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RESEARCH NEWS

The pre-M1 helix controls NMDA receptor gating

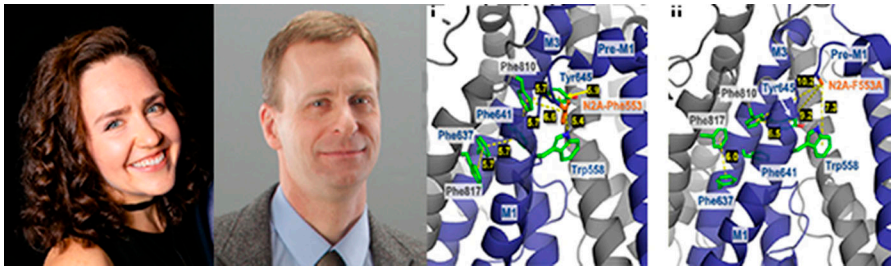
Ben Short

Researchers identify key residue in GluN2A subunit that may regulate channel opening by organizing a network of aromatic amino acids.

NMDA receptors (NMDARs) are glutamate-activated cation-selective channels that mediate fast excitatory neurotransmission and are required for synaptic plasticity, learning, and memory. These receptors are highly permeable to Ca^{2+}, in addition to Na^{+} and K^{+}. The tetrameric receptors are composed of two glutamate-binding GluN2 subunits and two GluN1 subunits that bind the coagonist glycine. Based on studies of the structurally similar AMPA receptor family, the opening of NMDAR channels is thought to involve motion of the transmembrane M3 helix. But how glutamate binding triggers M3 movement and channel opening is unclear. In this issue of *JGP*, McDaniel et al. now reveal that NMDAR gating is controlled by the pre-M1 helix of GluN2 subunits, potentially through its interaction with a network of aromatic amino acids within the receptor's transmembrane domain (1).

The pre-M1 helix lies parallel to the plasma membrane and is part of a linker region within all NMDAR subunits that connects the extracellular agonist-binding domain to the transmembrane domain. Based on this arrangement, and the fact that it is in van der Waals contact with the M3 helix, the pre-M1 helix has been proposed to regulate NMDAR gating (2, 3). Multiple mutations within the pre-M1 helix have been identified in patients with epilepsy and intellectual disabilities (4), indicating the functional importance of this region. "We wanted to demonstrate that the pre-M1 helix plays a functional role in NMDAR gating, as suggested by the structural data," says Stephen Traynelis, Professor of Pharmacology and Chemical Biology at Emory University.

Traynelis and colleagues, including first author Miranda McDaniel, generated a series of mutant versions of the GluN2A subunit in which different residues within the pre-M1 helix were mutated to alanine. Most of the mutations had little effect on channel function but one—F553A—dramatically altered the channel's behavior when the mutant subunit was coexpressed with wild-type GluN1 in *Xenopus* oocytes and HEK293 cells. GluN2A-F553A strongly enhanced the potency of not only glutamate but also glycine as activators of NMDARs, and slowed the time course of receptor deactivation by approximately ninefold. Somewhat counterintuitively, single-channel recordings revealed that the F553A mutation reduced the open probability of channels by a factor of 10 and caused an approximately sevenfold decrease in mean open duration.



Miranda McDaniel (left), Stephen Traynelis (right), and colleagues reveal that the pre-M1 helix of GluN2 subunits influences the gating of NMDA receptors. MD simulations suggest that a phenylalanine residue (F553) in the pre-M1 helix of GluN2A is a central component of a network of aromatic amino acids that may stabilize the channel in its closed state (panel i). This network is disrupted when F553 is mutated to alanine (panel ii).

To understand how F553 might control channel gating, Traynelis and colleagues constructed a homology model of GluN2A-containing NMDARs. MD simulations revealed that GluN2A F553 interacts with several other aromatic amino acids in both the GluN2A and GluN1 subunits, including residues within the M3 transmembrane helix important for channel gating. F553 is near the center of this aromatic network, and simulations showed that the F553A mutation could dramatically disrupt it. "This suggests that the aromatic network might stabilize the closed state of NMDARs," Traynelis says. "When the pre-M1 helix and F553 move in response to glutamate binding, the network could rearrange or come apart, and this may facilitate channel opening."

The aromatic network appears to be less extensive in GluN1, in keeping with previous data suggesting that the mechanism by which GluN1 contributes to gating is different from GluN2 (3, 5). The network is conserved in other GluN2 subunits, though, and Traynelis and colleagues provide evidence that the presence of tyrosine, instead of phenylalanine, in the pre-M1 helices of GluN2C and GluN2D may provide an additional hydrogen bond that further stabilizes the network and causes NMDARs containing these two subunits to have lower open probabilities than receptors containing GluN2A.

"We therefore hypothesize that the pre-M1 helix of GluN2 subunits contributes a critical residue to a network of aromatic amino acids that is well-positioned to control channel gating," Traynelis says. "We're currently designing experiments to test this intriguing idea."

References

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bshort@rockefeller.edu.