspring of alcoholics tend to be less sensitive to the effects of ethanol than control groups, and this was a strong predictor for the development of alcoholism.[148] Genetic analysis of this group revealed a polymorphism in the $GABA_A$ receptor α6 subunit, specifically a switch from proline at position 385 to serine.[149] Again, as was noted in the ANT mouse line, this mutation seems to be more important in benzodiazepine modulation and has not been shown to affect modulation of the receptor by ethanol. Results of another study in 2 psychiatric populations suggest that the serotonin1B gene might be linked to alcoholism in which aggression is displayed.[150]

The role of $GABA_A$ receptors in fetal alcohol syndrome

Fetal alcohol syndrome is a collection of abnormalities, including mental retardation, behavioural and developmental problems, as well as physical abnormalities (e.g., facial malformations).[151] The developing CNS appears to be extremely sensitive to the effects of ethanol, especially during a period of rapid brain growth in the third trimester.[152] There is evidence that $GABA_A$ and NMDA receptors in the developing brain are involved in mediating the damage brought about by ethanol.[153,154] With respect to GABA receptors, it appears that the potentiating effects of ethanol initiate a pro-apoptotic cascade that leads to neuronal cell death.[155] The pattern of cell death brought about by ethanol matched that caused by exposure to benzodiazepines and barbiturates, both positive allosteric modulators of $GABA_A$ receptors.[154] Relatively brief exposure of the developing brain to ethanol during periods of neuronal growth can lead to the deaths of millions of neurons in animal models. This neuronal death is likely the cause of the reduced brain mass and thinner cerebral cortex typically noted in those with fetal alcohol syndrome. The mechanism by which the apoptotic cascade begins through $GABA_A$ receptor activation is unclear, but a link between these receptors and calcium influx via L-type voltage-gated calcium channels has been shown in a model system composed of proliferating neural precursor cells.[156]

Conclusions

The effects of ethanol on proteins in the CNS are complex and involve many different systems. It appears that ligand-gated ion channels and voltage-gated calcium channels are important targets for this drug because their function, type and numbers are altered by short- and long-term exposure to ethanol. A large body of work ranging from biochemical to genetic studies points to the importance of $GABA_A$ receptors in mediating the effects of ethanol in the CNS. It is possible that drugs targeting these receptors could be a component of therapies designed to battle alcohol abuse and dependence.

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References

1. Holden C. Alcoholism and the medical cost crunch. *Science* 1987;235:1132-3.
2. Marshall EJ, Edwards G, Taylor C. Mortality in men with drinking problems: a 20-year follow up. *Addiction* 1994;89: 1293-8.
3. Lipson T. The fetal alcohol syndrome in Australia. *Med J Aust* 1994;161(8):461-2.
4. Loney EA, Habbick BF, Nanson JL. Hospital utilization of Saskatchewan people with fetal alcohol syndrome. *Can J Public Health* 1998;89(5):333-6.
5. Tanaka H. Fetal alcohol syndrome: a Japanese perspective. *Ann Med* 1998;30(1):21-6.
6. Warren KR, Calhoun FJ, May PA, Viljoen DL, Li TK, Tanaka H,

et al. Fetal alcohol syndrome: an international perspective. *Alcohol Clin Exp Res* 2001;25(5 Suppl):202S-206S.
7. Williams RJ, Odaibo FS, McGee JM. Incidence of fetal alcohol syndrome in northeastern Manitoba. *Can J Public Health* 1999; 90(3):192-4.
8. Olson HC, Morse BA, Huffine C. Development and psychopathology: fetal alcohol syndrome and related conditions. *Semin Clin Neuropsychiatry* 1998;3(4):262-84.
9. Stromland K. Present state of the fetal alcohol syndrome. *Acta Ophthalmol Scand Suppl* 1996;(219):10-2.
10. Sellers EM, Busto U. Benzodiazepines and ethanol: assessment of the effects and consequences of psychotropic drug interactions. *J Clin Psychopharmacol* 1982;2(4):249-62.
11. Ciraulo DA, Barnhill J. Pharmacokinetic mechanisms of ethanol-psychotropic drug interactions. *NIDA Res Monogr* 1986;68:73-88.
12. Poikolainen K. Estimated lethal ethanol concentrations in relation to age, aspiration, and drugs. *Alcohol Clin Exp Res* 1984; 8(2):223-5.
13. Katzung BG. *Basic and clinical pharmacology*. 8th ed. Toronto: Lange Medical Books/McGraw-Hill; 2001.
14. Grilly DM. *Drugs and human behavior*. 4th ed. Toronto: Allyn and Bacon; 2002.
15. Harris RA. Ethanol actions on multiple ion channels: which are important? *Alcohol Clin Exp Res* 1999; 23:1563-70.
16. Tabakoff B, Hoffman P. Alcohol addiction: an enigma among us. *Neuron* 1996;16:909-12.
17. Chandler LJ, Harris RA, Crews FT. Ethanol tolerance and synaptic plasticity. *Trends Pharmacol Sci* 1998;19(12):491-5.
18. Littleton J, Little H. Current concepts of ethanol dependence. *Addiction* 1994;89(11):1397-412.
19. Macdonald RL. Ethanol, γ-aminobutyrate type A receptors, and protein kinase C phosphorylation. *Proc Natl Acad Sci U S A* 1995;92:3633-5.
20. Pandey SC. Neuronal signaling systems and ethanol dependence. *Mol Neurobiol* 1998;17(1-3):1-15.
21. Narahashi T, Kuriyama K, Illes P, Wirkner K, Fischer W, Muhlberg K, et al. Neuroreceptors and ion channels as targets of alcohol. *Alcohol Clin Exp Res* 2001;25(5 Suppl):182S-188S.
22. Valenzuela CF, Cardoso RA, Wick MJ, Weiner JL, Dunwiddie TV, Harris RA. Effects of ethanol on recombinant glycine receptors expressed in mammalian cell lines. *Alcohol Clin Exp Res* 1998;22(5):1132-6.
23. Cardoso RA, Brozowski SJ, Chavez-Noriega LE, Harpold M, Valenzuela CF, Harris RA. Effects of ethanol on recombinant human neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *J Pharmacol Exp Ther* 1999;289(2):774-80.
24. Lovinger DM. 5-HT$_3$ receptors and the neural actions of alcohols: an increasingly exciting topic. *Neurochem Int* 1999;35(2): 125-30.
25. Mihic SJ. Acute effects of ethanol on GABA$_A$ and glycine receptor function. *Neurochem Int* 1999;35(2):115-23.
26. Hoffman PL, Rabe CS, Moses F, Tabakoff B. *N*-methyl-D-aspartate receptors and ethanol: inhibition of calcium flux and cyclic GMP production. *J Neurochem* 1989;52(6):1937-40.
27. Widmer H, Lemos JR, Treistman SN. Ethanol reduces the duration of single evoked spikes by a selective inhibition of voltage-gated calcium currents in acutely dissociated supraoptic neurons of the rat. *J Neuroendocrinol* 1998;10(6):399-406.
28. Wang X, Wang G, Lemos JR, Treistman SN. Ethanol directly modulates gating of a dihydropyridine-sensitive Ca^{2+} channel in neurohypophysial terminals. *J Neurosci* 1994;14(9):5453-60.
29. Anton RF. Pharmacologic approaches to the management of alcoholism. *J Clin Psychiatry* 2001;62(Suppl 20):11-7.
30. Spanagel R, Zieglgansberger W. Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. *Trends Pharmacol Sci* 1997;18(2):54-9.
31. Lipsky JJ, Shen ML, Naylor S. Overview—in vitro inhibition of aldehyde dehydrogenase by disulfiram and metabolites. *Chem Biol Interact* 2001;130-2(1-3):81-91.
32. Lipsky JJ, Shen ML, Naylor S. In vivo inhibition of aldehyde dehydrogenase by disulfiram. *Chem Biol Interact* 2001;130-2(1-3): 93-102.
33. Wright C, Moore RD. Disulfiram treatment of alcoholism. *Am J Med* 1990;88(6):647-55.
34. Gianoulakis C. Implications of endogenous opioids and dopamine in alcoholism: human and basic science studies. *Alcohol Alcohol* 1996;31(Suppl 1):33-42.
35. O'Malley SS. Opioid antagonists in the treatment of alcohol dependence: clinical efficacy and prevention of relapse. *Alcohol Alcohol* 1996;31(Suppl 1):77-81.
36. Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP. Naltrexone in the treatment of alcohol dependence. *Arch Gen Psychiatry* 1992;49(11):876-80.
37. Wilde MI, Wagstaff AJ. Acamprosate. A review of its pharmacology and clinical potential in the management of alcohol dependence after detoxification. *Drugs* 1997;53(6):1038-53.
38. Berton F, Francesconi WG, Madamba SG, Zieglgansberger W, Siggins GR. Acamprosate enhances *N*-methyl-D-apartate receptor-mediated neurotransmission but inhibits presynaptic GABA$_B$ receptors in nucleus accumbens neurons. *Alcohol Clin Exp Res* 1998;22(1):183-91.
39. Allgaier C, Franke H, Sobottka H, Scheibler P. Acamprosate inhibits Ca^{2+} influx mediated by NMDA receptors and voltage-sensitive Ca^{2+} channels in cultured rat mesencephalic neurones. *Naunyn Schmiedebergs Arch Pharmacol* 2000;362(4-5):440-3.
40. Johnson BA, Roache JD, Ait-Daoud N, Zanca NA, Velazquez M. Ondansetron reduces the craving of biologically predisposed alcoholics. *Psychopharmacology (Berl)* 2002;160(4): 408-13.
41. Yost DA. Alcohol withdrawal syndrome. *Am Fam Physician* 1996;54(2):657-64, 669.
42. Morris JC, Victor M. Alcohol withdrawal seizures. *Emerg Med Clin North Am* 1987;5(4):827-39.
43. Grobin AC, Matthews DB, Devaud LL, Morrow AL. The role of GABA$_A$ receptors in the acute and chronic effects of ethanol. *Psychopharmacology (Berl)* 1998;139(1-2):2-19.
44. Sieghart W. Structure and pharmacology of gamma-aminobutyric acid$_A$ receptor subtypes. *Pharmacol Rev* 1995;47(2):181-234.
45. Soltesz I, Roberts JD, Takagi H, Richards JG, Mohler H, Somogyi P. Synaptic and nonsynaptic localization of benzodiazepine/GABA$_A$ receptor/Cl^- channel complex using monoclonal antibodies in the dorsal lateral geniculate nucleus of the cat. *Eur J Neurosci* 1990;2(5):414-29.
46. Nayeem N, Green TP, Martin IL, Barnard EA. Quaternary structure of the native GABA$_A$ receptor determined by electron microscopic image analysis. *J Neurochem* 1994;62(2):815-8.
47. Barnard EA, Bateson AN, Darlison MG, Glencorse TA, Harvey RJ, Hicks AA, et al. Genes for the GABA$_A$ receptor subunit types and their expression. *Adv Biochem Psychopharmacol* 1992; 47:17-27.
48. Bonnert TP, McKernan RM, Farrar S, le Bourdelles B, Heavens RP, Smith DW, et al. θ, a novel γ-aminobutyric acid type A receptor subunit. *Proc Natl Acad Sci U S A* 1999;96(17):9891-6.
49. Sieghart W. Structure and pharmacology of gamma-aminobutyric acid$_A$ receptor subtypes. *Pharmacol Rev* 1995;47(2):181-234.
50. Hadingham KL, Wingrove PB, Wafford KA, Bain C, Kemp JA, Palmer KJ, et al. Role of the beta subunit in determining the

pharmacology of human gamma-aminobutyric acid type A receptors. *Mol Pharmaco*l 1993;44(6):1211-8.
51. Davies M, Bateson AN, Dunn SMJ. Molecular biology of the $GABA_A$ receptor: Domains implicated by mutational analysis. *Front Biosci* 1996;1:d214-d233.
52. McKernan RM, Whiting PJ. Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci* 1996;19(4):139-43.
53. 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.
54. Sigel E, Buhr A. The benzodiazepine binding site of $GABA_A$ receptors. *Trends Pharmacol Sci* 1997;18(11):425-9.
55. Changeux JP, Edelstein SJ. Allosteric receptors after 30 years. *Neuron* 1998;21:959-80.
56. Smith GB, Olsen RW. Functional domains of $GABA_A$ receptors. *Trends Pharmacol Sci* 1995;16(5):162-8.
57. Olsen RW. Drug interactions at the GABA receptor-ionophore complex. *Ann Rev Pharmacol Toxicol* 1982;22:245-77.
58. Sieghart W. $GABA_A$ receptors: ligand-gated Cl^- ion channels modulated by multiple drug-binding sites. *Trends Pharmacol Sci* 1992;13(12):446-50.
59. Doble A, Martin ILM. Multiple benzodiazepine receptors: No reason for anxiety. *Trends Pharmacol Sci* 1992;13:76-81.
60. Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, et al. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric $acid_A$ receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev* 1998;50(2):291-313.
61. Blair LA, Levitan ES, Marshall J, Dionne VE, Barnard EA. Single subunits of the $GABA_A$ receptor form ion channels with properties of the native receptor. *Science* 1988;242(4878):577-9.
62. Suzdak PD, Paul SM. Ethanol stimulates GABA receptor-mediated Cl^- ion flux in vitro: possible relationship to the anxiolytic and intoxicating actions of alcohol. *Psychopharmacol Bull* 1987;23(3):445-51.
63. Marszalec W, Kurata Y, Hamilton BJ, Carter DB, Narahashi T. Selective effects of alcohols on gamma-aminobutyric acid A receptor subunits expressed in human embryonic kidney cells. *J Pharmacol Exp Ther* 1994;269(1):157-63.
64. Siggins GR, Pittman QJ, French ED. Effects of ethanol on CA1 and CA3 pyramidal cells in the hippocampal slice preparation: an intracellular study. *Brain Res* 1987;414:22-34.
65. Frye GD, Fincher AS, Grover CA, Griffith WH. Interaction of ethanol and allosteric modulators with $GABA_A$-activated currents in adult medial septum/diagonal band neurons. *Brain Res* 1994;635(1-2):283-92.
66. Wafford KA, Whiting PJ. Ethanol potentiation of $GABA_A$ receptors requires phosphorylation of the alternatively spliced variant of the gamma 2 subunit. *FEBS Lett* 1992;313(2):113-7.
67. Zhai J, Stewart RR, Friedberg MW, Li C. Phosphorylation of the $GABA_A$ receptor γ2L subunit in rat sensory neurons may not be necessary for ethanol sensitivity. *Brain Res* 1998;805(1-2):116-22.
68. Mihic SJ, Whiting PJ, Harris RA. Anaesthetic concentrations of alcohols potentiate $GABA_A$ receptor-mediated currents: lack of subunit specificity. *Eur J Pharmacol* 1994;268(2):209-14.
69. Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, et al. Hormonally regulated α4β2δ $GABA_A$ receptors are a target for alcohol. *Nat Neurosci* 2002;5(8):721-2.
70. Roth SH. Physical mechanisms of anesthesia. *Annu Rev Pharmacol Toxicol* 1979;19:159-78.
71. Seeman P. The membrane actions of anesthetics and tranquilizers. *Pharmacol Rev* 1972;24(4):583-655.
72. Janoff AS, Pringle MJ, Miller KW. Correlation of general anesthetic potency with solubility in membranes. *Biochim Biophys Acta* 1981;649(1):125-8.
73. Pringle MJ, Brown KB, Miller KW. Can the lipid theories of anesthesia account for the cutoff in anesthetic potency in homologous series of alcohols? *Mol Pharmacol* 1981;19(1):49-55.
74. Franks NP, Lieb WR. Mapping of general anaesthetic target sites provides a molecular basis for cutoff effects. *Nature* 1985;316(6026):349-51.
75. Franks NP, Lieb WR. Partitioning of long-chain alcohols into lipid bilayers: implications for mechanisms of general anesthesia. *Proc Natl Acad Sci U S A* 1986;83(14):5116-20.
76. Harris BD, Moody EJ, Basile AS, Skolnick P. Volatile anesthetics bidirectionally and stereospecifically modulate ligand binding to GABA receptors. *Eur J Pharmacol* 1994;267(3):269-74.
77. Franks NP, Lieb WR. Stereospecific effects of inhalational general anesthetic optical isomers on nerve ion channels. *Science* 1991;254(5030):427-30.
78. Buck KJ, Allan AM, Harris RA. Fluidization of brain membranes by A2C does not produce anesthesia and does not augment muscimol-stimulated $^{36}Cl^-$ influx. *Eur J Pharmacol* 1989;160(3):359-67.
79. Dildy-Mayfield JE, Mihic SJ, Liu Y, Deitrich RA, Harris RA. Actions of long chain alcohols on $GABA_A$ and glutamate receptors: relation to in vivo effects. *Br J Pharmacol* 1996;118(2):378-84.
80. Nakahiro M, Arakawa O, Nishimura T, Narahashi T. Potentiation of GABA-induced Cl^- current by a series of n-alcohols disappears at a cutoff point of a longer-chain n-alcohol in rat dorsal root ganglion neurons. *Neurosci Lett* 1996;205(2):127-30.
81. Godden EL, Harris RA, Dunwiddie TV. Correlation between molecular volume and effects of n-alcohols on human neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. *J Pharmacol Exp Ther* 2001;296(3):716-22.
82. Akinshola BE. Straight-chain alcohols exhibit a cutoff in potency for the inhibition of recombinant glutamate receptor subunits. *Br J Pharmacol* 2001;133(5):651-8.
83. Peoples RW, Ren H. Inhibition of *N*-methyl-D-aspartate receptors by straight-chain diols: implications for the mechanism of the alcohol cutoff effect. *Mol Pharmacol* 2002;61(1):169-76.
84. Tatebayashi H, Motomura H, Narahashi T. Alcohol modulation of single $GABA_A$ receptor-channel kinetics. *Neuroreport* 1998;9:1769-75.
85. Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MA, Finn SE, et al. Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. *Nature* 1997;389:385-9.
86. Ye Q, Koltchine VV, Mihic SJ, Mascia MP, Wick MJ, Finn SE, et al. Enhancement of glycine receptor function by ethanol is inversely correlated with molecular volume at position alpha267. *J Biol Chem* 1998;273(6):3314-9.
87. Chester JA, Cunningham CL. $GABA_A$ receptor modulation of the rewarding and aversive effects of ethanol. *Alcohol* 2002;26(3):131-43.
88. Boyle AE, Segal R, Smith BR, Amit Z. Bidirectional effects of GABAergic agonists and antagonists on maintenance of voluntary ethanol intake in rats. *Pharmacol Biochem Behav* 1993;46(1):179-82.
89. Petry NM. Benzodiazepine-GABA modulation of concurrent ethanol and sucrose reinforcement in the rat. *Exp Clin Psychopharmacol* 1997;5(3):183-94.
90. Rassnick S, D'Amico E, Riley E, Koob GF. GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. *Alcohol Clin Exp Res* 1993;17(1):124-30.
91. Balakleevsky A, Colombo G, Fadda F, Gessa GL. Ro 19-4603, a

benzodiazepine receptor inverse agonist, attenuates voluntary ethanol consumption in rats selectively bred for high ethanol preference. *Alcohol Alcohol* 1990;25(5):449-52.
92. Wegelius K, Honkanen A, Korpi ER. Benzodiazepine receptor ligands modulate ethanol drinking in alcohol-preferring rats. *Eur J Pharmacol* 1994;263(1-2):141-7.
93. Shelton KL, Balster RL. Effects of gamma-aminobutyric acid agonists and *N*-methyl-D-aspartate antagonists on a multiple schedule of ethanol and saccharin self-administration in rats. *J Pharmacol Exp Ther* 1997;280(3):1250-60.
94. Rimondini R, Sommer W, Heilig M. Effects of tiagabine and diazepam on operant ethanol self-administration in the rat. *J Stud Alcohol* 2002;63(1):100-6.
95. Kalivas PW, Duffy P, Eberhardt H. Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. *J Pharmacol Exp Ther* 1990;253(2):858-66.
96. Ortiz J, Fitzgerald LW, Charlton M, Lane S, Trevisan L, Guitart X, et al. Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. *Synapse* 1995;21(4):289-98.
97. June HL, Harvey SC, Foster KL, McKay PF, Cummings R, Garcia M, et al. GABA$_A$ receptors containing α5 subunits in the CA1 and CA3 hippocampal fields regulate ethanol-motivated behaviors: an extended ethanol reward circuitry. *J Neurosci* 2001;21(6):2166-77.
98. Kokka N, Sapp DW, Taylor AM, Olsen RW. The kindling model of alcohol dependence: similar persistent reduction in seizure threshold to pentylenetetrazol in animals receiving chronic ethanol or chronic pentylenetetrazol. *Alcohol Clin Exp Res* 1993;17(3):525-31.
99. Kang M, Spigelman I, Sapp DW, Olsen RW. Persistent reduction of GABA$_A$ receptor-mediated inhibition in rat hippocampus after chronic intermittent ethanol treatment. *Brain Res* 1996;709(2):221-8.
100. Kang MH, Spigelman I, Olsen RW. Alteration in the sensitivity of GABA$_A$ receptors to allosteric modulatory drugs in rat hippocampus after chronic intermittent ethanol treatment. *Alcohol Clin Exp Res* 1998;22(9):2165-73.
101. Buck KJ, Harris RA. Benzodiazepine agonist and inverse agonist actions on GABA$_A$ receptor-operated chloride channels. II. Chronic effects of ethanol. *J Pharmacol Exp Ther* 1990;253(2):713-9.
102. Devaud LL, Purdy RH, Morrow AL. The neurosteroid, 3 α-hydroxy-5 α-pregnan-20-one, protects against bicucculline-induced seizures during ethanol withdrawal in rats. *Alcohol Clin Exp Res* 1995;19(2):350-5.
103. Devaud LL, Smith FD, Grayson DR, Morrow AL. Chronic ethanol consumption differentially alters the expression of gamma-aminobutyric acid$_A$ receptor subunit mRNAs in rat cerebral cortex: competitive, quantitative reverse transcriptase-polymerase chain reaction analysis. *Mol Pharmacol* 1995;48(5):861-8.
104. Devaud LL, Fritschy JM, Sieghart W, Morrow AL. Bi-directional alterations of GABA$_A$ receptor subunit peptide levles in rat cortex during chronic ethanol consumption and withdrawal. *J Neurochem* 1997;69:126-30.
105. Arnot MI, Davies M, Martin IL, Bateson AN. GABA$_A$ receptor gene expression in rat cortex: differential effects of two chronic diazepam treatment regimes. *J Neurosci Res* 2001;64(6):617-25.
106. Smith SS, Gong QH, Li X, Moran MH, Bitran D, Frye CA, et al. Withdrawal from 3alpha-OH-5alpha-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal GABA$_A$-gated current and increases the GABA$_A$ receptor α4 subunit in association with increased anxiety. *J Neurosci* 1998; 18(14):5275-84.
107. Walter HJ, McMahon T, Dadgar J, Wang D, Messing RO. Ethanol regulates calcium channel subunits by protein kinase C delta-dependent and -independent mechanisms. *J Biol Chem* 2000;275(33):25717-22.
108. Weiner JL, Valenzuela CF, Watson PL, Frazier CJ, Dunwiddie TV. Elevation of basal protein kinase C activity increases ethanol sensitivity of GABA$_A$ receptors in rat hippocampal CA1 pyramidal neurons. *J Neurochem* 1997;68(5):1949-59.
109. Hodge CW, Mehmert KK, Kelley SP, McMahon T, Haywood A, Olive MF, et al. Supersensitivity to allosteric GABA$_A$ receptor modulators and alcohol in mice lacking PKCε. *Nature Neurosci* 1999;2(11):997-1002.
110. Harris RA, McQuilkin SJ, Paylor R, Abeliovich A, Tonegawa S, Wehner JM. Mutant mice lacking the gamma isoform of protein kinase C show decreased behavioral actions of ethanol and altered function of gamma-aminobutyrate type A receptors. *Proc Natl Acad Sci U S A* 1995;92(9):3658-62.
111. Freund RK, Palmer MR. Beta adrenergic sensitization of γ-aminobutyric acid receptors to ethanol involves a cyclic AMP/protein kinase A second-messenger system. *J Pharmacol Exp Ther* 1997;254:1192-200.
112. Ortiz J, Fitzgerald LW, Charlton M, Lane S, Trevisan L, Guitart X, et al. Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. *Synaps*e 1995;21(4):289-98.
113. Dohrman DP, Diamond I, Gordon AS. Ethanol causes translocation of cAMP-dependent protein kinase catalytic subunit to the nucleus. *Proc Natl Acad Sci U S A* 1996;93(19):10217-21.
114. Gordon AS, Yao L, Dohrman DP, Diamond I. Ethanol alters the subcellular localization of cAMP-dependent protein kinase and protein kinase C. *Alcohol Clin Exp Res* 1998;22(5 Suppl): 238S-242S.
115. Thiele TE, Willis B, Stadler J, Reynolds JG, Bernstein IL, McKnight GS. High ethanol consupmtion and low sensitivity to ethanol-induced sedation in protein kinase A-mutant mice. *J Neurosci* 2000;20(10):RC75.
116. Homanics GE, Harrison NL, Quinlan JJ, Krasowski MD, Rick CE, De Blas AL, et al. Normal electrophysiological and behavioral responses to ethanol in mice lacking the long splice variant of the γ2 subunit of the gamma-aminobutyrate type A receptor. *Neuropharmacol* 1999;38(2):253-65.
117. Homanics GE, Ferguson C, Quinlan JJ, Daggett J, Snyder K, Lagenaur C, et al. Gene knockout of the α6 subunit of the gamma-aminobutyric acid type A receptor: lack of effect on responses to ethanol, pentobarbital, and general anesthetics. *Mol Pharmacol* 1997;51(4):588-96.
118. Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL, et al. GABA-A receptor δ subunit knockout mice have multiple defects in behavioral responses to ethanol. *Alcohol Clin Exp Res* 2001;12:1708-18.
119. Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, McKernan RM. Preferential coassembly of α4 and δ subunits of the gamma-aminobutyric acid$_A$ receptor in rat thalamus. *Mol Pharmacol* 1999;56(1):110-5.
120. Suzdak PD, Glowa JR, Crawley JN, Schwartz RD, Skolnick P, Paul SM. A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science* 1986;234(4781):1243-7.
121. Mulla Hummadi YM, Najim RA, Farjou IB. Benzodiazepines protect against ethanol-induced gastric mucosal damage in vitro. *Fundam Clin Pharmacol* 2001;15(4):247-54.
122. June HL, Devaraju SL, Eggers MW, Williams JA, Cason CR, Greene TL, et al. Benzodiazepine receptor antagonists modulate the actions of ethanol in alcohol-preferring and -nonpreferring rats. *Eur J Pharmacol* 1998;342(2-3):139-51.
123. June HL, Zuccarelli D, Torres L, Craig KS, DeLong J, Allen A, et al. High-affinity benzodiazepine antagonists reduce responding

maintained by ethanol presentation in ethanol-preferring rats. *J Pharmacol Exp Ther* 1998;284(3):1006-14.
124. Chan AW, Leong FW, Schanley DL, Langan MC, Penetrante F. Flumazenil (Ro15-1788) does not affect ethanol tolerance and dependence. *Pharmacol Biochem Behav* 1991;39(3):659-63.
125. Buck KJ, Heim H, Harris RA. Reversal of alcohol dependence and tolerance by a single administration of flumazenil. *J Pharmacol Exp Ther* 1991;257(3):984-9.
126. Masur J, Silva-Filho AR, de Souza ML, Pires ML. Lack of effect of the benzodiazepine receptor antagonist Ro 15-1788 on ethanol-induced intoxication in mice. *Alcohol* 1987;4(6):425-7.
127. Klotz U, Ziegler G, Rosenkranz B, Mikus G. Does the benzodiazepine antagonist Ro 15-1788 antagonize the action of ethanol? *Br J Clin Pharmacol* 1986;22(5):513-20.
128. Nutt DGP, Wilson S, Groves S, Coupland N, Bailey J. Flumazenil in alcohol withdrawal. *Alcohol Alcohol Suppl* 1993; 2:337-41.
129. Weerts EM, Tornatzky W, Miczek KA. Prevention of the pro-aggressive effects of alcohol in rats and squirrel monkeys by benzodiazepine receptor antagonists. *Psychopharmacology (Berl)* 1993;111(2):144-52.
130. Mendelson WB. The sleep-inducing effect of ethanol microinjection into the medial preoptic area is blocked by flumazenil. *Brain Res* 2001;892(1):118-21.
131. Koechling UM, Smith BR, Amit Z. Effects of GABA antagonists and habituation to novelty on ethanol-induced locomotor activity in mice. *Alcohol Alcohol* 1991;26(3):315-22.
132. Hellevuo K, Kiianmaa K, Korpi ER. Effect of GABAergic drugs on motor impairment from ethanol, barbital and lorazepam in rat lines selected for differential sensitivity to ethanol. *Pharmacol Biochem Behav* 1989;34(2):399-404.
133. Chester JA, Cunningham CL. $GABA_A$ receptors modulate ethanol-induced conditioned place preference and taste aversion in mice. *Psychopharmacology (Berl)* 1999;144(4):363-72.
134. Radel M, Goldman D. Pharmacogenetics of alcohol response and alcoholism: the interplay of genes and environmental factors in thresholds for alcoholism. *Drug Metab Dispos* 2001;29(4 Pt 2):489-94.
135. Li TK. Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. *J Stud Alcohol* 2000;61(1):5-12.
136. Thomasson HR, Crabb DW, Edenberg HJ, Li TK, Hwu HG, Chen CC, et al. Low frequency of the ADH2*2 allele among Atayal natives of Taiwan with alcohol use disorders. *Alcohol Clin Exp Res* 1994;18(3):640-3.
137. Thomasson HR, Edenberg HJ, Crabb DW, Mai XL, Jerome RE, Li TK, et al. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet* 1991; 48(4):677-81.
138. Wafford KA, Burnett DM, Dunwiddie TV, Harris RA. Genetic differences in the ethanol sensitivity of $GABA_A$ receptors expressed in *Xenopus* oocytes. *Science* 1990;249(4966):291-3.
139. Korpi ER, Kleingoor C, Kettenmann H, Seeburg PH. Benzodiazepine-induced motor impairment linked to point mutation in cerebellar $GABA_A$ receptor. *Nature* 1993;361(6410):356-9.
140. Wieland HA, Luddens H, Seeburg PH. A single histidine in $GABA_A$ receptors is essential for benzodiazepine agonist binding. *J Biol Chem* 1992;267(3):1426-9.
141. Dunn SM, Davies M, Muntoni AL, Lambert JJ. Mutagenesis of the rat alpha1 subunit of the gamma-aminobutyric $acid_A$ receptor reveals the importance of residue 101 in determining the allosteric effects of benzodiazepine site ligands. *Mol Pharmacol* 1999;56(4):768-74.
142. Buck KJ, Finn DA. Genetic factors in addiction: QTL mapping and candidate gene studies implicate GABAergic genes in alcohol and barbiturate withdrawal in mice. *Addiction* 2001; 96(1):139-49.
143. Buck KJ, Hood HM. Genetic association of a $GABA_A$ receptor γ2 subunit variant with severity of acute physiological dependence on alcohol. *Mamm Genome* 1998;9(12):975-8.
144. Hood HM, Buck KJ. Allelic variation in the $GABA_A$ receptor γ2 subunit is associated with genetic susceptibility to ethanol-induced motor incoordination and hypothermia, conditioned taste aversion, and withdrawal in BXD/Ty recombinant inbred mice. *Alcohol Clin Exp Res* 2000;24(9):1327-34.
145. Buck K, Metten P, Belknap J, Crabbe J. Quantitative trait loci affecting risk for pentobarbital withdrawal map near alcohol withdrawal loci on mouse chromosomes 1, 4, and 11. *Mamm Genome* 1999;10(5):431-7.
146. Crabbe JC, Belknap JK, Mitchell SR, Crawshaw LI. Quantitative trait loci mapping of genes that influence the sensitivity and tolerance to ethanol-induced hypothermia in BXD recombinant inbred mice. *J Pharmacol Exp Ther* 1994;269(1):184-92.
147. Crabbe JC, Buck KJ, Metten P, Belknap JK. Strategies for identifying genes underlying drug abuse susceptibility. *NIDA Res Monogr* 1996;161:201-19.
148. Schuckit MA, Mazzanti C, Smith TL, Ahmed U, Radel M, Iwata N, Goldman D. Selective genotyping for the role of 5-HT_{2A}, 5-HT_{2C}, and GABA α6 receptors and the serotonin transporter in the level of response to alcohol: a pilot study. *Biol Psychiatry* 1999;45(5):647-51.
149. Iwata N, Cowley DS, Radel M, Roy-Byrne PP, Goldman D. Relationship between a $GABA_A$ α6 Pro385Ser substitution and benzodiazepine sensitivity. *Am J Psychiatry* 1999;156(9):1447-9.
150. Lappalainen J, Long JC, Eggert M, Ozaki N, Robin RW, Brown GL, et al. Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. *Arch Gen Psychiatry* 1998;55(11):989-94.
151. Larkby C, Day N. The effects of prenatal alcohol exposure. *Alcohol Health Res World* 1997;21:192-8.
152. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev* 1979;3(1):79-83.
153. Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, et al. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science* 2000;287(5455):1056-60.
154. Olney JW, Wozniak DF, Farber NB, Jevtovic-Todorovic V, Bittigau P, Ikonomidou C. The enigma of fetal alcohol neurotoxicity. *Ann Med* 2002;34(2):109-19.
155. Ikonomidou C, Bittigau P, Koch C, Genz K, Hoerster F, Felderhoff-Mueser U, et al. Neurotransmitters and apoptosis in the developing brain. *Biochem Pharmacol* 2001;62(4):401-5.
156. Ma W, Pancrazio JJ, Andreadis JD, Shaffer KM, Stenger DA, Li BS, et al. Ethanol blocks cytosolic Ca^{2+} responses triggered by activation of $GABA_A$ receptor/Cl^- channels in cultured proliferating rat neuroepithelial cells. *Neuroscience* 2001;104(3):913-22.