RAPID REPORT

The δ2 'ionotropic' glutamate receptor functions as a non-ionotropic receptor to control cerebellar synaptic plasticity

Wataru Kakegawa, Kazuhisa Kohda and Michisuke Yuzaki

Department of Physiology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

The δ2 glutamate receptor (GluRδ2) belongs to the ionotropic glutamate receptor (iGluR) family and plays a crucial role in the induction of cerebellar long-term depression (LTD), a form of synaptic plasticity underlying motor learning. Nevertheless, the mechanisms by which GluRδ2 regulates cerebellar LTD have remained elusive. Because a mutation occurring in *lurcher* mice causes continuous GluRδ2 channel activity that can be abolished by 1-naphtylacetylspermine (NASP), a channel blocker for Ca^{2+}-permeable iGluRs, GluRδ2 is thought to function as an ion channel. Here, we introduced a mutant *GluRδ2* transgene, in which the putative channel pore was disrupted, into *GluRδ2*-null Purkinje cells using a virus vector. Surprisingly and similar to the effect of the wild-type *GluRδ2* transgene, the mutant *GluRδ2* completely rescued the abrogated LTD in *GluRδ2*-null mice. Furthermore, NASP did not block LTD induction in wild-type cerebellar slices. These results indicate that GluRδ2, a member of the iGluR family, does not serve as a channel in the regulation of LTD induction.

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Corresponding author M. Yuzaki: Department of Physiology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Email: myuzaki@sc.itc.keio.ac.jp

Glutamate receptors come in two types: ionotropic and metabotropic. Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that mediate fast excitatory neurotransmission in the mammalian central nervous system. They are subdivided into four subfamilies: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate receptors, *N*-methyl-D-aspartate (NMDA) receptors, and δ glutamate receptors (Hollmann & Heinemann, 1994). The δ2 glutamate receptor (GluRδ2) (Araki *et al.* 1993; Lomeli *et al.* 1993), which is predominantly expressed at the post-synaptic sites of parallel fibre (PF)–Purkinje cell synapses (Landsend *et al.* 1997), plays a crucial role in cerebellar functions: *GluRδ2*-null mice display ataxia and synapse malformation, such as a reduced number of PF–Purkinje cell synapses and the sustained innervation of Purkinje cells by multiple climbing fibres (CFs). In addition, long-term depression (LTD), a form of synaptic plasticity thought to underlie motor coordination and information storage (Ito, 1989), is abrogated at PF–Purkinje cell synapses in *GluRδ2*-null cerebella (Kashiwabuchi *et al.* 1995). Despite its importance, GluRδ2's role in cerebellar function has remained elusive – mainly because of the lack of specific agonists for GluRδ2 (Yuzaki, 2004). For example, although currents have never been evoked using wild-type GluRδ2, this situation might be due to a lack of information regarding GluRδ2's agonists or the conditions necessary to induce currents. Thus, whether GluRδ2 functions as an ion channel has been a long-standing question.

A point mutation at the end of the third transmembrane region of GluRδ2, which was originally identified in ataxic mutant *lurcher* mice (Zuo *et al.* 1997), causes the continuous activation of its channels ($GluR\delta2^{Lc}$). Like AMPA and kainate receptors, $GluR\delta2^{Lc}$ exhibited a rectified current–voltage relationship, was sensitive to the polyamine antagonist 1-naphtylacetylspermine (NASP), and exhibited moderate Ca^{2+} permeability (Kohda *et al.* 2000; Wollmuth *et al.* 2000). In addition, similar to wild-type AMPA receptors, the Ca^{2+}-permeability of $GluR\delta2^{Lc}$ was abolished by the replacement of glutamine (Q) by arginine (R) at the putative channel pore region (the Q/R site; Fig. 1*A* and *B*). Although these findings suggested that endogenous GluRδ2 may serve as a Ca^{2+}-permeable channel during LTD-inducing stimulation (Wollmuth *et al.* 2000), we recently used a *GluRδ2* transgene containing a mutation at the Q/R site

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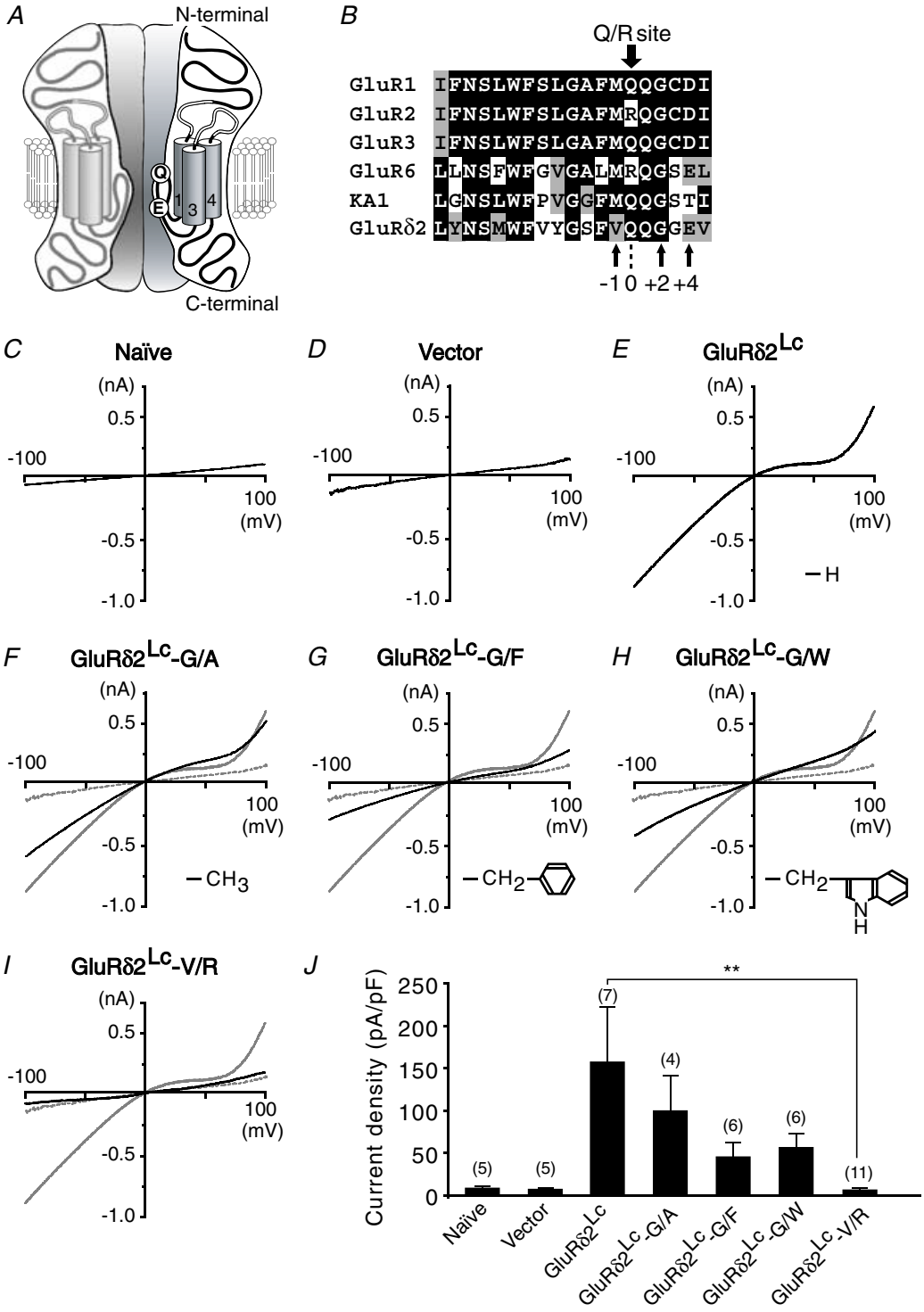

Figure 1. Mutations known to disrupt the channel pores of AMPA/kainate receptors effectively suppressed the current flow through GluRδ2^{Lc} channels in HEK 293 cells

A, presumed membrane topology of GluRδ2. Glutamine (Q) at the Q/R site and a negatively charged glutamate (E) are shown, which are presumed to be exposed to the channel pore and to determine its sensitivity to spermine analogues. *B*, alignment of the amino acid sequences of the channel pore regions of representative iGluRs. GluRδ2 has a Q at the Q/R site (position 0). The conserved glycine (G) residue at position +2 from the Q/R site contributes to the narrow constriction of the iGluR channel. Letters are shaded according to the similarity of the amino acids at each position. *C*–*I*, representative current–voltage relationships recorded from naïve HEK 293 cells (Naïve; panel *C*), or HEK 293 cells expressing an empty vector (Vector; panel *D*), GluRδ2^{Lc} (panel *E*), GluRδ2^{Lc} in which alanine (G/A, panel *F*), phenylalanine (G/F, panel *G*) or tryptophan (G/W, panel *H*) were substituted for glycine (G) at position +2,

to demonstrate that the Ca^{2+} permeability of GluRδ2 was not essential for its functions (Kakegawa *et al.* 2007). Nevertheless, a fundamental question regarding the ion channel activity of wild-type GluRδ2 has remained unanswered. If GluRδ2 functions as a channel under certain conditions, it may activate voltage-gated Ca^{2+} channels and contribute to the Ca^{2+} signals necessary for LTD induction.

To address this long-standing question, we exploited the fact that the putative channel pore region of GluRδ2 shares considerable similarity with that of AMPA and kainate receptors (Fig. 1*A* and *B*). We demonstrated that a mutation in the GluRδ2's putative channel pore domain did not interfere with GluRδ2's function to induce LTD, while similar mutations completely blocked the channel activities of GluRδ2^{Lc} and wild-type AMPA/kainate receptors. Furthermore, although currents passing through GluRδ2^{Lc} channels and glial AMPA receptors, both of which are composed of subunits containing Q at the Q/R site, were potently blocked by NASP, LTD was normally induced in cerebellar slices in the presence of NASP. Therefore, we propose that GluRδ2 does not serve as an ion channel in the regulation of LTD induction.

Methods

Clones and transfection

Site-directed mutagenesis was accomplished by overlap extension using polymerase chain reaction (PCR) with *Pfu* DNA polymerase (Stratagene, La Jolla, CA, USA). The nucleotide sequences of the amplified open reading frames were confirmed using bidirectional sequencing. GluRδ2 cDNAs were cloned into the expression vector pTracer-enhanced green fluorescent protein (Invitrogen, Carlsbad, CA, USA), and 4 μg of the plasmid was transfected into human embryonic kidney 293 (HEK 293) cells using the CellPhect transfection kit (Amersham Biosciences, Piscataway, NJ, USA). The incubation of the cells, the processing of the samples, and the cell-surface biotinylation assay were performed as previously described (Matsuda *et al.* 2003).

Recombinant Sindbis virus and *in vivo* injection

The recombinant Sindbis virus for the expression of GluRδ2 in combination with a yellow fluorescent protein containing a nuclear localization signal (nYFP) in the same neuron was constructed as previously described (Matsuda *et al.* 2003). Postnatal day 21–36 *GluRδ2*-null mice (*ho5J* mice; Jackson Laboratory, Bar Harbor, ME, USA) were anaesthetized using an intraperitoneal injection of ketamine/xylazine (80/20 mg kg^{-1}; Sigma, St Louis, MO, USA), and 2 μl of the solution containing the recombinant Sindbis virus (titre, 1.0×10^9 TU ml^{-1}) was injected into the vermis of cerebellar lobules V–VIII using a glass pipette (30 μm in diameter) and a microinjector (Nanoliter; World Precision Instruments, Sarasota, FL, USA). Parasagittal slices (100 μm) were prepared and subjected to immunohistochemical analysis under a confocal laser-scanning microscope (Fluoview; Olympus, Tokyo, Japan), as previously described (Kohda *et al.* 2007). All procedures relating to the care and treatment of the animals were performed in accordance with NIH guidelines.

Electrophysiology

Patch-clamp recordings were made from HEK 293 cells and cerebellar slices using an Axopatch 200B amplifier (Axon Instruments, Union City, CA, USA) and the pCLAMP system (version 9.2, Axon Instruments), as previously described (Kohda *et al.* 2000; Kakegawa *et al.* 2007). The detailed procedure is described in the Supplementary information. In some experiments, spermine (100 μM, Sigma) was added to the internal solution to maintain the voltage-dependent blockage of Ca^{2+}-permeable AMPA/kainate receptors during the recordings (Bowie & Mayer, 1995). A synthetic peptide that mimicked the C-terminus of GluRδ2 (pep-GluRδ2CT7: PDRGTSI) or a control peptide in which the sequence of pep-GluRδ2CT7 was scrambled (pep-GluRδ2SCR: RIPTDSG) was also included in the internal solution in some experiments, as previously described (Kohda *et al.* 2007). In the pharmacological experiments, NASP (Sigma) was extracellularly applied to block Ca^{2+}-permeable AMPA/kainate receptors and GluRδ2. For the statistical analysis, the amplitudes of the PF-evoked excitatory postsynaptic currents (PF-EPSCs) were averaged every 1 min and normalized according to a baseline measurement performed for 1 min immediately before the conjunctive stimulation.

Data analysis and statistics

Data are presented as the mean ± S.E.M., and statistical significance was defined as $P < 0.05$, as determined using the Mann–Whitney *U*-test.

or GluRδ2^{Lc} in which arginine was substituted for valine (V) at position −1 (V/R, panel *I*). The thin traces shown in panels *F–I* indicate the current–voltage relationship of cells expressing GluRδ2^{Lc} (taken from panel *E*); the thin dashed traces indicate the current–voltage relationship of cells expressing empty vector (taken from panel *D*). Insets of panel *E–H*: side-chain structures of the replaced amino acids. *J*, bar graph showing the mean current density at −80 mV. The number of experiments is given in parentheses. $^{**}P < 0.01$.

Results

Common mutations disrupting iGluR channel pores

GluRδ2 contains a glutamine (Q) residue at the Q/R site (position 0; Fig. 1*A* and *B*) in the putative channel pore domain; this residue determines the Ca^{2+} permeability of AMPA and kainate receptors (Burnashev *et al.* 1992). In addition, the +4 position of GluRδ2 contains a negatively charged glutamate (E; Fig. 1*A* and *B*) that contributes to the inward rectification of AMPA and kainate receptor currents (Panchenko *et al.* 1999). Indeed, GluR$\delta2^{Lc}$ channels exhibited moderate Ca^{2+} permeability and a rectified current–voltage relationship (Kohda *et al.* 2000; Wollmuth *et al.* 2000) in HEK 293 cells. Therefore, we expected that mutations rendering AMPA and kainate receptor channels non-conductive would also disrupt the GluRδ2 channel pores.

To evaluate the effects of mutations on the GluRδ2 channel pore, we introduced various mutations into GluR$\delta2^{Lc}$, which exhibited continuous channel activity in the absence of added ligands. A glycine (G) residue at position +2 (Fig. 1*B*) is thought to form the narrow constriction of the iGluR channel pore (Kuner *et al.* 2003). Indeed, the replacement of glycine with amino acids containing bulkier side chains, such as alanine (A; GluR$\delta2^{Lc}$-G/A), phenylalanine (F; GluR$\delta2^{Lc}$-G/F) or tryptophan (W; GluR$\delta2^{Lc}$-G/W), reduced GluR$\delta2^{Lc}$ currents in HEK 293 cells (Fig. 1*E–H* and *J*). Although these mutations substantially reduced the GluR$\delta2^{Lc}$ currents, we decided to continue searching for mutations that could block the currents. Thus, we next replaced valine (V) at position −1 (Fig. 1*B*) with arginine (R; GluR$\delta2^{Lc}$-V/R); similar mutations have been reported to disrupt the channel pores of AMPA (Dingledine *et al.* 1992; Robert *et al.* 2002) and kainate receptors (Robert *et al.* 2002). We found that the currents in cells expressing GluR$\delta2^{Lc}$ (158 ± 65 pA pF^{-1} at −80 mV, $n = 7$; Fig. 1*E* and *J*) were almost completely blocked by this mutation (7.9 ± 1.5 pA pF^{-1} at −80 mV, $n = 11$; $P = 0.009$; Fig. 1*I* and *J*); the remaining current levels were indistinguishable from those observed in naïve HEK 293 cells (9.0 ± 2.2 pA pF^{-1} at −80 mV, $n = 5$; $P = 0.257$; Fig. 1*C* and *J*) or those expressing empty vectors (7.7 ± 1.9 pA pF^{-1} at −80 mV, $n = 5$; $P = 0.423$;

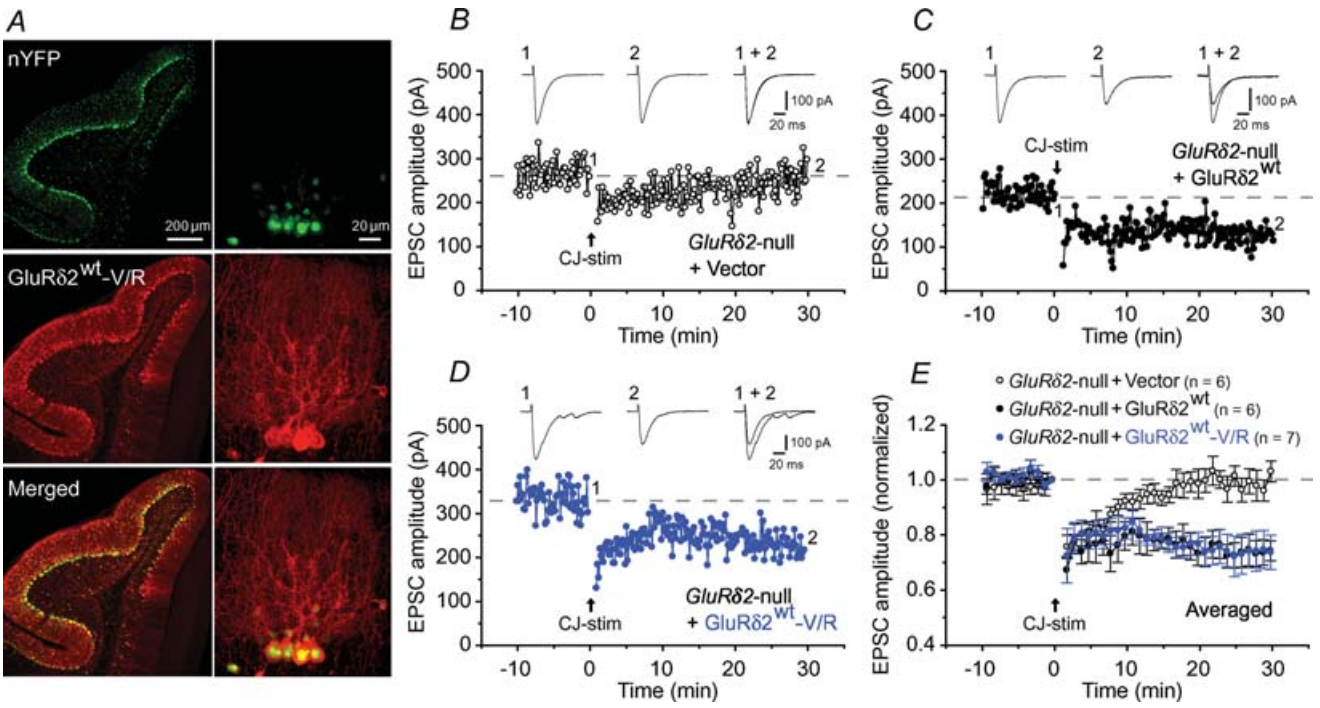

Figure 2. Virally mediated expression of GluRδ2 mutants with disrupted channel pores rescued abrogated cerebellar LTD in *GluRδ2*-null mice

A, confocal images of the virus-infected *GluRδ2*-null cerebellum. Infected Purkinje cells were identified using nYFP fluorescence (top). Transduced GluR$\delta2^{wt}$-V/R was visualized using anti-GluRδ2 antibodies and Alexa 546-conjugated secondary antibodies (middle) and merged with the nYFP signals (bottom). Each right panel shows a magnified view. *B–E*, PF-EPSC amplitudes following LTD-inducing conjunctive stimulation (CJ-stim) recorded from *GluRδ2*-null Purkinje cells expressing empty vector (+ Vector; *B*), GluR$\delta2^{wt}$ (+ GluR$\delta2^{wt}$; *C*), or GluR$\delta2^{wt}$-V/R (+ GluR$\delta2^{wt}$-V/R; *D*). Averaged data are shown in *E*. Inset traces: PF–EPSCs just before (1) and 30 min after (2) CJ-stim and their superimposition (1 + 2).

Fig. 1*D* and *J*). Because cell surface biotinylation assays revealed that GluR$\delta2^{Lc}$-V/R was transported to the cell surface in a manner similar to that of GluR$\delta2^{Lc}$ (see online supplemental material, Supplemental Fig. 1), this mutation is likely to disrupt the channel pore. In addition, these findings strongly support the view that the putative channel pore region of GluRδ2 shares considerable structural similarity with that of AMPA and kainate receptors.

GluR$\delta2^{wt}$-V/R rescued LTD in GluRδ2-null Purkinje cells. Since the V/R mutation probably disrupted the channel pore of GluR$\delta2^{Lc}$, we next introduced the V/R mutation into wild-type GluRδ2 (GluR$\delta2^{wt}$-V/R). To examine the effect of this mutation on the function of GluR$\delta2^{wt}$, we expressed GluR$\delta2^{wt}$-V/R together with nYFP in *GluRδ2*-null Purkinje cells using a Sindbis virus vector and performed whole-cell voltage-clamp recordings in acute cerebellar slices. Similar to a previous report (Kohda *et al.* 2007), we observed nYFP signals and GluRδ2 immunoreactivity in many Purkinje cells (Fig. 2*A*); Sindbis virus infection itself did not significantly affect the basic membrane properties of the Purkinje cells or the kinetics of PF–EPSCs in the Purkinje cells (data not shown). Next, we examined whether LTD could be restored in *GluRδ2*-null Purkinje cells transduced with GluR$\delta2^{wt}$, GluR$\delta2^{wt}$-V/R, or nYFP only. In *GluRδ2*-null Purkinje cells transduced with GluR$\delta2^{wt}$, the conjunctive stimulation, which consisted of 30 single PF stimuli together with a 200 ms depolarizing pulse, successfully induced LTD in PF-EPSCs ($74 \pm 4\%$ at $t = 30$ min, $n = 6$; Fig. 2*C* and *E*), but it did not induce LTD in *GluRδ2*-null Purkinje cells expressing nYFP only ($103 \pm 4\%$ at $t = 30$ min, $n = 6$; Fig. 2*B* and *E*); these results support the view that the impaired LTD in *GluRδ2*-null Purkinje cells was not caused by developmental deficits, but by a lack of GluRδ2 activity at the PF synapses (Yuzaki, 2004). Surprisingly, the expression of GluR$\delta2^{wt}$-V/R also rescued the abrogated LTD ($74 \pm 6\%$ at $t = 30$ min, $n = 7$; $P = 0.025$ *versus* vector, and $P = 0.999$ with GluR$\delta2^{wt}$; Fig. 2*D* and *E*), a result suggesting that channel activities were not required for GluRδ2's role in the regulation of LTD induction.

To further examine the function of GluR$\delta2^{wt}$-V/R, we introduced pep-GluRδ2CT7, a peptide that matched

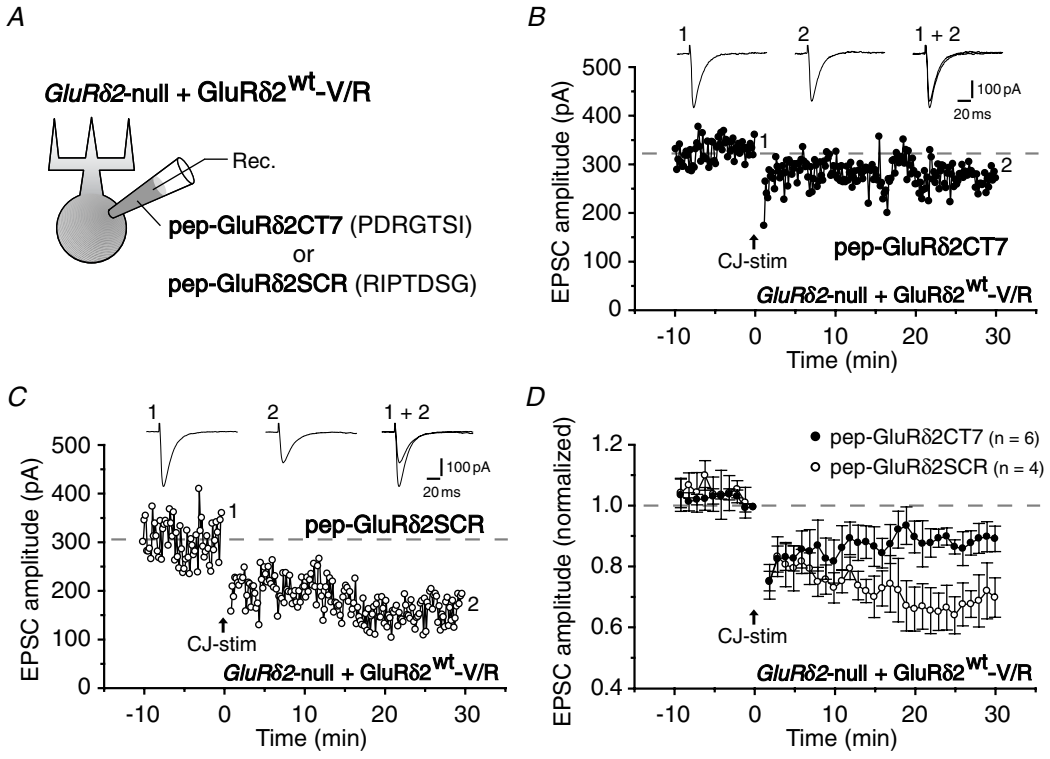

Figure 3. Suppression of LTD in *GluRδ2*-null Purkinje cells expressing GluR$\delta2^{wt}$-V/R by a peptide mimicking the C-terminus of GluRδ2

A, schematic diagram of the experimental set-up. *GluRδ2*-null Purkinje cells transduced with GluR$\delta2^{wt}$-V/R were perfused with pep-GluRδ2CT7, a peptide that matched the C-terminal 7 amino acids of GluRδ2, and pep-GluRδ2SCR, a peptide in which the sequence of pep-GluRδ2CT7 was scrambled, via a recording (Rec.) patch pipette. *B* and *C*, representative LTD data recorded from *GluRδ2*-null Purkinje cell expressing GluR$\delta2^{wt}$-V/R perfused with pep-GluRδ2CT7 (500 μM; *B*) or pep-GluRδ2SCR (500 μM; *C*). Inset traces: PF-EPSCs recorded at times 1 and 2. *D*, averaged LTD data.

the C-terminal seven amino acids of GluRδ2, and pep-GluRδ2SCR, a peptide in which the sequence of pep-GluRδ2CT7 was scrambled, to *GluRδ2*-null Purkinje cells expressing GluR$\delta2^{wt}$-V/R (Fig. 3*A*). We previously showed that pep-GluRδ2CT7 interfered with LTD induction in wild-type Purkinje cells by interfering with PDZ proteins that bind to the C-terminal end of endogenous GluRδ2 (Kohda *et al.* 2007). Similarly, LTD induction was significantly inhibited by pep-GluRδ2CT7 (500 μM) but not by pep-GluRδ2SCR (500 μM) in *GluRδ2*-null Purkinje cells transduced with GluR$\delta2^{wt}$-V/R; the EPSC amplitudes at 30 min after conjunctive stimulation were $90 \pm 4\%$ ($n = 6$) in Purkinje cells perfused with pep-GluRδ2CT7 (Fig. 3*B* and *D*) and $70 \pm 6\%$ ($n = 4$) in cells perfused with pep-GluRδ2SCR ($P = 0.033$; Fig. 3*C* and *D*). These results suggest that the C-terminus of GluRδ2, rather than the channel domain, plays an essential role in LTD induction.

A GluR$\delta2^{Lc}$ channel blocker, NASP, did not affect LTD induction

The *lurcher* mutation may allosterically affect the pore domain structure in a manner such that the V/R mutation does not disrupt the channel pore of wild-type GluRδ2. To exclude this possibility, we employed a complimentary pharmacological approach using a spermine analogue, NASP, that selectively blocks the channel pores of Ca^{2+}-permeable AMPA/kainate receptors composed of subunits containing Q at the Q/R site (Koike *et al.* 1997). As expected from its amino acid sequence at the Q/R site (Fig. 1*A* and *B*), GluR$\delta2^{Lc}$-invoked currents were significantly blocked by 10 μM of NASP (109 ± 25 pA pF^{-1} without NASP and 23 ± 7 pA pF^{-1} with NASP; holding potential, -80 mV; $n = 5$; $P = 0.015$; Fig. 4*A* and *B*). Thus, wild-type GluRδ2, which exists as a homomeric receptor containing Q at the Q/R site *in vivo* (Kohda *et al.* 2000), is likely to be blocked by NASP if it functions as a channel.

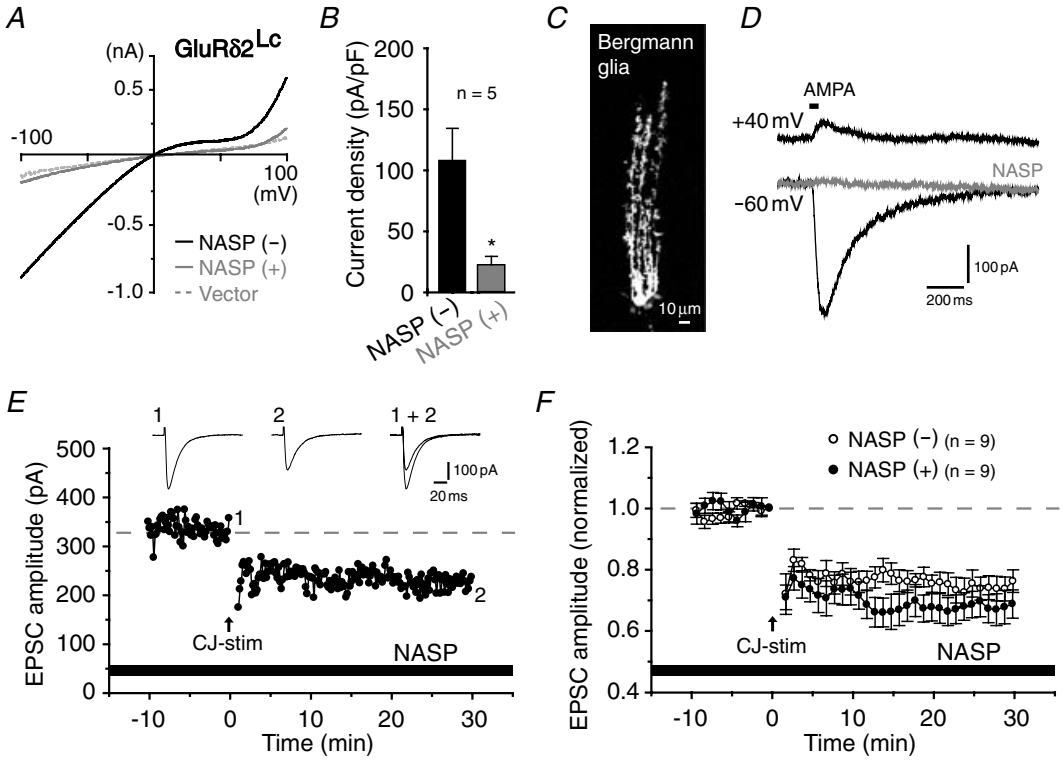

Figure 4. Channel blockers of GluRδ2 did not hamper LTD induction in wild-type cerebellum
A, current–voltage curves recorded from HEK 293 cells expressing GluR$\delta2^{Lc}$ before (−) and after (+) the application of NASP (10 μM). The thin dashed trace indicates the representative current–voltage relationship of cells expressing an empty vector (taken from Fig. 1*D*) for comparison. *B*, bar graph showing the mean current density at −80 mV; $^*P < 0.05$. *C*, confocal image of Bergmann glia visualized by the inclusion of Alexa 488-conjugated dextran in a patch pipette. *D*, AMPA-induced current responses from Bergmann glia before (thick traces; holding potential at +40 mV and −60 mV) and after (thin trace; holding potential at −60 mV) the application of 100 μM of NASP. AMPA currents were evoked by puffing (*RS*)-AMPA (10 mM) to the soma of Bergmann glia in the presence of cyclothiazide (50 μM). *E* and *F*, representative (*E*) and summarized (*F*) LTD data recorded from wild-type cerebellar slices in the presence of NASP (100 μM). Inset traces: PF-EPSCs recorded at times 1 and 2.

Indeed, both wild-type and *lurcher*-type AMPA receptors were effectively blocked by NASP (Kohda *et al.* 2000). Taking diffusion through the tissues into account, we used a higher concentration of NASP in the cerebellar slice preparations; we confirmed that 100 μM of NASP effectively blocked the AMPA-induced currents in Bergmann glial cells, which express Ca^{2+}-permeable AMPA receptors (Müller *et al.* 1992; Iino *et al.* 2001) (Fig. 4*C* and *D*). Nevertheless, conjunctive stimulation elicited robust LTD in wild-type cerebellar slices in the presence of 100 μM NASP ($69 \pm 5\%$ at $t = 30$ min, $n = 9$; Fig. 4*E* and *F*), similar to its effect in the absence of NASP ($76 \pm 4\%$ at $t = 30$ min, $n = 9$, $P = 0.233$; Fig. 4*F*); thus, this result strongly supports the view that wild-type GluRδ2 does not function as a channel in the LTD-induction process. In addition, it suggests that AMPA receptors in Bergmann glial cells do not participate in LTD induction.

Discussion

Whether GluRδ2 functions as an ion channel has been a long-standing question, but the lack of specific agonists has precluded an unequivocal conclusion. Thus, although we previously suggested that GluRδ2 does not function as a Ca^{2+}-permeable channel (Kakegawa *et al.* 2007), we were unable to examine its ion channel activities directly. In this study, we demonstrated that a V/R mutation in GluRδ2's putative channel pore domain did not interfere with its role in the induction of LTD (Fig. 2), while a V/R mutation and similar mutations at position −1 almost completely blocked the channel activities of GluRδ2^{Lc} (Fig. 1) and wild-type AMPA/kainate receptors (Dingledine *et al.* 1992; Robert *et al.* 2002). Furthermore, although currents passing through the GluRδ2^{Lc} channels (Fig. 4*A* and *B*) and glial AMPA receptors (Fig. 4*C* and *D*), both of which are composed of subunits containing Q at the Q/R site, were potently blocked by NASP, LTD was normally induced in cerebellar slices in the presence of NASP (Fig. 4*E* and *F*). Therefore, although we cannot rule out the possibility that GluRδ2 may function as an ion channel in other phenotypes observed in *GluRδ2*-null mice, we propose that GluRδ2, which belongs to the 'ionotropic' glutamate receptor family, does not serve as an ion channel in the regulation of LTD induction.

The roles of GluRδ2 in regulating the number of postsynaptic AMPA receptors (Hirai *et al.* 2003) are consistent with the view that GluRδ2 may rather serve as a non-ionotropic receptor, which mediates signals by interacting with intracellular molecules. Many PDZ proteins, like PSD-93 (Roche *et al.* 1999), PTPMEG (Hironaka *et al.* 2000), S-SCAM (Yap *et al.* 2003) and delphilin (Miyagi *et al.* 2002), have been reported to interact with the C-terminal end of GluRδ2. We demonstrated that the decoy peptide that hampered the interaction of GluRδ2 with these PDZ proteins interfered with LTD induction not only in wild-type Purkinje cells (Kohda *et al.* 2007), but also in *GluRδ2*-null Purkinje cells expressing GluRδ2^{wt}-V/R (Fig. 3). Interestingly, such non-ionotropic functions, which challenge classical thinking about ligand-gated ion channels, are not completely unprecedented; for example, the GluR2 subunit of AMPA receptors is reported to regulate dendritic spine formation in cultured hippocampus neurons independently of its ion channel activities (Passafaro *et al.* 2003). AMPA receptors have also been shown to regulate Lyn tyrosine kinase in the absence of channel activities (Hayashi *et al.* 1999). Similarly, kainate receptors have been reported to inhibit post-spike potassium currents to increase the excitability of hippocampal CA1 pyramidal neurons, independently of their ion channel activities (Melyan *et al.* 2002). Thus, we speculate that iGluRs may generally have both ionotropic and non-ionotropic functions; GluRδ2 is unique in that it may have lost its ionotropic functions during evolution. Therefore, further studies on the mechanisms by which GluRδ2 exerts its functions should provide insights into the non-ionotropic functions of iGluRs.

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Supplemental material

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http://jp.physoc.org/cgi/content/full/jphysiol.2007.141291/DC1
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