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Unlocking the secrets of the δ2 glutamate receptor

A gatekeeper for synaptic plasticity in the cerebellum

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Long-term changes in synaptic transmission in the central nervous system, such as long-term potentiation and long-term depression (LTD), are believed to underlie learning and memory in vivo. Despite intensive research, the precise molecular mechanisms underlying these phenomena have remained unclear. LTD is most commonly caused by the endocytosis of postsynaptic AMPA-type glutamate receptors, triggered by activity-induced serine phosphorylation of the GluA2 subunit. Interestingly, cerebellar LTD, which occurs at synapses between parallel fibers (PFs; axons of granule cells) and Purkinje cells, is unique in requiring an additional type of glutamate receptor, the δ2 receptor (GluD2). Cbln1 was recently identified as a GluD2 ligand that regulates PF synapse formation and maintenance. However, how GluD2 induces downstream signaling in Purkinje cells to regulate LTD induction is unknown. We here present evidence that GluD2 reduces the tyrosine phosphorylation level of the GluA2 subunit via PTPMEG, a protein tyrosine phosphatase that binds to GluD2's C-terminus. We also found that the serine phosphorylation of GluA2, a crucial step for AMPA-receptor endocytosis, requires prior tyrosine dephosphorylation. Thus, GluD2 may serve as a gatekeeper for LTD induction by coordinating interactions between GluA2's 2 phosphorylation sites.

Keywords: δ2 glutamate receptor (GluD2), AMPA receptor, cerebellum, Purkinje cell, long-term depression (LTD), synaptic plasticity, protein tyrosine phosphatase, endocytosis

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The activity-induced long-term potentiation and long-term depression (LTD) of synaptic transmission are thought to mediate higher brain functions such as learning and memory. Such plastic synapse changes exist in various regions of the brain. LTD at synapses between parallel fibers (PFs; axons of granule cells) and Purkinje cells is thought to mediate motor learning,[1] although its exact role remains controversial.[2,3] LTD is most commonly caused by the clathrin-dependent endocytosis of postsynaptic AMPA-type glutamate receptors. The activity-dependent phosphorylation of serine 880 (S880) of the AMPA receptor's GluA2 subunit is the initial step of LTD in both hippocampal[4,5] and cerebellar[6,7] synapses. This phosphorylation is thought to be necessary to allow AMPA receptors to remove anchoring proteins, such as glutamate receptor interacting protein (GRIP), before diffusing into the endocytic zone located at perisynaptic sites during LTD. Interestingly, PF-LTD in the cerebellum is unique in that it absolutely requires an additional type of glutamate receptor, the δ2 glutamate receptor (GluD2). However, GluD2's role in PF-LTD has not been clarified.

GluD2 is highly and predominantly expressed at the postsynaptic sites of PF–Purkinje cell synapses. Although GluD2 belongs to the ionotropic glutamate receptor family, it has long been called an orphan receptor, because it does not bind to glutamate analogs.[8] *GluD2*-null mice display 2 clear phenotypes: the number of PF–Purkinje cell synapses is reduced by 40–50%,[9,10] and LTD cannot be induced in the remaining, morphologically normal PF synapses.[11] Interestingly, Cbln1, a C1q-family protein released from PFs, was shown to bind the most N-terminal domain of GluD2 and to regulate the PF–Purkinje cell synapse formation and maintenance.[12,13] For this function,

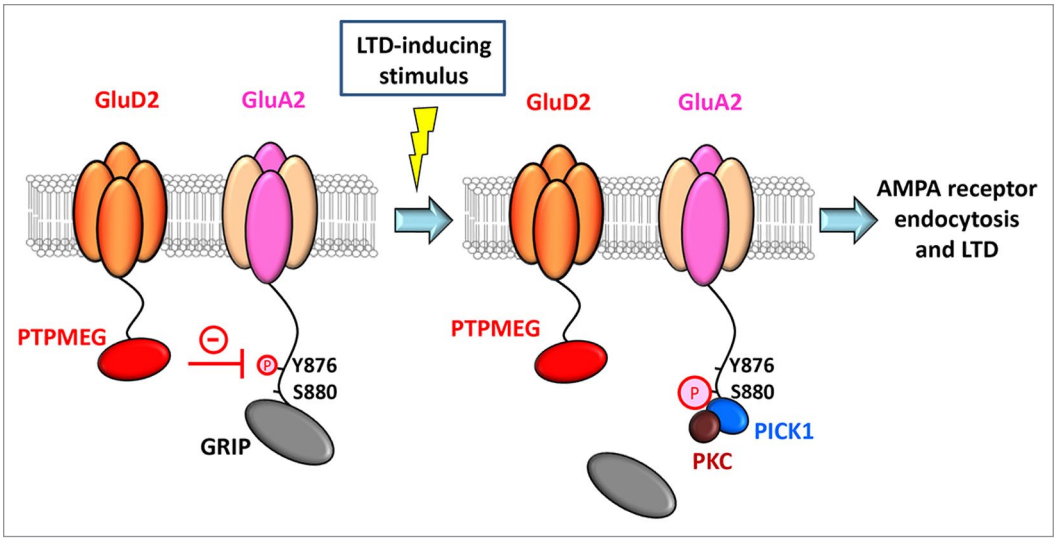

Figure 1. A model for GluD2's role in LTD induction. GluD2 acts as a gatekeeper for LTD induction by maintaining a low phosphorylation level at Y876 of the AMPA-receptor GluA2 subunit through PTPMEG, which binds to GluD2's C-terminus. LTD-inducing stimuli further dephosphorylate Y876 by unknown mechanisms. GluA2 Y876 dephosphorylation allows S880 to be phosphorylated by protein kinase C (PKC) and enables the glutamate receptor interacting protein (GRIP), a membrane anchoring protein, to be replaced by PICK1, leading to AMPA-receptor endocytosis and LTD.

GluD2's N-terminal domain is both necessary and sufficient.[14] On the other hand, GluD2's C-terminal intracellular region is indispensable for the PF-LTD induction. GluD2's C-terminal end contains a postsynaptic density-95/discs large/zonula occludens-1 (PDZ) ligand domain. Notably, PF-LTD is abolished in Purkinje cells that have been acutely perfused with a short peptide that corresponds to GluD2's PDZ ligand domain.[15] Although the PF synapse malformation in the *GluD2*-null cerebellum can be rescued by expressing a mutant GluD2 transgene lacking the PDZ ligand domain, the PF-LTD remains impaired.[16] However, expressing a mutant GluD2 transgene in which the channel pore domain is mutated to disrupt Ca^{2+} permeability[17] or ion conductance[18] rescues both the synapse malformation and the impaired LTD at PF–Purkinje cell synapses in *GluD2*-null mice. These results indicate that 2 of GluD2's primary functions at PF synapses, synapse formation/maintenance and LTD induction, are differentially regulated by its N-terminus and C-terminus, respectively.[19] However, the mechanism by which GluD2's C-terminus mediates the intracellular signaling necessary to induce LTD has remained a mystery.

A clue to this puzzle was revealed by a study of phosphorylation levels of the GluA2 subunit in the *GluD2*-null cerebellum. To determine at which step LTD is disrupted in the *GluD2*-null cerebellum, we examined whether a chemical LTD (chem-LTD) stimulus mimicking the depolarization of Purkinje cells and the activation of PF inputs[20] could induce the phosphorylation of GluA2 at S880. The chem-LTD stimulus induced S880 phosphorylation in the wild-type but not *GluD2*-null cerebellum. Thus, the S880 phosphorylation, an initial and essential step in AMPA-receptor endocytosis, was blunted in the *GluD2*-null mice.

We next asked why the absence of GluD2 signaling led to the failure of S880 phosphorylation. Since the C-terminal PDZ ligand domain is essential for inducing LTD, we considered molecules known to bind to this domain. One molecule, megakaryocyte protein tyrosine phosphatase (PTPMEG), particularly drew our attention. Although PTPMEG's target molecules for dephosphorylation were unclear, cerebellar LTD and motor learning are impaired in *PTPMEG*-null mice.[21] Furthermore, the phosphorylation of GluA2 at Y876 by the Src family kinase (SFK) has been shown to regulate AMPA-receptor endocytosis during certain forms of LTD at hippocampal synapses.[22-24] Therefore, we hypothesized that the GluA2 Y876 phosphorylation level was regulated by GluD2 via PTPMEG's phosphatase activity, to affect PF-LTD in the cerebellum.

Indeed, the basal level of GluA2 phosphorylation at Y876 was significantly elevated in the *GluD2*-null cerebellum, whereas the S880 phosphorylation level was comparable in the wild-type and *GluD2*-null cerebellum.[25] Furthermore, while chem-LTD induced S880 phosphorylation and decreased Y876 phosphorylation in the wild-type cerebellum, the GluA2 phosphorylation levels remained unchanged at both sites in the *GluD2*-null cerebellum. These basal and activity-induced GluA2-phosphorylation profiles were quite similar to those in the *PTPMEG*-null cerebellum.[25] These results indicated that in *GluD2*-null and *PTPMEG*-null mice, an increase in GluA2 phosphorylation at Y876 might be responsible for impairing the activity-induced GluA2 phosphorylation at S880, and consequently for the impaired LTD in these mice.

To establish a causal relationship between the increased GluA2 Y876 phosphorylation and the impaired S880 phosphorylation and LTD, we next examined whether reducing the Y876 phosphorylation could restore LTD in the *GluD2*-null cerebellum.[25] Transfer of an SFK-specific antagonist into *GluD2*-null Purkinje cells through patch electrodes enabled LTD to be induced in the cells by a standard electrical stimulation protocol. Similarly, the chem-LTD stimulus induced GluA2 S880 phosphorylation in *GluD2*-null cerebellum that was preincubated with an SFK-specific antagonist. Furthermore, the virus-mediated overexpression of a mutant GluA2 in which the Y876 phosphorylation site was replaced with phenylalanine restored the LTD induction in *GluD2*-null Purkinje cells. Finally, an in

vitro phosphorylation assay using a synthetic peptide corresponding to GluA2's C-terminus revealed that prior Y876 phosphorylation by Src significantly attenuated the subsequent S880 phosphorylation by protein kinase C. Together, these results indicated that increased Y876 phosphorylation was responsible for impairing the activity-induced GluA2 S880 phosphorylation and LTD in *GluD2*-null mice.

Next, we asked why the Y876 phosphorylation was increased in the *GluD2*-null Purkinje cells. While the virus-mediated expression of GluD2$^{\Delta CT7}$, which lacks the C-terminus, could not restore LTD in *GluD2*-null Purkinje cells, GluD2$^{\Delta CT7}$ was able to restore LTD when it was directly fused to the PTPMEG phosphatase domain. In addition, when phosphatase-inactive PTPMEG (PTPMEG^{DA}) was overexpressed in wild-type Purkinje cells to inhibit the endogenous PTPMEG activity, LTD was abrogated. In contrast, overexpressing a mutant PTPMEG^{DA} in which the PDZ domain necessary for GluD2 binding was disrupted did not inhibit LTD in wild-type Purkinje cells. Finally, substrate-trap and dephosphorylation assays in vitro revealed that GluA2 Y876 is a substrate that is directly dephosphorylated by PTPMEG. These findings indicated that the direct interaction of PTPMEG and GluD2 reduces the GluA2 Y876 phosphorylation level in Purkinje cells, thereby enabling activity-induced GluA2 S880 phosphorylation and LTD.[25]

The question of how GluD2 regulates PF-LTD in the cerebellum is one of long standing. Here we propose that GluD2 serves as a master switch, regulating LTD inducibility in the cerebellum by coordinating unique interactions between GluA2's 2 phosphorylation sites (**Fig. 1**). Because GluD1, which is closely related to GluD2, and PTPMEG are coexpressed in regions outside the cerebellum, similar signaling mechanisms may operate to regulate LTD inducibility at other synapses. Future studies are warranted to clarify whether and how the GluD2-PTPMEG signaling is regulated by neuronal activities, and how GluA2's 2 phosphorylation sites, Y876 and S880, interact with each other.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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