Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 319 (2004) 120–129
BBRC
www.elsevier.com/locate/ybbrc

Metabotropic glutamate receptors are expressed in adult human glial progenitor cells

Karen Luyt,[a] Aniko Varadi,[b,1] Christopher A. Halfpenny,[c] Neil J. Scolding,[c] and Elek Molnar[a,*]

[a] *MRC Centre for Synaptic Plasticity, Department of Anatomy, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK*
[b] *Department of Biochemistry, University of Bristol, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK*
[c] *Department of Neurology, Institute of Clinical Neurosciences, University of Bristol, Frenchay Hospital, Bristol BS16 1LE, UK*

Received 4 April 2004
Available online 10 May 2004

Abstract

Glial precursor cells (GPCs) are present in the adult human central nervous system (CNS) and they can be isolated and maintained in culture for in vitro studies. This study analysed expression of mGluR3 and mGluR5 metabotropic glutamate receptor (mGluR) mRNAs in GPCs. A2B5 surface antigen positive GPCs were isolated using immunomagnetic selection from dissociated temporal lobe subcortical white matter cells. The separated GPCs were maintained in cultures and characterised by immunoreactivity for the differentiation markers A2B5 and human platelet-derived growth factor-α receptor (PDGFαR). Reverse transcription followed by multiplex PCR analysis showed that the GPCs expressed both mGluR3 and mGluR5a mRNAs. Double immunostaining for glial progenitor markers and mGluR5 proteins demonstrated that all A2B5 and PDGFαR-positive cells were also positive for mGluR5. The results indicate that GPCs present in the adult human CNS express mGluR3 and mGluR5a. These neurotransmitter receptors may be involved in the proliferation and differentiation of glial cells.

Keywords: Glutamate; Metabotropic glutamate receptor; Human glial lineage; Myelin; RT-PCR; Immunocytochemistry; A2B5; PDGFα receptor

Glial progenitor cells (GPCs) persist in the adult nervous system and provide a limited capacity for the restoration of structure and function in myelinated pathways damaged by injury or disease [1]. Previous in vitro studies have isolated GPCs from adult rat CNS. These cells can be identified by A2B5 antibodies and, in appropriate in vitro environments, can give rise to oligodendrocytes [2]. GPCs also express the platelet-derived growth factor-α receptor (PDGFαR) and the integral membrane proteoglycan NG2 [3]. PDGFαR and NG2 are detected on GPCs, but not on differentiated oligodendrocytes, both in vitro [4] and in developing rodent brain [5]. PDGFαR-positive cells remain abundant throughout the adult rodent CNS and have the potential to generate oligodendrocytes [5,6]. Cells with phenotypic markers of rodent GPCs have also been detected in adult human brain [7–13] and multiple sclerosis lesions [14–16]. Human GPCs may be extracted and purified from adult human white matter and maintained in culture for in vitro analysis [9,11,12,17]. These endogenous GPCs in the adult human brain may represent a viable target for future therapies intended to enhance remyelination in multiple sclerosis patients. However, this will rely on a detailed understanding of the clinical biology of the disease and human GPCs in particular. While a considerable amount is known about the development and differentiation of rodent glial cells, fundamental properties of human GPCs are poorly understood. Moreover, it is widely accepted that information from work with rodent cells cannot be reliably

* Corresponding author. Fax: +44-117-9291687.
E-mail address: Elek.Molnar@bristol.ac.uk (E. Molnar).
[1] Present address: Centre for Research in Biomedicine, Bristol Genomics Research Institute, Faculty of Applied Sciences, University of the West of England, Bristol BS16 1QY, UK.

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.04.158

extrapolated to humans without direct experimental support using human cells.

While recent evidence supports a role for ionotropic glutamate receptors (iGluRs) in oligodendrocytes and demyelinating diseases (reviewed in [18]), the expression and function of metabotropic glutamate receptors (mGluRs) have not been established in adult human GPCs. mGluRs are a major family of class III G-protein-coupled receptors. They form a family of eight subtypes (mGluR1-8) that regulate a variety of intracellular signalling systems via activation of GTP-binding proteins. They have been classified into three groups on the basis of their sequence homology, pharmacological profile, and transduction pathways [19]. Group I includes mGluR1 and mGluR5, which are coupled to phosphoinositide hydrolysis, while group II (mGluR2 and mGluR3) and group III (mGluR4, -6, -7, and -8) mGluRs are negatively coupled to adenylyl cyclase [19]. Previous studies suggest that mGluR3 [20–23] and mGluR5 [22–28] are the predominant mGluR subtypes expressed in rodent astrocytes and human glioma cells. The expression and function of mGluRs in GPCs are less clear. Using the CG-4 rodent cell line [29] we have recently identified that functional mGluR3 and mGluR5 with intact intracellular signal transduction pathways are expressed in cells of the oligodendroglial lineage [30]. While hypothetically mGluRs could represent an exciting new mechanism involved in glial proliferation, differentiation, and glial–neuronal communication in pathological conditions, the presence of mGluRs in human GPCs has not been reported yet due to the extremely limited supply of suitable source tissue and poor cellular yield.

In the present study we used reverse transcription, multiplex PCR analysis, and immunocytochemistry to identify and characterise mGluR expression in human $A2B5^+$ and $PDGF\alpha R^+$ glial precursor cells. We found that immunomagnetically selected $A2B5^+$ primary adult GPC cultures express both mGluR3 and mGluR5a mRNAs, which agrees with our previous study of the CG-4 rodent clonal oligodendrocyte progenitor cell line [30]. In accordance with our RT-PCR analysis, immunocytochemical investigation also revealed the expression of mGluR5 protein in $A2B5^+$ and $PDGF\alpha R^+$ GPCs.

Materials and methods

Materials. Male Wistar rats were from Harlan UK (Bicester, UK). Tissue culture materials were obtained from Sigma (Poole, Dorset, UK). DNA oligonucleotide primers were purchased from Invitrogen Life Technologies (Paisley, UK). The Advantage-GC 2 PCR kit was from BD Biosciences Clontech (Palo Alto, CA, USA). Restriction endonucleases were from Roche Diagnostics (Lewes, UK). All other chemicals were of analytical grade. The mouse monoclonal anti-human PDGFαR affinity purified IgG was from R&D Systems Europe (Abingdon, UK). The rabbit anti-mGluR5 affinity purified IgG was obtained from Upstate Biotechnology (Lake Placid, NY, USA). The rabbit anti-GFAP antibody was purchased from DAKO (Glostrup, Denmark). Alexa fluor 568 goat anti-rabbit IgG, Alexa Fluor 488 goat anti-mouse IgM, and Alexa fluor 568 goat anti-mouse IgG were from Molecular Probes (Eugene, OR, USA).

Adult human white matter dissociation, magnetic separation of $A2B5^+$ cells, and culture of GPCs. GPCs were dissociated and cultured as previously described [7,9,12,31] from normal-appearing white matter removed at the time of anterior temporal lobe resection for intractable epilepsy. Consent was obtained for resected tissue to be used for research purposes, and ethical approval was obtained from both Local and Regional Ethics Committees. Previous studies had confirmed that comparable tissue, dissected from sites distant from the epileptic focus, was histologically normal [9]. Small fragments of tissue were dissected to remove meninges and identifiable blood vessels, digested using trypsin, collagenase III, and DNAseI enzymes, and triturated [7,9,31]. Undissociated tissue pieces were eliminated by passage through a 40 μm stainless steel mesh followed by density centrifugation through 9% (v/v) Optiprep I medium to remove myelin debris. Dissociated cells were then suspended in NS-A medium (Euroclone, Paignton, UK) with N2 supplements, L-glutamine (2 mM), insulin (25 mg/ml) with PDGF-AA (20 ng/ml; Peprotech, London, UK), FGF-2 (10 ng/ml; Peprotech, London, UK), and NT-3 (2 ng/ml; Peprotech, London, UK) and plated in 75 cm^2 tissue culture flasks (Nunc, Rochester, NY). After 48 h in culture, cells dissociated from adult human white matter were collected by washing the plates with Ca^{2+}/Mg^{2+}-free Hanks balanced salt solution (HBSS) [12]. The cells were incubated with filtered supernatant of hybridoma cells expressing the monoclonal IgM antibody A2B5 (clone 105; European Collection of Cell Cultures, Salisbury, UK). Incubation proceeded for 30 min at 4 °C on a rotating mixer. The cells were washed three times with 10 times the labelling volume in phosphate buffered saline (PBS) containing 0.5% (w/v) bovine serum albumin (BSA) and 2 mM EDTA. The cells were incubated with pre-washed anti-mouse IgM coated magnetic beads (Dynabeads, Dynal; [32]) for a further 30 min at 4 °C. A2B5-positive cells were separated by repeated selection using a Dynal magnet [32]. A2B5-positive human white matter cells were seeded onto poly-D-lysine-coated coverslips in fresh NS-A/N2 medium containing PDGF-AA (20 ng/ml), FGF-2 (10 ng/ml), and NT-3 (2 ng/ml) [12]. Cells were analysed at 5 days in vitro (3 days after magnetic selection).

CG-4 oligodendrocyte progenitor cell cultures. CG-4-cells [29] were used between passages 13 and 22 and grown in modified Sato medium containing 30% (v/v) B104 conditioned medium as described previously [29]. The modified Sato medium consisted of: Dulbecco's modified Eagle's medium (DMEM), 0.1% (w/v) bovine serum albumin fraction V, 60 μg/l progesterone, 16.1 mg/l putrescine, 5 μg/l sodium selenite, 400 μg/l tri-iodothyronine (T3), 400 μg/l L-thyroxine (T4), 50 mg/l holo-transferrin, 5 mg/l insulin, and 2 mM L-glutamine. Cells were cultured in a humidified atmosphere at 37 °C with 7% (v/v) CO_2. Glass coverslips and plastic flasks were coated with poly-L-lysine (100 mg/l). Cells were passaged every 2–3 days, harvested using porcine trypsin and trypsin inhibitor, and cultured for 2 days prior to RNA and membrane preparations or imaging. B104 cells were grown in DMEM containing 10% (v/v) heat-inactivated fetal calf serum (FCS) and 2 mM L-glutamine until 70% confluent and then conditioned with modified Sato medium (see above) for a period of 4 days. This conditioned medium was aspirated, filtered, and frozen for use in the 30% (v/v) B104 medium for maintaining the CG-4 cells in the proliferative phase [29].

Human embryonic kidney cell and HeLa cell cultures. Human embryonic kidney cell (HEK-293) and HeLa cells (American Type Culture Collection, Manassas, VA, USA) were cultured in DMEM supplemented with 10% (v/v) FCS, penicillin (100 μg/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM) at 37 °C in an atmosphere of humidified air (95%) and CO_2 (5%).

Hippocampal cell cultures. Primary hippocampal neuronal cultures were prepared from 3- to 5-day-old rat pups as previously described [33]. The CA3–CA1 region of the hippocampus was dissected at 4 °C, and neurons were recovered by enzymatic digestion with trypsin and mechanical dissociation. Cells were then plated at a density of ~50,000 cells per 22 mm glass coverslip coated with poly-L-ornithine (25 μg/ml, Sigma) and matrigel (1:50 dilution, Becton–Dickinson). Cultures were maintained at 37 °C in an atmosphere of humidified air (95%) and CO_2 (5%). The culture media were composed of minimal essential medium (Gibco, Life Technologies); 38.9 mM glucose, 2 mM glutamine, 15 mM Hepes, 100 μg/ml bovine transferrin, 30 μg/ml insulin, 0.1 μg/ml biotin, 1.5 μg/ml vitamin B12, 2 μg/ml gentamicin, and 10% (v/v) FCS. From the second day in culture, the media were supplemented with 2.5 μM cytosine-β-D-arabinofuranoside to prevent glial cell proliferation. Culture media were changed three times per week and after 10 days the concentration of fetal calf serum was reduced to 5% (v/v). Neurons were used for experiments 10–14 days after plating.

RNA isolation and cDNA synthesis. Ten magnetically selected $A2B5^+$ human GPCs were used for RNA extraction and reverse transcription (RT), which was performed in one step with the Cells-to-cDNAII kit (Ambion Europe, Huntingdon, UK) according to the manufacturer's instructions. Total RNA from human frontal cortex (Ambion Europe, Huntingdon, UK) was also reverse transcribed using methods previously described [30]. Potential contamination by genomic DNA was excluded in every sample tested by performing RT reactions in the absence of reverse transcriptase and subjecting the product to identical PCRs used for GPC and human cerebral cortex cDNA (described below).

Polymerase chain reaction. cDNA was synthesised from 10 $A2B5^+$ human GPCs. Multiple sequences (mGluR3, mGluR5, GFAP, and β-actin) were amplified simultaneously in one tube by PCR. This was followed by a second round of PCR during which individual sequences were amplified simultaneously in different tubes. RT product (3.75 μl) was used as template for the first round of PCR in a final volume of 25 μl. In this PCR, the primers for all the cDNA sequences of interest (Table 1) were included. Final concentrations of the reaction mix were as follows: 1× Advantage-GC2 PCR buffer (BD Biosciences Clontech, Palo Alto, USA), 0.5 M GC-Melt (BD Biosciences Clontech, Palo Alto, USA), 200 μmol/l of each dNTP (Roche Diagnostics, Lewes, UK), 2.5 pmol of each primer (Invitrogen Life Technologies, Paisley, UK), 0.5 μl Advantage-GC 2 Polymerase Mix (BD Biosciences Clontech, Palo Alto, USA), and purified water. PCR was carried out in a programmable thermal controller (PTC-100, MJ Research, Essex, UK) with an initial denaturation step of 3 min at 94 °C, 25 cycles with stable annealing temperature of 60 °C (56 °C for mGluR5). Each cycle consisted of 30 s at 94 °C, 30 s at annealing temperature, and 1 min at 72 °C. PCR was finished by a final elongation step of 3 min at 72 °C. Two microliters of aliquots of the product of this first round of PCR were used as template in a second PCR containing the primer pair of only one of the cDNA sequences of interest in a final volume of 25 μl reaction mix (see above). For the second round of PCR the initial denaturation step (3 min at 94 °C) was followed by 40 cycles of 30 s at 94 °C, 30 s at annealing temperature of 60 °C (56 °C for mGluR5), and 1 min at 72 °C. PCR was finished by a final elongation step of 3 min at 72 °C. PCR products were separated on a 1.5% (w/v) agarose gel stained with 0.5 mg/ml ethidium bromide and visualised on a UV screen. The separated bands were extracted from the gel slices and analysed by restriction digestion as previously described [34].

We used the following multiple controls to check for possible amplification of contaminant RNA or genomic DNA by PCR: (1) RNA blanks taken throughout the cDNA synthesis step in the absence of reverse transcriptase were used in every PCR, for each set of primers; (2) samples without templates were run for every primer pair for each PCR experiment; and (3) human frontal cortex cDNA as positive control was used in each experiment.

Immunocytochemistry and confocal microscopy. The following primary antibodies were used: (1) mouse monoclonal anti-human PDGFαR affinity purified IgG (16 μg/ml); (2) rabbit anti-mGluR5 affinity purified IgG (1.6 μg/ml); and (3) rabbit anti-GFAP antibody (1:200 dilution).

Human GPCs, HEK 293, HeLa cells, and hippocampal neurons cultured on glass coverslips were washed twice with phosphate buffered saline (PBS). The cells were fixed for 20 min in 4% (w/v) paraformaldehyde in PBS at room temperature followed by washing in PBS (5 min). Cells were washed in 100 mM glycine in PBS (pH 8.5) for 5 min and then in 10% (v/v) FCS in PBS (5 min) before permeabilisation in 0.2% (v/v) Triton X-100 in PBS (20 min) at room temperature. This was followed by blocking in 3% (w/v) BSA in PBS for 15 min. The cells were then incubated with the primary antibodies for 1.5 h at room temperature in 3% (w/v) BSA in PBS. The primary antibodies were visualised using the appropriate fluorochrome-conjugated secondary antibody: Alexa fluor 568 goat anti-rabbit IgG antibody (1:200 dilution), Alexa fluor 488 goat anti-mouse IgM (1:200 dilution) or Alexa fluor 568 goat anti-mouse IgG (1:200), which was applied in PBS with 3% (w/v) BSA for 30 min at room temperature. Coverslips were washed with PBS and mounted in Moviol mounting medium (Merck Biosciences, Nottingham, UK).

Images were captured on a Leica (Heidelberg, Germany) TCS-NT confocal laser-scanning microscope attached to a DM/RBE epifluorescence microscope using a 63× PL Apo 1.4 NA oil-immersion objective (Leica, Heidelberg, Germany). The 488 and 568 nm laser bands of a Kr–Ar laser were used for dual dye excitation and fluorescein isothiocyanate/tetramethylrhodamine isothiocyanate filters for fluorescence emission. With the imaging conditions used, there was no detectable bleed through of fluorescence from one channel to the other when we studied single-labelled specimens. Microscope settings were adjusted so that imaging conditions for both red and green channels were kept constant.

Results

Magnetic sorting and characterisation of $A2B5^+$ GPCs from adult human white matter

Previous studies established that GPCs are present in cultures prepared from adult human subcortical white

Table 1
Oligonucleotides used as PCR primers

Isoform specificity of primers	Primer region		Length of PCR product (bp)	Annealing temperature (°C)
	Forward	Reverse		
mGluR3 (NM_000840)	1919–1938	2481–2499	581	60
mGluR5a (NM_000842)	2487–2506	3117–3135	649	56
mGluR5b (D28539)			745	
β-Actin (NM_001101)	937–956	1174–1193	257	60
GFAP (AF419299)	150–170	478–498	349	60

The primers were based on human cDNA sequences. The EMBL/GenBank database accession numbers are indicated in the first column.

matter [9,11,12]. These small bipolar cells were generally classified as oligodendrocyte progenitors on the basis of their characteristic morphology, expression of the A2B5 and PDGFαR antigens, and their predominant differentiation into oligodendrocytes in serum-free culture medium [11,12]. We used immunomagnetic sorting [32] to select A2B5$^+$ cells from dissociated adult human subcortical white matter [12]. These primary cultures were negative for the neuronal marker βIII tubulin (not shown). Purified human A2B5$^+$ precursor cells were maintained as attached cells on poly-D-lysine-coated coverslips in serum-free medium [12]. We analysed morphological and immunocytochemical characteristics of cultured cells to confirm their GPC properties. Our A2B5$^+$ GPC cultures (5 days in vitro and 3 days after immunomagnetic sorting) consisted of round, phase-bright bi- and tri-polar cells with long processes which can be distinguished from the characteristically flat type 2 astrocytes. We used immunocytochemistry to confirm the presence of GPC markers in our cultures. In addition to A2B5 antigen [35], rodent GPCs express PDGFαR [36], and NG2 chondroitin sulphate proteoglycan, and antibodies against these three antigens have been used extensively as rodent GPC markers in vitro and in situ studies [6]. A series of double and triple staining experiments established that in our cultures all bi- and tri-polar cells expressed either NG2, A2B5 or PDGFαR, and the majority expressed all three of these GPC markers at 5 days in vitro. While PDGFαR, A2B5, and NG2 are all markers of developing GPCs, significant differences in their staining characteristics are apparent: in the adult human brain, there are substantially greater numbers of NG2$^+$ cells than PDGFαR$^+$. One suggestion emerging from human developmental studies is that PDGFαR is expressed by early human GPCs, A2B5 by early and intermediate precursors, and NG2 by intermediate and late GPCs plus early preoligodendrocytes [13]. Because under our experimental conditions the PDGFαR antibodies worked more consistently than NG2 antibodies, we used the presence of PDGFαR immunoreactivity in A2B5$^+$ cell population to identify GPCs for subsequent RT-PCR and immunocytochemical analysis. While about 40% of the bi- and tri-polar A2B5$^+$ and NG2$^+$ GPCs were labelled with the GFAP-specific antibody at 5 days in vitro (3 days after immunomagnetic sorting), there was generally an increase in GFAP$^+$ cells (characteristically with multiple processes) between 5 and 8 days in vitro. In this study bi- and tri-polar A2B5 and PDGFαR immunopositive GPC cells were used (at 5 days in vitro and 3 days after immunomagnetic sorting) for all subsequent experiments. Magnetically selected A2B5$^+$ and PDGFαR$^+$ cells incorporated bromodeoxyuridine (not shown), which confirms their proliferation under our experimental conditions.

Identification of mGluR mRNAs in A2B5-positive human GPCs using reverse transcription and multiplex PCR analysis

To identify and characterise mGluR mRNA expression in GPCs, 10 cells were visually selected and analysed by multiplex PCR following reverse transcription. Each of the selected cells displayed characteristic GPC morphology with round, phase-bright bi- and tri-polar cells with long processes. The surfaces of these cells were covered with magnetic bead-tagged anti-mouse IgM antibodies, which allowed the visualisation of bound anti-A2B5 antibody.

Two steps of multiplex PCR were performed. First the cDNAs were amplified simultaneously by using all of the primer pairs described in Table 1. For each primer pair the sense and antisense primers were positioned on two different exons [28,37]. The second round of PCR was performed by using 2 μl of the first PCR product as a template. In this second round each PCR product was further amplified individually with its specific primer pair (Table 1). We found that mRNAs for mGluR3 and mGluR5 were present in human GPCs (Fig. 1A). In adult rodent cerebral cortex mGluR5 is expressed as two splice variants, mGluR5a and mGluR5b, which differ in that mGluR5b has a 32-amino acid insert in the intracellular C-terminal domain [38]. To determine the splice variant present in human GPCs, we used a primer pair, which amplifies the alternatively spliced region of human mGluR5 (Table 1). The primers for mGluR5a and mGluR5b mRNAs were designed to flank introns in the genomic sequence [28]. Although both mGluR5a and mGluR5b mRNAs were identified in human cerebral cortex, only the shorter mGluR5a splice variant was detected in A2B5$^+$ GPCs (Fig. 1A, right panel). In agreement with previous rodent studies, the mGluR5b splice variant appeared as the dominant form in the adult human cerebral cortex [39,40]. The expression of GFAP mRNA was detected in both A2B5$^+$ GPC and human cerebral cortical samples (Fig. 1B). The integrity of each cDNA sample was confirmed by the detection of β-actin mRNA (data not shown). Restriction enzyme digestion confirmed that the amplified PCR products corresponded to the correct mRNA sequences (Fig. 1C). All the PCR experiments were done in parallel with multiple controls as described in 'Materials and methods.'

Immunocytochemical analysis of mGluR expression in A2B5 and PDGFαR-positive human GPCs

In addition to the RT-PCR investigations, parallel A2B5$^+$ sister cultures were analysed using double immunofluorescence labelling and confocal microscopy to confirm the expression of mGluR proteins in individual GPCs. Magnetically separated A2B5$^+$ cell cultures were immunostained with the anti-PDGFαR and

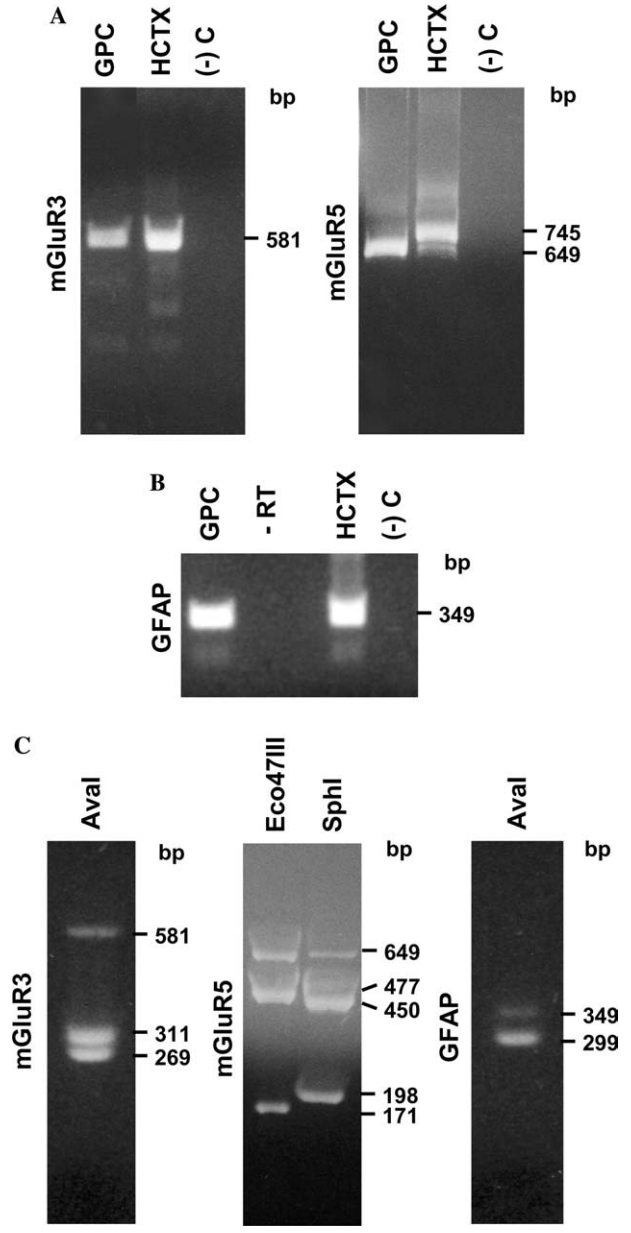

Fig. 1. Detection of mGluR transcripts in human GPCs following two rounds of PCR. (A) Receptor-specific primers (Table 1) were used to detect mGluR3 and mGluR5 mRNA expression in A2B5$^+$ human GPCs. The positive controls were mRNA prepared from human cerebral cortex (HCTX). Negative control PCRs were performed without template [(−)C]. (B) RT-PCR analysis identified GFAP in mRNAs isolated from primary GPC cultures. The positive controls were mRNA prepared from human cerebral cortex (HCTX). Negative control PCRs were performed without reverse transcription [−RT] or template [(−)C]. (C) PCR products were verified by their restriction enzyme digestion patterns using the following enzymes: *Ava*I (mGluR3, GFAP), *Eco*47III, and *Sph*I (mGluR5). The sizes of the PCR products and their digestion fragments are indicated on the right.

anti-mGluR5 antibodies 3 days after immunomagnetic sorting (Fig. 2). The characteristic morphological features together with clear A2B5 and PDGFαR immunoreactivity were used to identify GPCs in cultures for the assessment of mGluR5 staining. The antibody

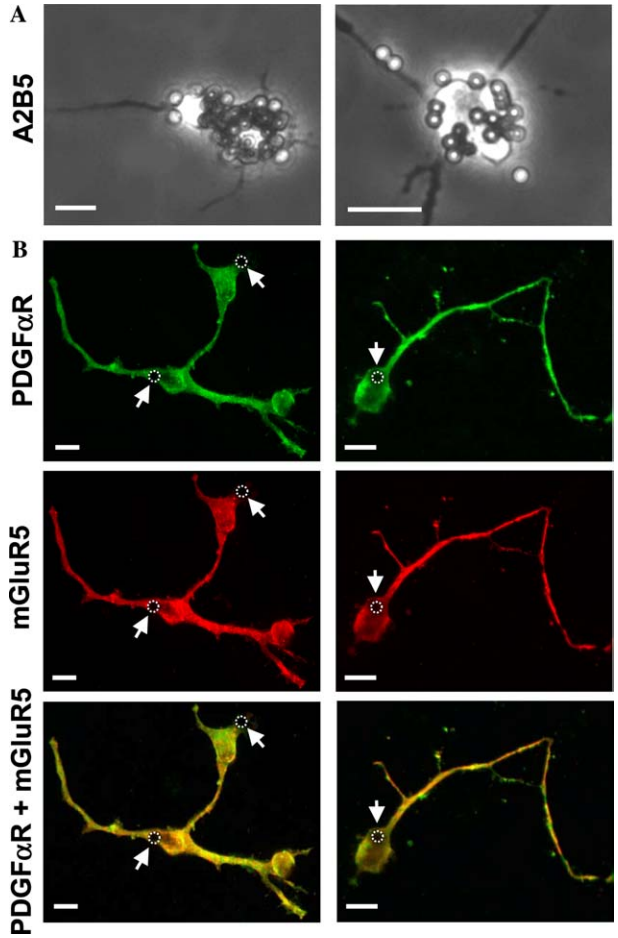

Fig. 2. Morphological and immunocytochemical characterisation of human GPCs in culture. (A) GPCs were separated using A2B5 antibody coated magnetic beads and cultured for 3 days as described under 'Materials and methods.' (B) GPCs were immunostained with mouse anti-PDGFα (green) and rabbit anti-mGluR5 (red) antibodies. Overlay of PDGFα and mGluR5 immunoreactivity (yellow) is shown on bottom panels. Anti-A2B5 antibody coated beads remain tightly associated with GPCs despite extensive washing (B, arrows). Scale bars, 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

against the conserved C-terminal 13 amino acids of rat mGluR5 is known to cross react with the corresponding human protein [27]. All A2B5$^+$ and PDGFαR$^+$ GPCs with characteristic oligogendrocyte progenitor morphology were labelled with the anti-mGluR5 antibody (Fig. 2B). Previous detailed double-labelling studies established that astrocytes in vitro were not labelled by the PDGFαR antibody [14]. To validate the specificity of each immunoreaction, oligodendrocyte progenitor cells derived from the rodent CG-4 cell line [29], primary hippocampal neuronal cultures [33], and HEK 293, and HeLa cells were used as positive and negative controls in parallel experiments. The anti-A2B5 IgM antibody selectively labelled bipolar CG-4 oligodendrocyte progenitor cells (Fig. 3A). As previously reported, the A2B5 immunoreactivity is reduced in more developed, multipolar oligodendrocytes (Fig. 3B, cell indicated with

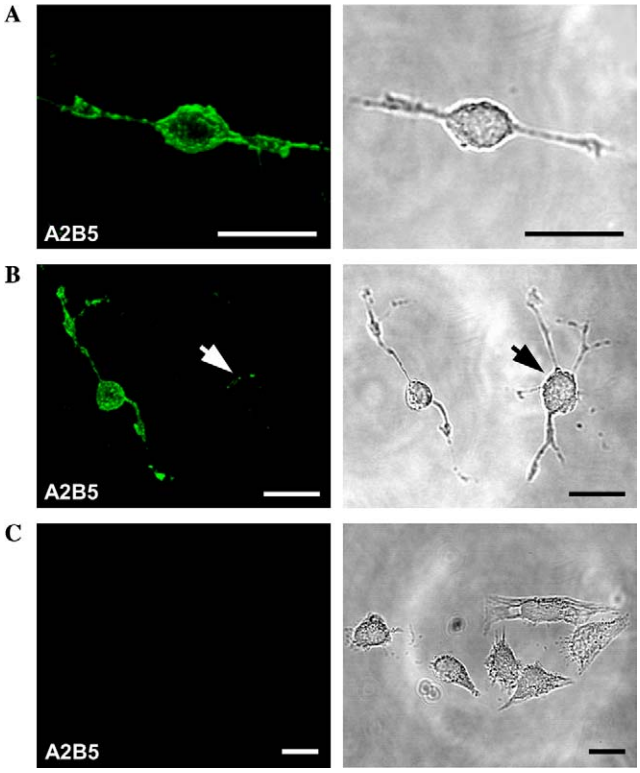

Fig. 3. Characterisation of the anti-A2B5 antibody using CG-4 GPC cultures. A2B5 immunoreactivity is clearly visible in bipolar GPCs (A,B), while multipolar cells remain negative in the same culture (B, arrow). HEK 293 cells were used as negative controls for the anti-A2B5 immunolabelling experiments (C). Panels on the left illustrate three-dimensional reconstructions of A2B5 surface-stained cells. Scale bars, 20 μm.

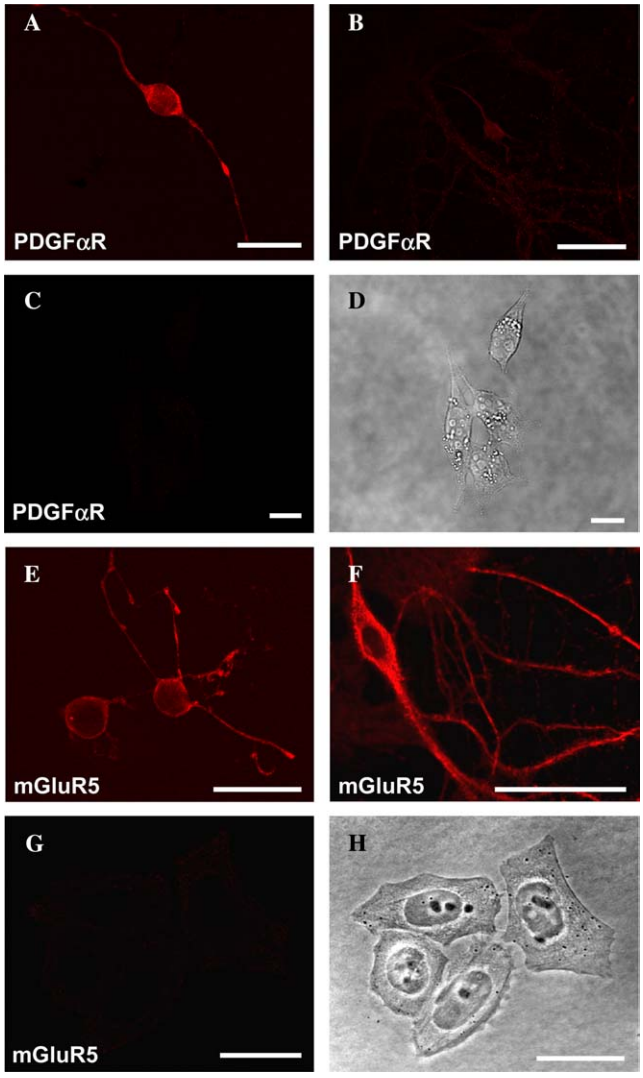

Fig. 4. Characterisation of anti-PDGFα and anti-mGluR5 antibodies using CG-4 GPCs and primary hippocampal neuronal cultures. While strong PDGFαR immunoreactivity is visible in GPCs (A); hippocampal neurons (B); and HEK 293 cells (C,D) show no detectable labelling. The anti-mGluR5 antibody labelled both CG-4 GPCs (E) and hippocampal neurons (F) but HeLa cells, used as negative controls, remained unlabelled (G,H). Scale bars, 20 μm.

arrow) and control HEK 293 cells also remained negative (Fig. 3C). While the mouse anti-PDGFαR IgG antibody strongly stains CG-4 cells (Fig. 4A), hippocampal neurons (Fig. 4B) and HEK 293 cells (Figs. 4C and D) remain unlabelled under the same experimental conditions. The rabbit anti-mGluR5 polyclonal antibody labelled both CG-4 oligodendrocyte progenitor cells (Fig. 4E) and hippocampal neurons (Fig. 4F). HeLa cells were used as negative controls to confirm the reaction specificity of the mGluR5 antibody in our experiments (Figs. 4G and H). These experiments provide confirmation of mGluR5 protein expression in GPCs. We have not been able to obtain corresponding data with either of the rabbit anti-GluR2/3 antibodies we tested (from Chemicon and Upstate Biotechnology), because both of these reagents produced high background staining in immunocytochemistry (data not shown) and identified additional bands in our immunoblot experiments [30].

Discussion

The present study identified mGluR3 and mGluR5a mRNAs in immunomagnetically separated A2B5$^+$ primary GPC cultures obtained from adult human subcortical white matter. Immunocytochemical analysis also revealed the expression of mGluR5 proteins in A2B5$^+$ and PDGFαR$^+$ GPCs.

GPCs immunomagnetically selected from adult human brain samples maintain their characteristic features in vitro

While a body of evidence suggests that GPCs are present in the adult subcortical white matter of both rodent and human brains [1,2,6,9–11,14], it proved to be very difficult to study these cells in situ within the CNS due to the lack of suitably specific markers. However,

these mitotically competent GPCs can be selectively isolated and maintained in vitro and recognized using immunophenotypic markers. Rodent GPCs can be identified by A2B5 antibodies and, in appropriate in vitro environments, they can give rise to oligodendrocytes [2]. In addition to A2B5, these cells typically express PDGFαR and the integral membrane proteoglycan NG2 [3]. While markers for cultured human GPCs are not well established, some of the extensively used rodent GPC markers also label human precursors [9,13,17]. In the present study we analysed primary $A2B5^+$ cell cultures prepared from adult human subcortical white matter. Similar $A2B5^+$ cell preparations possess oligodendrocyte progenitor characteristics, because they develop into myelinating oligodendrocytes following transplantation [12,41]. Our immunochemical characterisation of these cells is consistent with previous studies of GPCs, where the co-expression of the A2B5 antigen with NG2 and PDGFαR was reported [13,16]. The previously described potential cross-reactivities of the anti-A2B5 and anti-PDGFαR antibodies with neurons are not relevant to primary cultures from adult human CNS, because neurons do not survive the dissociation process [31]. Moreover, our $A2B5^+$ and $PDGF\alpha R^+$ cell cultures were completely negative for the neuronal marker βIII tubulin and they incorporated bromodeoxyuridine, which also exclude their neuronal origin. Astrocytes in vitro are not labelled by the PDGFαR antibody [14]. As they matured, the number of $GFAP^+$ cells increased even under serum-free conditions. Previous studies have also reported similar differences in GFAP expression in human GPCs compare to rat GPCs [42]. In the rat conditions have been identified in which freshly isolated GPCs are GFAP negative and remain so for several weeks [35], however, human GPC cultures express GFAP under similar conditions [42], which is consistent with the results of our RT-PCR and immunocytochemical experiments. More recent studies convincingly indicate that GFAP is not an exclusive marker of astrocytes and can be expressed in GPCs without astrocytic phenotype or function [43].

Metabotropic glutamate receptor isoforms mGluR3 and mGluR5 are expressed in adult human GPCs

Recent evidence supports a role for glutamate receptors in the pathophysiology of oligodendroglial death in demyelinating diseases (reviewed in [18]). Oligodendroglial cells have been shown to be highly vulnerable to glutamate-mediated toxicity both in vitro and in vivo [44,45]. Previous studies have focused specifically on the role of iGluRs, which are ligand gated ion channels [including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) receptors]. While these studies reported the presence of AMPA, kainate and the absence of NMDA type iGluRs in rat oligodendrocytes (reviewed in [18]), the expression and function of mGluRs are less clear. In a recent study of the CG-4 clonal rodent oligodendrocyte progenitor cell line we have identified and characterised functional mGluR3 and mGluR5 isoforms [30]. None of the other mGluRs were present. Because the expression pattern of mGluRs in adult human GPCs is unknown, and also to validate our previous data obtained using the CG-4 rodent cell line [30], we used immunomagetically separated, highly homogeneous $A2B5^+$ GPC cultures for the present study. This strategy allows the isolation of GPCs based on cell-surface markers and morphology. RT-PCR from very limited number of cells requires a highly sensitive and efficient system. The multiplex PCR procedure allowed us to reliably characterise mGluR3 and mGluR5a mRNA expression despite the extremely limited amount of starting tissue, which was obtained during anterior temporal lobe resection for intractable epilepsy.

It is interesting to note that mGluR5 exists in two splice variants, mGluR5a and mGluR5b [38]. The mGluR5b contains a 32 amino acid fragment inserted into the cytoplasmic tail and is the major type expressed in different regions of adult brain [46]. There is a developmental switch from mGluR5a to mGluR5b splicing during the early postnatal period in both neurons and astrocytes [22,39,40,47,48]. Our multiplex RT-PCR analysis selectively identified the mGluR5a splice variant in adult GPCs, which is consistent with the developmentally more immature state of these cells.

We used immunofluorescence double-labelling to confirm the expression of mGluR proteins in characterised GPCs. Our immunocytochemical analysis identified mGluR5 immunoreactivity in $A2B5^+$ and $PGDF\alpha R^+$ GPCs. Parallel positive and negative controls confirmed the reaction specificity of each of the antibodies under our experimental conditions. While we have not been able to analyse the presence of mGluR3 in human GPCs at the protein level due to the lack of suitable mGluR3-specific antibody, previous in situ hybridisation studies of normal rat brain identified mRNAs for mGluR3 in glial cells in various brain regions [49,50].

Astrocytes also express the mGluR3 and mGluR5 isoforms [21,23,24,26]. The astroglial expression of these mGluRs appears to be dynamic, with expression levels changing in response to different types of brain injury in vivo [23,26,27,51,52] and upon exposure of cultured astrocytes to growth factors [53,54]. The white matter used for the present study was dissected from regions distant from the epileptic focus in temporal lobe. While previous studies confirmed that comparable tissue remains histologically normal [9], we cannot completely eliminate the possibility that GPCs were exposed to pathological glutamate concentrations in the brain, which may lead to changes in mGluR expression levels as seen in astrocytes.

Possible functional roles of metabotropic glutamate receptors in human GPCs

Several observations suggest that glial mGluRs can regulate glial function and may be involved in the interaction between glia and neurons in both physiological and pathological conditions [55–57]. It is possible that mGluRs may play a developmental role in GPCs. Activation of mGluR5 enhances proliferation in cultured astrocytes, whereas activation of mGluR3 inhibits this process [58]. Therefore, the newly identified mGluR3 and mGluR5 may also be involved in the regulation of GPC division. The mGluR5 isoform has been shown to be involved in neurite extension in NG108-15 cells, with the mGluR5 specific antagonist MPEP preventing arborisation in these cells [59]. The same receptor could be involved in the differentiation process in oligodendroglial cells where complex ramification takes place.

The presence of mGluR3 and mGluR5 in GPCs and their recognised role in cell differentiation and proliferation in rodent glial cells may represent a novel system for the regulation of the spontaneous remyelination process observed in multiple sclerosis and other demyelinating conditions [6,12,14,16]. Previous studies reported enhanced mGluR5 and mGluR2/3 immunoreactivity in multiple sclerosis lesions [60]. It is plausible that glutamate released from injured axons may activate GPCs to begin the remyelination process. The increased mGluR2/3 and mGluR5 signal may in part be due to newly recruited GPCs migrating into the demyelinated area in addition to reactive astrocytes [60]. Since these receptors may be involved in the signalling process required to initiate remyelination in the human brain, they could represent novel potential pharmacological targets for therapeutic interventions in multiple sclerosis and other demyelinating diseases.

Acknowledgments

This work was supported by the Department of Health, Medical Research Council, the Wellcome Trust, and the Multiple Sclerosis Society. We thank Dr. Maria M. Usowicz for her advice on multiplex RT-PCR methodology. We thank the MRC for providing an Infrastructure Award to establish the School of Medical Sciences Cell Imaging Facility.

References

[1] N. Scolding, Glial precursor cells in the adult human brain, Neuroscientist 4 (1998) 264–272.
[2] C. ffrench-Constant, M.C. Raff, Proliferating bipotential glial progenitor cells in adult rat optic nerve, Nature 319 (1986) 499–502.
[3] A. Nishiyama, A. Chang, B.D. Trapp, NG2+ glial cells: a novel glial cell population in the adult brain, J. Neuropathol. Exp. Neurol. 58 (1999) 1113–1124.
[4] A. Nishiyama, X.H. Lin, N. Giese, C.H. Heldin, W.B. Stallcup, Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain, J. Neurosci. Res. 43 (1996) 299–314.
[5] A. Nishiyama, X.H. Lin, N. Giese, C.H. Heldin, W.B. Stallcup, Interaction between NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells is required for optimal response to PDGF, J. Neurosci. Res. 43 (1996) 315–330.
[6] J.M. Levine, R. Reynolds, J.W. Fawcett, The oligodendrocyte precursor cell in health and disease, Trends Neurosci. 24 (2001) 39–47.
[7] R.C. Armstrong, H.H. Dorn, C.V. Kufta, E. Friedman, M. Dubois-Dalcq, Pre-oligodendrocytes from adult human CNS, J. Neurosci. 12 (1992) 1538–1547.
[8] N. Gogate, L. Verma, J.M. Zhou, E. Milward, R. Rusten, M. Oconnor, C. Kufta, J. Kim, L. Hudson, M. Duboisdalcq, Plasticity in the adult human oligodendrocyte lineage, J. Neurosci. 14 (1994) 4571–4587.
[9] N.J. Scolding, P.J. Rayner, J. Sussman, C. Shaw, D.A. Compston, A proliferative adult human oligodendrocyte progenitor, Neuroreport 6 (1995) 441–445.
[10] N.J. Scolding, P.J. Rayner, D.A.S. Compston, Identification of A2B5-positive putative oligodendrocyte progenitor cells and A2B5-positive astrocytes in adult human white matter, Neuroscience 89 (1999) 1–4.
[11] N.S. Roy, S. Wang, C. Harrison-Restelli, A. Benraiss, R.A.R. Fraser, M. Gravel, P.E. Braun, S.A. Goldman, Identification, isolation, and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter, J. Neurosci. 19 (1999) 9986–9995.
[12] M.S. Windrem, N.S. Roy, J. Wang, M. Nunes, A. Benraiss, R. Goodman, G.M. McKhann II, S.A. Goldman, Progenitor cells derived from the adult human subcortical white matter disperse and differentiate as oligodendrocytes within demyelinated lesions of the rat brain, J. Neurosci. Res. 69 (2002) 966–975.
[13] H.C. Wilson, C. Onischke, C.S. Raine, Human oligodendrocyte precursor cells in vitro: phenotypic analysis and differential response to growth factors, Glia 44 (2003) 143–165.
[14] N. Scolding, R. Franklin, S. Stevens, C.-H. Heldin, A. Compton, J. Newcombe, Oligodendrocyte progenitors are present in the normal adult human CNS and in the lesions of multiple sclerosis, Brain 121 (1998) 2221–2228.
[15] G. Wolswijk, Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells, J. Neurosci. 18 (1998) 601–609.
[16] A. Chang, A. Nishiyama, J. Peterson, J. Prineas, B.D. Trapp, NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions, J. Neurosci. 20 (2000) 6404–6412.
[17] S.-C. Zhang, B. Ge, I.D. Duncan, Tracing human oligodendroglial development in vitro, J. Neurosci. Res. 59 (2000) 421–429.
[18] C. Matute, E. Aberdi, M. Domercq, F. Perez-Cerda, A. Perez-Samartin, M.V. Sanchez-Gomez, The link between excitotoxic oligodendroglial death and demyelinating diseases, Trends Neurosci. 24 (2001) 224–230.
[19] D.D. Schoepp, D.E. Jane, J.A. Monn, Pharmacological agents acting at subtypes of metabotropic glutamate receptors, Neuropharmacology 38 (1999) 1431–1476.
[20] C.M. Testa, D.G. Standaert, A.B. Young, J.B. Penney Jr., Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat, J. Neurosci. 14 (1994) 3005–3018.
[21] R.S. Petralia, Y.X. Wang, A.S. Niedzielski, R.J. Wenthold, The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations, Neuroscience 71 (1996) 949–976.
[22] G.P. Schools, H.K. Kimelberg, mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor mRNAs expressed

in hippocampal astrocytes acutely isolated from young rats, J. Neurosci. Res. 58 (1999) 533–543.
[23] E. Aronica, E.A. van Vliet, O.A. Mayboroda, D. Troost, F.H. da Silva, J.A. Gorter, Upregulation of metabotropic glutamate receptor subtype mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial temporal lobe epilepsy, Eur. J. Neurosci. 12 (2000) 2333–2344.
[24] C. Romano, M.C.T.M. Sesma, K. O'Malley, A.N. Van den Pol, J.W. Olney, Distribution of metabotropic glutamate receptor mGluR immunoreactivity in rat brain, J. Comp. Neurol. 355 (1995) 455–469.
[25] R. Balazs, S. Miller, C. Romano, A. de Vries, Y. Chun, C.W. Cotman, Metabotropic glutamate receptor mGluR5 in astrocytes: pharmacological properties and agonist regulation, J. Neurochem. 69 (1997) 151–163.
[26] E. Aronica, M.V. Catania, J. Geurts, B. Yankaya, D. Troost, Immunohistochemical localization of group I and II metabotropic glutamate receptors in control and amyotrophic lateral sclerosis human spinal cord: upregulation in reactive astrocytes, Neuroscience 105 (2001) 509–520.
[27] E. Aronica, J.A. Gorter, H. Ijlst-Keizers, A.J. Rozemuller, B. Yankaya, S. Leenstra, D. Troost, Expression and functional role of mGluR3 and mGluR5 in human astrocytes and glioma cells: opposite regulation of glutamate transporter proteins, Eur. J. Neurosci. 17 (2003) 2106–2118.
[28] C. Corti, R.W.E. Clarkson, L. Crepaldi, C.F. Sala, J.H. Xuereb, F. Ferraguti, Gene structure of the human metabotropic glutamate receptor 5 and functional analysis of its multiple promoters in neuroblastoma and astroglioma cells, J. Biol. Chem. 278 (2003) 33105–33119.
[29] J.C. Louis, E. Magal, D. Muir, M. Manthorpe, S. Varon, CG-4, a new bipotential glial cell line from rat brain, is capable of differentiating in vitro into either mature oligodendrocytes or type-2 astrocytes, J. Neurosci. Res. 31 (1992) 193–204.
[30] K. Luyt, A. Varadi, E. Molnar, Functional metabotropic glutamate receptors are expressed in oligodendrocyte progenitor cells, J. Neurochem. 84 (2003) 1452–1464.
[31] V.W. Young, J.P. Antel, Culture of glial cells from human brain biopsies, in: S. Federoff, A. Richardson (Eds.), Protocols for Neural Cell Culture, third ed., Humana Press, Totowa, NJ, USA, 2001, pp. 129–138.
[32] A.P. Wright, J.J. Fitzgerald, R.J. Colello, Rapid purification of glial cells using immunomagnetic separation, J. Neurosci. Methods 74 (1997) 37–44.
[33] L. Pickard, J. Noel, J.M. Henley, G.L. Collingridge, E. Molnar, Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons, J. Neurosci. 20 (2000) 7922–7931.
[34] N.L. Brice, A. Varadi, S.J. Ashcroft, E. Molnar, Metabotropic glutamate and GABA(B) receptors contribute to the modulation of glucose-stimulated insulin secretion in pancreatic beta cells, Diabetologia 45 (2002) 242–252.
[35] M.C. Raff, R.H. Miller, M. Noble, A glial progenitor cell that develops in vitro an astrocyte or an oligodendrocyte depending on culture medium, Nature 303 (1983) 390–396.
[36] N.P. Pringle, H.S. Mudhar, E.J. Collarini, W.D. Richardson, PDGF receptors in the rat CNS: during late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage, Development 115 (1992) 535–551.
[37] C. Corti, C.F. Sala, F.T. Yang, M. Corsi, J.H. Xuereb, F. Ferraguti, Genomic organization of the human metabotropic glutamate receptor subtype 3, J. Neurogenet. 14 (2000) 207–225.
[38] R. Minakami, F. Katsuki, H. Sugiyama, A variant of metabotropic glutamate receptor subtype 5: an evolutionally conserved insertion with no termination codon, Biochem. Biophys. Res. Commun. 194 (1993) 622–627.
[39] C. Romano, S. Smout, J.K. Miller, K.L. O'Malley, Developmental regulation of metabotropic glutamate receptor 5b protein in rodent brain, Neuroscience 111 (2002) 693–698.
[40] S.L. Harris, K. Cho, Z.I. Bashir, E. Molnar, Metabotropic glutamate receptor signalling in perirhinal cortical neurons, Mol. Cell. Neurosci. 25 (2004) 275–287.
[41] M.S. Windrem, M.C. Nunes, W.K. Rashbaum, T.H. Schwartz, R.A. Goodman, G. McKhann II, N.S. Roy, S.A. Goldman, Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain, Nat. Med. 10 (2004) 93–97.
[42] J. Dietrich, M. Noble, M. Mayer-Proschel, Characterization of $A2B5^+$ glial precursor cells from cryopreserved human fetal brain progenitor cells, Glia 40 (2002) 65–77.
[43] S. Goldman, Glia as neuronal progenitor cells, Trends Neurosci. 26 (2003) 590–596.
[44] C. Matute, M.V. Sanchez-Gomez, L. Martinez-Millan, R. Miledi, Glutamate receptor-mediated toxicity in optic nerve oligodendrocytes, Proc. Natl. Acad. Sci. USA 94 (1997) 8830–8835.
[45] J.W. McDonald, S.P. Althomsons, K.L. Hyrc, D.W. Choi, M.P. Goldberg, Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity, Nat. Med. 4 (1998) 291–297.
[46] C. Joly, J. Gomeza, I. Brabet, K. Curry, J. Bockaert, J.P. Pin, Molecular, functional, and pharmacological characterization of the metabotropic glutamate receptor type 5 splice variants: comparison with mGluR1, J. Neurosci. 15 (1995) 3970–3981.
[47] C. Romano, A.N. van den Pol, K.L. O'Malley, Enhanced early developmental expression of the metabotropic glutamate receptor mGluR5 in rat brain: protein, mRNA aplice variants, and regional distribution, J. Comp. Neurol. 367 (1996) 403–412.
[48] Z. Cai, G.P. Schools, H.K. Kimelberg, Metabotropic glutamate receptors in acutely isolated hippocampal astrocytes: developmental changes of mGluR5 mRNA and functional expression, Glia 29 (2000) 70–80.
[49] Y. Tanabe, M. Masu, T. Ishii, R. Shigemoto, S. Nakanishi, A family of metabotropic glutamate receptors, Neuron 8 (1992) 169–179.
[50] H. Ohishi, R. Shigemoto, S. Nakanishi, N. Mizuno, Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an in situ hybridization study, J. Comp. Neurol. 335 (1993) 252–266.
[51] J. Ulas, T. Satou, K.J. Ivins, J.P. Kesslak, C.W. Cotman, R. Balazs, Expression of metabotropic glutamate receptor 5 is increased in astrocytes after kainate-induced epileptic seizures, Glia 30 (2000) 352–361.
[52] F. Ferraguti, C. Corti, E. Valerio, S. Mion, J. Xuereb, Activated astrocytes in areas of kainate-induced neuronal injury upregulate the expression of the metabotropic glutamate receptors 2/3 and 5, Exp. Brain Res. 137 (2001) 1–11.
[53] S. Miller, C. Romano, C.W. Cotman, Growth factor upregulation of a phosphoinositide-coupled metabotropic glutamate receptor in cortical astrocytes, J. Neurosci. 15 (1995) 6103–6109.
[54] T. Minoshima, S. Nakanishi, Structural organization of the mouse metabotropic glutamate receptor subtype 3 gene and its regulation by growth factors in cultured cortical astrocytes, J. Biochem. (Tokyo) 126 (1999) 889–896.
[55] D.G. Winder, P.S. Ritch, R.W. Gereau IV, P.J. Conn, Novel glia-neuronal signalling by coactivation of metabotropic glutamate and beta-adrenergic receptors in rat hippocampus, J. Physiol. 494 (1996) 743–755.
[56] J. Cartmell, D.D. Schoepp, Regulation of neurotransmitter release by metabotropic glutamate receptors, J. Neurochem. 75 (2000) 889–907.
[57] M. D'Onofrio, L. Cuomo, G. Battaglia, R.T. Ngomba, M. Storto, A.E. Kingston, F. Orzi, A. De Blasi, P. Di lorio, F. Nicoletti, V.

Bruno, Neuroprotection mediated by glial group-II metabotropic glutamate receptors requires the activation of the MAP kinase and the phosphatidylinositol-3-kinase pathways, J. Neurochem. 78 (2001) 435–445.

[58] R. Ciccarelli, F.X. Sureda, G. Casabona, P. DiIorio, A. Caruso, F. Spinella, D.F. Condorelli, F. Nicoletti, F. Caciagli, Opposite influence of the metabotropic glutamate receptor subtypes mGluR3 and -5 on astrocyte proliferation in culture, Glia 21 (1997) 390–398.

[59] S. Mion, C. Corti, A. Neki, R. Shigemoto, M. Corsi, G. Fumagalli, F. Ferraguti, Bidirectional regulation of neurite elaboration by alternatively spliced metabotropic glutamate receptor 5 (mGluR5) isoforms, Mol. Cell. Neurosci. 17 (2001) 957–972.

[60] J.J.G. Geurts, G. Wolswijk, L. Bo, P. van der Valk, C.H. Polman, D. Troost, E. Aronica, Altered expression patterns of group I and II metabotropic glutamate receptors in multiple sclerosis, Brain 126 (2003) 1755–1766.