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RESEARCH NEWS

Resensitizing AMPA receptors

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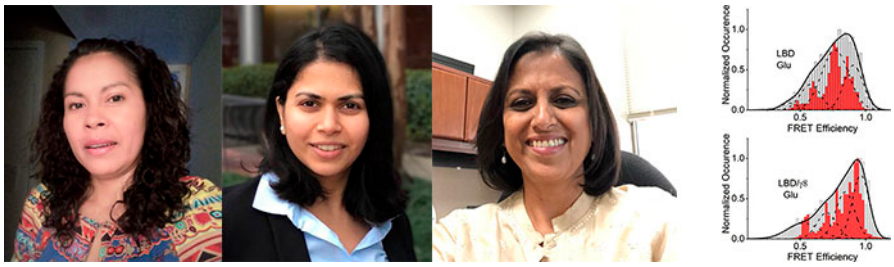
***JGP* study suggests how the regulatory protein γ8 reopens AMPA receptor channels in the continued presence of glutamate.**

AMPA receptors mediate fast excitatory synaptic transmission in the mammalian central nervous system when they are activated by the neurotransmitter glutamate on the postsynaptic membrane. The receptors are composed of four subunits, GluA1–GluA4, that can assemble together in various combinations to form glutamate-activated ion channels with diverse physiological properties. But the function of AMPA receptors is also influenced by associated factors such as the TARP family of Transmembrane AMPA receptor Regulatory Proteins. The TARP γ8, for example, allows AMPA receptors that have become desensitized in the continuous presence of glutamate to revert to an open state (1). In this issue of *JGP*, Carrillo et al. use single molecule approaches to provide new insights into how γ8 induces this receptor resensitization (2).

Understanding how γ8 resensitizes AMPA receptors is important because a number of drugs can selectively inhibit γ8-associated AMPA receptors and reduce neuronal excitability in epileptic forebrains (3,4). This suggests that γ8/AMPA receptor complexes can adopt unique conformations. Yet the only available structure of an AMPA receptor in complex with γ8 shows the receptor in an antagonist-bound, closed conformation (5). "We wanted to know more about the conformation of the complex in its resensitized state in the presence of glutamate, and understand how this relates to receptor function," explains Vasanthi Jayaraman, a professor at the University of Texas Health Science Center at Houston.

Jayaraman and colleagues, including co-first authors Elisa Carrillo and Sana Shaikh, began by performing single-channel recordings of GluA2 AMPA receptors. Upon exposure to 10 mM glutamate, the receptors became desensitized and displayed only brief, low conductance openings. In the presence of γ8, however, the majority of receptors transitioned to a high conductance state similar to that seen when GluA2 receptors are artificially stabilized in an open state by a drug called cyclothiazide.



Left to right: Elisa Carrillo, Sana Shaikh, Vasanthi Jayaraman, and colleagues reveal that the regulatory protein γ8 resensitizes AMPA receptors in the continuous presence of glutamate by inducing a tighter conformational coupling between receptor subunits that is associated with a highly conductive, open state. Single molecule FRET histograms of desensitized receptors in the absence of γ8 (top) and resensitized receptors in the presence of γ8 (bottom) show that γ8 increases the FRET efficiency between the ligand-binding domains of neighboring receptor subunits, indicative of tighter conformational coupling.

The researchers then used single molecule FRET to investigate the conformations of these different functional states, focusing on two extracellular regions of the receptor that undergo dramatic changes in response to activation or desensitization. In desensitized receptors, the N-terminal and ligand-binding domains of each subunit become separated, or decoupled, from the corresponding regions of neighboring subunits. In contrast, cyclothiazide stabilizes the open state of AMPA receptors by bringing the ligand-binding domains of neighboring subunits close together. Jayaraman and colleagues found that, in resensitized receptors bound to γ8, the N-terminal and ligand-binding domains were tightly coupled, similar to the coupling seen in receptors activated by cyclothiazide.

Moreover, the proportion of γ8-associated receptors in this tightly coupled conformation, and the time they spent in this state before transitioning to the decoupled conformation was similar to the frequency and kinetics of channel opening observed in the single-channel recordings. "That suggests a direct correlation between the conformation of the extracellular domains and channel function," Jayaraman says.

"Our data therefore indicate that γ8, which has a large extracellular domain of its own, is somehow interacting with the ligand-binding domains of the receptor and holding them together," Jayaraman continues. "This prevents the subunits from decoupling and favors the resensitized state."

As well as understanding the details of this interaction, one question that the researchers want to address is why γ8-associated receptors undergo a transient desensitization before γ8 can induce the tighter subunit coupling that restores channel activity.

1. Kato, A.S., et al. 2010. *Neuron*. https://doi.org/10.1016/j.neuron.2010.11.026
2. Carrillo, E., et al. 2019. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201912451
3. Kato, A.S., et al. 2016. *Nat. Med.* https://doi.org/10.1038/nm.4221
4. Lee, M.R., et al. 2017. *ACS Chem. Neurosci.* https://doi.org/10.1021/acschemneuro.7b00186
5. Herguedas, B., et al. 2019. *Science*. https://doi.org/10.1126/science.aav9011

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