Journal Club

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Phosphatidylinositol-4,5-Bisphosphate and α -Actinin: Two-Component Hinge for the NMDA Receptor

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Review of Michailidis *et al.* (<http://www.jneurosci.org/cgi/content/full/27/20/5523>)

Membrane ion channels are regulated through tightly coupled signaling complexes/microdomains that often include the channels themselves and membrane phospholipids. In many cases, scaffolding proteins, cytoskeletal components, and phospholipids together provide structural backbones supporting a small community, where effectors and regulators can communicate locally and privately. The membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2) is an example of these structural backbones. This phospholipid can bind to channels and regulate their conformation, thereby changing channel activities. KCNQ/M current modulation by PIP2 is an example of such a phenomenon. When PIP2 is depleted from the inner leaflet of the membrane in response to stimuli such as activation of muscarinic receptors, M currents are suppressed (Brown and Adams, 1980). Although many voltage-gated ion channels can be regulated by PIP2, much less is known about PIP2 in regulation of neurotransmitter-gated channels.

NMDA receptors are potential targets of PIP2, based on two tantalizing hints: PIP2 may bind directly to NMDA receptors (McLaughlin and Murray, 2005) and phospholipase C γ (PLC γ ; an enzyme that

catalyzes the hydrolysis of PIP2) interacts with NMDA receptor subunits NR2A and NR2B *in vitro* (Gurd and Bissoon, 1997). A recent paper published in *The Journal of Neuroscience* (Michailidis *et al.*, 2007) provides evidence that PIP2 regulates NMDA receptor activity through a cytoplasmic interaction with α -actinin. The authors could not detect any effect on NMDA receptor activity with direct application of PIP2 or PIP2 antibody to excised inside-out patches, presumably because cytoplasmic components such as α -actinin are easily lost in excised inside-out patches.

Xenopus oocytes injected with NMDA receptor RNA display robust and sustained responses to NMDA and glycine and are ideal for studying the effect of PIP2. In the first set of experiments, the authors showed that PLC-catalyzed PIP2 hydrolysis suppressed NMDA currents in oocytes [Michailidis *et al.* (2007), their Figs. 1A (<http://www.jneurosci.org/cgi/content/full/27/20/5523/F1>), 2A (<http://www.jneurosci.org/cgi/content/full/27/20/5523/F2>)]. One complication of this experiment is that hydrolysis of PIP2 leads to production of inositol trisphosphate and diacylglycerol, which mobilize intracellular Ca²⁺ release. However, even after “dumping” most of the Ca²⁺ from internal stores, NMDA receptor currents were still inhibited by PLC-coupled receptor stimulation [Michailidis *et al.* (2007), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/27/20/5523/F4>)]. This implies that PIP2 hydrolysis itself, not

downstream signaling pathways, produces the NMDA receptor current inhibition.

The authors then identified sites on NMDA receptors responsible for “sensing” PIP2, consistent with α -actinin binding sites on NMDA receptors [Michailidis *et al.* (2007), their Figs. 5 (<http://www.jneurosci.org/cgi/content/full/27/20/5523/F5>), 6 (<http://www.jneurosci.org/cgi/content/full/27/20/5523/F6>)]. The interaction of α -actinin with NMDA receptors prevents the intracellular C termini from tethering to the actin cytoskeleton and keeps the channel in an open state (Krupp *et al.*, 1999). Because PIP2 also binds to α -actinin (Fukami *et al.*, 1992), Michailidis *et al.* (2007) investigated whether α -actinin mediated the PIP2 effect on NMDA receptors.

The most convincing evidence comes from Figure 7 [Michailidis *et al.* (2007), their Fig. 7 (<http://www.jneurosci.org/cgi/content/full/27/20/5523/F7>)]. The authors used a “dominant-negative” method, constructing single-site (K184Q) and double-site (R172Q–K184Q) mutant α -actinin, which lack the ability to interact with negatively charged phospholipids such as PIP2. Overexpression of either of these two α -actinin mutants in oocytes inhibits endogenous α -actinin. Coexpression of these two PIP2-insensitive mutants with NMDA receptors was sufficient to block suppression of NMDA receptor currents induced by replenishment of PIP2. These results confirm that α -actinin binds PIP2 and NMDA receptors at dif-

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ferent sites and that these interactions mediate regulation by PIP2.

In their final experiment, the authors set out to examine whether α -actinin also mediates the PIP2 regulation of native NMDA receptor currents in cultured primary hippocampal neurons. Either sequestering PIP2 or overexpressing PIP2-insensitive mutants of α -actinin in neurons had the same result: reduction in NMDA receptor currents and acceleration of inactivation, suggesting that the same mechanism takes place in neurons [Michailidis et al. (2007), their Fig. 8 (<http://www.jneurosci.org/cgi/content/full/27/20/5523/F8>), supplemental Fig. 4 (<http://www.jneurosci.org/content/vol27/issue20/images/data/5523/DC1/sf4.gif>)].

As with many good papers, these findings raise additional questions: Does this PIP2-regulated mechanism of NMDA receptors play a role in synaptic plasticity? Inhibition of PLC-catalyzed hydrolysis of PIP2 blocks NMDA receptor-dependent long-term depression (Horne and Dell'Acqua, 2007); thus, this mechanism proposed by Michailidis et al. (2007) could be involved in NMDA receptor-dependent plasticity. Could other neurotransmitter-gated channels such as AMPA receptors also be regulated by PIP2 in a similar manner? Accumulating evidence suggests that α -actinin associates with AMPA receptors (Schulz et al., 2004; Nuriya et al., 2005). It would be interesting to know whether PIP2 might also affect AMPA receptor activity, through α -actinin.

In summary, this work clearly demonstrates that PIP2 supports the open state of NMDA receptors via the adaptor protein α -actinin. In this model, PIP2 and α -actinin act like a two-component hinge keeping the channel gate in its open state (Fig. 1). This hinge appears to be chemically controlled by the hydrolysis of PIP2 in response to different stimuli. Certain stimuli will uncouple the components of the hinge and allow gate closure. Michailidis et al. (2007) expand our knowledge of

Figure 1. Schematic diagram summarizing the results presented by Michailidis et al. (2007). **A**, α -Actinin tethers to C-terminal regions of NMDA receptors (NMDAR) and PIP2 (shown in red) in the plasma membrane. In this way, PIP2 and α -actinin together keep the channel fully open. **B**, When PIP2 are cleaved by PLC into inositol trisphosphate (IP_3) and diacylglycerol in response to various stimuli such as activating muscarinic M1 acetylcholine (ACh) receptor, a G-protein-coupled receptor that couples to PLC, α -actinin is detached from membrane and no longer able to hold the channel open. The opening conformation of NMDA receptors is shifted to a restrained state. As a result, NMDA receptor currents are strongly suppressed.

PIP2 regulation and make an important step toward understanding the regulation of NMDA receptors by membrane lipid and intracellular cytoskeletal protein.

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