Alcohol-sensitive GABA receptors and alcohol antagonists

Steven M. Paul*
Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

Alcohol is one of the oldest and most widely used and abused of all psychoactive drugs. Although alcohol ingestion impacts most organ systems, its effects on the brain are of considerable interest, given alcohol’s many neuropharmacological actions, including its intoxicating, sedative, anxiolytic, reinforcing, and addictive properties (1). However, elucidating the cellular and molecular targets for alcohol’s important pharmacological actions has proven challenging. Alcohol has a relatively simple chemical structure, it has pleiotropic effects in disordering membrane lipids and proteins, and relatively high clinically relevant (5–30 mM) tissue concentrations are required for its actions. Thus, it is unlikely that any single molecular mechanism (or target for that matter) will explain all of the relevant pharmacology of this important drug. Despite these caveats, research over the past two decades has identified a number of potential alcohol targets in brain, including various G protein-coupled receptors and ligand-gated ion channels (1). In some cases, alcohol has been shown to modify these targets at pharmacologically relevant concentrations; however, with few exceptions, the concentrations (or doses) of alcohol studied have been well above those that cause acute intoxication in animals, including humans. Despite considerable work in this area, two important questions remain largely unanswered. Can any of the clinically relevant neuropharmacological actions of alcohol be attributed to a direct interaction with one or more “specific” protein targets, such as a receptor or ion channel? If so, can the neuropharmacological actions of alcohol be mimicked, modified, or even blocked by a much more specific drug acting at this same target(s)? In this issue of PNAS, Hanchar et al. (2) and Wallner et al. (3) provide compelling data for a rather specific and pharmacologically relevant alcohol binding site on a subtype of GABA_A receptor composed of α4/β3δ subunits. Remarkably, they show that the behavioral alcohol antagonist Ro15–4513 binds to the same (or overlapping) site and competes with and antagonizes the actions of low to moderate concentrations of alcohol in potentiating GABA-induced Cl− currents. Thus, the authors provide exciting new evidence for a highly specific interaction of alcohol with a subtype of GABA_A receptor that may mediate (at least in part) some of this drug’s most important behavioral effects.

Alcohol and GABA

GABAergic neurotransmission and GABA_A receptors in particular have long been implicated in mediating at least some of the pharmacological actions of alcohol (1). GABA_A receptors are also the molecular targets for benzodiazepines and anesthetic barbiturates (4), both of which share neuropharmacological properties and show cross-tolerance and cross-dependence with alcohol (1). Despite much circumstantial in vivo evidence, demonstrating consistent direct effects of alcohol on GABA_A receptors, especially at clinically relevant concentrations, has proven problematic (5). In the mid-1980s, several laboratories including my own (6, 7), using an in vitro biochemical assay that measures GABA_A receptor-mediated 36Cl− flux in synaptoneurosomes, showed that alcohol potentiates GABA_A receptor activity at low (<20 mM) intoxicating concentrations (6). We also found that a novel imidazobenzodiazepine, Ro15–4513, completely blocked the ability of alcohol (but not pentobarbital) to enhance GABA_A receptor-mediated 36Cl− flux (8). Our work on this compound was prompted by earlier work by scientists at Roche demonstrating that Ro15–4513 had unique “anti-alcohol” properties in vivo (9, 10). We extended these findings and also observed that Ro15–4513 possessed potent anti-alcohol actions in vivo that could be differentiated from other benzodiazepine receptor antagonists and inverse agonists (11). In fact, in our hands, benzodiazepine receptor antagonists and most inverse agonists actually blocked the anti-alcohol actions of Ro15–4513 (11). However, it was also clear from these early studies that Ro15–4513 blocks only some of the behavioral effects of low to moderate doses of ethanol (8–12) and that its intrinsic inverse agonist properties could confound the interpretation of the behavioral data (11, 12). Consequently, these findings were met with considerable skepticism, and at least two controversies emerged. First, did alcohol bind directly to GABA_A receptors, or did it somehow potentiate GABA_A indirectly? Although several groups observed similar effects of alcohol on GABA_A receptor activity measured using 36Cl− flux in synaptoneurosomes (7, 13), many, if not most, electrophysiological studies simply failed to find direct alcohol-induced augmentation of GABA-mediated synaptic events (5, 14). Second, was the imidazobenzodiazepine “alcohol antagonist” Ro15–4513 really a “selective” alcohol antagonist, or did it merely reverse some of the biochemical, electrophysiological, and behavioral effects of alcohol by virtue of its inverse agonist properties (15, 16), i.e., by simply producing the opposite effects of alcohol? As to the former, little was known in those days of the rather remarkable heterogeneity of GABA_A receptor subunits, their assembly into functional heteropentameric GABA_A receptors, and the pharmacological implications of this receptor subunit heterogeneity (17). In this regard, several laboratories have recently reported a critical role for the δ subunit (expressed together with α4 or α6 and β3 subunits) in conferring heightened sensitivity to alcohol on GABA_A receptors. Indeed, the work of Hanchar et al. (2) and Wallner et al. (3) builds on earlier work by their laboratory (18) and others (19, 20) demonstrating that low concentrations of alcohol augment GABA acting at δ subunit-containing GABA_A receptors. Significantly, these GABA_A receptors have been shown by several laboratories to be extrasynaptic in location, meaning that, in contrast to synaptic GABA_A receptors, they are activated in a “tonic” as opposed to “phasic” manner by very low extrasynaptic concentrations of GABA (21). Parenthetically, one might postulate that the synaptoneurosomes that we and others used to measure alcohol’s effects on GABA_A receptor-mediated 36Cl− flux (6, 7, 13) are enriched in these extrasynaptic GABA_A receptors.

Alcohol and Ro15–4513-Sensitive GABA_A Receptors

Given the marked sensitivity of extrasynaptic α4/β3δ GABA_A receptors to alcohol (18), Hanchar et al. (2) first studied the binding of [3H]Ro15–4513 to both native and recombinant GABA_A receptors containing α4/β3δ subunits. Previous work had suggested that benzodiazepine binding to GABA_A receptors required γ*E-mail: paul.steven.m@lilly.com.

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hibits [3H]Ro15–4513 binding to δ subunit-containing native and recombinant GABA\(_A\) receptors. Remarkably, ethanol inhibited [3H]Ro15–4513 binding to δ subunit-containing receptors with an IC\(_{50}\) of \(\sim 12\) mM but was without effect on [3H]Ro15–4513 binding to α4β3γ2 GABA\(_A\) receptors. Significantly, ethanol completely inhibited [3H]Ro15–4513 binding to α4β3γ2 GABA\(_A\) receptors, and subsequent kinetic analysis using saturating and nonsaturating concentrations of [3H]Ro15–4513 and ethanol, respectively, strongly suggests a direct competitive (as opposed to allosteric) binding interaction between the two molecules. Finally, a series of β-carboline and β-carboline receptor ligands were tested as inhibitors of [3H]Ro15–4513 binding to α4β3γ2 GABA\(_A\) receptors. Only those compounds (FG7142, Ro15–1788, β-carboline-3-carboxyethyl ester (β-CCCE) previously reported to block the anti-alcohol effects of Ro15–4513 in vivo (11) inhibited [3H]Ro15–4513 binding to α4β3γ2 GABA\(_A\) receptors.

In their companion paper, Wallner et al. (3) extend these intriguing findings by showing that the enhancement of GABA\(_A\) receptor-mediated Cl\(^-\) currents (measured in α4β3γ2 GABA\(_A\) receptors expressed recombinantly in Xenopus oocytes) by ethanol (30 mM) was completely reversed by Ro15–4513 (300 nM). Moreover, Ro15–4513 did not reduce “basal” GABA-induced currents mediated by α4β3γ2 GABA\(_A\) receptors and thus did not behave as an inverse agonist at concentrations that blocked ethanol. The IC\(_{50}\) for Ro15–4513’s actions in blocking ethanol’s GABA-enhancing effects on α4β3γ2 GABA\(_A\) receptors was \(\sim 10\) nM, very similar to its K\(_d\) for binding to these same receptors (2). At higher alcohol concentrations (\(>100\) mM), a proportion of the ethanol-induced enhancement was not blocked, even by high concentrations of Ro15–4513, and therefore, α4β3γ2 GABA\(_A\) receptors appear to respond to high alcohol concentrations in a Ro15–4513-insensitive manner. The latter was abolished in recombinantly expressed receptors where the β3 wild-type subunit was replaced with a mutated β3N265M subunit, resulting in only Ro15–4513-sensitive alcohol enhancement. Finally, both β-carboline inverse agonists, β-CCCE and FG7142 (but not the potent inverse agonist DMC4M), as well as Ro15–1788 were shown to reverse the inhibition by Ro15–4513 of alcohol-induced enhancement of δ subunit-containing GABA\(_A\) receptors, results that are strikingly reminiscent of the earlier biochemical and behavioral work (8, 11). Of note, β-CCCE actually potentiated the effects of low concentrations of ethanol (3 mM) on α4β3γ2 GABA\(_A\) receptors and even stimulated these receptors in the absence of alcohol (3).

Taken together, these findings suggest that a subtype of extrasynaptic GABA\(_A\) receptor that contains δ subunits and gives rise to tonic (sustained) GABAergic inhibition in brain (21) is indeed an important molecular target for alcohol, especially at alcohol concentrations achieved during social alcohol ingestion. Moreover, the data suggest the presence of a rather specific “alcohol binding site” on these same GABA\(_A\) receptors that is shared by the behavioral alcohol antagonist Ro15–4513. If correct, these findings help clarify 20 years of puzzling and often contradictory findings on alcohol, GABA\(_A\) receptors, and the alcohol antagonist Ro15–4513. Nonetheless, as the authors themselves point out, these controversies are unlikely to be resolved soon because a recent report by Borgese et al. (22) claims that δ subunit-expressing GABA\(_A\) receptors do not respond to alcohol, a discrepancy that hopefully can be resolved.

There are several obvious implications and questions that derive from these exciting findings. What other pharmacological effects of alcohol might be mediated by these alcohol and Ro15–4513-sensitive extrasynaptic GABA\(_A\) receptors? Could the reinforcing or additive properties of alcohol be mediated by these receptors? Could highly specific drugs be developed (hopefully devoid of other undesirable intrinsic properties) that would selectively block or even mimic alcohol’s effects on these GABA\(_A\) receptors? In this regard, it is tempting to speculate that the neuroactive steroids (alotetrahydroDOC or allopregnanolone) (23, 24), which have been shown recently to augment δ subunit-containing GABA\(_A\) receptors (25), may represent endogenous “alcohol-like” agonists at these same receptors. Do naturally occurring δ (or related) GABA\(_A\) receptor subunit polymorphisms exist in animals, including humans, and do they alter alcohol-related behaviors? Finally, similar pharmacologically relevant alcohol-binding sites undoubtedly exist on other important neuronal proteins. Are there common structural motifs for these alcohol-binding sites like that recently revealed by the high-resolution crystal structure of the Drosophila melanogaster alcohol-binding protein LUSH (26), and could such motifs be used to find other alcohol targets in brain? Despite these exciting findings, it seems highly likely that the clinically relevant behavioral effects of alcohol will involve multiple CNS targets; however, teasing these apart may be more feasible than once thought.

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