Alcohol and inhibitory receptors: Unexpected specificity from a nonspecific drug

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It is remarkable that a simple two-carbon molecule like ethyl alcohol produces the many neurological, physiological, and societal effects that result from alcohol (ethanol) use and misuse. It is the least potent of all drugs, requiring concentrations of 10–20 mM to produce intoxication in humans and 100–200 mM to produce anesthesia in experimental animals (1, 2). Because of the limited structural information that can be obtained from such a simple molecule and the high concentrations required, a nonspecific or nonreceptor-mediated mechanism of action was considered likely, and, as a result, early studies focused on the actions of alcohol on physical properties of membrane lipids. More recently, the search for sites of action shifted from lipids to proteins, but the identification of brain proteins that are clearly affected by ethanol at concentrations of 10–20 mM has proven remarkably elusive. For reference, a moderately intoxicating blood alcohol level of 0.08% (80 mg/dl) is equivalent to an ethanol concentration of 17 mM. In 1986 an important first step toward defining targets of alcohol action was taken when three groups demonstrated that intoxicating concentrations of ethanol enhance the function of γ-aminobutyric acid type A ($GABA_A$) receptors, the major inhibitory neurotransmitter receptors in the brain (3–5). One of these studies also showed that this action was missing in a line of mice (short-sleep mice) exhibiting genetic resistance to alcohol actions, suggesting the existence of alcohol-sensitive and alcohol-resistant subtypes of $GABA_A$ receptors (3). Despite the numerous electrophysiological studies stimulated by these studies, there is no clear understanding of what makes a $GABA_A$ receptor sensitive to low millimolar concentrations of ethanol and indeed many studies find either no effect of ethanol or effects only at concentrations of $\approx$100 mM or more (6).

Receptor Composition Determines Sensitivity to Ethanol

The report by Wallner *et al.* (7) in a recent issue of PNAS and a previous study by Sundstrom-Poromaa *et al.* (8) provide a glimmer of hope for understanding the molecular basis and physiological importance of alcohol-sensitive and -resistant receptors. The findings of Wallner *et al.* (7) demonstrate that high alcohol sensitivity of $GABA_A$ receptors requires the coexpression of both δ and $\beta3$ subunits. Replacing the δ subunit with $\gamma2$, or the $\beta3$ subunit with $\beta2$, markedly decreases the alcohol sensitivities of $GABA_A$ receptors. In the Wallner *et al.* (7) study, receptors composed of rat $\alpha4\beta2\delta$ subunits were insensitive to ethanol concentrations <10 mM, in contrast to the findings made by Sundstrom-Poromaa *et al.* (8) in which EtOH concentrations of 1 and 3 mM produced marked enhancement of $\alpha4\beta2\delta$ receptor function. In the latter study, mouse $\alpha4$, rat $\beta2$, and human δ subunits were used, whereas rat $\alpha4$ and δ subunits were used in the Wallner *et al.* (7) study. Thus, the differences in alcohol sensitivity observed may be caused by either species-dependent differences in subunit sequences or perhaps differences in the extent of subunit expression, which could affect receptor composition or density. Nevertheless, data from both studies lead to the surprising conclusion that alcohol has a more demanding receptor subunit composition requirement than sedative-hypnotic drugs such as barbiturates, which affect $GABA_A$ receptors composed of a wide variety of subunit combinations. The δ subunit may play an important role in determining the enhancing actions of modulatory agents other than alcohol. Lees and Edwards (9) showed that incorporation of δ subunits into receptors markedly enhance responses of $GABA_A$ receptors to the volatile anesthetic isoflurane.

Differences in alcohol sensitivity may be due to species-specific subunit sequences or receptor composition.

The key physiological implication, which is discussed in detail by Wallner *et al.* (7), is that alcohol likely acts on specific extrasynaptic responses, rather than on synaptic receptors (which contain γ and not δ subunits). This work raises two key questions for future study: (*i*) what is the molecular basis of the ethanol–protein interaction that results in alcohol modulation of $GABA_A$ receptor function? and (*ii*) which behavioral actions of alcohol are mediated by enhancement of the function of extrasynaptic $GABA_A$ receptors? The answer to the first question may well provide tools to answer the second. There is considerable evidence that the effects of high (anesthetic) concentrations of n-alcohols on $GABA_A$ receptors are caused by their binding in a water-filled protein cavity between the second and third transmembrane segments of the receptor subunits (10, 11). The α subunits appear to be particularly important for this interaction, but there is also a role for β subunits and perhaps also for γ (12). The high alcohol sensitivities of receptors containing $\beta3$ and δ subunits provide the opportunity to determine whether this same binding cavity is found in all or some of the subunits of this receptor. In addition, such studies also raise the possibility of constructing mutant receptors in which alcohol sensitivity is removed. These mutant receptors can then be introduced into "knock-in" mice to provide a powerful tool for associating alcohol effects on a particular receptor subtype with specific behavioral actions of alcohol. As reviewed by Wallner *et al.* (7), this approach has linked $\beta3$ subunits to the anesthetic actions of etomidate.

Knockouts and Knock-ins

Although less elegant than the knock-in approach, traditional knockout mice may also be useful in linking behavioral actions to receptors. For example, ethanol enhancement of GIRK2 channel function was linked to the analgesic actions, but not other effects, of ethanol by using GIRK2 null mutant mice (13). In this regard, it might be useful to con-

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struct mice lacking the α4 subunit of the $GABA_A$ receptor. One could also breed together existing mutant mice lacking the δ and β3 subunits and the α4 knockout mice when they are available to create multiple subunit knockouts of the $GABA_A$ receptor. Despite the considerable ethanol sensitivity of $GABA_A$ receptors containing the α6 subunit seen by Wallner *et al*. (7), results obtained from α6 knockout mice thus far are not encouraging, with the subunit exerting little effect on alcohol-induced sleep time, acute functional tolerance, withdrawal hyperexcitability, or protracted tolerance (14, 15). At this point in time, there appears to be no behavioral correlate for the enhancement of the α6-containing $GABA_A$ receptors by low concentrations of ethanol.

One of the overall goals of studies such as that of Wallner *et al.* (7) is to first identify molecular targets that are sensitive to concentrations of alcohol commonly achieved *in vivo*. Once such targets are identified, the next step is to determine whether those targets underlie specific actions of alcohol, such as reward, craving, tolerance, and dependence. Such information should allow targeted intervention to limit the neuronal damage, craving, loss of control, and relapse that characterize chronic alcoholism.

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