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REVIEW

Physiology and pharmacology of alcohol: the imidazobenzodiazepine alcohol antagonist site on subtypes of $GABA_A$ receptors as an opportunity for drug development?

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Alcohol (ethanol, EtOH) has pleiotropic actions and induces a number of acute and long-term effects due to direct actions on alcohol targets, and effects of alcohol metabolites and metabolism. Many detrimental health consequences are due to EtOH metabolism and metabolites, in particular acetaldehyde, whose high reactivity leads to nonspecific chemical modifications of proteins and nucleic acids. Like acetaldehyde, alcohol has been widely considered a nonspecific drug, despite rather persuasive evidence implicating inhibitory $GABA_A$ receptors ($GABA_A$Rs) in acute alcohol actions, for example, a $GABA_A$R ligand, the imidazobenzodiazepine Ro15-4513 antagonizes many low-to-moderate dose alcohol actions in mammals. It was therefore rather surprising that abundant types of synaptic $GABA_A$Rs are generally not responsive to relevant low concentrations of EtOH. In contrast, δ-subunit-containing $GABA_A$Rs and extrasynaptic tonic GABA currents mediated by these receptors are sensitive to alcohol concentrations that are reached in blood and tissues during low-to-moderate alcohol consumption. We recently showed that low-dose alcohol enhancement on highly alcohol-sensitive $GABA_A$R subtypes is antagonized by Ro15-4513 in an apparently competitive manner, providing a molecular explanation for behavioural Ro15-4513 alcohol antagonism. The identification of a Ro15-4513/EtOH binding site on unique $GABA_A$R subtypes opens the possibility to characterize this alcohol site(s) and screen for compounds that modulate the function of EtOH/Ro15-4513-sensitive $GABA_A$Rs. The utility of such drugs might range from novel alcohol antagonists that might be useful in the emergency room, to drugs for the treatment of alcoholism, as well as alcohol-mimetic drugs to harness acute positive effects of alcohol.
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Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; BZ, benzodiazepine; EtOH, ethanol; GABA, γ-aminobutyric acid; $GABA_A$R, $GABA_A$ receptor; MEOS, microsomal ethanol-oxidizing system; Ro15-4513, ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol(1,5-*a*)benzodiazepine-3-carboxylate; Ro15-1788, ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol(1,5-*a*)benzodiazepine-3-carboxylate (also known as flumazenil)

Introduction

Alcohol is by far the most frequently used and abused addictive drug, and therefore a detailed understanding of the molecular mechanisms of alcohol actions is important to human health and well-being. The aim of this review is to discuss our current view of the biochemical mechanisms by which alcohol consumption influences mammalian physiology. The widespread use of alcohol is likely due to its anxiolytic, mood-enhancing and rewarding effects in mammalian brains. In addition, there are numerous studies showing that regular low-to-moderate alcohol consumption has significant beneficial effects, in particular on the cardiovascular system (Friedman and Klatsky, 1993). In contrast, alcohol abuse and alcoholism cause tremendous human suffering with severe detrimental health effects such as alcoholic liver and heart disease, increased risk for stroke, chronic diarrhoea and alcohol dementia (Zernig *et al*., 2000; Fleming *et al*., 2005). There is evidence that alcohol metabolism, and in particular the metabolite acetaldehyde, is an important mediator of acute and long-term alcohol toxicity. We also discuss acute direct actions of alcohol on the many putative 'alcohol receptors' in mammalian brains,

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with emphasis on highly alcohol-sensitive γ-aminobutyric acid $(GABA)_A$ receptor ($GABA_AR$) subtypes at which low-to-moderate dose alcohol enhancement can be reversed in mammals with the benzodiazepine (BZ) behavioural alcohol antagonist Ro15-4513. Our finding that the BZ Ro15-4513 is a competitive alcohol antagonist and can be displaced by a few other BZ-site ligands suggests that the alcohol site on δ-subunit-containing $GABA_ARs$ is a binding pocket 'homologous' to classical BZ sites. This enables us to leverage the vast knowledge and the huge pharmacopeia of compounds that has been developed for classical BZ sites to develop alcohol/Ro15-4513 site ligands with alcohol antagonist as well as alcohol-mimetic activity. This might lead to novel anxiolytic, sedative/hypnotic drugs and to useful novel alcohol antagonists, which have the potential to reduce drinking in patients who suffer from alcohol abuse and alcoholism.

Pleiotropic effects of alcohol

Alcohol in society has a long history (Vallee, 1998), and is, besides caffeine and nicotine, by far the most widely used and abused addictive drug, and it has a number of effects on our body. In order of increasing dose (or number of drinks), alcohol is anxiolytic, mood-enhancing and sedative, slows reaction time, produces motor incoordination, and impairs judgment (making it dangerous and illegal to drive a car). At very high doses alcohol produces loss of consciousness (that is, it acts as an anaesthetic). In addition, overindulgence frequently produces nausea and vomiting as early symptoms of acute alcohol intoxication, and the next day one wakes up with a hangover, characterized by headache accompanied by thirst and general misery. Alcohol abuse is a major public health problem, and leads to increased risk of injury and death, as well as poor functioning in society (family, work and law). Also, chronic alcohol abuse over years and decades leads to liver damage (Lieber, 1988) and heart disease (for example, alcoholic cardiomyopathy), and causes a small, yet significant, increase in the risk for many types of cancer (Room *et al*., 2005; Boffetta *et al*., 2006).

On the other hand, it has been noted as early as 1904 that alcohol consumption is associated with positive effects on the cardiovascular (Cabot, 1904) system. There is now almost unanimous agreement (but see Fillmore *et al*., 2006) that regular low-to-moderate alcohol consumption leads to significant positive effects on health and longevity (Zakhari and Gordis, 1999; Klatsky, 2003; Mukamal *et al*., 2003). Regular low-to-moderate alcohol consumption (defined as 1–3 standard drinks a day) is associated with a decreased risk for cardiovascular disease (that is, coronary heart disease and ischaemic stroke). The so-called 'French paradox' posits that regular alcohol consumption, with red wine possibly particularly beneficial (Szmitko and Verma, 2005), compensates for an otherwise unhealthy lifestyle with high fat consumption and a high prevalence of smoking (Klatsky, 2003). However, beneficial EtOH effects at low-to-moderate doses are eliminated already at only slightly higher intakes (>2–3 standard drinks a day). These detrimental alcohol effects lead to a J-shaped dose–mortality curve, where initial protective effects at low-to-moderate intake reverse into negative effects with further increase in EtOH intake. Because of this J-shaped dose–mortality curve and the risk that encouraging alcohol consumption could lead to (or might encourage continued) alcohol abuse, medical advice from the American Heart Association at this time does not generally recommend moderate alcohol consumption to patients who currently do not drink (Klatsky, 2001). However, a personalized risk/benefit analysis might suggest that persons at risk for atherosclerotic cardiovascular disease, but with low risk for alcohol abuse and alcoholism, could draw substantial health benefits from regular low-to-moderate alcohol consumption.

Alcohol metabolism and acetaldehyde are responsible for much of the short- and long-term alcohol toxicity

Alcohol elimination is accomplished to a large extent in the liver, through the oxidation of alcohol into acetaldehyde. There are two main alcohol elimination pathways: (1) a constitutively active aldehyde dehydrogenases (ADHes) pathway (Figure 1a) and (2) an inducible alcohol elimination pathway also called the **m**icrosomal **e**thanol-**o**xidizing **s**ystem (MEOS; Figure 1b). ADHes have a higher affinity for alcohol (low Michaelis–Menten, K_m) than the MEOS, and therefore are responsible for much of the elimination at low blood alcohol concentration, whereas the inducible MEOS

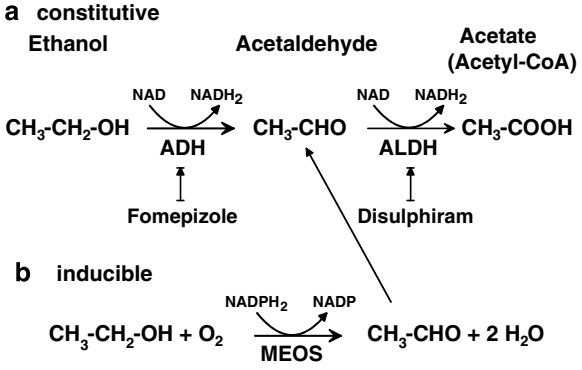


Figure 1 Alcohol metabolism and alcohol metabolites are to a large extent responsible for toxicity of alcohols (ethanol (EtOH), methanol and ethylene glycol). (**a**) The constitutive alcohol dehydrogenase (ADH) pathway produces acetaldehyde that is further metabolized by aldehyde dehydrogenase (ALDH). Decreased ALDH activity in individuals with low-activity version of ALDH, as well as block of ALDH by drugs such as disulphiram is associated with acetaldehyde accumulation and aversive reactions to EtOH ingestion. The competitive blocker of the most abundant forms of ADH, fomepizole, is now used (instead of EtOH) to aid in the management of methanol and ethylene glycol poisoning by slowing the conversion of methanol and ethylene glycol into their highly toxic metabolites. (**b**) The inducible microsomal ethanol-oxidizing system (MEOS) utilizes the cytochrome *P*450 system to convert EtOH into acetaldehyde. After chronic alcohol consumption, the activity of this system increases due to a rise in, especially, the CYP2E1 isoform, of cytochrome *P*450. The upregulation of this system contributes to alcohol tolerance, and it is likely that oxidative stress associated with the activation of the MEOS makes an important contribution to alcohol-induced liver damage.

pathway becomes more important at high alcohol levels and in regular alcohol consumers (Lieber, 1999). Acetaldehyde produced by ADHes and the MEOS is further metabolized by aldehyde dehydrogenases (ALDH) to acetate, which in turn is used for fatty acid synthesis or burned in the citric acid cycle for energy production (see Figure 1). Assuming a daily caloric intake of about 2000 kcal and a caloric content of alcohol of 7.1 kcal g^{-1}, moderate alcohol consumption of two daily standard drinks (a standard drink is 14 g of EtOH), would raise the total caloric intake by about 200 kcal or around 10% (not including additional nutrients present in many alcoholic beverages). Therefore, alcohol consumed in alcoholic beverages makes a significant contribution, even at low-to-moderate consumption levels, to our daily caloric food intake. This led to the suggestion that alcohol consumption might be a risk factor for weight gain and obesity. However, the issue whether or not alcohol consumption is associated with weight gain is still unanswered, and, in fact, epidemiological studies suggested that in many cases alcohol consumption is paradoxically associated with lower body weight (Jequier, 1999; Suter, 2005).

The EtOH metabolite acetaldehyde mediates much of the unpleasant 'side effects' and the hangover experienced after excessive alcohol consumption. Acute acetaldehyde toxicity is illustrated in individuals who carry an inactive form of the ALDH2*2 (mitochondrial aldehyde dehydrogenase 2). The ALDH2*2 polymorphism, a frequent allele in some East Asian populations, is associated with the 'flushing reaction' immediately following alcohol intake. The 'flushing reaction' is due to increased levels of acetaldehyde (Mizoi *et al*., 1979; Thomasson *et al*., 1991, 1993) and individuals carrying the inactive form of the aldehyde-degrading enzyme (ALDH2*2) show a drastically reduced risk of alcoholism (Crabb *et al*., 1993). The toxicity of acetaldehyde is 'utilized' by blockers of ALDH such as disulphiram (antabuse) and leads to acetaldehyde accumulation (Lipsky *et al*., 2001; Ramchandani *et al*., 2001) (Figure 1). An increased acetaldehyde concentration in aldehyde dehydrogenase-deficient individuals as well in alcoholic patients treated with disulphiram, causes facial flushing, tachycardia, palpitations, dizziness, nausea, vomiting and headache, and can even lead to death by acetaldehyde poisoning if heavy drinking is continued.

It seems likely that short- and long-term toxicity associated with chronic alcohol use and abuse are caused to a large extent by acetaldehyde, oxidative stress (particularly via MEOS) and $NADH_2$ (nicotinamide adenine dinucleotide (reduced form)) production that are related to alcohol and acetaldehyde metabolism (Lieber, 2005). The increased risk for cancer is probably at least in part a consequence of nucleic acid modifications by the alcohol metabolite acetaldehyde (Brooks and Theruvathu, 2005). In addition, alcoholic cardiomyopathy and liver damage are likely primarily caused by the EtOH metabolite acetaldehyde, and the oxidative stress and NAD depletion associated with alcohol metabolism (Zhang *et al*., 2004). Acetaldehyde can react nonspecifically with proteins and DNA, and it seems, therefore, likely that acute as well as chronic acetaldehyde toxicity is due to a summation of effects on numerous targets.

Alcohol dehydrogenases have a fairly broad substrate specificity and convert, besides EtOH, otherwise relatively benign alcohols such as methanol and ethylene glycol into dangerous toxicants. In the absence of ADHs, methanol and ethylene glycol are merely inebriating, and their toxicity is almost exclusively due to their catabolites: formaldehyde and formic acid in the case of methanol, and glycolate and oxalate in the case of ethylene glycol. In the past, EtOH administration was the logical choice in cases of methanol and ethylene glycol poisoning, as EtOH is the preferred (competitive) substrate for ADH. Moreover, it works because at blood levels of $\geqslant$20 mM EtOH blocks the metabolism of ethylene glycol and methanol in their usual overdose concentrations (Casavant, 2001). However, EtOH as an antidote has disadvantages: besides its own toxicity at these inebriating levels, its narrow therapeutic range combined with varying oral absorption and elimination rates necessitates frequent monitoring of blood alcohol levels (Casavant, 2001; Mycyk and Leikin, 2003). In recent years, the competitive ADH blocker fomepizole (4-methylpyrazole or 4-MP, Antizol, see Figure 1a) has been approved by the US Food and Drug Administration and is now used to aid in the management of suspected ethylene glycol and methanol poisonings (Mycyk and Leikin, 2003).

EtOH/Ro15-4513-sensitive $GABA_A$ receptor subtypes mediate important aspects of acute alcohol actions

Unlike acetaldehyde, EtOH is chemically rather inert under physiological conditions. However, the high alcohol concentrations needed for physiological effects ($\geqslant$3 mM), combined with the difficulties identifying targets that respond to relevant inebriating EtOH concentrations, has led to the view that EtOH must be a nonspecific drug, and that the intoxicating actions of alcohol are simply due to a summation of effects on numerous molecular targets (Eckardt *et al*., 1998). This view is contrasted by reports that one of the major effects of alcohol is because of enhancing the function of $GABA_A$Rs, the major inhibitory neurotransmitter receptors in mammalian brain (Nestoros, 1980; Liljequist and Engel, 1982, 1984; Engblom *et al*., 1991; Korpi, 1994; Grobin *et al*., 1998). Particularly interesting is that a $GABA_A$R ligand, the imidazobenzodiazepine Ro15-4513, originally reported as an alcohol antagonist by scientists at Hoffman-La Roche, Basel, Switzerland (Bonetti *et al*., 1985), antagonized behavioural alcohol action (Suzdak *et al*., 1986a; Lister and Nutt, 1987), as well as EtOH-induced Cl^- flux enhancement (Kolata, 1986; Suzdak *et al*., 1986a, b). The acute intoxicating effects of alcohol at a highly inebriating EtOH dose of 2 g kg^{-1} are essentially completely reversed by 3 mg kg^{-1} Ro15-4513, a dose that has little or no effect on the behaviour of these animals by itself (see Figure 2c) (Suzdak *et al*., 1986a). Evidence like this, that EtOH affects $GABA_A$Rs, was so convincing that many authors of textbooks in pharmacology listed alcohol as a drug that enhanced $GABA_A$Rs, despite conflicting observations that in many neurons, synaptic $GABA_A$Rs were enhanced by EtOH only at very high concentrations (White *et al*., 1990; Weiner *et al*.,

1997). At least part of the solution is that low doses of EtOH might be rather specific for extrasynaptic $GABA_AR$ subtypes that mediate a nonsynaptic form of 'background' inhibition in neurons (Richerson and Wu, 2003; Farrant and Nusser, 2005). Such uniquely alcohol-sensitive receptor subtypes, including extrasynaptic δ-subunit-containing $GABA_ARs$ subtypes, have been reported in recombinant expression using α4βδ receptors (Sundstrom-Poromaa *et al*., 2002; Wallner *et al*., 2003, 2006b; Hanchar *et al*., 2004, 2005). While we now have routinely expressed highly alcohol-sensitive δ-subunit-containing receptors in recombinant systems for more than 5 years, negative results published by others (Borghese *et al*., 2006) have led to controversy. It is difficult to troubleshoot experiments of others; however, the possible reasons why it is difficult to express highly alcohol-sensitive receptors are the low GABA efficacy of these types of receptors that leads to rather low current levels (Bianchi and Macdonald, 2003; Wallner *et al*., 2003). Furthermore, the α4-subunit, which together with the cerebellar α6-subunit is the main subunit found assembled with the δ-subunit in mammalian brain, has been notoriously difficult to express in recombinant systems, presumably because the α4-subunit mRNA contains inhibitory sequences in the 5′ untranslated region that leads to low levels of functional expression (M Wallner, unpublished). In addition, the reconstitution of δ-subunit-containing receptors is challenging, because of the propensity of recombinant expression systems to produce alcohol-insensitive functional $GABA_ARs$ formed by α- and β-subunits alone, without the incorporation of δ-, γ2- or ε-subunits, even if the nucleic acids that code for these subunits are coinjected or cotransfected (Boileau *et al*., 2002). Making matters worse, receptors formed by α- and β-subunits alone superficially resemble, in many of their functional properties, δ-subunit-containing receptors, although a closer inspection shows that apart from alcohol sensitivity, α4β3 and α4β3δ receptors can be distinguished by differential sensitivity to GABA, β-carboline-3-carboxylate ethyl ester (β-CCE) and Zn^{2+}. The function of δ-subunit-containing receptors is enhanced by the β-carboline, β-CCE (Adkins *et al*., 2001; Wallner *et al*., 2006b), δ-subunit-containing GABA receptors are more sensitive to GABA than those formed by α- and β-subunits alone, and αβ-receptors show substantial block by 1 μM Zn^{2+}, whereas receptors with δ-subunits are insensitive to blockade by 1 μM Zn^{2+}. Readers interested in further detail can consult a special issue available in the journal *Alcohol* (Mody *et al*., 2007; Olsen *et al*., 2007; Santhakumar *et al*., 2007).

Although technical difficulties with the expression of δ-subunit-containing receptors have led to controversy, there is now essentially a consensus that sustained/tonic GABA currents in neurons that are mediated by δ-subunit-containing $GABA_ARs$ show the same low alcohol sensitivity as recombinant αβ3δ receptors (Wei *et al*., 2004; Hanchar *et al*., 2005; Liang *et al*., 2006; Fleming *et al*., 2007; Glykys *et al*., 2007b; Mody *et al*., 2007; Santhakumar *et al*., 2007). The notion that unique types of extrasynaptic $GABA_ARs$ are low-to-moderate dose alcohol targets also explains and is consistent with many previous reports that implicated $GABA_ARs$ in mediating low-dose alcohol actions, including reports that alcohol enhancement in some neurons is reversed by Ro15-4513 (Reynolds *et al*., 1992). In particular under conditions of low [GABA] that would lead to a preferential activation of these highly GABA-sensitive extrasynaptic receptors, low-dose EtOH effects were reported (for reviews see Aguayo *et al*., 2002 and Wallner *et al*., 2006a).

About 20 years ago, the debate was whether the negative modulation of certain types of classical $GABA_ARs$ by Ro15-4513 (also known as partial inverse agonist activity) could be responsible for alcohol antagonistic actions of Ro15-4513 (Lister and Nutt, 1988), in a manner similar to actions of general $GABA_AR$ blockers such as bicuculline and picrotoxin, which block actions of $GABA_AR$ agonists, including alcohol (Liljequist and Engel, 1982). However, the finding that Ro15-4513, at alcohol antagonistic concentrations, is specific for EtOH (that is, Ro15-4513 does not reverse barbiturate actions at the same doses that reverse EtOH actions), and that other, even more efficacious inverse agonists, are not alcohol antagonists argued against the notion that negative modulation (partial inverse agonist actions) of $GABA_ARs$ by Ro15-4513 is responsible for the behavioural alcohol antagonism (Suzdak *et al*., 1988). In full support for such specific Ro15-4513 alcohol antagonist actions on $GABA_AR$ subtypes, we have recently shown that 100 nM Ro15-4513 antagonizes 3–30 mM alcohol enhancement on recombinant αβ3δ $GABA_ARs$ (Wallner *et al*., 2006b) (see Figures 2a and b) and also reverses the alcohol enhancement of tonic GABA currents in the cerebellum (Santhakumar *et al*., 2007). In addition, we showed that [^{3}H]Ro15-4513 binds to α4/6β3δ receptors and is displaced, in a competitive manner, not only by alcohol, but also by other selected BZ-site ligands (flumazenil, also known as Ro15-1788, and β-CCE) (Hanchar *et al*., 2006). Tellingly, flumazenil (the clinically used BZ antagonist) and β-CCE (an inverse agonist on classical BZ receptors without alcohol antagonist actions) both have been shown to antagonize alcohol antagonist actions of Ro15-4513 *in vivo* and *in vitro* (Suzdak *et al*., 1986b; Paul, 2006; Wallner *et al*., 2006b). Although the presence of a unique Ro15-4513/flumazenil/β-CCE/EtOH-binding site on δ-subunit-containing receptors was rather surprising, it is in agreement with an early study that showed that [^{3}H]flumazenil bound with high affinity to immunopurified δ-subunit-containing receptors (Benke *et al*., 1991). However, these detailed positive findings by Benke and Möhler were challenged and it was proposed that [^{3}H]flumazenil and [^{3}H]Ro15-4513 binding in immunoprecipitated δ-subunit-containing receptors might be explained by contamination with γ-subunit-containing receptors (Araujo *et al*., 1998). A γ-subunit in $GABA_ARs$ is required for high-affinity binding of, and modulation by, classical BZ-site agonists such as diazepam (Pritchett *et al*., 1989), and this led to the dogma that δ-subunit-containing receptors cannot have high-affinity binding sites for BZ-site ligands. However, given the large number of $GABA_AR$ isoforms with BZ-ligand-binding sites formed by 'homologous' binding pockets at subunit interfaces (that are also 'homologous' to the GABA-binding site), it may not be surprising that some BZ-site ligands also bind with high affinity and exert efficacy at sites other than the classical BZ sites at the α+/γ− subunit interfaces in $GABA_AR$ pentamers.

The identification of an alcohol/BZ site on δ-subunit-containing receptors also provides an explanation for the

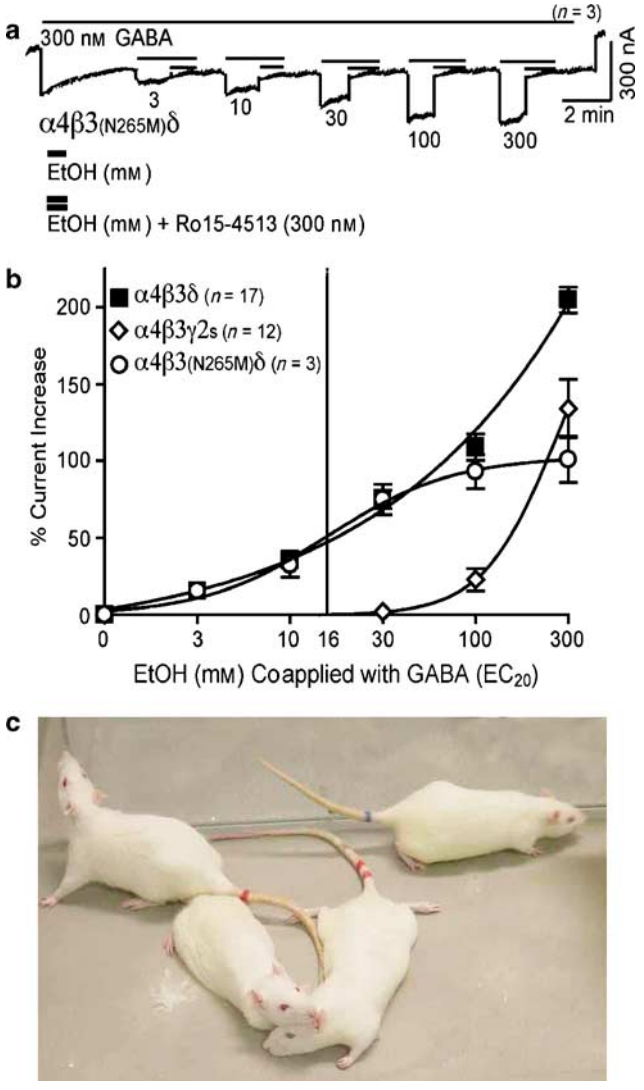


Figure 2 The alcohol antagonist Ro15-4513 (ethyl 8-azido-5, 6-dihydro-5-methyl-6-oxo-4*H*-imidazol(1,5-*a*)benzodiazepine-3-carboxylate) blocks alcohol actions *in vitro* on α4β3δ $GABA_A$ receptors ($GABA_A$Rs) expressed in *Xenopus* oocytes and *in vivo*. (**a**) Ethanol (EtOH) from 3 to 300 mm leads to a dose-dependent increase in α4β3N265Mδ currents evoked by application of 300 nM GABA (tonic current mimicking mode) that is blocked by 300 nM Ro15-4513. The β3N265M mutation eliminates EtOH actions at concentrations ⩾100 mM that are not blocked by Ro15-4513. (**b**) In contrast to α4β3δ receptors, receptors containing the mutated β3N265M subunit show EtOH saturation at ∼100 mM. The EtOH EC_{50} in α4β3N265Mδ $GABA_A$R is 16 mM, close to the legal US driving limit of 17.4 mM. Receptors in which the δ-subunit is replaced with the γ2-subunit are sensitive only to concentrations ⩾100 mM (a lethal concentration for most humans). The experiments were performed with coapplication of GABA (300 nM for α4βδ-receptors and 30 mM for α4β3γ2) with the indicated concentrations of EtOH (for original publication see Wallner *et al.*, 2006b). (**c**) Experiment to confirm and illustrate the reported reversal of user-rated intoxication by Ro15-4513 in Suzdak *et al.* (1986b). This picture shows four adult female rats 20 min after i.p. injection of 2 g kg^{-1} EtOH, with or without 3 mg kg^{-1} Ro15-4513. Two rats (middle) that received 2 g kg^{-1} EtOH alone are severely impaired and lay flat on their bellies, whereas two animals that received 3 mg kg^{-1} Ro15-4513 in addition to 2 g kg^{-1} EtOH show essentially no signs of visible intoxication (left and right).

initially quite puzzling finding that a BZ-site mutation in the cerebellar α6-subunit (α6R100Q) leads to alcohol hypersensitivity of recombinant α6β3δ-receptors, to increased EtOH sensitivity of tonic cerebellar granule cell GABA currents in slices and to increased alcohol-induced motor impairment (Hanchar *et al.*, 2005). The α6R100Q phenotype, combined with the finding that the α6100Q is a frequent allele in rats, now explains why the α6100Q allele has been enriched in three independent breeding studies that selected rats for behavioural alcohol hypersensitivity (Uusi-Oukari and Korpi, 1991; Farrant and Cull-Candy, 1993; Carr *et al.*, 2003; Sanna *et al.*, 2003). Note that the hypothesis by Dr Valenzuela and colleagues is that in cerebellar granule cells, which express α6-, β3- and δ-subunit proteins at high levels (Pirker *et al.*, 2000) and show α6βδ-receptor-mediated highly alcohol-sensitive tonic currents, the increase in tonic current seen with low dose of EtOH is entirely due to increased extracellular GABA that may result from increased spillover, due to increased firing of GABAergic synapses (Carta *et al.*, 2004). In support of this tipsy terminal hypothesis, and in contrast to published data (Hanchar *et al.*, 2005), in their hands EtOH sensitivity of tonic current is not significantly different in animals that are homozygous for the α6R100Q allele (Botta *et al.*, 2007). Readers interested in further details are referred to a rebuttal (Otis, 2008). Increases in firing frequency of GABAergic inputs with low EtOH concentrations are unique for cerebellar granule cells, where tonic GABA currents are mediated by α6βδ-receptors rather than the more prevalent and closely related α4βδ $GABA_A$R subtypes which are more prevalent. No increase in GABA release (firing frequency) are observed that may explain the EtOH sensitivity of tonic GABA currents mediated by α4βδ-receptors in dentate gyrus granule cells (Wei *et al.*, 2003; Liang *et al.*, 2006; Fleming *et al.*, 2007), or in hippocampal interneurons, where EtOH-sensitive tonic currents are mediated by α1βδ-receptors (Glykys *et al.*, 2007b).

Alcohol actions that are not sensitive to reversal by Ro15-4513

There is essentially a consensus that Ro15-4513 reverses most of the obvious signs of alcohol intoxication (see Figure 2c) at low-to-moderate alcohol doses that correspond to blood EtOH level of ⩽30 mM (about twice the US legal driving limit of 17 mM). However, Ro15-4513 becomes much less effective at very high EtOH doses, and even at low doses there are EtOH effects that are insensitive to Ro15-4513 reversal. It was shown that the reversal of hypnotic alcohol and motor-impairing behavioural alcohol effects by Ro15-4513 was not accompanied by reversal of hypothermic alcohol effects (Hoffman *et al.*, 1987; Syapin *et al.*, 1987). This is consistent with earlier studies that concluded that motor impairment, narcosis and hypothermia are mediated by genetically distinct mechanisms (Eriksson and Sarviharju, 1984), and that EtOH-induced motor incoordination, but not hypothermia, is GABA mediated (Dar and Wooles, 1985). Inwardly rectifying G protein-gated K^+ channels are appealing candidates for mediating hypothermic alcohol actions (Kobayashi *et al.*, 1999), and inwardly rectifying

G protein-gated K^+ channels also might contribute to analgesic EtOH actions (Ikeda *et al*., 2002; Blednov *et al*., 2003). In addition, sedative/hypnotic alcohol effects at doses higher than 2 g kg^{-1} i.p. in rats (equivalent to $\geqslant$30 mM blood EtOH concentration) are not completely reversed by Ro15-4513, and this is in line with reports that Ro15-4513 cannot prevent lethality at massive alcohol doses (Nutt *et al*., 1988). A large number of potential alcohol targets have been identified in *in vitro* studies that show EtOH modulation, usually at concentrations >20 mM, and these are candidates for mediating Ro15-4513-insensitive EtOH actions. These targets include (among many others) NMDA-type glutamate receptors (Danysz *et al*., 1992), glycine receptors (Aguayo and Pancetti, 1994; Davies *et al*., 2004), effects mediated by alcohol modulation of neurosteroid synthesis (Morrow *et al*., 2001) and effects on the adenosine system (Mailliard and Diamond, 2004). In addition, many voltage-dependent ion channels such as Ca^{2+}-activated K^+ channels (Feinberg-Zadek and Treistman, 2007), Ca^{2+} channels (Messing *et al*., 1986) and Na^+ channels (Klein *et al*., 2007) have been shown to be influenced by alcohol at (usually) rather high concentrations.

In addition, most $GABA_A$R subtypes are enhanced by high EtOH concentrations (>50 mM), and mutations in a transmembrane 'site' in α- or β-subunits (for example, βN265M) dramatically reduces sensitivity to >100 mM EtOH in $GABA_A$R subtypes (Mihic *et al*., 1997). In fact, we showed that highly alcohol-sensitive αβ3δ $GABA_A$R subtypes have two distinct alcohol sites: (1) a 'high-affinity site' that is sensitive to Ro15-4513 reversal and (2) a 'low-affinity site' that is eliminated by the β3N265M mutation, located in the pore-forming transmembrane segment M2 (see Figures 2a and b). Mutations at certain positions in proposed transmembrane segments of certain subunits (for example, βN265M) in $GABA_A$Rs not only drastically reduce $\geqslant$100 mM EtOH sensitivity, but also the sensitivity to the anaesthetics etomidate, propofol and pentobarbital (Benson *et al*., 1998), suggesting a common mechanism of (anaesthetic) action at high EtOH doses. Mice with the knock-in point mutation in the β3-subunit (β3N265M) lose the immobilizing effects of etomidate and propofol *in vivo* (Jurd *et al*., 2003; Zeller *et al*., 2007), demonstrating that the β3-subunit-containing receptors are important for mediating the anaesthetic effects of etomidate and propofol (Grasshoff *et al*., 2006). It was, therefore, surprising to learn that β3N265M mice show little changes in sensitivity to high alcohol doses, which suggests that the 'transmembrane' alcohol site in β3-subunits might contribute little, if at all, to acute (Ro15-4513 insensitive) EtOH actions (Sanchis-Segura *et al*., 2007). A possibility that should be tested in β2N265M knock-in mice is that β2- and/or β1-subunit-containing $GABA_A$Rs are more important than those containing the β3-subunit for Ro15-4513-insensitive high-dose EtOH actions mediated by 'transmembrane/anaesthetic' EtOH sites in $GABA_A$Rs.

In addition to these potential direct alcohol targets described above, a number of receptors might indirectly modulate acute alcohol actions; this could, for example, include mechanisms that lead to acute alcohol tolerance. Receptors that might not by themselves be alcohol targets, include, among others, metabotropic adenosine (Dar, 1993), $GABA_B$ (Wan *et al*., 1996) and metabotropic glutamate receptors (Besheer *et al*., 2006), as well as ionotropic nicotinic acetylcholine receptors (Steensland *et al*., 2007). Furthermore, consistent with the notion that many acute alcohol actions are mediated by (extrasynaptic) GABA receptors, systems responsible to maintain and regulate the extracellular [GABA] (Richerson and Wu, 2003; Wu *et al*., 2006; Glykys and Mody, 2007a) might be expected to modify behavioural EtOH actions. For example, GABA transaminase inhibitors such as amino-oxyacetic acid and vigabatrin lead to increased alcohol sensitivity (Frye and Breese, 1982; Dar and Wooles, 1985), likely because they cause increased extracellular [GABA] resulting in increased tonic GABA currents (Overstreet and Westbrook, 2001; Wu *et al*., 2003, 2007). In addition, changes in the activity of GABA transporters, either in knockouts or during blockade by GABA transporter modulators such as tiagabine, lead to changes in alcohol sensitivity (Cai *et al*., 2006), although the effects are complex, in part, due to expected compensatory changes that occur in global knockout animals and under long-term drug treatment. Further, the proposed role of GABA transporters to regulate extrasynaptic [GABA] by both forward and reverse GABA transport in a dynamic equilibrium (Gaspary *et al*., 1998; Richerson and Wu, 2003) could be another reason that effects of $GABA_A$R-specific drug actions after interventions in GABA transporter activity are difficult to interpret (Fehr *et al*., 2007).

Consistent with the notion that extrasynaptic [GABA] and extrasynaptic $GABA_A$Rs are important mediators of EtOH effects, extrasynaptic [GABA] is reduced in alcohol-treated animals (Leitch *et al*., 1977), and acute EtOH administration causes a rapid internalization of δ-subunits (Liang *et al*., 2007). Both reduced extrasynaptic [GABA] and downregulation of δ-subunit-containing receptors likely contribute to alcohol tolerance and crosstolerance to $GABA_A$R-specific agonists as well as the hyperexcitability seen after alcohol withdrawal (Cagetti *et al*., 2003; Olsen *et al*., 2005).

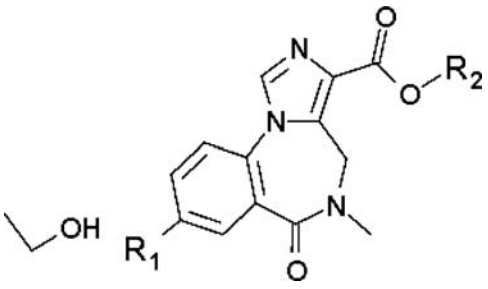
In summary, while there is converging evidence that EtOH/Ro15-4513-sensitive $GABA_A$Rs are important alcohol targets at low and moderate alcohol concentrations, effects such as EtOH-induced hypothermia and possibly analgesia are apparently not, or only partly, mediated by EtOH/Ro15-4513-sensitive alcohol sites. Also, at alcohol concentrations >30 mM, Ro15-4513-insensitive alcohol targets (or non-specific effects) are important contributors to sedative/hypnotic/anaesthetic and lethal acute EtOH actions, although the exact contributions of the many possible molecular targets for *in vivo* high-dose alcohol actions remains to be clarified.

Ro15-4513/EtOH sites as potential drug targets: alcohol antagonists

The behavioural alcohol antagonist Ro15-4513 is effective in many mammals and the high sequence conservation of mammalian $GABA_A$R orthologues makes it reasonable to assume that the Ro15-4513/EtOH sites are also conserved in humans. Consistent with this notion, we have confirmed that indeed recombinant human α4β3δ-receptors expressed

in human embryonic kidney cells are highly sensitive to EtOH, and that 30 mM EtOH actions are selectively reversed with 100 nM Ro15-4513 in a flumazenil-sensitive manner (P Meera *et al.*, unpublished). Given that other BZ-site ligands such as flumazenil and β-CCE also show high affinity for the EtOH/Ro15-4513 site and have been shown to reverse alcohol antagonism of Ro15-4513 (without acting as alcohol antagonists by themselves), it seems likely that behavioural alcohol antagonist actions of Ro15-4513 are mediated exclusively by BZ sites in $GABA_A$Rs. However, given the large number of $GABA_A$R subunits with the potential to form a large number of $GABA_A$Rs isoforms present in mammalian brain, we do not know whether δ-subunit-containing receptors are the only types of $GABA_A$Rs that mediate EtOH effects that are reversed by Ro15-4513. In fact, the observation that δ-subunit knockout animals show reduced alcohol sensitivity for some, but not all low-dose EtOH effects (Mihalek *et al.*, 2001) suggests that, as is frequently the case, there might be compensatory changes in global knockouts or that δ-subunit-containing $GABA_A$Rs may not be the only EtOH/Ro15-4513-sensitive $GABA_A$R subtypes present in mammalian brain, or both. Additional, yet to be discovered, EtOH/Ro15-4513-sensitive $GABA_A$R subtypes might provide an opportunity to target drugs to specific alcohol receptor subtypes.

Unfortunately, Ro15-4513 is not useful in humans because of its short half-life of only about 30 min and the partial inverse agonist activity at higher doses, which leads to tremor in monkeys (Miczek and Weerts, 1987). The observation that the imidazobenzodiazepines RY080, RY023 and RY024, initially developed as α5-specific partial inverse agonists (Lui *et al.*, 1996; Skolnick *et al.*, 1997), reverse behavioural alcohol actions *in vivo* in a similar way as Ro15-4513, led to speculations that inverse agonist actions on α5-subunit-containing receptors lead to alcohol antagonism (McKay *et al.*, 2004; Cook *et al.*, 2005). A comparison of the chemical structures of RY080, RY023 and RY024 reveals that these compounds are very close structural analogues of Ro15-4513 (see Figure 3 for a structural comparison). All four alcohol antagonist compounds have large bulky side chains on the C7 position of the BZ ring that we propose would occupy the alcohol-binding site and competitively displace alcohol from its 'high-affinity' binding site on αβ3δ-receptors. Our observation that these compounds displace Ro15-4513 from α4β3δ receptors (Hanchar *et al.*, 2006), suggest that the high affinity of Ro15-4513, and its close structural analogues (RY080, RY023 and RY024), to classical BZ sites located at the α5γ-subunit interface in α5-subunit-containing receptors (and the partial inverse agonist efficacy at α5-receptors) is possibly a coincidence and unrelated to their actions as alcohol antagonists. This is consistent with the observation that relatively high doses of these imidazobenzodiazepine drugs are needed for behavioural alcohol antagonism, and with a recent report that the α5-specific inverse agonist a5IA is not a general alcohol antagonist such as Ro15-4513 (Nutt *et al.*, 2007). However, Ro15-4513 and the structural analogues RY080, RY023 and RY024 are weak partial inverse agonists on abundant γ-subunit-containing $GABA_A$R subtypes, and it remains to be determined if, and how much, negative $GABA_A$R modulation contributes to alcohol antagonism.



Compound	R1	R2
Ro15-4513	$-N_3$	$-CH_2-CH_3$
Ro15-1788 (flumazenil)	-F	$-CH_2-CH_3$
RY023	$-C\equiv C-Si(CH_3)_3$	$-CH_2-CH_3$
RY024	$-C\equiv CH$	$-C(CH_3)_3$
RY080	$-C\equiv CH$	$-CH_2-CH_3$

Figure 3 Structures of the reported alcohol antagonists Ro15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol(1,5-*a*)benzodiazepine-3-carboxylate), RY024, RY023 and RY080, and that of Ro15-1788 (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazol(1,5-*a*)benzodiazepine-3-carboxylate; flumazenil). Flumazenil is not an alcohol antagonist but antagonizes the alcohol antagonist activity of Ro15-4513 in behavioural (and *in vitro*) assays. Because the only difference between flumazenil and Ro15-4513 is the identity of the R1 moiety, we propose a model where the R1 moiety at position 8 of the imidazobenzodiazepine ring of Ro15-4513 as well as RY023, RY024 and RY080 occupies the alcohol-binding pocket and therefore blocks in a competitive manner alcohol actions on α4/6β3δ-receptors. We suggest that fluorine in flumazenil is small enough so that it can fit together with ethanol (EtOH) into a side-by-side binding pocket. Note that the compounds listed are the only 'partial inverse agonists' reported to show alcohol antagonism. In fact, the rather efficacious partial inverse agonist β-carboline-3-carboxylate ethyl ester (β-CCE; a β-carboline) has been shown to block the alcohol antagonist activity of the Ro15-4513, and displaces [^{3}H]Ro15-4513 from recombinant α4β3δ-receptors (Hanchar *et al.*, 2006). Also, note that position 8 of the imidazobenzodiazepine ring is equivalent to position 7 in the benzodiazepine (BZ) ring, a position found to be close to an amino-acid residue (α1-101R and α6-100Q) critical for BZ as well as EtOH sensitivity of $GABA_A$R subtypes.

Ro15-4513 eliminates alcohol preference and alcohol self-administration in rats (Samson *et al.*, 1989; June *et al.*, 1994), suggesting the involvement of EtOH/Ro15-4513-sensitive $GABA_A$Rs in mediating the hedonic, and possibly addictive alcohol actions. Therefore, novel alcohol antagonists could hold great promise for the treatment of alcohol abuse and alcoholism. In addition, given the high incidence of alcohol intoxication in emergency rooms, a combined alcohol/BZ antagonist with longer half-life might be useful, despite the expected limitations of such novel potential alcohol antagonists active on the EtOH/Ro15-4513 receptors in reversing very high-dose alcohol intoxication. In addition, alcohol antagonists with longer half-life and without inverse agonist side effects seen with Ro15-4513 and related compounds could be tremendously useful for future alcohol research, for example, to distinguish alcohol effects on Ro15-4513-insensitive from those on Ro15-4513-sensitive alcohol targets. This might help, for example, to find the molecular

targets and mechanism that mediate the beneficial effects of long-term low-to-moderate alcohol consumption discussed above, research that could have tremendous consequences for public health.

Whether alcohol antagonist drugs should be made publicly available, for example, to allow people to drive home safely after an evening of indulging, not only will depend on their efficacy and safety, but also on legislation that would redefine legal alcohol intoxication by criteria other than blood alcohol levels.

Ro15-4513/EtOH sites as potential drug targets: alcohol mimetics (Synthehol)

In humans, alcohol 'self-administration' rarely leads to blood alcohol levels that exceed levels where alcohol antagonists such as Ro15-4513 would be effective in reversing the motor-in-coordinating, sedative, anxiolytic and rewarding effects (about 30 mM blood alcohol concentrations or about twice the US legal driving limit, see Figure 2c). Conversely, one might expect that a drug that binds with high affinity and specificity to the EtOH/Ro15-4513 site, and possesses alcohol-like efficacy might be rather effective in mimicking effects of acute alcohol effects seen at low-to-moderate EtOH doses. Such an alcohol-mimetic drug would not only be without the aversive and toxic effects of the alcohol metabolite acetaldehyde and alcohol metabolism, but as a drug taken in milligram quantities, would also lack the caloric 'value' of alcohol consumption. In addition, it seems likely that such alcohol mimetics as BZ-site allosteric-positive modulators could be safer than alcohol itself, because lethal toxicity that appears associated with Ro15-4513-insensitive alcohol actions might be missing. Furthermore, in a similar way as flumazenil is reported to reverse the alcohol antagonism of Ro15-4513, flumazenil (FDA approved and available in the clinic) would displace such novel alcohol mimetics from their site of action, and thereby pharmacologically reverse their actions. In some ways, synthetic alcohol mimetics might therefore be expected to resemble the fictional alcohol substitute 'Synthehol' of the TV series Star Trek; Synthehol intoxication is said to lack hangovers and is readily reversible. Speculations about such synthetic alcohol mimetics have been made recently, with some emphasis on the notion that inverse agonism on the α5-subunit-containing $GABA_A$Rs and inverse agonist activity on these receptor subtypes are responsible for alcohol antagonism by the imidazobenzodiazepines Ro15-4513 (Nutt, 2006). However, as discussed above, the relatively high doses of Ro15-4513, as well as the specific alcohol antagonism that involves a competitive mechanism on $GABA_A$R subtypes, argues against the notion that alcohol antagonism is due to specific inverse agonist actions on high-affinity ($K_d < 1$ nM) α5-subunit-containing receptors, but agrees with the previous conclusion that Ro15-4513 specifically reverses important alcohol actions (Suzdak *et al.*, 1988).

An important question is how addictive synthetic alcohol mimetics might be, considering that they might activate the reward pathway in a similar way as alcohol, but without the 'aversion break' that is associated with acetaldehyde formation. Against the notion that such drugs would be highly addictive, one could posit that the majority of individuals who consume alcohol do not show physical dependence and/or become addicted. However, the addictive potential of such alcohol-mimetic drugs is certainly an issue that would benefit from further insights into receptor subtypes and neuronal pathways that mediate rewarding alcohol actions.

Pharmacological research has long focused on finding specific BZ-site ligands that might only bind to and show agonist efficacy on certain subtypes of classical BZ receptors. In particular, there is hope to find selective anxiolytic drugs, possibly specific for α2γ2-subunit-containing $GABA_A$Rs (Low *et al.*, 2000). As mentioned above, there is evidence that δ-subunit-containing receptors might only be a subfraction of highly alcohol/Ro15-4513-sensitive receptors. While this incomplete knowledge of EtOH/Ro15-4513-sensitive $GABA_A$Rs may currently limit our understanding of *in vivo* alcohol actions on these receptors, it provides hope that in the future we might be able to target alcohol-mimetic compounds to specific EtOH/Ro15-4513-sensitive receptor subtypes. This might allow to specifically mimic, for example, anxiolytic, sedative mood-elevating and anti-convulsive alcohol actions while hopefully being able to avoid addictive and motor-in-coordinating 'side effects'. In addition, alcohol is effective in reducing essential tremor in patients (Klebe *et al.*, 2005; Lorenz and Deuschl, 2007), and therefore alcohol-mimetic compounds might be useful as antitremor medications.

As mentioned above, there are a number of expected efficacies (anxiolysis, sedation, anticonvulsive and anti-depressive) that might make drugs that mimic alcohol effects useful. Whether such drugs could ever replace alcohol for recreational use, not only will depend on the safety and efficacy of such potential drugs, but also if authorities, such as the EMEA (European Medicines Agency) or FDA (US Food and Drug Administration), would approve such drugs. Clearly, there are, besides issues of pharmacology and drug development, a number of psychosocial, legal and moral issues. Perhaps the most likely scenario is that alcohol-mimetic compounds would be developed for indications, such as tremor, anxiety or as anticonvulsant drugs. Once such drugs are established as safe and efficacious, and their addictive potential can be evaluated, maybe then societies can consider them as potentially healthier alternatives to 'good old booze' and make them, like alcohol, available for recreational purposes.

Summary

Given the simple structure and the high concentrations of EtOH that are needed for intoxication, it is not surprising that no single molecular mechanism can explain all the pleiotropic effects that alcohol consumption has on the human body. In fact, many toxic effects of alcohol on our body are actually not mediated by alcohol itself but by alcohol metabolism and alcohol metabolites such as acetaldehyde. In this review, we suggest that acute alcohol effects in mammals should be separated into effects that are reversed by particular types of imidazobenzodiazepine alcohol antagonists (Ro15-4513, RY023, RY024 and RY080)

and those that cannot be reversed by alcohol antagonists. Alcohol effects reversed by the imidazobenzodiazepine alcohol antagonist are likely mediated through subtypes of $GABA_A$Rs such as $\alpha4/6\beta3\delta$ receptors, whereas Ro15-4513-insensitive alcohol actions apparently involve a number of different alcohol targets, which may include $GABA_A$Rs. Finally, we discuss the usefulness of novel alcohol antagonists as well as alcohol mimetics that could specifically target EtOH/Ro15-4513-sensitive $GABA_A$Rs, and how these could be used to develop novel drugs with unique anxiolytic, sedative and anticonvulsive properties as well as potential treatments of alcohol abuse and alcoholism.

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References

Adkins CE, Pillai GV, Kerby J, Bonnert TP, Haldon C, McKernan RM *et al.* (2001). $\alpha_4\beta_3\delta$ $GABA_A$ receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. *J Biol Chem* **276**: 38934–38939.

Aguayo LG, Pancetti FC (1994). Ethanol modulation of GABA- and glycine-activated Cl^- current in cultured mouse neurons. *J Pharmacol Exp Ther* **270**: 61–69.

Aguayo LG, Peoples RW, Yeh HH, Yevenes GE (2002). $GABA_A$ receptors as molecular sites of ethanol action. Direct or indirect actions? *Curr Top Med Chem* **2**: 869–885.

Araujo F, Ruano D, Vitorica J (1998). Absence of association between delta and gamma2 subunits in native GABA(A) receptors from rat brain. *Eur J Pharmacol* **347**: 347–353.

Benke D, Mertens S, Trzeciak A, Gillessen D, Möhler H (1991). Identification and immunohistochemical mapping of $GABA_A$ receptor subtypes containing the delta-subunit in rat brain. *FEBS Lett* **283**: 145–149.

Benson JA, Low K, Keist R, Möhler H, Rudolph U (1998). Pharmacology of recombinant $GABA_A$ receptors rendered diazepam-insensitive by point-mutated α-subunits. *FEBS Lett* **431**: 400–404.

Besheer J, Stevenson RA, Hodge CW (2006). mGlu5 receptors are involved in the discriminative stimulus effects of self-administered ethanol in rats. *Eur J Pharmacol* **551**: 71–75.

Bianchi MT, Macdonald RL (2003). Neurosteroids shift partial agonist activation of $GABA_A$ receptor channels from low- to high-efficacy gating patterns. *J Neurosci* **23**: 10934–10943.

Blednov YA, Stoffel M, Alva H, Harris RA (2003). A pervasive mechanism for analgesia: activation of GIRK2 channels. *Proc Natl Acad Sci USA* **100**: 277–282.

Boffetta P, Hashibe M, La Vecchia C, Zatonski W, Rehm J (2006). The burden of cancer attributable to alcohol drinking. *Int J Cancer* **119**: 884–887.

Boileau AJ, Baur R, Sharkey LM, Sigel E, Czajkowski C (2002). The relative amount of cRNA coding for $\gamma2$ subunits affects stimulation by benzodiazepines in $GABA_A$ receptors expressed in *Xenopus* oocytes. *Neuropharmacology* **43**: 695–700.

Bonetti EP, Burkhard WP, Gabl M, Möhler H (1985). A partial inverse benzodiazepine agonist Ro15-4513 antagonizes acute ethanol effects in mice and rats. *Br J Pharmacol* **86**: 463P.

Borghese CM, Storustovu S, Ebert B, Herd MB, Belelli D, Lambert JJ *et al.* (2006). The delta subunit of $GABA_A$ receptors does not confer sensitivity to low concentrations of ethanol. *J Pharmacol Exp Ther* **316**: 1360–1368.

Botta P, Mameli M, Floyd KL, Radcliffe RA, Valenzuela CF (2007). Ethanol sensitivity of GABAergic currents in cerebellar granule neurons is not increased by a single amino acid change (R100Q) in the alpha6 GABAA receptor subunit. *J Pharmacol Exp Ther* **323**: 684–691.

Brooks PJ, Theruvathu JA (2005). DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. *Alcohol* **35**: 187–193.

Cabot R (1904). The relation of alcohol to arteriosclerosis. *JAMA* **43**: 774–775.

Cagetti E, Liang J, Spigelman I, Olsen RW (2003). Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of $GABA_A$ receptors. *Mol Pharmacol* **63**: 53–64.

Cai YQ, Cai GQ, Liu GX, Cai Q, Shi JH, Shi J *et al.* (2006). Mice with genetically altered GABA transporter subtype I (GAT1) expression show altered behavioral responses to ethanol. *J Neurosci Res* **84**: 255–267.

Carr LG, Spence JP, Peter Eriksson CJ, Lumeng L, Li TK (2003). AA and ANA rats exhibit the R100Q mutation in the $GABA_A$ receptor $\alpha6$ subunit. *Alcohol* **31**: 93–97.

Carta M, Mameli M, Valenzuela CF (2004). Alcohol enhances GABAergic transmission to cerebellar granule cells via an increase in Golgi cell excitability. *J Neurosci* **24**: 3746–3751.

Casavant MJ (2001). Fomepizole in the treatment of poisoning. *Pediatrics* **107**: 170.

Cook JB, Foster KL, Eiler II WJ, McKay PF, Woods II J, Harvey SC *et al.* (2005). Selective $GABA_A$ $\alpha5$ benzodiazepine inverse agonist antagonizes the neurobehavioral actions of alcohol. *Alcohol Clin Exp Res* **29**: 1390–1401.

Crabb DW, Dipple KM, Thomasson HR (1993). Alcohol sensitivity, alcohol metabolism, risk of alcoholism, and the role of alcohol and aldehyde dehydrogenase genotypes. *J Lab Clin Med* **122**: 234–240.

Danysz W, Dyr W, Jankowska E, Glazewski S, Kostowski W (1992). The involvement of NMDA receptors in acute and chronic effects of ethanol. *Alcohol Clin Exp Res* **16**: 499–504.

Dar MS (1993). Brain adenosinergic modulation of acute ethanol-induced motor impairment. *Alcohol Alcohol Suppl* **2**: 425–429.

Dar MS, Wooles WR (1985). GABA mediation of the central effects of acute and chronic ethanol in mice. *Pharmacol Biochem Behav* **22**: 77–84.

Davies DL, Crawford DK, Trudell JR, Mihic SJ, Alkana RL (2004). Multiple sites of ethanol action in alpha1 and alpha2 glycine receptors suggested by sensitivity to pressure antagonism. *J Neurochem* **89**: 1175–1185.

Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL *et al.* (1998). Effects of moderate alcohol consumption on the central nervous system. *Alcohol Clin Exp Res* **22**: 998–1040.

Engblom AC, Holopainen I, Akerman KE (1991). Ethanol-induced Cl^- flux in rat cerebellar granule cells as measured by a fluorescent probe. *Brain Res* **568**: 55–60.

Eriksson CJ, Sarviharju M (1984). Motor impairment, narcosis and hypothermia by ethanol: separate genetic mechanisms. *Alcohol* **1**: 59–62.

Farrant M, Cull-Candy S (1993). GABA receptors, granule cells and genes. *Nature* **361**: 302–303.

Farrant M, Nusser Z (2005). Variations on an inhibitory theme: phasic and tonic activation of $GABA_A$ receptors. *Nat Rev Neurosci* **6**: 215–229.

Fehr C, Hohmann N, Grunder G, Dielentheis TF, Buchholz HG, Chechko N *et al.* (2007). Tiagabine does not attenuate alcohol-induced activation of the human reward system. *Psychopharmacology (Berl)* **191**: 975–983.

Feinberg-Zadek PL, Treistman SN (2007). Beta-subunits are important modulators of the acute response to alcohol in human BK channels. *Alcohol Clin Exp Res* **31**: 737–744.

Fillmore KM, Kerr WC, Stockwell T, Chikritzhs T, Bostrom A (2006). Moderate alcohol use and reduced mortality risk: systematic error in prospective studies. *Addiction Res Theor* **14**: 101–132.

Fleming M, Mihic SJ, Harris RA (2005). III. Drugs acting on the central nervous system; Chapter 22. Ethanol. In: Brunton LL, Lazo JS, Parker KL (eds). *Goodman & Gilman's Pharmacology*, 11th edn. The McGraw-Hill Companies Inc.: San Diego, CA.

Fleming RL, Wilson WA, Swartzwelder HS (2007). Magnitude and ethanol sensitivity of tonic $GABA_A$ receptor-mediated inhibition in dentate gyrus changes from adolescence to adulthood. *J Neurophysiol* **97**: 3806–3811.

Friedman GD, Klatsky AL (1993). Is alcohol good for your health? *N Engl J Med* **329**: 1882–1883.

Frye GD, Breese GR (1982). GABAergic modulation of ethanol-induced motor impairment. *J Pharmacol Exp Ther* **223**: 750–756.

Gaspary HL, Wang W, Richerson GB (1998). Carrier-mediated GABA release activates GABA receptors on hippocampal neurons. *J Neurophysiol* **80**: 270–281.

Glykys J, Mody I (2007a). The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. *J Physiol* **582**: 1163–1178.

Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I (2007b). A new naturally occurring $GABA_A$ receptor subunit partnership with high sensitivity to ethanol. *Nat Neurosci* **10**: 40–48.

Grasshoff C, Drexler B, Rudolph U, Antkowiak B (2006). Anaesthetic drugs: linking molecular actions to clinical effects. *Curr Pharm Des* **12**: 3665–3679.

Grobin AC, Matthews DB, Devaud LL, Morrow AL (1998). The role of $GABA_A$ receptors in the acute and chronic effects of ethanol. *Psychopharmacology (Berl)* **139**: 2–19.

Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M *et al.* (2006). Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to $\alpha_{4/6}\beta_3\delta$ $GABA_A$ receptors. *Proc Natl Acad Sci USA* **103**: 8546–8550.

Hanchar HJ, Dodson PD, Olsen RW, Otis TS, Wallner M (2005). Alcohol-induced motor impairment caused by increased extrasynaptic $GABA_A$ receptor activity. *Nat Neurosci* **8**: 339–345.

Hanchar HJ, Wallner M, Olsen RW (2004). Alcohol effects on $GABA_A$ receptors: are extrasynaptic receptors the answer? *Life Sci* **76**: 1–8.

Hoffman PL, Tabakoff B, Szabo G, Suzdak PD, Paul SM (1987). Effect of an imidazobenzodiazepine, Ro15-4513, on the incoordination and hypothermia produced by ethanol and pentobarbital. *Life Sci* **41**: 611–619.

Ikeda K, Kobayashi T, Kumanishi T, Yano R, Sora I, Niki H (2002). Molecular mechanisms of analgesia induced by opioids and ethanol: is the GIRK channel one of the keys? *Neurosci Res* **44**: 121–131.

Jequier E (1999). Alcohol intake and body weight: a paradox. *Am J Clin Nutr* **69**: 173–174.

June HL, Hughes RW, Spurlock HL, Lewis MJ (1994). Ethanol self-administration in freely feeding and drinking rats: effects of Ro15-4513 alone, and in combination with Ro15-1788 (flumazenil). *Psychopharmacology (Berl)* **115**: 332–339.

Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F *et al.* (2003). General anesthetic actions *in vivo* strongly attenuated by a point mutation in the $GABA_A$ receptor β3 subunit. *FASEB J* **17**: 250–252.

Klatsky AL (2001). Commentary: could abstinence from alcohol be hazardous to your health? *Int J Epidemiol* **30**: 739–742.

Klatsky AL (2003). Drink to your health? *Sci Am* **288**: 74–81.

Klebe S, Stolze H, Grensing K, Volkmann J, Wenzelburger R, Deuschl G (2005). Influence of alcohol on gait in patients with essential tremor. *Neurology* **65**: 96–101.

Klein G, Gardiwal A, Schaefer A, Panning B, Breitmeier D (2007). Effect of ethanol on cardiac single sodium channel gating. *Forensic Sci Int* **171**: 131–135.

Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T *et al.* (1999). Ethanol opens G-protein-activated inwardly rectifying K^+ channels. *Nat Neurosci* **2**: 1091–1097.

Kolata G (1986). New drug counters alcohol intoxication. *Science* **234**: 1198–1199.

Korpi ER (1994). Role of $GABA_A$ receptors in the actions of alcohol and in alcoholism: recent advances. *Alcohol Alcohol* **29**: 115–129.

Leitch GJ, Backes DJ, Siegman FS, Guthrie GD (1977). Possible role of GABA in the development of tolerance to alcohol. *Experientia* **33**: 496–498.

Liang J, Suryanarayanan A, Abriam A, Snyder B, Olsen RW, Spigelman I (2007). Mechanisms of reversible $GABA_A$ receptor plasticity after ethanol intoxication. *J Neurosci* **27**: 12367–12377.

Liang J, Zhang N, Cagetti E, Houser CR, Olsen RW, Spigelman I (2006). Chronic intermittent ethanol-induced switch of ethanol actions from extrasynaptic to synaptic hippocampal $GABA_A$ receptors. *J Neurosci* **26**: 1749–1758.

Lieber CS (1988). Biochemical and molecular basis of alcohol-induced injury to liver and other tissues. *N Engl J Med* **319**: 1639–1650.

Lieber CS (1999). Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998)—a review. *Alcohol Clin Exp Res* **23**: 991–1007.

Lieber CS (2005). Metabolism of alcohol. *Clin Liver Dis* **9**: 1–35.

Liljequist S, Engel J (1982). Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. *Psychopharmacology (Berl)* **78**: 71–75.

Liljequist S, Engel JA (1984). The effects of GABA and benzodiazepine receptor antagonists on the anti-conflict actions of diazepam or ethanol. *Pharmacol Biochem Behav* **21**: 521–525.

Lipsky JJ, Shen ML, Naylor S (2001). *In vivo* inhibition of aldehyde dehydrogenase by disulfiram. *Chem Biol Interact* **130–132**: 93–102.

Lister RG, Nutt DJ (1987). Is Ro15-4513 a specific alcohol antagonist? *Trends Neurosci* **10**: 223–225.

Lister RG, Nutt DJ (1988). Ro15-4513 and its interaction with ethanol. *Adv Alcohol Subst Abuse* **7**: 119–123.

Lorenz D, Deuschl G (2007). Update on pathogenesis and treatment of essential tremor. *Curr Opin Neurol* **20**: 447–452.

Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA *et al.* (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* **290**: 131–134.

Lui R, Hu RJ, Zhang H, Skolnick P, Cook JM (1996). Synthesis and pharmacological properties of novel 8-substituted imidazobenzodiazepines: high-affinity, selective probes for α5-containing $GABA_A$ receptors. *J Med Chem* **39**: 1928–1934.

Mailliard WS, Diamond I (2004). Recent advances in the neurobiology of alcoholism: the role of adenosine. *Pharmacol Ther* **101**: 39–46.

McKay PF, Foster KL, Mason D, Cummings R, Garcia M, Williams LS *et al.* (2004). A high affinity ligand for $GABA_A$-receptor containing α5 subunit antagonizes ethanol's neurobehavioral effects in Long-Evans rats. *Psychopharmacology (Berl)* **172**: 455–462.

Messing RO, Carpenter CL, Diamond I, Greenberg DA (1986). Ethanol regulates calcium channels in clonal neural cells. *Proc Natl Acad Sci USA* **83**: 6213–6215.

Miczek KA, Weerts EM (1987). Seizures in drug-treated animals. *Science* **235**: 1127–1128.

Mihalek RM, Bowers BJ, Wehner JM, Kralic JE, VanDoren MJ, Morrow AL *et al.* (2001). $GABA_A$-receptor δ subunit knockout mice have multiple defects in behavioral responses to ethanol. *Alcohol Clin Exp Res* **25**: 1708–1718.

Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE *et al.* (1997). Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. *Nature* **389**: 385–389.

Mizoi Y, Ijiri I, Tatsuno Y, Kijima T, Fujiwara S, Adachi J *et al.* (1979). Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. *Pharmacol Biochem Behav* **10**: 303–311.

Mody I, Glykys J, Wei W (2007). A new meaning for 'Gin & Tonic': tonic inhibition as the target for ethanol action in the brain. *Alcohol* **41**: 145–153.

Morrow AL, VanDoren MJ, Fleming R, Penland S (2001). Ethanol and neurosteroid interactions in the brain. *Int Rev Neurobiol* **46**: 349–377.

Mukamal KJ, Conigrave KM, Mittleman MA, Camargo Jr CA, Stampfer MJ, Willett WC *et al.* (2003). Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. *N Engl J Med* **348**: 109–118.

Mycyk MB, Leikin JB (2003). Antidote review: fomepizole for methanol poisoning. *Am J Ther* **10**: 68–70.
Nestoros JN (1980). Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. *Science* **209**: 708–710.
Nutt DJ (2006). Alcohol alternatives—a goal for psychopharmacology? *J Psychopharmacol* **20**: 318–320.
Nutt DJ, Besson M, Wilson SJ, Dawson GR, Lingford-Hughes AR (2007). Blockade of alcohol's amnestic activity in humans by an α5 subtype benzodiazepine receptor inverse agonist. *Neuropharmacology* **53**: 810–820.
Nutt DJ, Lister RG, Rusche D, Bonetti EP, Reese RE, Rufener R (1988). Ro15-4513 does not protect rats against the lethal effects of ethanol. *Eur J Pharmacol* **151**: 127–129.
Olsen RW, Hanchar HJ, Meera P, Wallner M (2007). $GABA_A$ receptor subtypes: the 'one glass of wine' receptors. *Alcohol* **41**: 201–209.
Olsen RW, Liang J, Cagetti E, Spigelman I (2005). Plasticity of $GABA_A$ receptors in brains of rats treated with chronic intermittent ethanol. *Neurochem Res* **30**: 1579–1588.
Otis T (2008). Comment on 'Ethanol sensitivity of GABAergic currents in cerebellar granule neurons is not increased by a single amino acid change (R100Q) in the α6 $GABA_A$ receptor subunit'. *J Pharmacol Exp Ther* **324**: 399–400.
Overstreet LS, Westbrook GL (2001). Paradoxical reduction of synaptic inhibition by vigabatrin. *J Neurophysiol* **86**: 596–603.
Paul SM (2006). Alcohol-sensitive GABA receptors and alcohol antagonists. *Proc Natl Acad Sci USA* **103**: 8307–8308.
Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000). $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* **101**: 815–850.
Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR *et al.* (1989). Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. *Nature* **338**: 582–585.
Ramchandani VA, Bosron WF, Li TK (2001). Research advances in ethanol metabolism. *Pathol Biol (Paris)* **49**: 676–682.
Reynolds JN, Prasad A, MacDonald JF (1992). Ethanol modulation of GABA receptor-activated Cl^- currents in neurons of the chick, rat and mouse central nervous system. *Eur J Pharmacol* **224**: 173–181.
Richerson GB, Wu Y (2003). Dynamic equilibrium of neurotransmitter transporters: not just for reuptake anymore. *J Neurophysiol* **90**: 1363–1374.
Room R, Babor T, Rehm J (2005). Alcohol and public health. *Lancet* **365**: 519–530.
Samson HH, Haraguchi M, Tolliver GA, Sadeghi KG (1989). Antagonism of ethanol-reinforced behavior by the benzodiazepine inverse agonists Ro15-4513 and FG 7142: relation to sucrose reinforcement. *Pharmacol Biochem Behav* **33**: 601–608.
Sanchis-Segura C, Cline B, Jurd R, Rudolph U, Spanagel R (2007). Etomidate and propofol-hyposensitive $GABA_A$ receptor β3(N265M) mice show little changes in acute alcohol sensitivity but enhanced tolerance and withdrawal. *Neurosci Lett* **416**: 275–278.
Sanna A, Congeddu E, Porcella A, Saba L, Pistis M, Peis M *et al.* (2003). Characterization of wild-type (R100R) and mutated (Q100Q) $GABA_A$ α6 subunit in Sardinian alcohol non-preferring rats (sNP). *Brain Res* **967**: 98–105.
Santhakumar V, Wallner M, Otis T (2007). Ethanol acts directly on extrasynaptic subtypes of $GABA_A$ receptors to increase tonic inhibition. *Alcohol* **41**: 211–221.
Skolnick P, Hu RJ, Cook CM, Hurt SD, Trometer JD, Liu R *et al.* (1997). [^{3}H]RY 80: a high-affinity, selective ligand for $GABA_A$ receptors containing α5 subunits. *J Pharmacol Exp Ther* **283**: 488–493.
Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE (2007). Varenicline, an α4β2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc Natl Acad Sci USA* **104**: 12518–12523.
Sundstrom-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A *et al.* (2002). Hormonally regulated $\alpha_4\beta_2\delta$ $GABA_A$ receptors are a target for alcohol. *Nat Neurosci* **5**: 721–722.
Suter PM (2005). Is alcohol consumption a risk factor for weight gain and obesity? *Crit Rev Clin Lab Sci* **42**: 197–227.
Suzdak PD, Glowa JR, Crawley JN, Schwartz RD, Skolnick P, Paul SM (1986a). A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science* **234**: 1243–1247.
Suzdak PD, Paul SM, Crawley JN (1988). Effects of Ro15-4513 and other benzodiazepine receptor inverse agonists on alcohol-induced intoxication in the rat. *J Pharmacol Exp Ther* **245**: 880–886.
Suzdak PD, Schwartz RD, Skolnick P, Paul SM (1986b). Ethanol stimulates gamma-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. *Proc Natl Acad Sci USA* **83**: 4071–4075.
Syapin PJ, Gee KW, Alkana RL (1987). Ro15-4513 differentially affects ethanol-induced hypnosis and hypothermia. *Brain Res Bull* **19**: 603–605.
Szmitko PE, Verma S (2005). Cardiology patient pages. Red wine and your heart. *Circulation* **111**: e10–e11.
Thomasson HR, Crabb DW, Edenberg HJ, Li TK (1993). Alcohol and aldehyde dehydrogenase polymorphisms and alcoholism. *Behav Genet* **23**: 131–136.
Thomasson HR, Edenberg HJ, Crabb DW, Mai XL, Jerome RE, Li TK *et al.* (1991). Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet* **48**: 677–681.
Uusi-Oukari M, Korpi ER (1991). Specific alterations in the cerebellar $GABA_A$ receptors of an alcohol-sensitive ANT rat line. *Alcohol Clin Exp Res* **15**: 241–248.
Vallee BL (1998). Alcohol in the western world. *Sci Am* **278**: 80–85.
Wallner M, Hanchar HJ, Olsen RW (2003). Ethanol enhances α4β3δ and α6β3δ $GABA_A$ receptors at low concentrations known to affect humans. *Proc Natl Acad Sci USA* **100**: 15218–15223.
Wallner M, Hanchar HJ, Olsen RW (2006a). Low dose acute alcohol effects on $GABA_A$ receptor subtypes. *Pharmacol Ther* **112**: 513–528.
Wallner M, Hanchar HJ, Olsen RW (2006b). Low dose alcohol actions on α4β3δ $GABA_A$ receptors are reversed by the behavioral alcohol antagonist Ro15-4513. *Proc Natl Acad Sci USA* **103**: 8540–8545.
Wan FJ, Berton F, Madamba SG, Francesconi W, Siggins GR (1996). Low ethanol concentrations enhance GABAergic inhibitory post-synaptic potentials in hippocampal pyramidal neurons only after block of $GABA_B$ receptors. *Proc Natl Acad Sci USA* **93**: 5049–5054.
Wei W, Faria LC, Mody I (2004). Low ethanol concentrations selectively augment the tonic inhibition mediated by δ subunit-containing $GABA_A$ receptors in hippocampal neurons. *J Neurosci* **24**: 8379–8382.
Wei W, Zhang N, Peng Z, Houser CR, Mody I (2003). Perisynaptic localization of δ subunit-containing $GABA_A$ receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci* **23**: 10650–10661.
Weiner JL, Gu C, Dunwiddie TV (1997). Differential ethanol sensitivity of subpopulations of $GABA_A$ synapses onto rat hippocampal CA1 pyramidal neurons. *J Neurophysiol* **77**: 1306–1312.
White G, Lovinger DM, Weight FF (1990). Ethanol inhibits NMDA-activated current but does not alter GABA-activated current in an isolated adult mammalian neuron. *Brain Res* **507**: 332–336.
Wu Y, Wang W, Diez-Sampedro A, Richerson GB (2007). Nonvesicular inhibitory neurotransmission via reversal of the GABA transporter GAT-1. *Neuron* **56**: 851–855.
Wu Y, Wang W, Richerson GB (2003). Vigabatrin induces tonic inhibition via GABA transporter reversal without increasing vesicular GABA release. *J Neurophysiol* **89**: 2021–2034.
Wu Y, Wang W, Richerson GB (2006). The transmembrane sodium gradient influences ambient GABA concentration by altering the equilibrium of GABA transporters. *J Neurophysiol* **96**: 2425–2436.
Zakhari S, Gordis E (1999). Moderate drinking and cardiovascular health. *Proc Assoc Am Physicians* **111**: 148–158.
Zeller A, Arras M, Jurd R, Rudolph U (2007). Mapping the contribution of β3-containing $GABA_A$ receptors to volatile and intravenous general anesthetic actions. *BMC Pharmacol* **7**: 2.
Zernig G, Saria A, Kurz M, O'Malley SS (eds) (2000). *Handbook of Alcoholism*. CRC Press: Boca Raton, FL.
Zhang X, Li SY, Brown RA, Ren J (2004). Ethanol and acetaldehyde in alcoholic cardiomyopathy: from bad to ugly en route to oxidative stress. *Alcohol* **32**: 175–186.