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RESEARCH HIGHLIGHT

NMDA receptor internalization down-regulates NMDA receptor-mediated synaptic responses through the inhibition of remaining (non-internalized) surface NMDA receptors

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Cell-surface protein endocytosis is critically involved in the regulation of organismal homeostasis, immune responses, development and neurotransmission [1,2,3,4]. Mechanisms underlying the endocytosis of cell surface proteins have been extensively investigated. However, until very recently no study has reported how non-internalized cell surface proteins may behave following endocytosis of same type of proteins. Here, we highlight findings that regulated NMDA receptor (NMDAR) internalization not only reduces the amount of NMDARs expressed on neuronal surface but also through activating PKD1 pathway phosphorylates and down-regulates remaining (non-internalized) surface NMDARs. This down-regulation of remaining surface NMDARs plays a critical role in the modulation of NMDAR-mediated synaptic responses by NMDAR internalization.

Keywords: endocytosis; glutamate receptor; phosphorylation; PKD; neuroplasticity

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Cell-surface proteins including receptors/channels undergo internalization constitutively or induced by regulated stimulations. The cell-surface protein internalization plays critical roles in the regulation of organismal homeostasis, development, immune responses and neurotransmissions [1, 2, 3]. Depending upon whether clathrin or caveolae is involved, detailed mechanistic studies have shown that there are clathrin or caveolae dependent or independent endocytosis [1, 2, 3].

In constitutive internalization, the amount of internalized cell surface proteins is balanced by that of externalized proteins. Regulated internalization of cell-surface proteins such as receptors/channels can be induced by external (such as ligands) stimulation. This type of internalization has been accepted as a general mechanism for reducing the number of receptor proteins on cell surface, and thereby regulating the function of the receptors [1, 2, 3, 4]. Furthermore, the stimulation-induced protein internalization has been found to be an important mechanism triggering intracellular down-stream signaling pathways [5]. However, until very

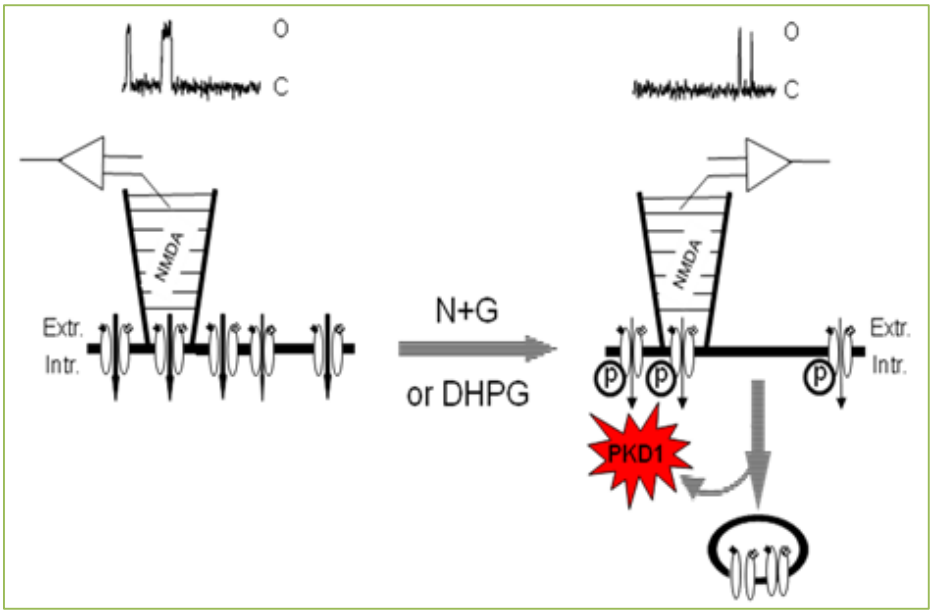

Figure. 1 Through the activation of PKD1, regulated NMDAR internalization down-regulates remaining surface NMDARs. A diagram shows recording of single NMDAR/channels in cell-attached patches before and after regulated internalization of cell surface NMDARs induced by bath application of high NMDA/glycine (N+G) or DHPG. Examples of NMDAR-mediated current traces recorded before (Left) and after (Right) N+G or DHPG application are shown above the diagram. Extr.: Extracellular, Intr: Intracellular. **P**: serine phosphorylation; C: closed level; O: opened level

recently no study was conducted to clarify how non-internalized cell surface proteins may behave following endocytosis of same type of proteins.

N-methyl-D-aspartate receptors (NMDARs) are a subtype of glutamate receptors and involved in many physiological and pathophysiological processes, such as synaptic plasticity, learning and memory, neuronal development and death [6,7,8,9,10,11]. Neuronal surface NMDARs are known to undergo homologous and heterologous internalization, which can be induced respectively by stimulating both the glutamate and glycine binding sites on the receptor [12, 13] and by stimulating group 1 metabotropic glutamate receptors (mGluRs) [14, 15, 16, 17, 18]. The dysregulation of NMDAR trafficking has been found to be related to the development of some neuropsychological disorders [19, 20, 21, 22].

Our previous studies have shown that through intracellular signaling mediated by Na^+ and/or Ca^{2+} neuronal surface NMDARs may cross talk to each other and therefore the activity of one NMDAR can be regulated by other NMDARs [11, 23, 24]. Based on these findings, we questioned: may endocytosis of some NMDARs affect the activity of remaining (non-internalized) neuronal surface NMDARs? To address this question we conducted investigations by using a combination of electrophysiological, biochemical, and genetic approaches. Our data demonstrate that through activating PKD1, NMDAR endocytosis down-regulates remaining surface NMDARs and thereby inhibits NMDAR-mediated synaptic transmissions [25].

I. Regulated internalization of NMDARs inhibits the activity of remaining (non- internalized) surface NMDARs [25]. NMDAR-mediated single-channel activity evoked with NMDA (10 μM) and glycine (3 μM) added into the patch pipette was recorded in cell-attached patches on cultured hippocampal neurons (Fig. 1). In this recording configuration (Fig. 1), the patch electrode isolates the recorded receptor/channels from the extracellular bath environment and therefore prevents the direct stimulation by bath-applied lipophobic agents such as NMDA and/or glycine [23, 24, 25]. The regulated endocytosis of NMDARs, which were located outside the membrane patches, was induced by bath application of the group 1 mGluR agonist, (*S*)-3,5-Dihydroxyphenylglycine (DHPG, 50 μM) [14, 15] or high concentration of NMDA (1 mM) and glycine (100 μM) (NMDA/glycine) [13]. NaCl and KCl in the extracellular bath solution were replaced by Na_2SO_4 and Cs_2SO_4. No damage of neurons was noted following bath application of high NMDA/glycine [23, 26].

Our data showed that bath application of either DHPG or NMDA/glycine not only caused significant reductions of NMDARs expressed on neuronal surface but also inhibited NMDAR activity recorded in cell-attached patches [25]. Application of dynamin inhibitory peptide (DIP), Myr-4–

QVPSRPNRAP (50 μM), which prevents dynamin-dependent receptor internalization [13,27,28], blocked the NMDAR down-regulation induced by either DHPG or high NMDA/glycine. We then examined effects of immobilizing NMDARs inside and/or outside the membrane patches by application of X-link consisting of 5 μg/ml goat-anti-mouse IgG and 2.5 μg/ml GluN1 antibody (mouse). We found that X-link application effectively prevented NMDAR endocytosis induced by high NMDA/glycine. However, immobilization of recorded NMDAR/channels within the membrane patch could not prevent the inhibition of the recorded NMDARs following endocytosis of NMDAR located outside of the patch except that X-link was also bath applied. Furthermore, immobilization of NMDARs outside of the membrane patch successfully prevented the reduction of the activity of recorded NMDARs which were within the membrane patches and not immobilized. Thus, we conclude that following bath application of either DHPG or high NMDA/glycine, the inhibition of recorded NMDARs which remained on the neuronal surface is induced by endocytosis of remote NMDARs outside of the membrane patch [25].

II. Through activation of PKD1 the regulated NMDAR internalization causes serine phosphorylation of surface NMDARs and down-regulates remaining surface NMDARs [25] (Fig. 1).

To understand the mechanisms underlying NMDAR endocytosis-induced inhibition of remaining surface NMDARs, we examined remaining surface NMDARs located at the synaptic plasma membrane (LP1) [29, 30, 31]. We found that the serine phosphorylation of the GluN2A and GluN2B subunits of membrane NMDARs increased significantly following regulated NMDAR endocytosis. Detailed investigations clarified that serine residue 1416 (s1416) of the GluN2A and the C-tail of GluN2B were critical phosphorylation sites on NMDARs. Furthermore, we found that PKD1 activity was enhanced following regulated NMDAR endocytosis and that PKD1 phosphorylated surface NMDAR proteins directly. Application of active recombinant PKD1 into cells depressed NMDAR activity. This suppression produced by PKD1 required s1416 in the GluN2A subunit or C-terminus in the GluN2B subunit. Neither application of the protein kinase inhibitor staurosporine nor knocking down PKD1 by infection of PKD1 shRNA affected NMDAR endocytosis. But both the phosphorylation and inhibition of NMDARs induced by NMDAR internalization were prevented. Taking all the findings together, we conclude that PKD1 plays a critical role in the regulation of remaining NMDARs by regulated NMDAR endocytosis [25] (Fig. 1).

III. Remaining surface NMDARs play a key role in the regulation of NMDAR-mediated synaptic activity by NMDAR internalization [25].

Miniature excitatory post-synaptic currents (mEPSCs) in cultured hippocampal neurons without or with knockdown of PKD1 were recorded. Similar to those reported previously [14, 15, 16, 17], both the NMDAR and AMPAR-mediated mEPSC components in neurons without shRNA infection were significantly reduced following bath application of DHPG. Intracellular application of DIP prevented the DHPG-induced inhibition of both the NMDAR- and AMPAR-mediated mEPSCs. While AMPAR-mediated synaptic responses remained no change, knockdown of PKD1 which blocks the inhibition of remaining surface NMDARs, substantially abolished the inhibition of NMDAR-mediated mEPSCs [25]. It has been generally accepted that through reducing the number of active receptor/channels expressed on the neuronal surface, the regulated receptor/channel internalization inhibits the receptor/channel-mediated synaptic responses. Our present work has demonstrated a novel and challenging concept that the inhibition of remaining surface NMDARs plays a key role in the down-regulation of NMDAR-mediated synaptic transmission by NMDAR endocytosis. As such, further clarifying how NMDAR internalization induces PKD1 activation and how remaining surface NMDA receptors regulate synaptic transmission will be essential for understanding functional changes in the central nervous system associated with receptor trafficking.

Conflicting interests

The authors have declared that no conflict of interests exist.

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References

1. Pfeffer SR. Unsolved mysteries in membrane traffic. Annu Rev Biochem 2007; 76: 629-645.
2. Kennedy MJ, Ehlers MD. Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci 2006; 29: 325-362.
3. Grant BD, Donaldson JG. Pathways and mechanisms of endocytic recycling. Nat Rev Mol Cell Biol 2009; 10: 597-608.
4. Watanabe S, Rost BR, Camacho-Perez M, Davis MW, Sohl-Kielczynski B, Rosenmund C, *et al*. Ultrafast endocytosis at mouse hippocampal synapses. Nature 2013; 504: 242-247.
5. Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling.

Pharmacol Rev 2001; 53: 1-24.

6. Newcomer JW, Krystal JH. NMDA receptor regulation of memory and behavior in humans. Hippocampus 2001; 11: 529-542.
7. Scannevin RH, Huganir RL. Postsynaptic organization and regulation of excitatory synapses. Nat Rev Neurosci 2000; 1: 133-141.
8. Sheng M, Kim MJ. Postsynaptic signaling and plasticity mechanisms. Science 2002; 298: 776-780.
9. Kumar J, Mayer ML. Functional insights from glutamate receptor ion channel structures. Annu Rev Physiol 2013; 75: 313-337.
10. Wisniewski K, Car H. (S)-3,5-DHPG: a review. CNS Drug Rev 2002; 8: 101-116.
11. Yu XM, Groveman BR, Fang XQ, Lin SX. The role of intracellular sodium (Na) in the regulation of calcium (Ca)-mediated signaling and toxicity. Health 2010; 2: 8-15.
12. Nong Y, Huang YQ, Salter MW. NMDA receptors are movin' in. Curr Opin Neurobiol 2004; 14: 353-361.
13. Nong Y, Huang YQ, Ju W, Kalia LV, Ahmadian G, Wang YT, *et al*. Glycine binding primes NMDA receptor internalization. Nature 2003; 422: 302-307.
14. Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF. Internalization of ionotropic glutamate receptors in response to mGluR activation. Nat Neurosci 2001; 4: 1079-1085.
15. Montgomery JM, Selcher JC, Hanson JE, Madison DV. Dynamin-dependent NMDAR endocytosis during LTD and its dependence on synaptic state. BMC Neurosci 2005; 6: 48-
16. Luscher C, Huber KM. Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron 2010; 65: 445-459.
17. Malenka RC Bear MF. LTP and LTD: an embarrassment of riches. Neuron 2004; 44: 5-21.
18. Palmer MJ, Irving AJ, Seabrook GR, Jane DE, Collingridge GL. The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. Neuropharmacology 1997; 36: 1517-1532.
19. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, at al. Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 2002; 71: 877-892.
20. Gu Z, Jiang Q, Fu AK, Ip NY, Yan Z. Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. J Neurosci 2005; 25: 4974-4984.
21. Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R, *et al*. Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. J Neurosci 2010; 30: 5866-5875.
22. Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, *et al*. Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci 2005; 8: 1051-1058.
23. Xin WK, Kwan CL, Zhao XH, Xu J, Ellen RP, McCulloch CA, *et al*. A functional interaction of sodium and calcium in the regulation of NMDA receptor activity by remote NMDA receptors. J Neurosci 2005; 25: 139-148.
24. Yu XM. The role of intracellular sodium (Na+) in the regulation of NMDA receptor-mediated channel activity and toxicity. Mol Neurobiol 2006; 3: 63-79.
25. Fang XQ, Qiao H, Groveman BR, Feng S, Pflueger M, Xin WK, *et al*. Regulated internalization of NMDA receptors drives PKD1-mediated suppression of the activity of residual cell-surface NMDA receptors. Mol Brain 2015; 8: 75- doi: 10.1186/s13041-015-0167-1
26. Xin WK, Zhao XH, Xu J, Lei G, Kwan CL, Zhu KM, *et al*. The removal of extracellular calcium: a novel mechanism underlying the recruitment of N-methyl-d-aspartate (NMDA) receptors in neurotoxicity. Eur J Neurosci 2005; 21: 622-636.
27. Gout I, Dhand R, Hiles ID, Fry MJ, Panayotou G, Das P, *et al*. The GTPase dynamin binds to and is activated by a subset of SH3 domains. Cell 1993; 75: 25-36.
28. Marks B, McMahon HT. Calcium triggers calcineurin-dependent synaptic vesicle recycling in mammalian nerve terminals. Curr Biol 1998; 8: 740-749.
29. Hallett PJ, Collins TL, Standaert DG, Dunah AW. Biochemical fractionation of brain tissue for studies of receptor distribution and trafficking. Curr Protoc Neurosci 2008; Chapter 1: Unit 1.16- doi: 10.1002/0471142301.ns0116s42.
30. Mao LM, Wang W, Chu XP, Zhang GC, Liu XY, Yang YJ, *et al*. Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. Nat Neurosci 2009; 12: 602-610.
31. Xu J, Weerapura M, Ali MK, Jackson MF, Li H, Lei G, *et al*. Control of excitatory synaptic transmission by C-terminal Src kinase. J Biol Chem 2008; 283: 17503-17514.