Alcohol actions on NMDA receptors

DR ROBERT PEOPLES

An estimated 18 million Americans abuse or are dependent on alcohol, with overconsumption leading to over 75,000 deaths each year. In this discussion, **Dr Robert Peoples** explains his research into alcohol's effect on NMDA receptor gating domains

How are you hoping to tackle these issues through your research?

My research deals with the mechanism by which alcohol acts on one of its major target proteins in the central nervous system (CNS). Although this may at first seem far-removed from human alcoholism and alcohol abuse, the actions of alcohol at the molecular level on its target proteins ultimately underlie the behavioural consequences of excessive alcohol consumption. Our hope is that a better understanding of the molecular mechanism will lead to better therapeutic approaches.

How does alcohol affect the N-methyl-D-aspartate (NMDA) receptor? What are the consequences of this?

The NMDA receptor is an ion channel in the membrane of neurons in the brain that is activated by the neurotransmitter glutamate. When glutamate molecules bind to the NMDA receptor, its ion channel begins to gate, or rapidly and repeatedly open and close in a complex manner. Because the NMDA receptor-ion channel allows calcium to enter the cell, as well as sodium, it is able to activate multiple intracellular signalling pathways, such as protein kinases, that have important consequences for cellular activity. The NMDA receptor is therefore essential for many aspects of CNS function, including some forms of learning and memory, cognition, and motor function.

Our lab and others showed that alcohol inhibits the gating of the NMDA receptor-ion channel, so that it spends less time in the open state, and as a result is less able to stimulate the cell. This contributes to the well-known effects of alcohol on human behaviour, such as motor incoordination and impairment of judgment and memory.

What are the subunits of this receptor and what have you discovered about the mechanisms of action of alcohol on these subunits?

The main type of NMDA receptor is composed of four subunits of two types: two GluN1 subunits and two GluN2 subunits. In addition, there are four subtypes of GluN2 subunits, GluN2A – D. Because the GluN2A subunit is the most abundant in the adult brain, most of our work to date has investigated sites of alcohol action in this subunit. At present we are also actively investigating the other GluN2 subunits, especially GluN2B, as a number of recent studies have pointed to an important role for this subunit in the behavioural and neurophysiological effects of alcohol.

Interestingly, even though both the amino acid sequences and the alcohol sensitivity of the GluN2 subunits are very similar to each other, the way in which alcohol acts on the different GluN2 subunits appears to differ. For example, mutations at one amino acid position in the GluN2A subunit that dramatically change alcohol sensitivity have little or no effect in the GluN2B subunit.

How are you creating models of alcohol action on the NMDA receptor? What purpose will these have?

We have both structural models of the sites of alcohol action, based on the X-ray crystallography studies of glutamate receptors by Eric Gouaux's laboratory, as well as kinetic models that incorporate the various conformational states of the protein, such as the open and closed ion channel. Both types of models allow us to test predictions about the influence of specific mutations on the gating and alcohol sensitivity of the NMDA receptor-ion channel, and together we hope that they will ultimately provide a detailed picture of how alcohol interacts with the protein to alter its function.

What approaches have you taken to overcoming the challenge presented by the fact that alcohol does not bind tightly to the proteins with which it interacts?

We have had to use multiple approaches, such as testing other alcohols structurally similar to ethyl alcohol, and testing the manner in which alcohol inhibition and receptor kinetics are affected by substitutions of various amino acids with different physical-chemical characteristics at a putative alcohol-sensitive position. We are always looking for new ways to answer our research questions.

What are the next steps for this project? Do you have a vision for any future projects that you would like to conduct related to this topic?

Our long-term, overarching goal is to have a detailed understanding of exactly how the alcohol molecule is able to interact with the NMDA receptor to regulate its function, and how this differs for each of the major subunit combinations.

DR ROBERT PEOPLES

Overcoming the anomalies of alcohol

Historically, alcohol has proven an irresistible drug of choice, yet its effects on the human brain are only now becoming clear. A study conducted at **Marquette University** is investigating the effect of alcohol at the molecular level, with the ultimate goal of informing new therapeutic strategies for the treatment of alcoholism

ALCOHOLISM AND ALCOHOL abuse has become an ever-increasing problem in US society. While illegal drug use attracts greater attention, the results of alcoholism are devastating. Abuse of alcohol is currently the third leading preventable cause of death in the US, costing the country approximately $220 billion each year. The cultural pathology of the condition is equally staggering. Consumption of alcohol contributes to a third of domestic abuses cases and a quarter of motoring deaths. Meanwhile, an estimated 25 per cent of children are already estimated to be exposed to alcoholism at home, and unless action is taken, this pathological trend will continue to grow.

The influence of alcohol on human behaviour is now widely understood to be mediated by brain proteins – among the most notable of which is the N-methyl-D-aspartate (NMDA) receptor: a receptor-ion channel triggered by the major excitatory neurotransmitter glutamate. This key receptor is particularly important, as it contributes to signal transmission in the central nervous system. Fundamentally, the functions of the NMDA receptor are hindered by alcohol, which has direct implications on behaviour. The inhibition of NMDA receptor function is intrinsic to development of alcohol addiction.

STUDYING NMDA RECEPTORS

Led by Dr Robert Peoples, researchers at Marquette University are attempting to characterise the ways in which alcohol interacts with the NMDA receptor at the molecular level and how it affects receptor function. "I originally became interested in the molecular mechanism of alcohol action as a subtopic of the broader discussion of central nervous system depressants, such as general anaesthetics," Peoples explains. At that time, he was drawn to the field by a lively debate that took place in the scientific literature he read in graduate school: "The prevailing view for nearly a century was that alcohol and general anaesthetics produced their effects on the brain by acting on cell membrane lipids. My early research played a role in establishing that membrane proteins at brain synapses – particularly ion channels gated by neurotransmitters – are the relevant targets of alcohol." The current study focuses more specifically on establishing how alcohol alters the function of the NMDA receptor.

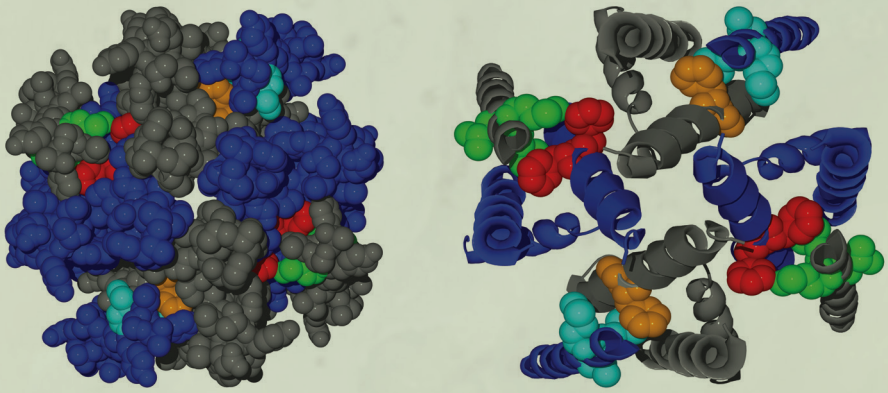
The research team is using advanced techniques – including single-channel recording – to observe the activity of proteins in real-time; concentration-jump recording and kinetic modelling is also utilised to characterise the kinetic activity of the NMDA receptor and its modulation by ethanol.

The main challenge in studying the interactions of alcohol with proteins is its low affinity, as Peoples highlights: "Compared to the majority of other drugs, alcohol does not bind tightly to its sites of action. Therefore, many approaches that can be used for other drugs, such as radiolabeled ligand binding, cannot be used to study alcohol".

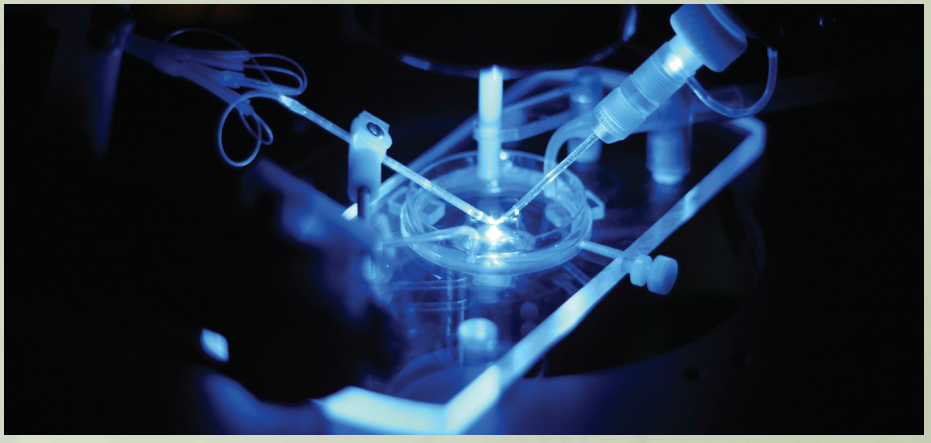
RECEPTIVE RESULTS

The laboratory has successfully identified and characterised specific sites in the NMDA receptor that interact with alcohol – the findings of which have been published in leading journals, including the *Journal of Biological Chemistry*. The research has also provided new and important information about the function of the membrane-associated domains of the NMDA receptor.

Results published in 2003 gave the first example of a physiological role for the fourth membrane-associated domain (or M4 domain) in a glutamate receptor. Membrane-associated domains refer to one of four regions of a NMDA receptor subunit – M1, M2, M3 and M4 – that cross and dip in and



The molecular models are of the membrane-associated domains of the NMDA receptor, looking down from the top. The ion channel is the open space in the centre, and the coloured molecules are the four clusters of amino acids making up the sites of alcohol action.



Electrophysiological recording in cells expressing mutant NMDA receptors. Cells containing NMDA receptors and a marker protein are visualised with fluorescent light.

out of the cell membrane. The NMDA receptor comprises four subunits: two GluN1 and two GluN2 subunits, of which there are four subtypes, GluN2A – D. Each subunit has multiple regions, including the four M domains.

Work on how alcohol affects gating was conducted in the early 1990s by Peoples among others. This demonstrated that alcohol inhibits the NMDA receptor by altering gating. The team then began searching for specific sites of alcohol action. To achieve this, the regions of the NMDA receptor that mediate gating were tested. Sections of the first three domains (M1, M2 and M3) were known to control gating, but further investigation showed that the M4 domain does too, as Peoples explains: "This was the first observation to attribute a physiological function to the M4 domain". At the same time, the lab found that a few amino acid positions in M4 could also alter alcohol sensitivity. "We have subsequently identified a number of individual amino acid positions in the M3 and M4 domains of the GluN2A subunit that modulate alcohol sensitivity, and have some evidence to support the view that the alcohol molecule is binding to these amino acids to produce its effects on the receptor". Indeed, the team quickly identified that most amino acid residues that control alcohol sensitivity regulate gating too.

The most recent published work has shown that, for each NMDA receptor, there are four 'putative sites' of alcohol action. These are created through groups of five alcohol-sensitive amino acids in the M3 and M4 domains, which meet at the subunit interfaces. Further findings suggest that the originally proposed position of GluN1 M4 is moved by one position. The team is still continuing to uncover the roles of the individual positions of the subunits.

IMPLICATIONS FOR THE FUTURE

Peoples' research may seem to focus solely on the effects of alcohol at the molecular level, but his overall aim is to improve the understanding and treatment of alcoholism and alcohol abuse. Beyond scientific interest, the fundamental effects of alcohol on human behaviour have a huge impact on US society, both economically and socially. With the knowledge gained from this research, Peoples hopes that a better understanding of the molecular implications of alcohol will ultimately lead to better therapeutic strategies for the treatment of alcoholism: "We need to have more pharmacological agents available for the therapy of alcohol use disorders," he concludes.

INTELLIGENCE

ALCOHOL ACTIONS ON NMDA RECEPTOR GATING DOMAINS

OBJECTIVES

To characterise how alcohol and other related compounds interact with the NMDA receptor at the molecular level to alter their function. The group are investigating sites in the NMDA receptor gating regions that modulate sensitivity to inhibition by alcohol, as well as looking at sites that are likely to directly bind alcohol. Both of these types of sites represent novel therapeutic targets for the treatment of alcohol use disorders.

KEY COLLABORATORS

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Professor Robert Lipsky, George Mason University, USA

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ROBERT PEOPLES completed his PhD in pharmacology in 1989 from Purdue University, and was awarded a National Research Council Fellowship at the US National Institutes of Health in 1990. From 1998-2003 he served as acting Chief of the Unit of Cellular Neuropharmacology at the National Institute on Alcohol Abuse and Alcoholism. Peoples joined the faculty at Marquette University in 2003 and was promoted to full professor in 2012.

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