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Immunohistochemistry of GluR1 subunits of AMPA receptors of rat cerebellar nerve cells

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ABSTRACT: The localization of GluR1 subunits of ionotropic glutamate receptors in the glial cells and inhibitory neurons of cerebellar cortex and their association with the climbing and parallel fibers, and basket cell axons were studied. Samples of P14 and P21 rat cerebellar cortex were exposed to a specific antibody against GluR1 subunit(s) of AMPA receptors and were examined with confocal laser scanning microscopy. GluR1 strong immunoreactivity was confined to Purkinje cell and the molecular layer. Weak GluR1 immunoreactivity was observed surrounding some Golgi cells in the granule cell layer. Intense GluR1 immunoreactivity was localized around Purkinje, basket, and stellate cells. Purkinje cells expressed strong GluR1 immunoreactivity surrounding the cell body, primary dendritic trunk and secondary and tertiary spiny dendritic branches. Marked immunofluorescent staining was also detected in the Bergmann glial fibers at the level of middle and outer third molecular layer. Positive immunofluorescence staining was also observed surrounding basket and stellate cells, and in the capillary wall. These findings suggest the specific localization of GluR1 subunits of AMPA receptors in Bergmann glial cells, inhibitory cerebellar neurons, and the associated excitatory glutamatergic circuits formed by climbing and parallel fibers, and by the inhibitory basket cell axons.

Introduction

GluR1 is one of the several subunits of the quisqualate receptors coupled to cationic ionic channel, also termed AMPA receptors (Crepel *et al*., 1996; Michaelis, 1996). The characterization of the glutamate receptor subunit GluR1 was earlier reported in the adult rats by Rogers *et al*. (1991) in neurons of the cerebellum and some structures of limbic system, including hippocampus, the central nucleus of amygdala, and portion of the septum. Blackstone *et al*. (1992) made the biochemical characterization and localization of a non-N-methyl-D-aspartate glutamate receptor in rat brain. Molnar *et al*. (1993) studied the immuno-cytochemical characterization of antipeptide antibodies to a cloned GluR1 glutamate receptor subunit showing immunoreactivity for the GluR1 subunit selectively distributed to the rat forebrain. The hippocampus, septum, amygdala and olfactory bulb exhibited the strongest immunoreactivity.

Van den Pool *et al*. (1994) studied ionotropic glutamate-receptor gene expression using *in situ* hybridization and Northern blot technique for demonstrating the ionotropic subtypes of the glutamate receptors in the hypothalamus. Day *et al*. (1995) showed the distribution of AMPA-selective glutamate receptor subunits in human cerebellum and hippocampus in human postmortem brain. Breese *et al*. (1996) described the regional gene expression of the glutamate receptor subtypes GluR1, GluR2, and GluR3 in human postmortem brain.

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Garyfallou *et al*. (1996) demonstrated the distribution of NMDA and AMPA receptors in the cerebellar cortex of Rhesus macaques. Petralia *et al*. (1996) found the ionotropic and metabotropic glutamate receptors at the pre- and postsynaptic, and glial localizations in the dorsal cochlear nucleus. Wu *et al*. (1996) made the molecular analysis of cDNA molecules encoding glutamate receptor subunits, fGluR1 alpha and fGluR1 beta in the telencephalon, optic tectum and cerebellum of adult fish brain, *Oreochromis* sp. Perry and Henley (1997) studied the half-life analysis of the alpha-amino-3-hydroxy-5-methyl-4-isoazolepropionate receptor subunit GluR1 in primary cultured rat cerebellar granule cells. Archibald *et al*. (1998) demonstrated the surface expression and metabolic half-life of AMPA receptors in cultured rat cerebellar granule cells.

Martin *et al*. (1993, 1998) reported brain glutamate receptor-1 expression and localization change at regional, cellular, and subcellular levels with maturation. Ripellino *et al*. (1998) examined the expression and heteromeric interactions of non-N-methyl-D-aspartate glutamate receptor subunits in the developing and adult cerebellum. Zhao *et al*. (1998) postulated that glutamate receptor targeting to synaptic populations on Purkinje cells is developmentally regulated. Keifer and Carr (2000) showed the immunocytochemical localization of glutamate receptor subunits in the brain stem and cerebellum of the turtle *Chrysemys picta.* Wang *et al*. (2000) made a light and electron microscopic study of glutamate receptors in the monkey subthalamic nucleus, and demonstrated the presence of GluR1 at the cell body, dendrites, and synaptic region. Kondo *et al*. (2000) showed that a high Glur1: GluR2 ratio is correlated with expression of Ca^{2+}-binding proteins in rat forebrain neurons. Inglis *et al*. (2002) studied the role of GluR1 on dendrite morphogenesis in motor neurons. Douyard *et al*. (2007) demonstrated by means of light and electron microscopy the expression of GluR1 and its scaffolding protein SAP97 during cerebellar development. These authors have shown by double labeled IF coupled to confocal microscopy that GluR1 expression is exclusively expressed in Bergmann glia at postnatal ages. More recently, Passeto *et al*. (2008) made a morphometric analysis of the AMPA-type neurons in the Deiter´s vestibular complex of the chick brain. However, little is known about the specific GluR-1 subunit receptor distribution pattern in the rat cerebellar cortex using confocal laser scanning microscopy and specific labeling immunohistochemistry.

In the present study we report by means of confocal laser scanning immunocytochemistry the localization of GluR1 subunits of AMPA receptors in Bergmann glial cells and inhibitory neurons of rat cerebellar cortex and their association with excitatory cerebellar afferent fibers formed by climbing fibers, granule cell axons or parallel fibers, and also with the inhibitory basket cell axons. The P14 developing cerebellar cortex is ideally suited for this purpose because of the exquisite level of laminar segregation of both afferent mossy, climbing and parallel fibers, as well as by the peak of synap-togenesis at this maturation stage, and by the already organized recipient neurons in the three-layered cerebellar cortex. From this perspective we have analyzed the distribution of GluR1 in P14 Wistar rats using a specific rabbit anti-phospho-GluR1 (SER 831) polyclonal unconjugated antibody, followed by Alexa-488 goat anti-rabbit (GAR) as a secondary antibody, and examined with confocal laser scanning microscope equipped with Argon (Ar; 488 nm), Krypton (Kr; 568 nm) and Helium-Neon (He-Ne; 633 nm) lasers, in order to detect if such receptors are organized in a region-specific and circuit-specific manner.

Material and Methods

GluR1 immunohistochemistry

Animals were used in accordance with both NIH and our own institutional guidelines. For GluR1 immunohistochemistry, cerebellar cortex slices were derived from 14-day-old postnatal (P14) Wistar rats anesthetized with CO_2. After decapitation, the lateral lobules of cerebellum were removed from posterior fossa and placed in ice-cold (4ºC) Hanks balanced salt solution supplemented with dextrose (6 mg/ml). Fragments of cerebellar cortex were transversally sliced (300 µm thick sections) using a manual tissue chopper (Stoelting). The slices were immediately fixed in 4% paraformaldehyde in ice-cold 0.1M phosphate-buffered saline (PBS). All samples for GluR1 immunolabeling were chemically fixed in ice cold paraformadehyde for no more than 30 min to preserve antigenicity. Free-floating slices were placed in a multiwell plate for subsequent rinsing, blocking and labeling steps. Slices were rinsed in PBS (2 x 5 minutes) and then extracted overnight in 1% Triton X-100 in PBS (Polysciences). Blocking was done with 50 mM NH_4Cl in PBS (30 min), followed by 20% horse serum in PBS (30 min). Washing was done with 1% horse serum in PBS (5 min). All subsequent steps were carried out in 1% horse serum-PBS. Slices were labeled with a rabbit anti-phospho-GluR1 (SER 831) polyclonal

unconjugated antibody specific to rat GluR1 (diluted 1:500; Molecular Probes (Invitrogen), Eugene, OR). This primary antibody was applied overnight (4ºC), and then tissues were rinsed again (3 x 15 minutes) in horse serum-PBS. The secondary antibody used was Alexa-488 goat anti-rabbit IgG (Molecular Probes), diluted 1:300, and applied overnight (4ºC). Tissues were then rinsed 3 x 15 min in 1% horse serum-PBS, mounted on microscope slides, sealed with vacuum grease in a closed chamber containing the same buffer, and covered with a coverslip.

Glial fibrillary acidic protein immunohistochemistry

For identification of Bergman glial cell brain slices were incubated overnight with the first antibody to GFAP (Electron Microscopy Sciences, Hatfield.USA), diluted 1:400; washed three times in PBS 1% serum (15 min each) and then incubated overnight in the Alexa fluor conjugates for secondary detection: Alexa fluor 488 goat antimouse and Alexa-IB_4 568 (Molecular Probes, Or. USA). The slices were then rinsed twice in PBS 1% serum (5 min each) and mounted on microscope slides. A small closed chamber containing PBS 1% serum, was made using vacuum grease before placing the coverslip, thus leaving the slices in a humid chamber during confocal observations (Castejón *et al*., 2002).

Synapsin-I, PSD95 and calbindin immunohistochemistry

For identification of cerebellar nerve cells and their synaptic relationship we used Synapsin-I and PSD-95 immunohistochemistry. Cerebellar cortex slices were derived from postnatal day 14 of wistar rats. After decapitation, the cerebellar lateral lobules were removed from posterior fossa and placed in ice-cold (4ºC) Hanks balanced salt solution supplemented with dextrose (6mg/ml). Fragments of cerebellar cortex were transversally sliced (300 µm thick sections) using a manual tissue chopper (Stoelting). The slices were immediately fixed in 4% paraformaldehyde in ice-cold 0.1M PBS. All samples for PSD95 immunolabeling were chemically fixed in ice cold paraformadehyde for no more than 15 min to preserve antigenicity. Free floating slices were placed in a multi-well plate for subsequent rinsing, blocking and labeling steps. Slices were rinsed in PBS (3 x 5 minutes) and then extracted overnight in 1% Triton X-100 in PBS (Polysciences). Blocking was done with 50 mM NH4Cl in PBS (30 min), followed by 20% horse serum in PBS (30 min). Washing (5 min) was done with 1% horse serum-PBS. All subsequent steps were carried out in 1% horse serum-PBS. Slices were double labeled with anti-synapsin-I rabbit IgG polyclonal antibody (diluted 1:300; Molecular Probes, Eugene, OR) and anti-PSD-95 (mouse) diluted 1:200 (Alexis, San Diego, CA). These primary antibodies were applied overnight (4ºC), and then tissues were rinsed again (3 x 15 minutes) in horse serum-PBS. The secondary antibodies used were Alexa-488 goat anti-rabbit IgG and Alexa-568 goat-anti-mouse IgG (GAM; Molecular Probes), diluted 1:500, and applied overnight (4ºC). Tissues were then rinsed 3 x 15 min in 1% horse serum-PBS, mounted on microscope slides, sealed with vacuum grease in a closed chamber containing 1% horse serum-PBS, and covered with a coverslip. To visualize Purkinje cell bodies and dendrites, samples were immunolabeled with rabbit anti-calbindin antibodies (1:500-1:2000; Chemicon). Some tissue slices were triple-labeled with antibodies against PSD-95, synapsin-I, and calbindin. Primary and secondary antibodies against PSD-95 and synapsin-I were applied first, then primary antibodies against calbindin were applied followed by Cy5 a secondary antibody (Castejón *et al*., 2004).

Confocal laser scanning imaging

Images of fluorescent labeled cerebellar tissue were captured using a Leica TCS NT scanning laser confocal microscope equipped with Argon (Ar; 488 nm), Krypton (Kr; 568 nm) and Helium-Neon (HeNe; 633 nm) lasers. Alexa-488 goat antirabbit-labeled secondary antibody was visualized with Ar laser excitation and a fluorescein-like filter set (510 nm dichroic mirror, 515 nm long pass barrier filter).

To study the structure of rat cerebellar cortex at low magnification we used a 10X Plan Fluotar or 20X Plan Apo objective lens. To resolve individual synaptic puncta, we used a 63X/1.2NA water immersion PLAN APO objective lens with an additional electronic zoom factor of up to 3.6. The pinhole size was set to 1.0-1.8 times the Airy disk to maximally reject out-of-focus haze and to improve spatial resolution. For improved signal-to-noise ratio, up to eight scans were averaged at each optical section. Stacks of 8-15 optical sections (1,024 x 1,024 pixel array) yielded voxel dimensions between 0.15 and 0.4 for the X, Y and Z planes.

Image processing

Brightness and contrast were adjusted using Photoshop 5.0 (Adobe). Red-yellow stereo images (Figs.

4, 5 and 6) were generated from stacks of confocal optical sections using Scion Imaging (Scion Corp., Frederick, MD, USA).

Results

Cerebellar tissues were imaged by confocal laser immunofluorescence microscopy in rat cerebellar cortex, at P14 and P21 days, developmental stages marked by a prolific synaptogenesis. At these stages, there is a three-layer tissue structure composed of granular, Purkinje cell and molecular layers.

Confocal laser scanning microscopy observations at low magnification (10X) showed intense GluR1 immunofluorescence at the level of Purkinje cell and molecular layers (Fig. 1). At higher magnification (20X), a weak immunofluorescent staining, observed as few isolated GluR1 small hotspots, was also observed at the level of the granular layer (Fig. 2), corresponding to climbing fiber terminals making axosomatic contacts with Golgi cell bodies. By means of PSD-95 immunohistochemistry the Golgi cells in the granular layer were identified showing the postsynaptic receptor sites of axosomatic synapses of climbing fibers (Fig. 3). At the level of molecular layer an intense, GluR1 immuno-reactivity was concentrated along the Purkinje and basket cell bodies and their dendritic arborization, and at the level of the palisade pattern arrangement formed by Bergmann glial cell fibers (Fig. 4). Bergmann glial cells were identified using GFAP immunohistochemistry by the cell bodies intimately applied to Purkinje cell bodies, and by their typical palisade arrangement formed by the Bergmann fibers directed toward the molecular layer (Fig. 5). At a higher magnification (63X), in a stack of confocal optical sections, very large clusters of GluR1 hotspots were localized around Purkinje, basket, and stellate cells (Fig. 6). At this magnification intense GluR1 immunofluorescent staining was observed at the molecular layer following primary, secondary and tertiary dendrites of Purkinje cells, corresponding at the level of the primary trunk to the multiple innervation of climbing fibers, and at the secondary and tertiary spiny branches of Purkinje cells to the parallel fiber synapses. At a higher magnification, GluR1 strong immunofluorescent staining was observed surrounding the Purkinje cell bodies corresponding to the basket axonal endings forming the pericellular nest, and also their ascending collaterals surrounding Purkinje cell secondary and tertiary dendritic ramifications. Weak GluR1 immunoreactivity was found surrounding the

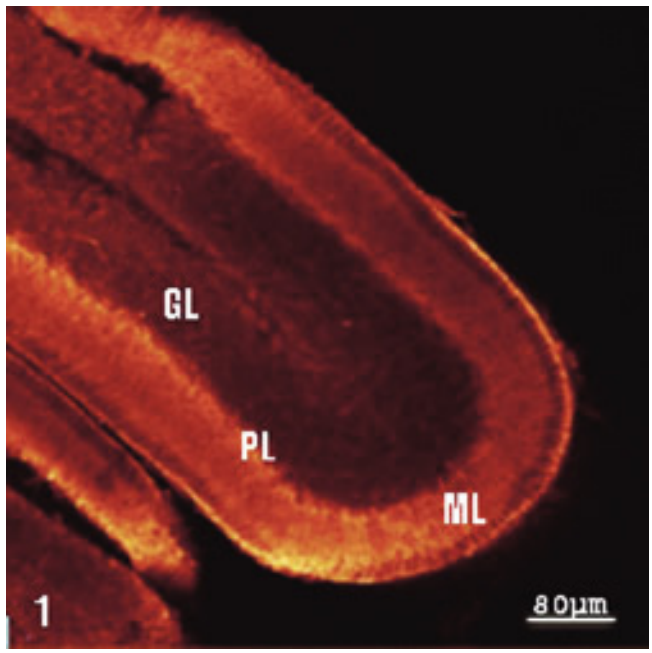

FIGURE 1. Low magnification view of rat cerebellar folia showing the three-layered structure of the cerebellar cortex: granular layer (GL), Purkinje cell layer (PL) and molecular layer (ML). Note the intense immunolocalization of GluR1 at the Purkinje cell and molecular layers and the weak immunoreactivity at the granular layer.

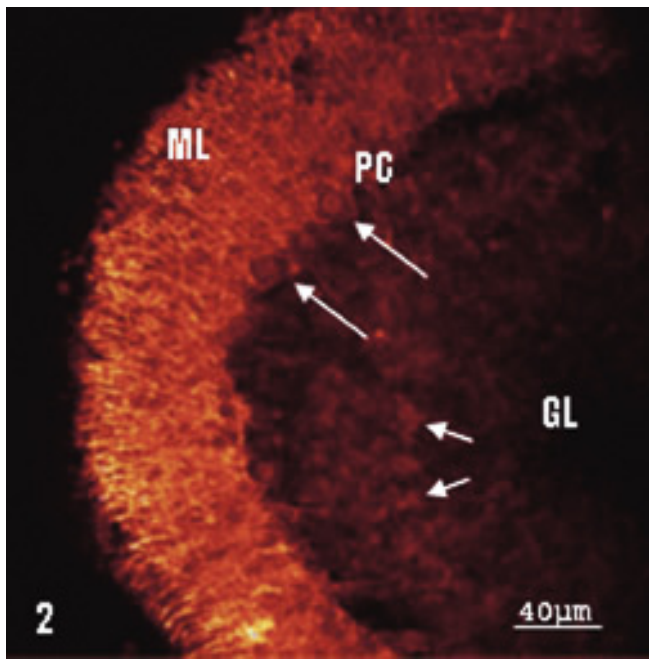

FIGURE 2. Higher magnification view of rat cerebellar cortex showing high, immunoreactivity of GluR1 around Purkinje cell (PC) bodies (long arrow) and their dendritic arborization. An intense inmmunolabeling is observed following the palisade arrangement of Bergmann glial fibers in the molecular layer (ML). A weak GluR1 immunoreactivity is expressed as isolated small hotspots (short arrow) in the granular layer (GL), surrounding Golgi cells.

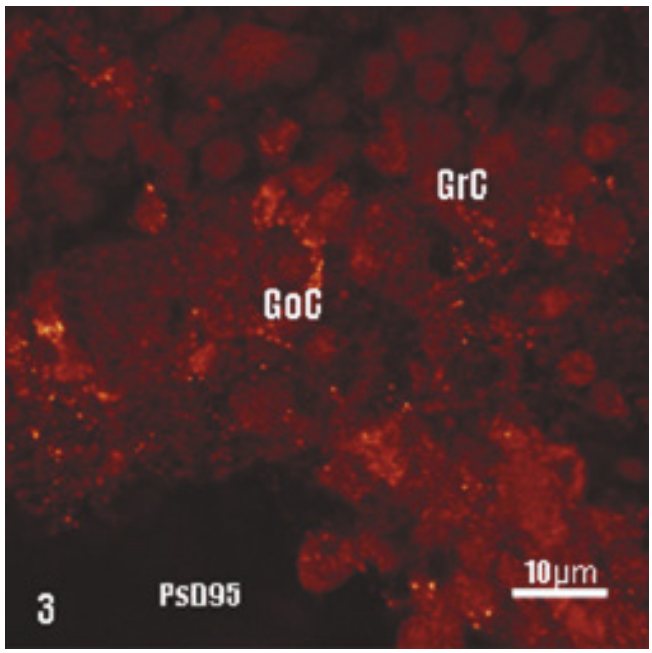

FIGURE 3. Higher magnification view of granular layer showing intense PSD95 immunochemistry (yellow) surrounding the macroneuron, identified as Golgi cell (GC) soma, corresponding to the axosomatic synapses of afferent climbing fibers. Note the unlabeled microneurons that correspond to granule cells (GrC) groups.

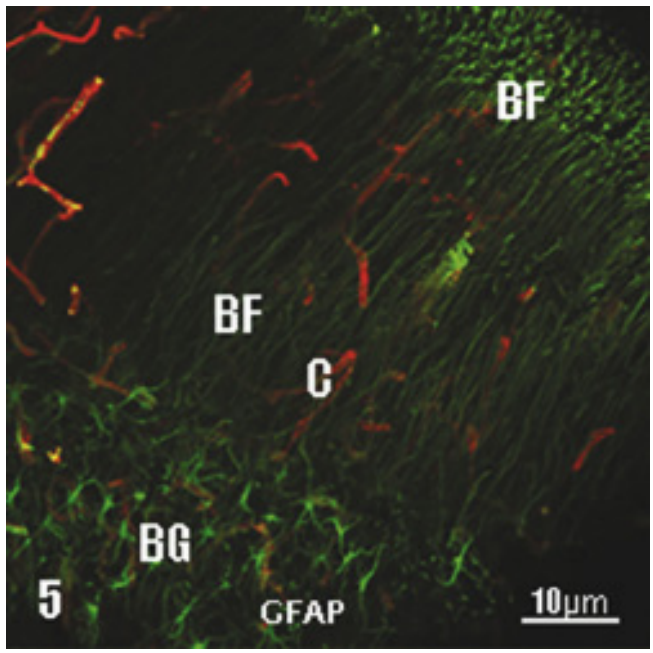

FIGURE 5. Low magnification view of cerebellar molecular layer immunolabeled with glial fibrillary acidic protein (green) to demonstrate the identification of Bergmann glial cell body (BG) localized at the Purkinje cell layer, and the Bergmannn glial fibers (BF) directed in parallel arrangement toward the molecular layer outer surface. The capillaries (C) appear stained in red.

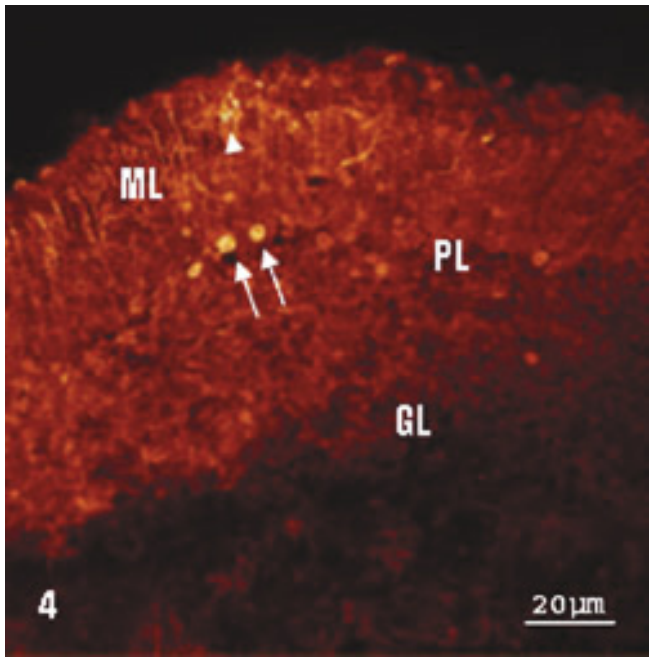

FIGURE 4. Higher magnification view of rat cerebellar cortex showing high, immunoreactivity of GluR1 at the level of basket cells (arrows) in the Purkinje cell layer (PL), and Bergmann glial fibers (arrowhead) in the molecular layer (ML). Note the weak immunoreactivity of the granular layer (GL).

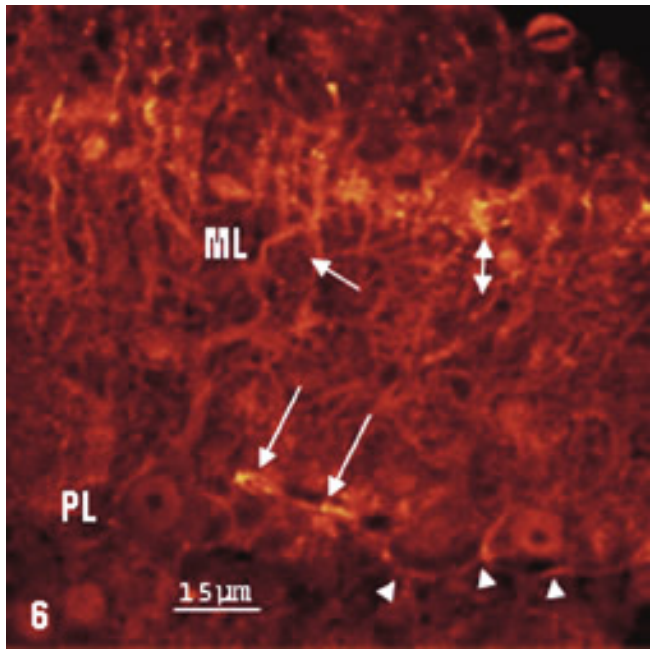

FIGURE 6. Higher magnification of Purkinje cell (PL) and molecular (ML) layers of rat cerebellar cortex. Note the yellow and high GluR1 immunoreactivity around basket cells (long arrows), Purkinje cell bodies (arrowheads), stellate cell soma and dendrites (short arrow), and Bergmann glial fibers (double head arrow). This image represents a composite of 15 optical image planes spanning a depth of 15µm.

Golgi cell bodies in the granular layer, corresponding to the axosomatic synapses of climbing fiber collaterals. Marked GluR1 immunoreactivity, expressed as small puncta, was also observed surrounding basket and stellate cells corresponding to their axosomatic and axodendritic synaptic connections with parallel fibers (Figs. 6 and 7). The identification of Purkinje cells in a single cell layer was made using calbindin immunostaining (Fig. 8). The precise identification of climbing fibers, basket cell axons, and parallel fibers with Purkinje cell bodies and their dendritic arborization was made using Synapsin-I and PSD-95 immunohistochemistry (Figs. 9 and 10).

Sinapsin-I inmmunostaining showed the numerous and wide distribution of small green puncta of the terminal endings of basket cell axons forming the pericellular nest, and the terminal ending of climbing fibers, parallel fibers, and stellate cell axons (Fig. 11). At the highest magnification, the strongest GluR1 immunoreactivity was distinguished surrounding Purkinje cell body, corresponding to basket cell axonal axosomatic synapses, and also covering the primary dendritic trunk corresponding to the terminal synapses of multiple innervation of climbing fibers (Fig. 12). Positive immunofluorescent staining was also found at the level of capillary wall.

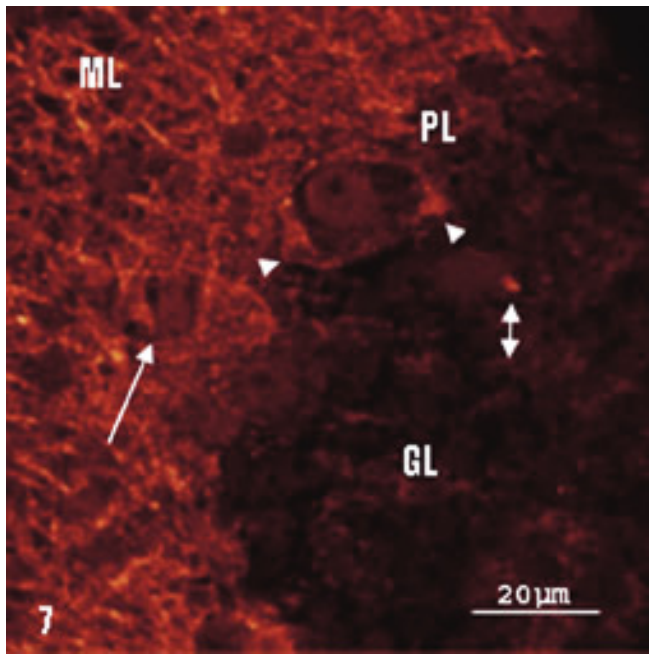

FIGURE 7. Higher magnification of granule cell layer (GL), Purkinje cell layer (PL) and inner and middle third molecular layer (ML) of rat cerebellar cortex. Intense GluR1 immunoreactivity is observed around Purkinje (arrowheads), and basket cell (long arrow) bodies. A few isolated and small puncta (double head arrow) is observed in the granule layer surrounding Golgi cells. This image represents a composite of 8 optical images planes spanning a depth of 16µm.

Discussion

Our observations of GluR1 subunit by confocal laser immunohistochemistry reveal the presence of the GluR1 subclass of AMPA receptors surrounding inhibitory cerebellar neurons (Golgi, Purkinje, basket and stellate cells). GluR1 subunit segregation was confined to the soma and dendritic processes of recipient cerebellar inhibitory neurons and this segregation is in register with different segregated afferent fibers, such as, climbing and parallel fibers, basket and stellate cell axons.

The GluR1 distribution pattern herein demonstrated by confocal laser scanning microscopy corresponds with the postsynaptic sites of the excitatory circuits formed by climbing and parallel fibers at the Purkinje cell and molecular layers, and with the inhibitory circuits formed by basket cell axons on Purkinje cell soma, as demonstrated in previous transmission and scanning electron microscopy, and confocal laser scanning microscopic using labeling markers and immunochemical studies (Eccles *et al*., 1967; Castejón, 1983; Castejón and Apkarian, 1993; Castejón and Sims, 2000; Castejón and Castejón, 2000; Castejón *et al*., 2001a,b; Castejón, 2003;

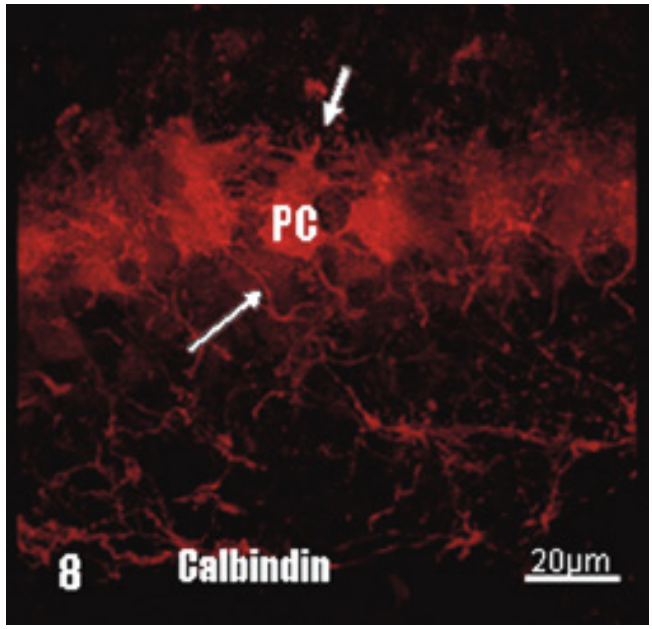

FIGURE 8. Calbindin inmmunolabeling of Purkinje cell (PC) layer showing the single row formed by Purkinje cell bodies, their axonal process directed toward the granular layer, and recurrent collaterals (long arrow), and the dendritic processes going to the molecular layer (short arrow).

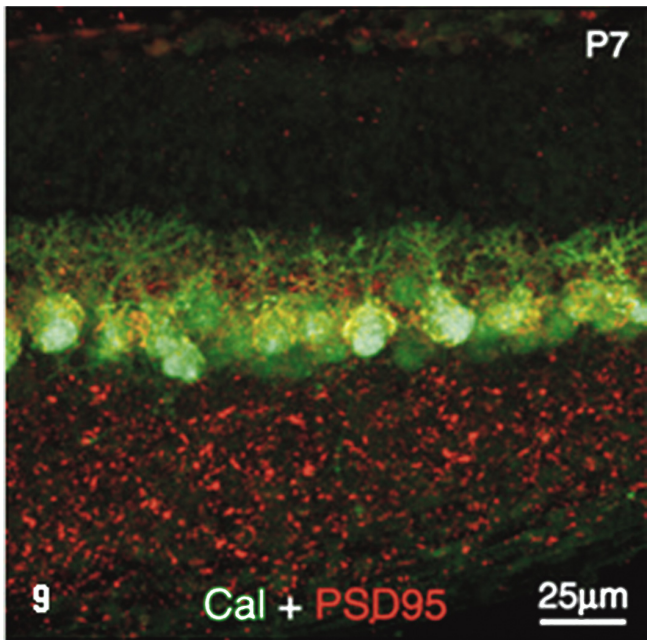

FIGURE 9. Low magnification image of calbindin and PSD-95 image of cerebellar cortex showing the distribution of postsynaptic sites of afferent mossy and climbing fibers in the granular layer (red hotspots), and the small puncta in the inner third molecular layer (small red puncta). The Purkinje cell bodies appear surrounded by the pericellular nest formed by basket cell axons (yellow).

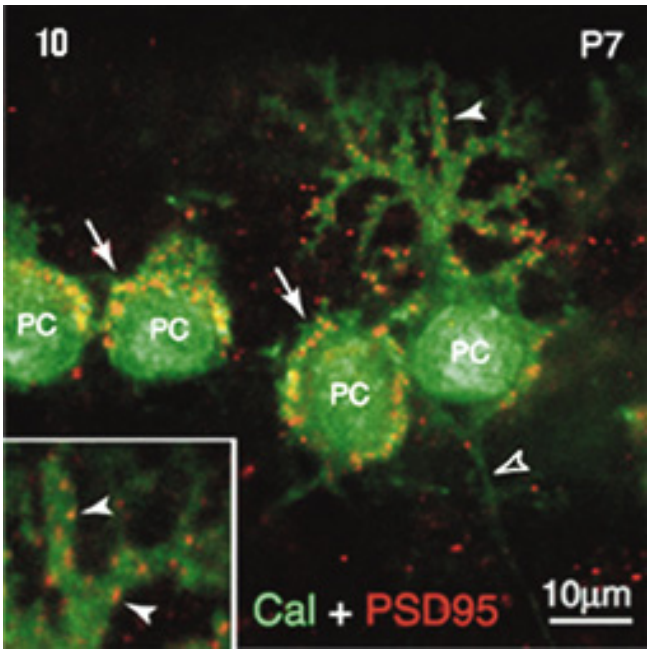

FIGURE 10. Higher magnification image of calbinding and PSD-95 immunostaining of Purkinje cell body (PC) showing the pericellular nest formed by basket cell synapses upon Purkinje cell body (small arrows). The secondary and tertiary Purkinje cell dendritic processes (insert) show the small puncta corresponding mainly to the synapses formed by granule cell axons or parallel fibers (dense arrowheads). The Purkinje cell axon hillock region also is observed (clear arrowhead).

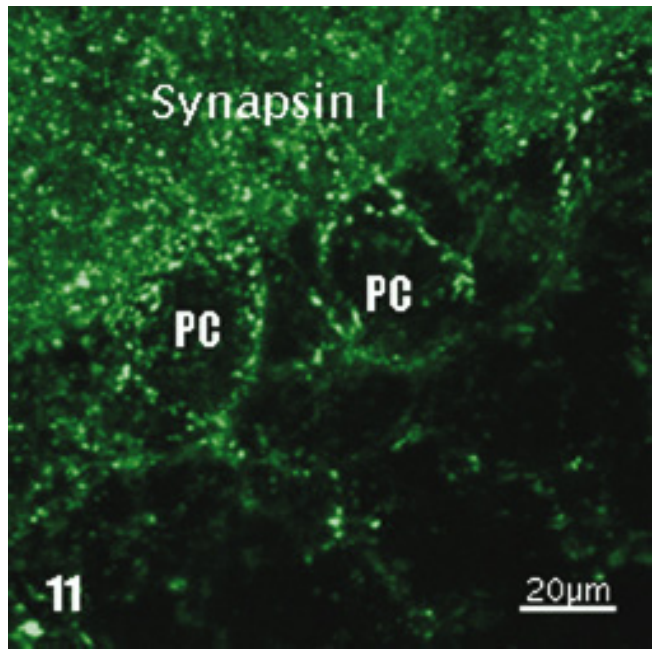

FIGURE 11. Projection imaging of Synapsin-I immuno-labeling (green) of Purkinje cell (PC) pericellular nest, and the distribution in the molecular layer of small puncta corresponding to presynaptic ending of climbing and parallel fiber terminals, and stellate axonal endings.

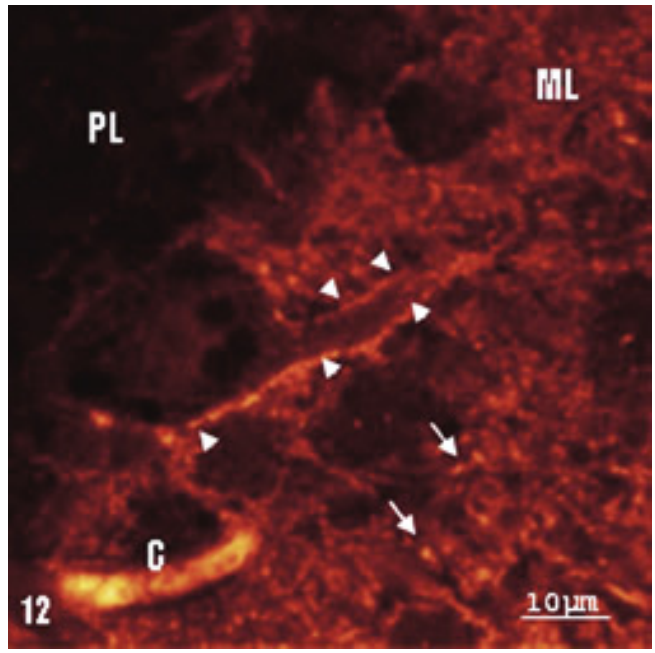

FIGURE 12. Higher magnification of GluR1 immunostaining of Purkinje cell (PL) and molecular (ML) layers showing the distribution of GluR1 subunits. Large hotspots clusters are observed surrounding Purkinje cell body corresponding to basket cell axonal terminals, and the primary dendritic trunk belonging to climbing fiber endings (arrowheads). The short arrows indicate small GluR1 puncta presumably corresponding to axonal ending of stellate cell axons in the inner third molecular layer. A capillary wall (C) also is heavily fluorescent. This image represents a composite of 13 optical image planes spanning a depth of 13µm.

Castejón *et al*., 2004). Earlier transmission electron microscopic and correlative confocal laser scanning studies have demonstrated that climbing fibers are segregated to Purkinje cell primary dendritic trunk (Chan-Palay and Palay, 1970; Castejón 1983; Palay and Chan-Palay, 1974; Castejón and Sims, 2000; Castejón *et al*., 2000), and the parallel fibers are topographically related to Purkinje cell secondary and tertiary spiny dendritic branches (Larramendi and Victor, 1967; Palay and Chan-Palay, 1974; Castejón and Apkarian, 1993, Castejón *et al*., 2004). According to previous transmission electron microscopic studies climbing and parallel fibers also establish axosomatic and axodendritic contacts with basket and stellate cells (Hamori and Szentágothai, 1965, 1966; Lemkey-Johnston and Larramendi, 1968; Palay and Chan-Palay, 1974; Castejón *et al*., 2001b).

The regional distribution of GluR1 subunits relates this postsynaptic AMPA receptor subclass to the excitatory circuits of the cerebellar cortex formed by climbing and parallel fibers, and to the inhibitory circuits formed by basket cell axons on Purkinje cell soma. A relationship of GluR1 subunits of AMPA receptors with excitatory circuits have also been reported by Morrisson *et al*. (1996) in the hippocampus and neocortex.

Parallel and climbing fibers are likely to use glutamate as neurotransmitter (Ito, 1984; Kano and Kato, 1987; Kano *et al*., 1988; Zhang *et al*., 1990; Otis *et al*., 1997). These two excitatory inputs to Purkinje cells mediate fast excitatory postsynaptic potentials via AMPA type ionotropic glutamate receptors (Knöpfel and Grandes, 2002). The AMPA receptors of Purkinje cells are indeed involved in induction and expression of long-term depression (LTD) (Crepel *et al*., 1996); and in cerebellar synaptic plasticity (Kano and Kato, 1987).

Martin *et al*. (1998) have reported by means of immunoelectron microscopy that GluR1 is expressed transiently at restricted times partially by granule cells (PO-P11) and Purkinje cells (P13-P19). These observations are partially in agreement with the present confocal laser scanning microscopy study, and support also the finding related to the weak GluR1 immunoreactivity in the granular layer, and the presence of GluR1 immunoreactivity in Purkinje cells of P14 rat cerebellar cortex herein examined. Keifer and Carr (2000) also showed the immunocytochemical localization of glutamate receptor subunits in the brain stem and in corresponding and correlative confocal laser scanning layers of turtle cerebellum (*Chrysemys picta*).

In the present study the strongest GluR1 immunoreactivity was localized in Purkinje cell body and dendritic ramifications, and in the Bergmann glial cell processes. Such findings support and extend those earlier reported by Martin *et al*. (1993), using antipeptide antibodies that recognize the C-terminal domains of GluR1 subunits in rat cerebellar cortex.

Our findings partially resemble those reported by Baude *et al*. (1994) by means of immunoelectron microscopy of peptides in the rat cerebellum, who described strong GluR1 immunoreactivity at the molecular layer. However, these authors did not show labeling in the granular layer. It is interesting to mention that GluR1 is surface expressed also in cultured rat cerebellar granule cells (Hack *et al*., 1995; Archibald *et al*., 1998; Mogensen and Jorgensen, 2000).

As illustrated in figures 2, 4, and 6, we have found strong GluR1 immunoreactivity in Bergmann glial cells processes surrounding Purkinje cell dendrites. Our findings support and extend those previously reported by Martin *et al*. (1993); Baude *et al*. (1994); Breese *et al*. (1996); Garyfallou *et al*. (1996); and Ripellino *et al*. (1998) using different but related microscopical and biochemical techniques. These findings mean that Bergmann glial cells have functional ionotropic glutamate receptors that regulate calcium levels. Glutamatergic regulation of astrocytic Ca^{2+} may be involved in synapse modeling, long term potentiation, excitotoxicity and others events dependent on glutamatergic neurotransmission (Shelton and McCarthy, 1999). AMPA receptors and glutamate transporter expressed by Bergmann glial cells are activated by glutamate neurotransmitter release from climbing fibers (Dzubay and Jahr, 1999). Glutamate regulates the morphology of glial processes by activating Ca^{2+} permeable AMPARs (Ishiuchi *et al*., 2001; Pellegrini-Giampietro *et al*., 1992; Iino *et al*., 2001). According to Burnashev *et al*. (1992), GluR channels of fusiform cerebellar glial cells (Bergmann glial cells) exhibit high calcium permeability, whereas GluR channels of cerebellar neurons have low calcium permeability. GluR-mediated Ca^{2+} signaling is essential to maintain functional connections between the glial cells and the glutamatergic synapses (Watanabe, 2002).

The presence of ionotropic glutamate receptors has been also observed in hippocampal astrocytes (Shelton and McCarthy, 1999). The above findings reveal that GluR1 subunit of AMPA receptors are expressed in a cell-specific and region-specific manner, consistent with its role in neuronal-glial communication. Similar conclusions have been postulated by Fan *et al*. (1999) in astrocytes cultured from hippocampus. The fact that Bergmann glial cells express AMPA receptors indicate

that synaptic information could flow through cerebellum via glial/neuronal networks and support the hypothesis of existing synaptic networks of neurons and glia (LoTurco, 2000), and that the role of glia may be to modulate the activity at neuron-to-neuron synapses.

In the present study, GluR1 subunit was also localized surrounding stellate and basket cells in the rat molecular layer. Thus far, the expression of another subunit, the GluR2 subunits-containing AMPA receptors has been reported only on cerebellar stellate cells (Liu and Cull-Candy, 2000, 2002).

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