Modeling the Alcohol Binding Site of the NMDA Receptor Using the GluA2-receptor Structure

Brookfield Academy SMART Team

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According to the National Institute of Alcohol Abuse and Alcoholism, about 18 million people have an alcohol use disorder. Alcohol binds to the N-Methyl-D-aspartate (NMDA) receptor, inhibiting cognition, short-term memory formation, motor coordination, and overall regular CNS function. The Brookfield Academy SMART (Students Modeling A Research Topic) Team used 3D printing technology to model the alcohol binding site on the NMDA receptor. This receptor is an ion channel in neurons of the CNS. Binding of the neurotransmitter, glutamate, allows the passage of calcium and sodium ions through the channel, thus controlling neuronal excitability and multiple intracellular signaling pathways. Alcohol inhibits the gating of the receptor, decreasing the flow of ions, leading to the symptoms of intoxication. The NMDA receptor is a heterotetramer, containing two GluN1 and two GluN2A subunits. Alcohol binds to the transmembrane domain of the receptor, interacting with the amino acids Gly638, Phe639, Phe639, Leu819, and Met818 (of subunit GluN1) and Met 823, Phe636, Leu824 and Phe637 (on GluN2A). Site-directed mutagenesis studies have identified the importance of these residues. Mutations in the same position on different subunits can drastically modulate the inhibition of the receptor by alcohol. Further understanding of the NMDA receptor mechanisms could lead to treatment for long-term alcohol abuse.

INTRODUCTION

The physical dependence or craving for alcohol, known as alcoholism, is a common problem in the United States today. Long term effects of alcohol abuse include increased risk of liver diseases, cardiovascular disease, and a plethora of cancers. With 2.96 gallons of ethanol consumed per capita, Wisconsin has one of the highest rates of alcohol abuse in the United States. According to the National Institute of Alcohol Abuse and Alcoholism, approximately 744,330 people are regularly blocking their N-Methyl-D-aspartate (NMDA) receptors with alcohol, inhibiting cognition, short-term memory formation, motor coordination, and overall central nervous system (CNS) function. Using structural data from the related glutamate receptor, GluA2, in combination with mutagenesis studies, Dr. Peoples' research has provided insight into the inner workings of this ion channel, shedding light upon the way it interacts with alcohol. This research may aid in the development of a drug for combating the withdrawal symptoms of alcoholism, making this addiction easier to quit.

THE NMDA RECEPTOR AND NEURONAL COMMUNICATION

NMDA receptors (NMDAR) are glutamate-dependent ion channels that mediate communication between neurons of the CNS involved in memory, motor coordination, and learning.

1. When a nervous signal reaches the end of a neuron, transport vesicles fuse with the cell membrane.
2. The neurotransmitter glutamate is released into the synaptic cleft.
3. Binding of glutamate to the NMDA Receptor triggers opening of the ion channel.
4. Na^{2+} and Ca^{2+} ions are allowed into the next neuron.
5. The change in membrane potential, due to Na^{2+} influx, triggers a new action potential in the postsynaptic neuron, continuing the communication pathway.
6. Ca^{2+} then acts as an important second messenger, activating several intracellular signaling cascades.

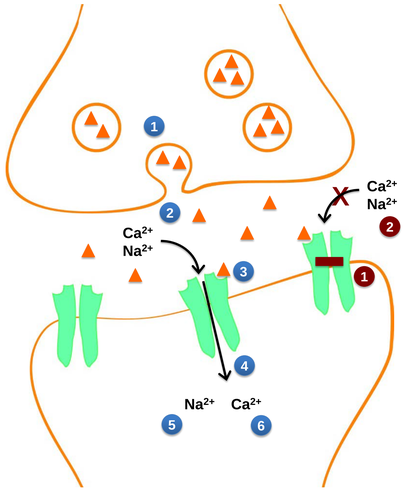

Fig 1. Glutamatergic synapse

ALCOHOL BINDS TO NMDAR, BLOCKING NMDAR-MEDIATED NEURONAL FUNCTION

The symptoms of intoxication, such as lack of coordination, faulty short-term memory, and impaired cognition are caused when ethanol molecules saturate NMDA receptors in the brain.

1. Ethanol binds to the transmembrane region of the NMDA receptor.
2. Ethanol binding does not prevent interaction of NMDAR with glutamate, but does inhibit opening of the ion channel by neurotransmitter, by decreasing the time spent in the 'open' position. This event lowers NMDAR-mediated activity in all areas of the brain.

THE NMDA RECEPTOR IS A TRANSMEMBRANE, HETEROTETRAMIC PROTEIN, CONTAINING TWO GLUN1 AND TWO GLUN2A SUBUNITS

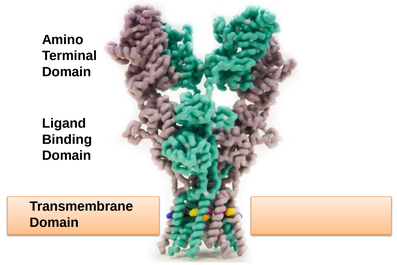

Fig 2. Structure of the NMDA Receptor

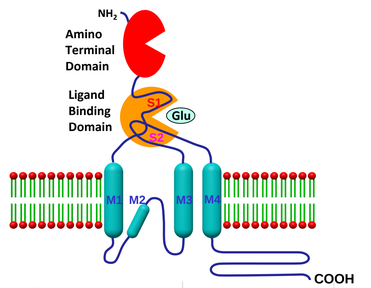

Fig 3. Folding topology of the GluN2A subunit

Fig. 2, The most thoroughly studied subunit GluN2 consists of 4 subtypes: GluN2A-D. Each subunit contains an amino terminal domain, a ligand binding domain, a transmembrane domain, and an intracelllular domain. Opening of ion channel requires concomitant binding of both glutamate and glycine to the LBDs of GluN2A (cyan) and GluN1 (grey), respectively. Model generated using JMol and PDB # 3KG2. ***Fig. 3***, Glutamate binds to the ligand binding domain of the NMDA receptor. Binding triggers conformational changes in the protein that lead to movement of helix M3 in the transmembrane domain, triggering the ion channel to open.

ETHANOL BINDS TO THE TRANSMEMBRANE DOMAIN OF NMDAR AT THE INTERFACE BETWEEN THE GLUN1 AND GLUN2A SUBUNITS

GluN1	635	VWAGFAMI
GluN2A	633	VWAFFAMI
GluN1	816	VFMLVAGGI
GluN2A	820	VFYMLAAAM

Fig 7. Amino acids proposed to constitute the ethanol binding site. Residues within ethanol binding site are colored-coded by side chain identity.

Structural and biochemical studies support the presence of four alcohol interaction sites in the NMDA receptor, each containing four to five amino acids residues. Binding sites 1 and 3 are composed of amino acids: M823, A825, L824 from subunit GluN2A and G638, F639 from subunit GluN1. Binding sites 2 and 4 are composed of amino acids: L819, M818, V820 from GluN1, and F636, F637 from GluN2A.

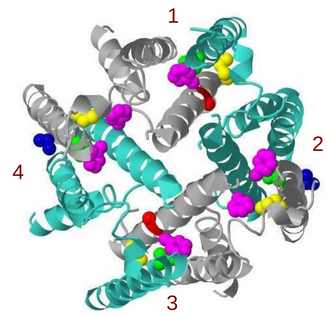

Fig 8. Ribbon diagram of the NMDAR transmembrane domain with ethanol binding sites in spacefill. Figure generated using JMol and PDB # 3KG2.

SITED-DIRECTED MUTAGENESIS STUDIES HAVE IDENTIFIED RESIDUES THAT MODULATE ETHANOL SENSITIVITY IN NMDAR AND PROVIDE POSITIONAL INFORMATION FOR AMINO ACIDS IN TMD OF GLUN1 AND GLUN2A SUBUNITS

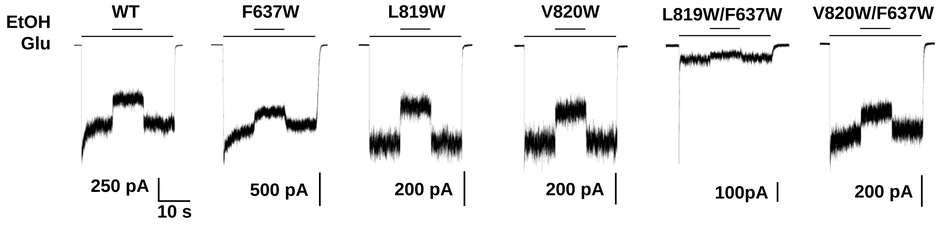

Fig 4. Current traces of WT and mutant NMDA Receptors. Traces are currents activated by 10 μM glutamate in the presence of 50 μM glycine in cells expressing wild-type (WT) and mutant GluN1 and GluN2A NMDA receptor subunits, as indicated.

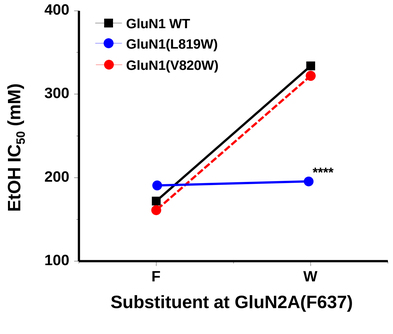

Fig 5. Ethanol IC_{50} values of NMDAR variants. Graph plots ethanol IC_{50} values *vs.* the amino acid substitution at position 637 in GluN2A for mutants at either GluN1 position 820 or 819. Asterisks indicate significant interactions detected using log-transformed IC_{50} values (***$P < 0.005$, two-way analysis of variance).

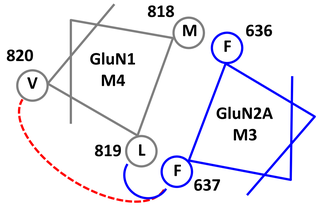

Fig 6. An example of a pair of positions in the M domains of the NMDA receptor GluN1 and GluN2A subunits that interact to regulate ethanol sensitivity. Ethanol IC_{50} values for single versus double mutants provide positional information of residues located at the interface between GluN1 and GluN2A subunits and help define the distinct ethanol interaction sites in the NMDA receptor.

CONCLUSION

Site-directed mutagenesis studies and structural alignments with a related glutamate-dependent receptor, have allowed for the elucidation of ethanol binding sites on the NMDA-receptor and greater understanding of NMDAR function. The NMDA receptor is a Na^{+} and Ca^{2+} ion channel that maintains the proper function of the central nervous system. Alcohol binds to the transmembrane domain of NMDAR, preventing the ion channel from opening. The blocked NMDAR receptor plays an important role in the well-known effects of intoxication: inhibited motor coordination, cognition, and even short-term memory loss. These studies have provided greater insight into the mechanism of alcohol intoxication, and offer the possibility of developing a pharmaceutical approach to therapeutically treating alcoholism and alcohol-related diseases.

REFERENCES AND ACKNOWLEDGEMENTS

Gouax, Rosconi, and Sobolevsky. (2009). X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature*. 462:745-755.
Ren, Zhao, Dwyer, and Peoples (2012). Interactions among Positions in the Third and Fourth Membrane-associated Domains at the Intersubunit Interface of the *N*-Methyl-D-aspartate Receptor Forming Sites of Alcohol Action*. *The Journal of Biological Chemistry*. 287:27302-27312.

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