RESEARCH HIGHLIGHT

The hidden face of GluD1 at inhibitory synapses

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The δ-glutamate receptor (GluD), comprising GluD1 and GluD2, is a member of the ionotropic glutamate receptor family, playing a pivotal role in the development and maturation of both excitatory and inhibitory synapses. In a recent study in *Science*, Piot et al. revealed that GABA, as well as D-Ser, binds to GluD1 to regulate long-term plasticity at inhibitory synapses in the hippocampus, revealing yet another face of GluD receptors.

In the mammalian central nervous system, glutamate and GABA function as major neurotransmitters by binding to ionotropic glutamate receptors (iGluRs) coupled to cation-selective channels and $GABA_A$ receptors coupled to anion-selective channels to achieve rapid excitatory and inhibitory neurotransmission, respectively. The iGluRs are classified into three types based on their selective ligands: AMPAR, kainateR, and NMDAR. The δ-glutamate receptor (GluD) was initially called an orphan receptor because its specific ligand was unknown for a long time. However, the discovery that mutations in the GluD coding genes contribute to neuropsychiatric and neurological disorders underscores the need for further exploration to elucidate the signaling pathway.[1]

GluD2, predominantly expressed in cerebellar Purkinje cells, forms a tripartite complex with presynaptic neurexin (Nrxn) through binding to Cbln1 at the amino-terminal domain (ATD) (Fig. 1). It plays a crucial role in regulating the formation and maintenance of excitatory synapses between granule cells and Purkinje cells. In addition, D-Ser, secreted by glial cells during neuronal activation, binds to the ligand-binding domain (LBD) of GluD2, thereby inducing long-term synaptic plasticity by reducing the number of AMPARs in the postsynaptic site.[2]

In contrast, GluD1 is widely expressed in various brain regions. In subiculum pyramidal cells, Cbln2, secreted by the pyramidal cells themselves, binds to the ATD of GluD1, forming a tripartite complex with Nrxn at the presynaptic site to determine the number of postsynaptic AMPARs and NMDARs.[3] Interestingly, in the cortex, Cbln4, secreted by somatostatin-positive neurons, binds to the ATD of GluD1 and regulates inhibitory synapse formation and maintenance.[4,5] However, it has remained unclear whether ligand binding to the LBD of GluD1 plays any role.

Recently, Piot et al.[6] demonstrated that GABA binds to the LBD of GluD1 in addition to D-Ser and Gly. First, conformational changes of full-length GluD1 upon ligand application was assessed in *Xenopus* oocytes. Although wild-type GluD1 and GluD2 do not exhibit channel activity, introducing a "Lurcher" mutation (Lc) in the transmembrane domain 3 results in spontaneous channel activity.[1] Administration of D-Ser or Gly, but not GABA, suppressed $GluD2^{Lc}$ channel activity, while both GABA and D-Ser enhanced $GluD1^{Lc}$ channel activity with EC_{50} of 3 mM and 320 μM, respectively. In addition, voltage clamp fluorometry revealed fluorescence changes at the LBD upon GABA administration with an EC_{50} of 12 mM, in addition to D-Ser and Gly. These results suggest that full-length GluD1 undergoes a conformational change upon GABA binding in addition to D-Ser and Gly.

Piot et al. further demonstrated, by thermal shift assays and isothermal titration calorimetry experiments, that GABA and D-Ser bind directly to GluD1-LBD with K_D of 2 mM and 248 μM, respectively. X-ray crystallography also revealed that binding of the GluD1-LBD to D-Ser and GABA results in a closed conformation of the LBD, similar to other iGluRs. Interestingly, the GluD2-LBD binds to D-Ser but not to GABA.

What happens at the synapse following the ligand binding to the LBD of GluD1? To address this question, Piot et al. investigated the stratum lacunosum-moleculare (SLM) of hippocampal CA1, where GluD1 is highly expressed. They recorded inhibitory postsynaptic currents (IPSCs) from pyramidal neurons using the whole-cell patch-clamp technique following electrical stimulation of the SLM. First, D-Ser (300 μM) treatment for 10 min increased IPSCs by 50%. This effect was abolished when GluD1 was knocked down. Second, high-frequency stimulation (HFS) of the SLM increased IPSCs by 25%, and the effect lasted 20 min. This effect was abolished by knockdown of GluD1 and restored by reintroduction of wild-type GluD1 or channel pore-dead mutant GluD1, but not by GluD1 mutant that does not bind to Cbln family proteins (i.e., Cbln1, Cbln2 and Cbln4) or GluD1 mutant with selectively reduced binding affinity to GABA. On the other hand, HFS-induced increases in IPSCs were also observed in mice deficient in serine racemase (SR), a major enzyme that produces D-Ser in the brain. These results suggest that GluD1, bound by Cbln family molecules, induces long-term inhibitory synaptic plasticity by binding GABA at the LBD.

It should be noted that the binding affinity of the GluD1-LBD for GABA is weak, requiring several millimolar. The exact concentration of GABA at inhibitory synapses is unknown, but is estimated to peak at 1.5–3 mM and decay over several hundred microseconds.[7] Consequently, to be activated by GABA, GluD1 needs to be localized at the same nanocolumn directly below the GABA release site. Considering that 10%–20% of D-Ser remains in the brain in SR knockout mice,[8] and Gly or D-Ser binding is also reduced in the GluD1 mutant in which GABA binding is inhibited,[6] D-Ser or Gly, released during HFS, may also bind to the GluD1-LBD and regulate inhibitory synaptic plasticity. Therefore, a detailed co-localization analysis of GluD1 with inhibitory synaptic and glial marker molecules using super-resolution microscopy and/or electron microscopy is warranted.

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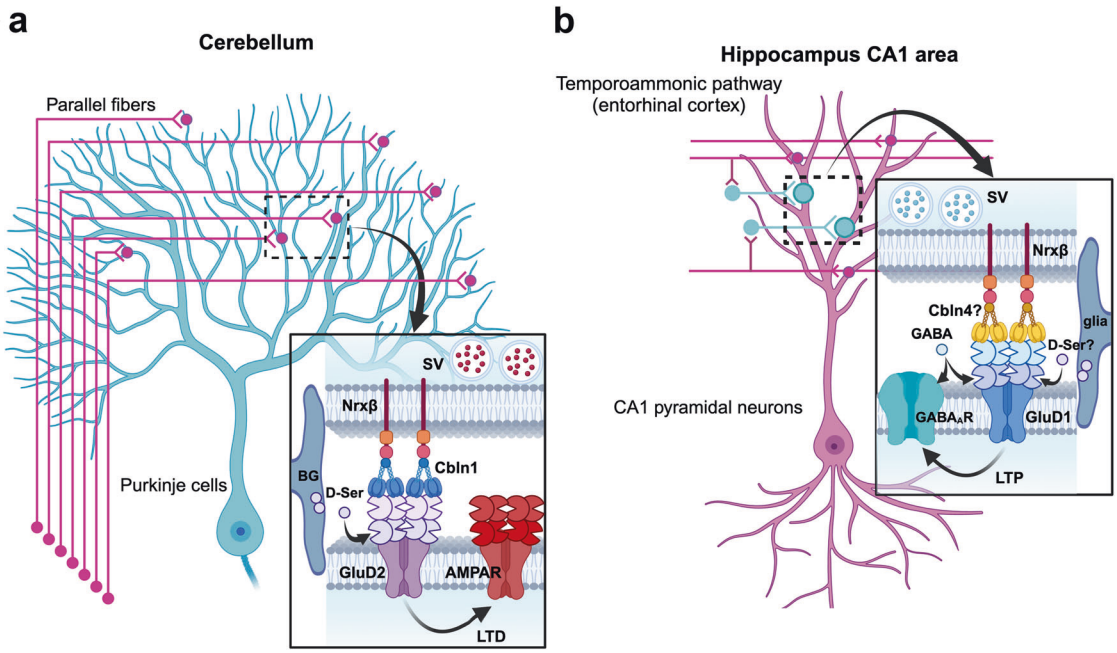

Fig. 1 GluD1 and GluD2 regulate synaptic plasticity through ligand binding. a In the juvenile cerebellum, D-Ser, released from Bergmann glia (BG), binds to the LBD of GluD2 and facilitates long-term depression (LTD) by inducing endocytosis of postsynaptic AMPARs at parallel fiber-Purkinje cell synapses. **b** In the CA1 hippocampus, GABA, and possibly D-Ser, binds to the LBD of GluD1 and facilitates long-term potentiation (LTP) of inhibitory synapses on pyramidal neurons. SV synaptic vesicle. Created with BioRender.com.

Inhibitory and excitatory synapses are not completely dichotomous despite their opposite signals to the postsynaptic site. Depending on the receptor, the same ligand can have different functions at excitatory and inhibitory synapses. For example, Gly acts at inhibitory synapses through the GlyR and at excitatory synapses as a co-agonist of the NMDAR or as an agonist of the excitatory GlyR.[9] However, GluD1 is unique in that the same receptor functions differently at excitatory and inhibitory synapses. While the channel activity of GluD1 is dispensable for regulating excitatory and inhibitory synaptic functions described above, GluD1 has also been reported to function as a channel under certain conditions.[10] With Piot's report, a new aspect of GluD1, which already exhibits multifaceted functions, is now unveiled. It remains to be elucidated what differences in the composition of synaptic molecules, including Cbln family proteins, are responsible for the various functions of GluD1. Another important question is how GluD1 and GluD2 regulate long-term synaptic plasticity at inhibitory and excitatory synapses, respectively, through ligand binding to the LBD.

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ADDITIONAL INFORMATION

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