The Protein-tyrosine Phosphatase PTPMEG Interacts with Glutamate Receptor δ2 and ε Subunits*

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Glutamate receptor (GluR) δ2 is selectively expressed in cerebellar Purkinje cells and plays a crucial role in cerebellum-dependent motor learning. Although GluRδ2 belongs to an ionotropic GluR family, little is known about its pharmacological features and downstream signaling cascade. To study molecular mechanisms underlying GluRδ2-dependent motor learning, we employed yeast two-hybrid screening to isolate GluRδ2-interacting molecules and identified protein-tyrosine phosphatase PTPMEG. PTPMEG is a family member of band 4.1 domain-containing protein-tyrosine phosphatases and is expressed prominently in brain. Here, we showed by in situ hybridization analysis that the PTPMEG mRNA was enriched in mouse thalamus and Purkinje cells. We also showed that PTPMEG interacted with GluRδ2 as well as with N-methyl-d-aspartate receptor GluR1 in cultured cells and in brain. PTPMEG bound to the putative C-terminal PDZ target sequence of GluRδ2 and GluR1 via its PDZ domain. Examination of the effect of PTPMEG on tyrosine phosphorylation of GluRδ1 unexpectedly revealed that PTPMEG enhanced Fyn-mediated tyrosine phosphorylation of GluR1 in its PTPase activity-dependent manner. Thus, we conclude that PTPMEG associates directly with GluRδ2 and GluR1. Moreover, our data suggest that PTPMEG plays a role in signaling downstream of the Glurs and/or in regulation of their activities through tyrosine dephosphorylation.
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**EXPERIMENTAL PROCEDURES**

**Yeast Two-hybrid Screening**—Yeast two-hybrid screens were performed using the L40 yeast strain harboring the reporter genes HIS3 and β-galactosidase under the control of upstream LexA-binding sites. According to the topological model of ionotropic glutamate receptors (20–22), the C terminus of GluR2 (amino acids 922–1007) was used to screen a human brain cDNA library (CLONTECH Laboratories) in vector pACT2.

**DNA Constructs**—The cDNAs encoding PTPMEG (11) and GluR2 (3) were subcloned into the mammalian expression vector containing Shs promoter, pME18S, or pME18S-Myc (23). We utilized the cDNA encoding rat NR2A instead of mouse Glur1 to construct the expression plasmid of Glur1 (24). To prepare the FLAG epitope-tagged GluR2 (GluR2FLAG) deletion mutants, Glur2 cDNA was inserted in-frame with the oligonucleotides encoding a FLAG epitope sequence DDKDDK between amino acid residues 51 and 52. To prepare the HA epitope-tagged Glur1 (GluR1HA) deletion mutants, Glur1 cDNA was inserted in-frame with the oligonucleotides encoding a HA epitope sequence YPYDVPDYASL between amino acid residues 56 and 57. For the constructions of the expression vectors of deletion mutants, the cDNA fragments encoding PTPMEG and GluR2FLAG deletion mutants were amplified by polymerase chain reaction techniques. After the validity is confirmed by DNA sequencing, the amplified fragment was subcloned into pME18S-Myc or pME18S. The PTPMEG deletion mutants in pME18S-Myc and GluR2FLAG deletion mutants in pME18S contain the following amino acid residues of the respective proteins: PTPMEG/wt, 1–926; PTPMEG/a, 1–658; PTPMEG/b, 1–603; PTPMEG/c, 1–516; PTPMEG/d, 1–367; PTPMEG/e, 368–926; PTPMEG/f, 517–926; GluR2/1–926; Glur2/1–1004; Glur2/2, 1–983. The plasmid encoding Y531F constitutive active form of Fyn PTK (FynF) is as described (23, 25). PTPase inactive mutant of PTPMEG (PTPMEG-DA), encoding Ala–840 instead of Asp–840, Glur2FLAG-IA (11007A) and Glur1HA-VA (V1464A) were generated by the method of Kunkel. The amino acid substitution was confirmed by DNA sequencing.

**In situ Hybridization**—In situ hybridization was performed as described previously (26, 27). In short, the cDNA fragments corresponding to the N terminus proximal region of mouse PTPMEG (amino acids 11–142) was amplified by the reverse transcription-polymerase chain reaction method. The amplified cDNA fragment was subcloned into the pBluescript II vector. After confirming the DNA sequence, we generated the constructs of the expression vectors of deletion mutants, the cDNA fragments encoding PTPMEG and Glur2FLAG deletion mutants were amplified by polymerase chain reaction techniques. After the validity is confirmed by DNA sequencing, the amplified fragment was subcloned into pME18S-Myc or pME18S. The PTPMEG deletion mutants in pME18S-Myc and Glur2FLAG deletion mutants in pME18S contain the following amino acid residues of the respective proteins: PTPMEG/wt, 1–926; PTPMEG/a, 1–658; PTPMEG/b, 1–603; PTPMEG/c, 1–516; PTPMEG/d, 1–367; PTPMEG/e, 368–926; PTPMEG/f, 517–926; Glur2/1–926; Glur2/1–1004; Glur2/2, 1–983. The plasmid encoding Y531F constitutive active form of Fyn PTK (FynF) is as described (23, 25). PTPase inactive mutant of PTPMEG (PTPMEG-DA), encoding Ala–840 instead of Asp–840, Glur2FLAG-IA (11007A) and Glur1HA-VA (V1464A) were generated by the method of Kunkel. The amino acid substitution was confirmed by DNA sequencing.

**Antibodies**—Rabbit anti-PTPMEG polyclonal antibodies were raised against the GST mouse PTPMEG (amino acids 436–926) and affinity purified. Rabbit anti-GluR2 polyclonal antibodies were raised against GST-GluR2 (amino acids 852–931) and affinity purified. Rabbit anti-PDF-95 polyclonal antibodies were used for immunoprecipitation were raised against the GluR-human PSD-95 (amino acids 1–45) and affinity purified. Mouse anti-PTPMEG monoclonal antibody used to immunoprecipitate Glur1 complex was described previously (24). Mouse anti-synaptophysin monoclonal antibody and rabbit anti-PDF-95 polyclonal antibodies used for Western blotting were described previously (28–30). Rabbit anti-Trk B polyclonal antibodies were purchased from Transduction Laboratories. Anti-Myc (9E10), rabbit anti-Fyn (Fyn3), and goat anti-GluR1 (C-17) polyclonal antibodies were purchased from Santa Cruz Biotechnology. Anti-PY (4G10) monoclonal antibodies were from Upstate Biotechnology. Anti-influenza HA monoclonal antibody and rabbit anti-PSD-95 polyclonal antibodies used for immunoprecipitation were raised against the GST mouse PTPMEG (amino acids 436–926) and affinity purified. Rabbit anti-GluR2 polyclonal antibodies were raised against GST-GluR2 (amino acids 852–931) and affinity purified. Rabbit anti-PDF-95 polyclonal antibodies were used for immunoprecipitation were raised against the GluR-human PSD-95 (amino acids 1–45) and affinity purified. Mouse anti-PTPMEG monoclonal antibody used to immunoprecipitate Glur1 complex was described previously (24). Mouse anti-synaptophysin monoclonal antibody and rabbit anti-PDF-95 polyclonal antibodies used for Western blotting were described previously (28–30). Rabbit anti-Trk B polyclonal antibodies were purchased from Transduction Laboratories. Anti-Myc (9E10), rabbit anti-Fyn (Fyn3), and goat anti-GluR1 (C-17) polyclonal antibodies were purchased from Santa Cruz Biotechnology. Anti-PY (4G10) monoclonal antibodies were from Upstate Biotechnology. Anti-influenza HA monoclonal antibody was from Roche Molecular Biochemicals. Anti-FLAG epitope monoclonal antibody was from Sigma.

**Cell Culture, Transfection, Immunoprecipitation, and Immunoblotting**—293T cells were maintained in 10% fetal bovine serum/Dulbecco’s modified Eagle’s medium under 37 °C, 5% CO2 condition. 293T cells (1 × 106 cells per dish) were transfected with combinations of expression plasmids (total, 20 μg) by the standard calcium phosphate method. Two days after transfection, cells were lysed in TNE buffer (50 mM Tris-HCl, 1% Nonidet P-40, 5 mM EDTA, 150 mM NaCl) containing 100 μM Na,VO3, 100 μM phenylmethylsulfonyl fluoride, 10 units/ml aprotinin, and 20 μM Pefabloc SC (Merck). Insoluble fraction was excluded by centrifugation at 15 K rpm for 20 min. Total cell lysates (TCLs) were boiled in the presence of sample buffer. Affinity purified antibodies (1–3 μg) were added to the precleared cell lysates to obtain protein immunoprecipitates. Immunoprecipitates and TCL were separated by SDS–polyacrylamide gel electrophoresis (7.5% gel) and transferred onto polyvinylidene difluoride membranes (Bio-Rad). The membrane was then blocked with 5% bovine serum albumin/TBST solution for 2 h at room temperature and treated with primary antibodies. Horseradish peroxidase-conjugated secondary antibodies and Renaissance Plus reagent (NEN Life Science Products) were used to visualize the immunoreactive proteins.

**Preparation of Subcellular Fractions and Lysate from Brain**—Isolation of subcellular fractions of brain was performed as described previously (31). Equal amounts (total protein, 15 μg) of fractions were separated by SDS–polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane. For immunoprecipitation, cerebellar or telencephalon of 12–18-week-old C57BL/6 or Glur2 knockout mice were homogenized with 10 volumes (w/v) of homogenization buffer (0.32 μM sucrose, 1 mM NaHCO3, 1 mM MgCl2, 100 μM Na,VO3, 100 μM phenylmethylsulfonyl fluoride, 10 units/ml aprotinin, and 20 μM Pefabloc SC). The supernatome and mitochondria fractions were prepared as described previously (31) and were lysed in 1% deoxycholate buffer (32). Immunoprecipitation of proteins from the lysates was performed as described above.

**RESULTS**

**Identification of PTPMEG as a GluR2-interacting Molecule**—To identify GluR2-interacting molecules, we employed yeast two-hybrid system using the C terminus proximal resi-
dues of GluRd2 (amino acids 922–1007) as a bait (Fig. 1A). We screened approximately 10⁶ yeast transformants and obtained six positive clones that activated expression of selection markers His3 and LacZ. Nucleotide sequence analysis revealed that they encoded a GTPase exchange factor, three PTPases, and two PDZ containing-scaffold proteins that did not belong to the PSD-95 family. One of the PTPase-encoding clones carried a sequence for C-terminal two-thirds of PTPMEG (Fig. 1B). PTPMEG belongs to a family of intracellular protein-tyrosine phosphatases that contain a band 4.1 domain, a PDZ domain, and a catalytic PTP domain (Ref. 11 and Fig. 1B). PTPMEG is expressed highly in brain (15), suggesting that PTPMEG may have important functions in the central nervous system. To confirm the interaction between GluRd2 and PTPMEG, 293T cells were transfected with Myc-tagged PTPMEG and/or GluRd2 expression plasmids, and then protein lysates were prepared from the transfected cells. By probing the anti-Myc immunoprecipitates of the lysates with anti-GluRd2 or anti-Myc antibody, we showed that GluRd2 co-precipitated with Myc-tagged PTPMEG only when both proteins were expressed (Fig. 1C, left panel). Conversely, PTPMEG was present in the GluRd2 immunoprecipitates (Fig. 1C, right panel), indicating that PTPMEG associated with GluRd2 in heterologous 293T cells.

Expression Pattern of PTPMEG in Brain—Previous reports indicated that expression of GluRd2 was confined to cerebellar Purkinje cells (3). In contrast, the precise distribution of PTPMEG in brain remained to be established. Because GluRd2 immunoreactivity in cerebellum increased dramatically during synaptogenesis (postnatal 2–3 weeks) (2), we compared expression pattern of PTPMEG with that of GluRd2 in brain during this same stage of development. Using the cRNA probes corresponding to mouse PTPMEG (amino acids 11–142), we performed in situ hybridization analysis of P17 mouse brain parasagittal sections (Fig. 2A). The antisense probe showed intense signals in the thalamus and the cerebellar Purkinje cell layer. Moderate signals were detected in the olfactory bulb, cerebral cortex, and hippocampus, and weak signals were detected broadly. No specific hybridization was detected using the...
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Fig. 4. Interaction sites between GluR\(_{22}\) and PTPMEG. A, schematic diagram of the PTPMEG deletion mutants. A hatched box at the N terminus of each mutant indicates the Myc tag. Numbers are amino acid positions of PTPMEG showing truncation site of each mutant. B, schematic diagram of the Glur\(_{22}\) deletion mutants. TM1-TM4 indicate transmembrane regions. Numbers are amino acid positions of the truncation site of each Glur\(_{22}\) mutant. C and D, binding of PTPMEG to the C-terminal PDZ target sequence of Glur\(_{22}\) through the PDZ domain. Various combinations of expression plasmids, as indicated above each lane, were transfected into 293T cells. The cells were subsequently lysed and immunoprecipitated with the indicated antibodies to test for co-precipitation of associated proteins. Immunoprecipitates and TCL sense cRNA probe (data not shown). Dark and bright field photomicrographs of cerebellum revealed that the PTPMEG mRNA was abundant in Purkinje cells (Fig. 2, B and C). These data suggested co-expression of Glur\(_{22}\) and PTPMEG mRNA in Purkinje cells during synaptogenesis, which was consistent with our observation that Glur\(_{22}\) interacted with PTPMEG. We also detected abundant PTPMEG mRNA in the regions of the anterior nucleus and the ventral anterior nucleus of the thalamus. The molecules that would interact with PTPMEG in these regions have not been known.

Subcellular Distribution of PTPMEG and Its Interaction with Glur\(_{22}\) in Cerebellum—GluR\(_{22}\) subunits are targeted to the postsynaptic fraction of Purkinje cells (4). To compare the subcellular localization of PTPMEG with that of Glur\(_{22}\) in the neural cells, we prepared subcellular fractions of adult mouse cerebellum and telencephalon and performed immunoblot analysis (33–35). As shown in Fig. 3A, PTPMEG was present in the postsynaptic density (PSD) fraction where glutamate receptors were concentrated (upper panel). PTPMEG in the PSD fraction was about 3% of PTPMEG in the total soluble fraction. Note that the proteins in the PSD fraction prepared as above corresponded to about 0.5% of total soluble proteins. The validity of subcellular fractionation was proved by immunoblotting the proteins in the subcellular fractions with antibodies against various brain specific proteins such as Glur\(_{22}\), GluR1, PSD-95, and synaptophysin (Fig. 3A). To determine whether Glur\(_{22}\) and PTPMEG interact in cerebellum, anti-GluR\(_{22}\) immunoprecipitates from cerebellar lysates were probed with anti-PTPMEG antibodies. The data clearly showed that PTPMEG was co-precipitated with Glur\(_{22}\) (Fig. 3B). In reciprocal co-immunoprecipitation experiments, anti-PTPMEG immunoprecipitates from cerebellar lysates were probed with anti-GluR\(_{22}\) antibodies. The data showed that anti-PTPMEG immunoprecipitates contained Glur\(_{22}\).

To identify the amino acid sequences responsible for the interaction between Glur\(_{22}\) and PTPMEG, we constructed deletion mutants of PTPMEG and Glur\(_{22}\) (Fig. 4, A and B), and combinations of Glur\(_{22}\) and PTPMEG constructs were transfected into 293T cells. Co-immunoprecipitation experiments with the lysates of the transfected cells showed that wild-type PTPMEG and its mutants containing the PDZ domain, except mutant "e" interacted with wild-type Glur\(_{22}\) (Fig. 4C). It is likely that the sequence between the band 4.1 domain and the PDZ domain had an inhibitory effect on the interaction when exposed at the N terminus. Co-immunoprecipitation experiments also revealed that removal of the putative PDZ target sequence Thr-Ser-Ile from the C terminus of Glur\(_{22}\) and the point mutation at the C terminus (Ala-1007 instead of Ile-1007) abolished interaction between the two proteins (Fig. 4D). Thus, we concluded that at least some PTPMEG proteins were co-localized with Glur\(_{22}\) and that these two proteins interacted with each other in brain. Our present data suggest that PTPMEG associates directly with the putative C-terminal PDZ target sequence of Glur\(_{22}\) via its PDZ domain.

Interaction between GluR1 and PTPMEG in Cultured Cells and in Telencephalon—Because the Ser-Asp-Val sequence of Glur\(_{22}\) is a typical target of the PDZ domain, GluR1 may also interact with PTPMEG. To examine this possibility, 293T cells were transfected with expression plasmids encoding Myc-tagged PTPMEG and/or GluR1, and the lysates of the transfected cells were subjected to co-immunoprecipitation experiments. By probing anti-Myc immunoprecipitation are as indicated in Fig. 1. The same filter was stripped and then reprobed with the indicated antibodies. wt, wild type; IP, immunoprecipitation.
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munoprecipitates and TCL are as indicated in Fig. 1. Immunoblotting were immunoprecipitated (IP) with anti-Myc monoclonal antibody. Immunoblotting were reprobed with the indicated antibodies. As shown in Fig. 5B, we detected PTPMEG in the anti-GluRe1 immunoprecipitates (left panel) and GluRe1 in the anti-PTPMEG immune-complex (right panel). The data suggested that PTPMEG interacted with GluRe1 in vivo. To address the mechanism of the interaction, we expressed various PTPMEG mutants (Fig. 4A) together with GluRe1 in 293T cells. The lysates of the transfectants, we could co-immunoprecipitate GluRe1 with wild-type PTPMEG and with mutants that carried the PDZ domain (Fig. 5D). Mutant e of PTPMEG, which did not interact with GluR82, could associate with GluRe1. We do not have good explanation for this observation. However, it is possible that the three-dimensional structure around the C terminus of GluRe1 is different from that of GluR82, which could cause different affinities of mutant e construct to the GluRs. In reciprocal immunoprecipitation experiments with various HA-tagged GluRe1 mutants (Fig. 5C), we could detect wild-type GluRe1 and its mutants containing the C-terminal PDZ target sequence in the PTPMEG immunoprecipitates (Fig. 5E). Furthermore, the point mutant of the C terminus of GluRe1 (GluRe1HA-V1464A) did not associate with PTPMEG in 293T cells. Thus, we conclude that the PDZ domain of PTPMEG and the C terminus of GluRe1 are critically important for the interaction between PTPMEG and GluRe1.

Enhancement of Fyn-mediated Tyrosine Phosphorylation of GluRe1 by PTPMEG—Because the biochemical and pharmacological features of NMDA receptors had been better characterized than those of GluR82, we decided to explore the biological significance of PTPMEG-GluRe1 interaction. We first examined the effect of PTPMEG on tyrosine phosphorylation of GluRe1. Because PTPMEG has PTPase activity, we expected that PTPMEG might compete with Fyn in tyrosine phosphorylating GluRe1. To test this possibility, the expression vectors encoding constitutively active Fyn (FynF) and/or PTPMEG were transfected into 293T cells together with GluRe1, and the level of tyrosine phosphorylation of GluRe1 was examined. Unexpectedly, coexpression of PTPMEG enhanced Fyn-mediated tyrosine phosphorylation of GluRe1 (Fig. 6). Furthermore, was performed using anti-Myc or anti-GluRe1 monoclonal antibody to detect the interacting proteins. The same filter was stripped and then reprobed with the indicated antibodies. B, identification of GluRe1 in PTPMEG immunoprecipitates (left panel) and PTPMEG in GluRe1 immunoprecipitates (right panel) from mice telencephalons. The lysates of mouse telencephalons were immunoprecipitated with the indicated antibodies. The immunoprecipitates and synaptosome and mitochondria fractions (Sm; 1/8 amount of lysate used for anti-GluRe1 immunoprecipitates and 1/6 amount of lysate used for anti-PTPMEG immunoprecipitates) were subjected to immunoblotting with anti-GluRe1, anti-PTPMEG, anti-Trk B, and anti-PSD-95 antibodies. C, schematic diagram of the GluRe1 deletion mutants. TM1–4 indicate transmembrane regions. HA indicates the influenza HA tag. Residue numbers correspond to those in the amino acid sequence of GluRe1. The ΔA mutant contains an internal deletion of 125 amino acids (amino acids 1220–1345). The ΔB and ΔC mutants lack 348 and 607 C-terminal amino acids, respectively. VA indicates the point mutant of GluRe1 (GluRe1HA-V1464A). D and E, binding of PTPMEG to the C terminus of GluRe1 through its PDZ domain. The expression constructs of PTPMEG deletion mutants were as shown in Fig. 4A. Various combinations of expression constructs, as indicated above each lane, were transfected into 293T cells. The lysates of the transfectants were subsequently immunoprecipitated with the indicated antibodies to test for co-precipitation of associated proteins. Immunoprecipitates and TCL are as indicated in Fig. 1. The positions of the PTPMEG deletion mutants are indicated by arrowheads. The same filter was stripped and then reprobed with the indicated antibodies. wt, wild type.
coexpression of PTPase inactive mutant of PTPMEG (termed PTPMEG-DA) did not increase Fyn-mediated tyrosine phosphorylation of GluR1. We also showed that the PTPase active mutant (Fig. 4A, construct f) facilitated phosphorylation of GluR1 more effectively than wild-type PTPMEG. These data showed that PTPMEG enhanced Fyn-mediated tyrosine phosphorylation of GluR1 in a PTPase activity-dependent manner. Furthermore, introduction of the V1464A point mutation of GluR1 significantly reduced Fyn-mediated GluR1 phosphorylation in the presence of PTPMEG. Because the GluR1 mutant was unable to interact with PTPMEG, the data supported our conclusion that Fyn-mediated tyrosine phosphorylation of GluR1 was enhanced by the interaction between PTPMEG and GluR1. A slight enhancement of tyrosine phosphorylation of the GluR1 mutant observed in the presence of PTPMEG could be due to nonspecific microenvironmental changes induced by the phosphatase activity. The overall level of protein-tyrosine phosphorylation in the cells expressing GluR1, FynF in the presence of PTPMEG was significantly lower than that in the absence of PTPMEG (data not shown), suggesting that PTPMEG was active in the cells. Moreover, the effect of PTPMEG on tyrosine-phosphorylation of GluR1 was not caused by the further activation of FynF, because in vitro kinase assays showed that the activity of FynF was not dependent on the PTPase activity of PTPMEG (data not shown).

**DISCUSSION**

In this report, we have shown that a protein-tyrosine phosphatase PTPMEG interacts with GluR2 as well as GluR1 both in vivo and in vitro. The interaction is mediated by the PDZ domain of PTPMEG and the C-terminal PDZ target sequences of these GluRs. Because the C termini of the four GluR subunits contain Glu-Ser-(Asp/Glu)-Val sequences, we assume that all of them would interact with PTPMEG. In fact, we observed the interaction between PTPMEG and GluR2 in 293T cells (data not shown). Both the GluR subunits and GluR2 also bind to other PDZ domain-containing proteins, such as PSD-95 family proteins (16, 17, 32, 36). These proteins are thought to be important to localize the GluR subunits to the postsynaptic cell membrane, serving as scaffold proteins. Unlike these, PTPMEG is a catalytical protein associated with PTPase activity. Therefore, the biological significance of its interaction with GluRs ought to be distinct from the scaffold function. We showed here that PTPMEG stimulated Fyn-mediated tyrosine phosphorylation of GluR1 in a manner dependent on its PTPase activity. Although we previously reported that PSD-95 could stimulate Fyn-mediated tyrosine phosphorylation of GluR1 (24), the underlying mechanisms of stimulation mediated by PSD-95 and by PTPMEG would be different from each other. Apparently, it is important to clarify the mechanism by which the interaction between GluRs and the PDZ domain-containing proteins, including PTPMEG, is regulated.

The band 4.1 domain is observed in several membrane-cytoskeleton linker proteins such as ezrin, radixin, and moesin (ERM proteins). ERM proteins associate with the transmembrane protein CD44 through their band 4.1 domains (37). The band 4.1 domain of ERM proteins can also interact with Rho GDI and Dbl and links ERM proteins to the signal transduction pathways controlled by Rho GTPases (38, 39). These results suggest that the band 4.1 domain of PTPMEG functions as the binding domain for another transmembrane protein or as the adapter domain involved in the Rho GTPase signaling cascade to regulate the organization of actin cytoskeleton. We propose that the rearrangement of the cytoskeleton around GluR1 might make GluR1 more susceptible to tyrosine phosphorylation by Fyn PTK. It would be a likely mechanism by which PTPMEG exerts its effect on Fyn-mediated tyrosine phosphorylation of GluR1. Tyrosine phosphorylation of GluR2 was not observed so far, suggesting that PTPMEG would play another role by interacting with GluR2.

NMDA receptors play crucial roles in the neuronal functions, such as development, synaptic plasticity, and neurotoxicity (28, 40). NMDA receptor activation induces calcium influx, followed by activation of Ca^{2+}-dependent enzymes including a calcium-activated neutral protease (calpain). In platelets, the phosphatase activity of PTPMEG is activated upon cleavage by calpain in response to calcium ionophore and thrombin (15). Thus, NMDA receptor stimulation may activate PTPMEG through the activation of calpain. This would result in further activation of NMDA receptor, because PTPMEG enhances Fyn-mediated GluR1 phosphorylation and because tyrosine phosphorylation of GluR1 could stimulate the channel activity of the NMDA receptor (41). However, these possibilities need clarification by experimentation in primary neurons.

We have shown that PTPMEG is prominently expressed in cerebellar Purkinje cells and in the cells at thalamus where GluR2 (3) and the four GluR subunits (42), respectively, are rich. Therefore, PTPMEG could interact with distinct GluRs depending on the cell types in which it is expressed. In addition, because PTPH1, a PTPase highly similar to PTPMEG, is also expressed significantly in thalamus, it is intriguing to address whether PTPH1 also interacts with GluR2 subunits. Finally, our present study suggests that PTPMEG could function as a regulator as well as a downstream signal transducer of GluR2 and GluR subunits. Further investigations, such as those aimed at elucidating the roles of PTPMEG in GluRs signaling pathway, will provide valuable insight into the molecular mechanisms of GluR2-dependent motor learning as well as NMDA receptor functions.

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