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REVIEW

AN ORPHAN IONOTROPIC GLUTAMATE RECEPTOR: THE δ2 SUBUNIT

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Abstract—The glutamate receptor δ2 (GluRδ2) subunit has been classified as an ionotropic glutamate receptor on the basis of the amino acid sequence. It is considered an orphan receptor since no physiological ligand has so far been identified. GluRδ2 is selectively localized at the parallel fiber–Purkinje cell (PF–PC) synapses in the adult cerebellar cortex, where it promotes and maintains the integrity of these synapses. Mutations of the gene coding for the GluRδ2 are also accompanied by reduced regression of the climbing fiber (CF) multiple innervation, loss of long term depression (LDT) and by specific cerebellar dysfunctions involving motor coordination, motor learning and impairment of fear memory consolidation. In addition, it participates in the competition between heterologous afferent fibers to PCs. On the whole, it appears that during evolution GluRδ2 has lost its channel properties to acquire the function of an activity-dependent adhesion molecule with the key role of orchestrating the architecture of the PC innervation to allow two different patterns of signal elaboration; the CF all-or-none depolarization in the proximal dendritic domain and a highly discriminative capacity in the distal domain.

Key words: cerebellum, axonal competition, Purkinje cell, activity-dependent plasticity, synaptogenesis.

Contents

The δ receptors are a subtype of glutamate receptors which have been cloned by homology screening (Yamazaki et al., 1992) and exist under two forms called δ1 and δ2. From the amino acidic sequence pattern, these subunits belong to the ionotropic type. In particular the δ2 has a 14–24% amino acidic sequence identity with the subunits of AMPA-kainate or *N*-methyl-D-aspartate (NMDA) receptors and shares a 56% identity with the δ1 subunit (Yamazaki et al., 1992; Lomeli et al., 1993). So far, the glutamate receptor delta (GluRδ) 2 and GluRδ1 remain the only receptor subunits without a known ligand. Excellent reviews on this topic have been recently published (Yuzaki, 2003a,b, 2004, 2005; Hirano, 2006).

THE GENE CODING THE GLURδ2: EXPRESSION AND PROTEIN LOCALIZATION

In mice the *G*lutamate *r*eceptor *i*onotropic *d*elta-2 (Grid2) gene is located on chromosome 6 (29.6 cM) and consists of 16 exons covering a region of approximately 1.4 Mb. Many spontaneous mutations occur in this gene (Wang et al., 2003). In fact, at least 18 ataxic mutant mice (Mouse Genome Informatics, 2007) are linked to the Grid2 locus (Lalouette et al., 2001). Recently, fragile sites have been identified in the mouse Grid2 gene and its human ortholog (Rozier et al., 2004; Robinson et al., 2005). In humans, the GRID2 gene is located on chromosome 4 (q22), and has a 97% identity in amino acidic sequence to the mouse gene (Hu et al., 1998). Although no neurological diseases have

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Abbreviations: aa, amino acid; AP-4, adaptor protein-4; C-terminal, carboxyl-terminal; CF, climbing fiber; CS, conditioned stimulus; delphilin, delta2-philic-protein; ER, endoplasmic reticulum; GluRδ, glutamate receptor delta; Grid2, glutamate receptor ionotropic delta-2; ho, hotfoot; iGluR, ionotropic glutamate receptor; KO, knockout; LAOBP, lysine/arginine/ornithine-binding protein; LIVBP, leucine/isoleucine/valine-binding protein; LTD, long term depression; MAGUK, membrane-associated guanylate kinase; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; nPIST, postsynaptic density-95/discs large/zona occludins-1 domain protein interacting specifically with TC10; N-terminus, amino-terminus; P, postnatal day; PC, Purkinje cell; PDZ, postsynaptic density-95/discs large/zona occludins-1; PF, parallel fiber; PICK1, protein interacting with C kinase 1; PKC, protein kinase C; PSD, postsynaptic density; PTPMEG, protein-tyrosine phosphatase megakaryoblastic; S-SCAM, synaptic scaffolding molecule; TM, transmembrane; TTX, tetrodotoxin; US, unconditioned stimulus; wt, wild type; 3-AP, 3-acetylpyridine.

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been so far genetically linked to this locus in human, it is very likely that at least one of the many cerebellar ataxias is due to this gene dysfunction.

It is of interest that patients with acute cerebellar ataxia present high levels of anti-GluRδ2 auto-antibodies in the plasma and in their cerebrospinal fluid. Furthermore, a correlation between the progressive decrease of the auto-antibodies against GluRδ2 and the clinical improvement of the patients has been found, thus suggesting a role of the auto-antibodies in inducing cerebellar ataxia (Shiihara et al., 2007; Shimokaze et al., 2007).

RNA blot, *in situ* hybridization and immunocytochemical labeling analysis have shown a selective cellular localization of δ2 subunit mRNA and protein in cerebellum, whereas it is hardly detectable in forebrain. GluRδ2 is expressed in the Purkinje cells (PC) before synaptogenesis and becomes concentrated at the synaptic membrane of dendritic spines according to the PC maturation. mRNA is detected in PCs in mice as early as embryonic day 15 (E15). Then, the expression increases markedly during the second and third week of postnatal development and it remains high throughout adulthood. At early postnatal period, the GluRδ2 protein is localized widely in both dendritic shafts and spines. From postnatal day 14 (P14), immunoreactive GluRδ2 is found predominantly in dendritic spines and at P21 it is detectable exclusively in spines (Takayama et al., 1996). Ultrastructural high-resolution techniques of cryosubstitution and postembedding showed that in adult mice high gold particle density occurs in all parallel fiber (PF) synapses with PC distal dendritic spines, whereas other synapses are consistently devoid of labeling, including the climbing fiber (CF) synapses on proximal spines (Landsend et al., 1997; Zhao et al., 1998). However, the analysis on developing PCs revealed that it is transiently detectable also in CF synapses and it is highly expressed between P10 and P14 (Zhao et al., 1998) (Fig. 1A).

Recently it has been shown that in adult rats following block of electrical activity by a chronic infusion of tetrodotoxin (TTX), GluRδ2 reappears in spines innervated by the CFs (Figs. 1B, 2A, B) and also in postsynaptic densities of the spines innervated by GABA-ergic neurons (Morando et al., 2001). Therefore, it has been proposed that GluRδ2 is intrinsically expressed in all PC spines independent from their innervation and that the activity of the CF exerts an inhibitory action on GluRδ2 expression in their synapses.

THE GLURδ2 PROTEIN STRUCTURE AND FUNCTION

The GluRδ2 subunit has an architecture similar to other ionotropic glutamate receptor (iGluR) subunits and consists of an extracellular amino-terminus (N-terminus) which harbors a bacterial periplasmic amino acid (aa) leucine/isoleucine/valine-binding protein (LIVBP) –like domain and a bipartite lysine/arginine/ornithine-binding protein (LAOBP)-like domain, three transmembrane domains (TM1, TM3 and TM4), an ion-channel-forming re-entrant loop segment (TM2) and a

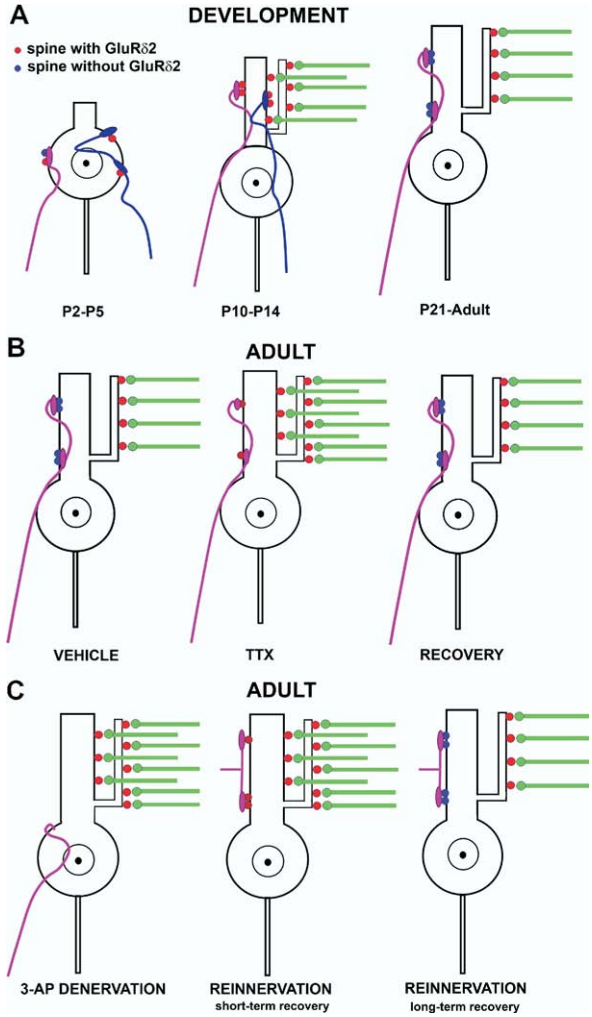

Fig. 1. GluRδ2 expression in the cerebellum. (A) Schematic localization of PC excitatory innervation and expression of this subunit in the dendritic spines during development. Multiple CFs innervate the PC body at early postnatal ages (~P2–P5). In a second stage (~P10–P14) the PC dendritic arborization expands and PFs and multiples CFs innervate these dendrites. A final stage (~P21–adult) involves the maturation of the excitatory synapses on PCs: adult PCs are innervated by many PFs and by numerous contacts from one CF. GluRδ2 is expressed in all PF synapses from early postnatal stages to adult and it is transiently detectable in CF synapses where it is later repressed. (B) Following TTX infusion in the adult cerebellum, the situation brings to mind the developmental pattern, since this subunit appears in the proximal dendritic domain. CF varicosities become smaller and lose contacts with the PC. During the recovery period, CF exerts a repressive action on the GluRδ2 expression and displaces the competitor afferent. (C) After inferior olive lesion new spines expressing the GluRδ2s appear on the proximal dendrites of the CF denervated PCs and they are innervated by the PFs. Surviving CFs extend new collateral sprouting toward the denervated PCs, and contact the GluRδ2-bearing spines of the denervated PCs (short-term recovery). At longer survival times the CFs stabilize their synapses and repress the GluRδ2 expression, the supernumerary spines disappear and PF synapses are withdrawn from the proximal dendrites (long-term recovery).

cytoplasmic carboxyl-terminal (C-terminal) region (Dingledine et al., 1999; Yuzaki, 2003a) (Fig. 3). Despite this similarity, the ionotropic function of the GluRδ2 seems to be lost during evolution (Yuzaki, 2004). The mouse