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PERSPECTIVES

Stargazing from a new vantage – TARP modulation of AMPA receptor pharmacology

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An extensive body of molecular, biochemical and biophysical evidence has firmly established that members of the transmembrane AMPA receptor regulatory protein (TARP) family are AMPA receptor (AMPAR) auxiliary subunits and the dominant modulators of their trafficking and function in the brain. Aside from their role in membrane trafficking and synaptic targeting, TARPs have potent and far-reaching effects on AMPAR gating and pharmacology. TARP family members, such as the canonical TARP γ-2 (or stargazin), have been shown to predominantly display salutary effects on AMPAR function, e.g. by slowing desensitization and deactivation kinetics and increasing mean channel conductance. In addition, TARPs can enhance the affinity of AMPARs to their full agonist glutamate, allow partial agonists (i.e. ones that induce submaximal channel activation at saturating concentrations) to act as full agonists and well-known competitive antagonists to behave as partial agonists (Milstein & Nicoll, 2008). However, in an insightful biophysical study in a recent issue of *The Journal of Physiology*, MacLean and Bowie demonstrated that the mechanism underlying this last TARP effect may not be as straightforward as initially surmised.

First identified by Honoré and colleagues, quinoxalinedione derivatives are widely used as potent competitive antagonists of AMPARs (Honoré *et al.* 1988) and have proven to be valuable tools for parsing out the functions of ionotropic glutamate receptors in the brain. The most commonly used derivative in this family is 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). However, the use of CNQX in brain slices generates a paradoxical phenomenon, first described by McBain and colleagues (1992), which puzzled investigators for over 15 years. Recording from CA3 pyramidal neurons in hippocampal slices, they found that bath-application of CNQX, at a concentration known to block excitatory synaptic transmission, dramatically increased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs). The same concentration of CNQX induced inward current ($V_{rev} \sim 0$ mV), and robust burst firing in a subpopulation of hippocampal interneurons. The structurally related compound NBQX had no such effects (McBain *et al.* 1992). This phenomenon was subsequently studied by others but without resulting in any clear mechanistic explanation.

Menuz and colleagues (2007) picked up the trail by showing that the induction of a slow, steady-state inward current by CNQX is a more general property of both interneurons and principal neurons in several brain regions. Furthermore, the fact that the CNQX-induced current was blocked by the highly selective, non-competitive AMPAR antagonist, GYKI 53655, and enhanced by the positive allosteric modulator trichloromethiazide (TCM), suggested that CNQX behaves as a weak partial agonist at AMPARs in native neurons. Importantly, CNQX-evoked AMPAR currents could only be reconstituted in heterologous cells in the presence of a TARP. Structural analysis of CNQX bound to the crystal structure of AMPAR ligand-binding domains suggested that it could induce partial cleft closure, consistent with the notion of partial agonist activity. However, there was no evidence for a resulting separation of the linker domains, suggesting that the modest CNQX-evoked cleft closure would be insufficient to actuate channel opening. Although it was hypothesized that the presence of a TARP would amplify this coupling (Menuz *et al.* 2007), validation of this idea awaits the crystal structure of an AMPAR–TARP complex. Nevertheless, as neuronal AMPARs are TARP-associated, these findings present a satisfactory molecular explanation for the puzzling phenomena observed in brain slices. Furthermore, TARP-dependent partial agonist activity of CNQX was thought to explain the substantially lowered potency of CNQX antagonism of glutamate-evoked currents from TARP–AMPAR complexes observed by others (Cokić & Stein, 2008; Kott *et al.* 2009).

This mechanism, however, may not adequately explain the effects of CNQX on AMPAR gating in the presence of TARPs, as such effects had been observed under steady-state or equilibrium conditions. In a series of elegant and penetrating biophysical experiments, MacLean and Bowie (2011) addressed this question using ultra-fast agonist application. This approach permits the study of channel gating under non-equilibrium conditions – arguably more pertinent to the function of AMPARs at excitatory synapses. The authors first showed that CNQX blocks glutamate-evoked AMPAR currents with a substantially higher affinity under non-equilibrium conditions (within the first ~1 ms of activation) than equilibrium conditions. This led them to re-evaluate the role of TARPs (using stargazin) in modulating the blocking affinity of CNQX. In contrast to experiments performed under equilibrium conditions, they found that the presence of stargazin only modestly diminished the affinity of CNQX block of glutamate-evoked currents. They then asked whether the partial agonist activity of CNQX could account for the significant TARP-dependent decrease in CNQX blocking affinity observed under equilibrium conditions. In other words, is the dose-dependent block of glutamate-evoked AMPAR current by CNQX significantly offset by its activation through partial agonist activity? Interestingly, despite the fact that CNQX gave rise to only small-amplitude currents from AMPAR–stargazin complexes, they were found to undergo rapid desensitization – a characteristic not shared with other partial agonists like kainate. Nevertheless, the modest partial agonist activity of CNQX was found to be insufficient to account for its dramatically diminished blocking affinity in the presence of TARPs, observed under equilibrium conditions. Finally, using kinetic simulations that broadly reproduce TARP effects on AMPAR gating and kinetics, they were able to show that the rightward shift in CNQX blocking affinity by TARPs is better explained as a secondary effect of a leftward shift in

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glutamate affinity. Overall, this report from MacLean and Bowie offers new and valuable insights into AMPAR pharmacology and makes testable predictions about the way in which TARP auxiliary subunits might modulate AMPAR function on a structural level.

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