Review

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Alcohol dependence: molecular and behavioral evidence

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Alcohol dependence is a complex condition with clear genetic factors. Some of the leading candidate genes code for subunits of the inhibitory GABA$_A$ and glycine receptors. These and related ion channels are also targets for the acute actions of alcohol, and there is considerable progress in understanding interactions of alcohol with these proteins at the molecular and even atomic levels. X-ray structures of open and closed states of ion channels combined with structural modeling and site-directed mutagenesis have elucidated direct actions of alcohol. Alcohol also alters channel function by translational and post-translational mechanisms, including phosphorylation and protein trafficking. Construction of mutant mice with either deletion of key proteins or introduction of alcohol-resistant channels has further linked specific proteins with discrete behavioral effects of alcohol. A combination of approaches, including genome wide association studies in humans, continues to advance the molecular basis of alcohol action on receptor structure and function.

Molecular targets of alcohol

The pathway from an initial drink of alcohol (ethanol) to dependence is a long and complex one. However, in recent years there has been much progress in understanding the complexity and dynamicity of the acute and chronic mechanisms at play. This review describes the acute actions and chronic, persistent adaptations of alcohol, encompassing the structural, protein, intracellular, and genomic targets implicated in alcohol dependence. First, some of the rapid-onset effects of alcohol are probably mediated via direct action on ion channels [1]. The strongest evidence for the receptors most responsible for intoxication comes from the family of Cys-loop pentameric ligand-gated ion channels (LGICs), including GABA$_A$, glycine, nicotinic cholinergic, and serotonin receptors, as well as other ion channels such as *N*-methyl-D-aspartate (NMDA) and G-protein-gated inwardly rectifying potassium (GIRK) [2]. Intoxicating concentrations of alcohol (10–25 mM) produce euphoria and decrease inhibitions, and approximately 17 mM is considered the legal definition of intoxication in the USA. Second, there are slow-onset adaptations to alcohol that may include phosphorylation of LGICs, interactions with intracellular messenger proteins, and alterations in channel proteins [3,4]. Third, there are long-term adaptations in brain gene expression caused by chronic alcohol administration, and these changes in expression are organized into defined modules of related genes that are brain region- and cell type-specific [5]. However, alcohol-mediated changes in gene regulation do not necessarily explain changes in proteins, thus underscoring the necessity for a combination of rigorous genomic and proteomic approaches for a more complete understanding of the chronic effects of alcohol abuse and how best to approach its treatment [6].

Locating sites of acute alcohol action that are critical for modulating ion channel function was first facilitated by chimera and site-directed mutagenesis studies. Chimeras between the alcohol-inhibited GABA$_A$ ρ receptor and the alcohol-potentiated GABA$_A$ and glycine receptors provided initial evidence for transmembrane sites of alcohol/anesthetic action on LGICs. This important finding was followed by single point mutations that localized the critical amino acid residues to the transmembrane domain of glycine and GABAA receptors. Mutagenesis and covalent labeling studies suggested that these receptors were modulated by actions at competitive binding sites for alcohols and anesthetics [2,7]. Subsequent studies of GLIC (a prokaryotic homolog of pentameric LGICs) provided a unique structural model for identifying both the potentiating and inhibitory effects of n-alcohols on LGICs and the corresponding amino acid residues involved [8]. Combining X-ray crystallography, structural modeling, and site-directed mutagenesis has further advanced resolution of alcohol-binding pockets in diverse classes of brain proteins [2,9]. Crystallography studies of the GIRK channel recently demonstrated that alcohol activation is mediated via a unique microprotein domain that is distinct from receptor-mediated channel gating [10], and the chemical properties revealed here might be relevant for other alcohol-sensitive proteins.

How does the small, weakly binding ethanol molecule affect the function of large proteins such as LGICs and other ion channels? An important point about weakly interacting ligands, such as alcohol, is that the term 'binding' should be used cautiously. Weber introduced the concept that the forward rate constant for binding of small molecules is diffusion controlled (approximately 10^7/mol $\times$ s), thereby allowing estimation of the unbinding rate for ethanol as $<1\ \mu$s [11]. Although surprising, this

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result is within the time scale of electron paramagnetic resonance [12] and nuclear magnetic resonance studies that estimated the equilibration time of halothane into membranes [13]. These results are also consistent with a 1 μs molecular dynamics simulation of ethanol molecules in a fully hydrated lipid membrane, showing ethanol hopping from site to site on a time scale of nanoseconds [14]. Thus, for alcohol and other weakly bound molecules such as inhaled anesthetics, the term 'binding' more accurately refers to 'probability of occupation'.

Given the low binding energy and low probability of occupation of an alcohol-binding site, we must determine how alcohol acts once it transiently occupies a site such as an internal cavity of a receptor protein [1]. A cavity can open or collapse during the resting/open/desensitized cycle of ion channel function, and alcohol could act via one of three mechanisms: (i) occupy the cavity and prevent its collapse; (ii) displace pre-existing water molecules that are essential for the transitions; and (iii) provide H-bond or van der Waals bridges that facilitate the transitions [2].

The transient presence and small size of alcohol in an internal cavity might be expected to have little effect on the static [15] or crystal structure of a protein [16]. However, alcohol molecules probably change the dynamics of LGICs [17,18]. Ethanol sites can be visualized through crystal structures as occupying a water-filled cavity, and replacement of water by ethanol results in a higher resolution structure for GLIC [19] and was also important for crystallization of the alcohol-binding protein LUSH from *Drosophila* [20]. Thus, it appears that ethanol can stabilize specific states or conformations of these proteins, and for ion channels, this may correspond to open or closed states, providing a basis for changes in channel function [19]. This point is shown in Figure 1, where the B-factor, a measure of structural flexibility, is increased by the mutation and decreased by ethanol binding.

Crystal structures are not ideal for assessing dynamic changes, and computational approaches (molecular dynamics) may provide greater insight. However, at present, even a 1 μs molecular dynamics simulation of alcohol in a

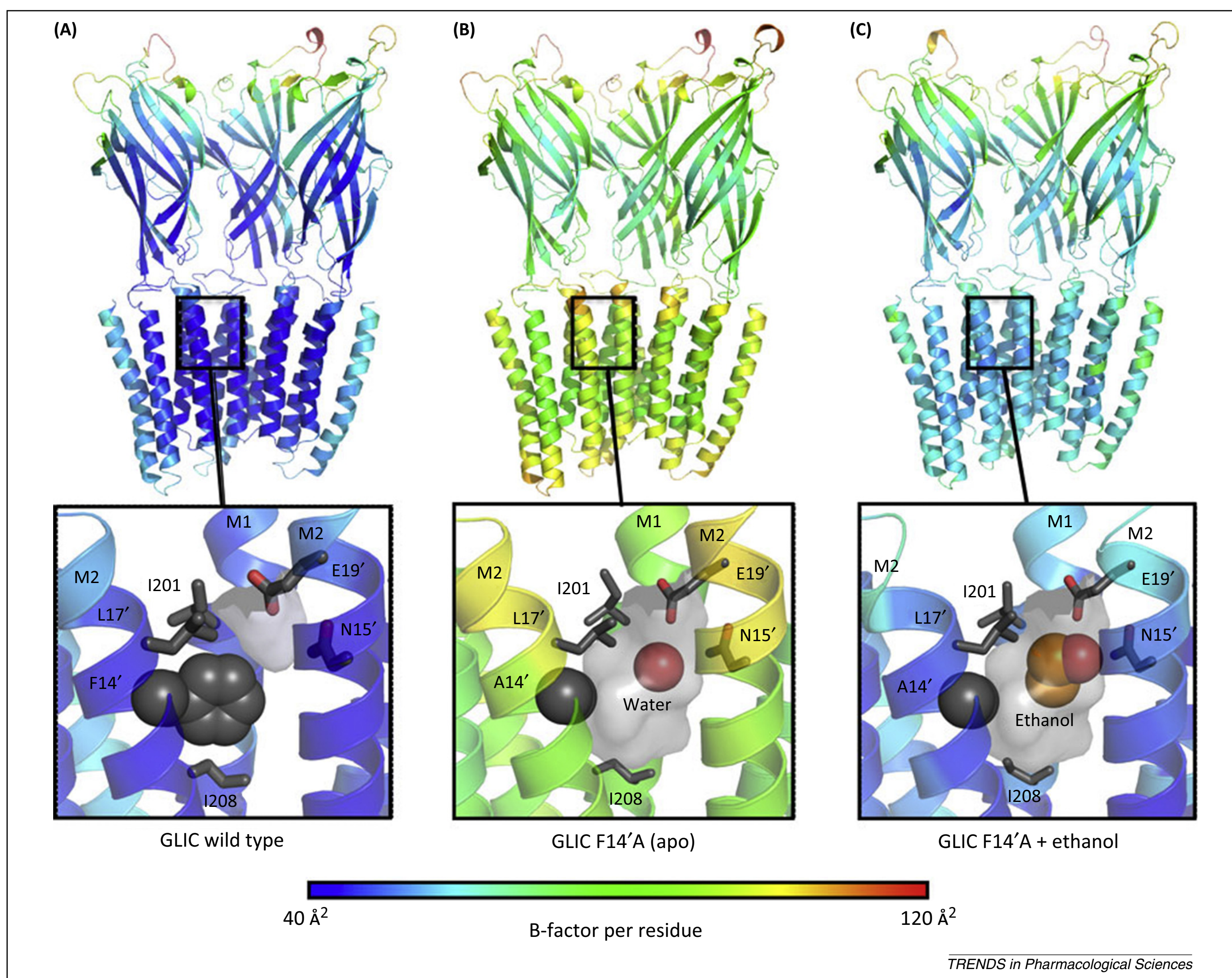

Figure 1. X-ray structures of wild type and ethanol-sensitized *Gloeobacter violaceus* ligand-gated ion channel (GLIC). **(A)** Wild type GLIC (Protein Data Bank identification: 4HFI), colored by residue B-factor according to scale at bottom. For clarity, two proximal subunits are hidden, revealing three distal subunits surrounding channel pore. Lower panel shows intersubunit transmembrane cavity (light gray surface) formed by the extracellular portions of M1–M2 from one subunit and M2–M3 from the neighboring subunit, and occluded on one end by the F14′ side chain (dark gray spheres). **(B)** Ethanol-sensitized GLIC variant (F14′A) (Protein Data Bank identification: 4HFB), depicted as in panel (A). Lower panel shows expanded intersubunit cavity containing resolved water (red sphere). **(C)** GLIC F14′A mutant co-crystallized with ethanol (Protein Data Bank identification: 4HFE), depicted as in panel (A). Lower panel shows intersubunit cavity containing ethanol (orange and red spheres). Figure and legend are from [19].

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