Oligodendrocyte Progenitor Cell Proliferation and Lineage Progression Are Regulated by Glutamate Receptor-Mediated K+ Channel Block

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We have analyzed the role of glutamate and its receptors (GluRs) in regulating the development of oligodendrocytes. Activation of AMPA-preferring GluRs with selective agonists inhibited proliferation of purified cortical oligodendrocyte progenitor (O-2A) cells cultured with different mitogens, as measured by [³H]thymidine incorporation or bromodeoxyuridine staining. In contrast, activation of GABA or muscarinic receptors did not affect O-2A proliferation. Cell viability and apoptosis assays demonstrated that the inhibition of O-2A proliferation was not attributable to a cytotoxic action of GluR agonists, and was reversible. Activation of GluRs prevented lineage progression from the O-2A (GD3+/nestin+) stage to the pro-oligodendroblast (O4+) stage, but did not affect O-2A migration. Additional experiments examined the membrane ionic channels mediating these GluR activation effects. We found that proliferating O-2A cells expressed functional delayed rectifier K+ channels, which were absent in pro-oligodendroblasts. GluR agonists and the K+ channel blocker tetraethylammonium (TEA) strongly inhibited delayed rectifier K+ currents in O-2A cells. TEA reproduced the effects of GluR activation on O-2A proliferation and lineage progression in the same concentration range that blocked delayed rectifier K+ currents. These results indicate that glutamate regulates oligodendrogenesis specifically at the O-2A stage by modulating K+ channel activity.

Key words: AMPA receptors; O-2A cells; glia; cell migration; tetraethylammonium; delayed rectifier K+ channels

Classical neurotransmitters, including amino acids, can influence neural cell development in the mammalian CNS (Komuro and Rakic, 1993; Lauder, 1993; Behar et al., 1994; Thomas et al., 1995). The physiological action of the major excitatory neurotransmitter glutamate is mediated through different receptor subtypes that are expressed in both neurons and glia (Mayer and Westbrook, 1987; Gallo and Russell, 1995). In the developing and mature CNS, glutamate receptors (GluRs) in neurons are involved in synaptic transmission and plasticity, but their role in glial cells is unknown.

Oligodendrocytes are generated from progenitor (O-2A) cells that proliferate in CNS germinal zones and migrate to formative white matter regions, where they further proliferate, differentiate, and form myelin sheaths around axons (Reynolds and Wilkin, 1988; Levison and Goldman, 1993; Luskin and McDermott, 1994; Zerlin and Goldman, 1995). Distinct and sequential stages of

oligodendrocyte maturation have been characterized, based on the expression of developmentally regulated antigens, which also identify proliferative versus nonproliferative stages of O-2A development (Sommer and Schachner, 1981; Raff et al., 1983; Goldman et al., 1984; Levi et al., 1986; Gard and Pfeiffer, 1990; Warrington and Pfeiffer, 1992; Gallo and Armstrong, 1995). The expression pattern of these antigens is reproduced in vitro as well as in vivo (Reynolds and Wilkin, 1988; Warrington and Pfeiffer, 1992; Gallo and Armstrong, 1995). For example, the intermediate filament protein nestin is highly expressed in dividing pre-O-2A and O-2A cells, but downregulated in postmitotic O4+ pro-oligodendroblasts and O1+ oligodendrocytes (Gallo and Armstrong, 1995). Distinct changes in the membrane channel phenotype of O-2A cells also occur during lineage progression (Sontheimer et al., 1989; Barres et al., 1990; von Blankenfeld et al., 1991), indicating that some of these membrane constituents may have a role in the regulation of O-2A cell differentiation.

Oligodendrocyte development takes place during the late gestational and early postnatal periods, after neuronal maturation has occurred (Curtis et al., 1988; LeVine and Goldman, 1988; Skoff, 1990). O-2A cells proliferate, migrate, and differentiate in an environment in which cells that can produce glutamate, i.e., neurons and astrocytes, are already present (Jacobson, 1991). O-2A cells express functional GluRs in vivo and in vitro (Gallo et al., 1989; Barres et al., 1990; Wyllie et al., 1991; Fulton et al., 1992; Borges et al., 1994; Holzwarth et al., 1994; Patneau et al., 1994; Berger et al., 1995). By using a combined molecular and electrophysiological approach, we have demonstrated previously that cultured O-2A cells coexpress functional kainate- and AMPA-preferring receptors (Patneau et al., 1994).

Figure 1. Glutamate receptor activation inhibits O-2A cell proliferation: [³H]thymidine incorporation assays. O-2A progenitor cells were cultured in DME-N1 + 0.5% FBS (*A*), with PDGF (*B*), bFGF (*C*), or PDGF + bFGF (*D*). Cells were plated in 24-well plates. After 2 hr, PDGF and/or bFGF (both 10 ng/ml) in combination with kainic acid (*KAI;* 200 μM), AMPA (200 μM), glutamate (*GLU;* 200 μM), or DNQX (30 μM) were added to the cultures as well as [³H]thymidine (0.5 μCi/ml). After 22 hr, [³H]thymidine incorporation was measured by trichloroacetic acid precipitation and scintillation counting. Control cells (*CTR*) in N1 incorporated 3508 ± 371 cpm/well/22 hr (average ± SEM, *n* = 9; 30,000 cells/well). Averages of three experiments in triplicate ± SEM are shown. *$p < 0.001$; **$p < 0.01$; ***$p < 0.05$ compared with their respective controls (Student's *t* test). Cells in kainate + DNQX were compared with cells in kainate alone for statistical analysis. Kainate and AMPA similarly inhibited proliferation of O-2A cells cultured in B104-CM (data not shown).
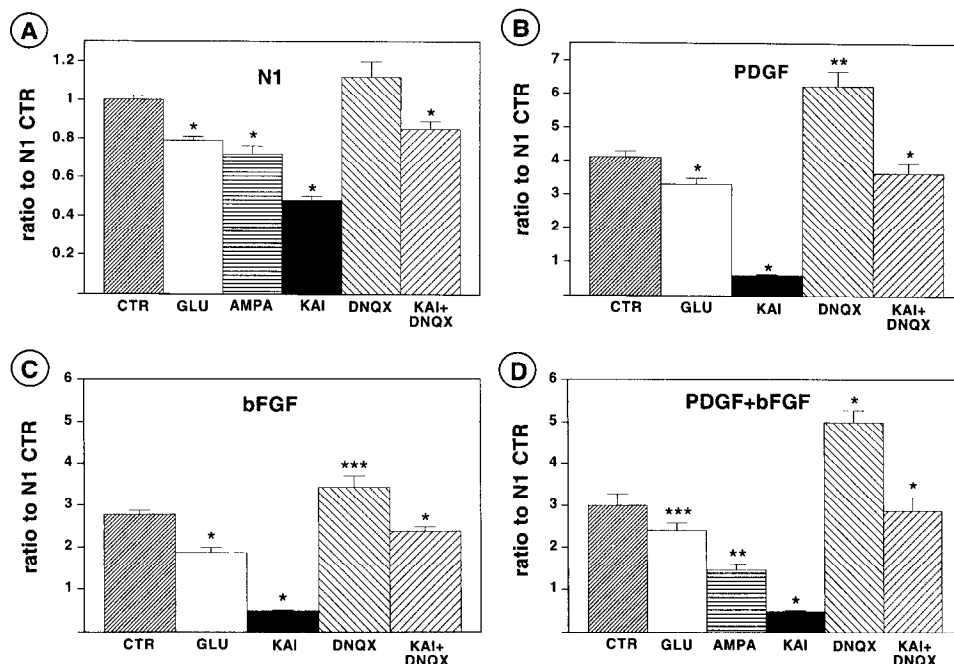
In the present study, we analyzed potential functions of GluRs in modulating proliferation, migration, and differentiation of O-2A progenitor cells during development. We used *in vitro* bioassays and soluble factors, which stimulate mitosis, motility, and maturation of O-2A cells, to reproduce the events that occur during CNS development. We report that activation of AMPA GluRs inhibits cortical O-2A cell division in a reversible manner and prevents differentiation to the pro-oligodendroblast stage by blocking delayed rectifier K⁺ channels. These cationic channels are expressed only in proliferative O-2A cells and are drastically downregulated in postmitotic pro-oligodendroblasts (see also Sontheimer et al., 1989; Barres et al., 1990; Borges et al., 1994). Our analysis not only reveals a physiological role for GluR in glial development, but uncovers a mechanism that involves other membrane channels the function of which in O-2A cells was previously unknown. Our findings indicate that neurotransmitters and their receptors can control glial proliferation and maturation and, therefore, may modulate the cellular development of the CNS.

MATERIALS AND METHODS

Cell cultures. Purified cortical O-2A progenitor cultures were prepared by modifications of previously described methods (Armstrong et al., 1990a; McKinnon et al., 1990). Briefly, embryonic day 20 (E20) Sprague–Dawley rats were killed following the National Institutes of Health Animal Welfare guidelines, and cortices were removed, mechanically dissociated, suspended in DMEM (Gibco, Gaithersburg, MD) containing 10% fetal bovine serum (FBS; HyClone, Logan, UT), and plated in plastic T75 flasks. After 12 d in culture, O-2A progenitor cells growing on top of a confluent monolayer of astrocytes were detached by overnight shaking (McCarthy and de Vellis, 1980). Contaminating microglial cells were eliminated further by plating this fraction on plastic culture dishes for 1 hr. The O-2A progenitor cells, which do not attach well to plastic, were collected by gently washing the dishes, replated (3 × 10⁴ cells/cm², unless otherwise specified) onto poly-D-ornithine-coated plates (0.1 mg/ml), and cultured in DME-N1 biotin-containing medium. After 2 hr, platelet-derived growth factor (PDGF; human AB, heterodimer form, 10 ng/ml), basic fibroblast growth factor (bFGF; human, 10 ng/ml), PDGF + bFGF (both from Upstate Biotechnology, Lake Placid, NY), or 30% B104-conditioned medium (B104-CM) was added to the culture medium. O-2A cells were cultured for 1–3 d and treated every 24 hr with PDGF and/or bFGF, or B104-CM. Differentiation into O4⁺, postmitotic pro-oligodendroblasts was promoted by withdrawing the mitogenic factors

and growing the O-2A progenitors for an additional 2–3 d in DME-N1 medium containing 0.5% FBS.

Cultures enriched in different cell types were characterized immunocytochemically by using cell type-specific antibodies (see below). Cell cultures used for immunostaining were grown on glass coverslips precoated with poly-D-ornithine. In cortical cultures enriched in O-2A progenitors, >95% of the cells were labeled by the LB1 (anti-GD3) antibody, after 24 hr *in vitro* with PDGF, PDGF + bFGF, or B104-CM. In pro-oligodendroblast-enriched cultures, >85% of the cells were O4⁺, after 48 hr of culture in the absence of mitogens.

Cell proliferation assays. Purified cortical O-2A cells were plated in DME-N1 biotin-containing medium with 0.5% FBS in 24-multiwell plates ([³H]thymidine incorporation assays), or on 25-mm-diameter coverslips [bromodeoxyuridine (BrdU) incorporation assays] at a density of 2 × 10⁴ cells/cm². After 2 hr, PDGF and/or bFGF, kainic acid (Sigma, St. Louis, MO), AMPA (Tocris Cookson, Bristol, UK), glutamate (Sigma), 6,7-dinitroquinoxaline-2,3-dione (DNQX; Tocris Cookson), GABA, muscimol, or carbachol were added to the cultures along with [methyl-³H]thymidine (0.5 μCi/ml, 85 Ci/mmol; Amersham, Arlington Heights, IL). Unless otherwise stated, after 22 hr cells were lysed and [³H]thymidine incorporation was measured by precipitation with 10% trichloroacetic acid and scintillation counting.

For BrdU staining, cells were pulsed with 25 μM BrdU for 20 hr, fixed in 4% paraformaldehyde for 10 min at room temperature, and incubated with 0.07N NaOH for 10 min at room temperature. After washing in PBS, cells were fixed in 4% paraformaldehyde for 10 min and permeabilized in 0.2% Triton X-100 in PBS for 10 min. After washing in PBS, cells were incubated with 10% heat-inactivated goat serum for 15 min, with monoclonal anti-BrdU antibodies (1:20; Dako, Carpinteria, CA) for 1 hr, and with fluorescein-conjugated goat anti-mouse (GAM) IgG for 30 min.

Cell viability and apoptosis assays. O-2A cells were grown for 1–2 d on glass coverslips under the conditions listed in Results. Cells were incubated with fluorescein diacetate (Sigma; 10 μg/ml) and propidium iodide (Sigma; 20 μg/ml) for 5 min. Cells contained in five to eight 40× objective fields were counted (80–150 total cells/field) per coverslip, using fluorescein and rhodamine filters on a Zeiss Axiophot (Thornwood, NY) fluorescence microscope (Jones and Senft, 1985). Viable O-2A cells appeared bright fluorescent green, whereas nonviable cells were stained in red.

Apoptotic cell death was determined by fluorescence microscopy with the nuclear dye Hoechst 33258 (Calbiochem, La Jolla, CA). O-2A cells were incubated with GluR agonists for 22 hr under different culture conditions and fixed in methanol/acetic acid (3:1) for 30 min. After incubation for 15 min at 37°C with 0.4 μg/ml Hoechst 33258, fluorescence was examined using a 100× Neofluar objective. Apoptotic O-2A cells were identified by nuclear condensation and/or fragmented chromatin.

Immunocytochemistry and counting of cell cultures. The following pri-

mary antibodies were used: LB1 (Levi et al., 1986; Curtis et al., 1988), O4 (Sommer and Schachner, 1981), O1 (Sommer and Schachner, 1981), anti-nestin (Tohyama et al., 1992), and anti-GFAP (Boehringer Mannheim, Indianapolis, IN). All secondary fluorochrome-conjugated antibodies were from Cappel-Organon Teknika (Durham, NC). Double indirect immunofluorescence experiments were performed as described previously (Gallo and Bertolotto, 1990). For staining with antibodies to cell surface components (LB1, O4, and O1), live cells were incubated for 30 min with primary antibodies diluted in DMEM, followed by fluorescein-conjugated GAM IgG (for LB1) or IgM (for O4 and O1) for 20 min. For subsequent staining with anti-nestin, cells were fixed in 4% paraformaldehyde and 0.2% glutaraldehyde (pH 7.4, in PBS) for 15 min, permeabilized in 95% ethanol/5% acetic acid for 10 min at $-20°C$, and incubated with anti-nestin polyclonal rabbit antibody (1:1000 in 1% FBS + 0.5% bovine serum albumin) overnight at 4°C. After incubation with goat anti-rabbit (GAR) rhodamine-conjugated IgG + IgM for 30 min, cells were mounted in Vectashield (Vector Laboratories, Burlingame, CA). For staining with monoclonal anti-GFAP antibody, cells were fixed and permeabilized as described above, and then incubated with anti-GFAP antibody (1:4), followed by fluorescein-conjugated GAM IgG and mounting in Vectashield. Controls for antibody specificity were performed by sequentially omitting each of the primary antibodies in the immunostaining protocols. The immunofluorescence micrographs presented are representative of at least three experiments.

Cell migration assays. Migration of O-2A cells was assayed in a two-part microchemotaxis chamber, as described in Armstrong et al. (1990b). Enriched populations of O-2A progenitor cells from neonatal rat brain were prepared as described above. To test for potential chemoattractant activity, GluR agonists (kainate, AMPA, or glutamate), a GluR antagonist (DNQX), a K$^+$ channel blocker [tetraethylammonium (TEA)], or a positive control (PDGF) (Armstrong et al., 1990b) was added to the lower well of the chemotaxis chamber. The lower wells were then covered with a poly-D-lysine-coated polycarbonate membrane with 8 μm pores. The upper wells were then secured in place over the filter, and each well was filled with a suspension containing 20,000 O-2A progenitor cells in 54 μl of defined medium (Armstrong et al., 1990b). In response to positive attractants diffusing from the lower to the upper well, the cells migrated through the pores during a 16 hr incubation period at 37°C. Migrated cells remained adhered to the filter on the lower surface and were labeled using Diff-Quik histological stain (American Scientific Products, McGaw Park, IL). To test for the ability to alter a chemotactic response to PDGF, the same test compounds (kainate, AMPA, glutamate, DNQX, and TEA) were each added with the cell suspension in the upper wells while PDGF (20 ng/ml) was present in the lower wells. Each condition was tested in triplicate wells for each of three separate experiments (except TEA, which was tested in a single experiment). For each well, five fields of cells were counted using a 25× objective on a Zeiss Photoscope III.

Electrophysiology. For electrophysiological experiments, cells were either cultured with 10 ng/ml PDGF (proliferating O-2A progenitor cells) or with PDGF for 2 d, and then in N1 + 0.5% FBS for 3 d (pro-oligodendroblasts). Cells were perfused with media of the following composition (in mM): NaCl 160, KCl 2.5, CaCl 1.5, MgSO$_4$ 1.5, glucose 10, HEPES 10, and 1 μM tetrodotoxin. In experiments in which Ca^{2+} was omitted from the recording solution, 1 mM Co^{2+} was substituted. Tight seal (>5 GΩ) whole cell recordings (Hamill et al., 1981; Edwards et al., 1989) were made from GD3$^+$ O-2A progenitors or O4$^+$ oligodendroblasts. Patch electrodes had resistances of 2–6 MΩ when filled with (in mM): K-gluconate 130, NaCl 10, Na$_2$-ATP 2, Na-GTP 0.3, HEPES 10, EGTA 0.6, glutathione 5, buffered to pH 7.4 and ~275 mOsm. The series resistance (6–12 MΩ) was compensated to at least 85%. Breakthrough into whole-cell mode was performed in current clamp; O-2A cells had a resting membrane potential of -33.5 ± 1.8 mV ($n = 14$), and pro-oligodendroblasts had a resting potential of -54.3 ± 4.4 mV ($n = 8$). Cells were voltage-clamped at -70 mV and test pulses delivered to -60 to $+45$ mV ($+15$ mV increments, 0.1 Hz). Linear leak current and the capacitive artifacts were digitally subtracted off-line using Clampfit before analysis. Records were filtered at 2 kHz and digitized at 5 kHz.

RESULTS

GluR agonists inhibit O-2A cell proliferation

A majority of freshly purified rat cortical oligodendrocyte lineage cells expressed the O-2A phenotype, as determined by double

Table 1. Glutamate receptor activation inhibits O-2A cell proliferation

	BrdU$^+$ cells (% of total ± SEM)			
	N1	PDGF	bFGF	PDGF + bFGF
Control	21 ± 1	62 ± 3	72 ± 1	62 ± 3
Kainate	4 ± 1*	5 ± 2*	7 ± 1*	10 ± 1*
AMPA	10 ± 2*	12 ± 2*	27 ± 3*	20 ± 1*
DNQX	36 ± 3*	77 ± 3*	83 ± 2**	80 ± 3**

O-2A progenitor cells were purified and cultured on coverslips as previously described at a density of 3×10^4 cells/cm^2 in DME-N1 biotin-containing medium with 0.5% FBS (Gallo and Armstrong, 1995). After 2 hr, PDGF and/or bFGF (both 10 ng/ml), kainic acid (200 μM), AMPA (200 μM), or DNQX (30 μM) was added to the culture medium with 25 μM BrdU. After 20 hr, cells were stained with anti-BrdU antibodies as previously described (Gallo and Armstrong, 1995). A total of three coverslips (10 fields/coverslip) were counted (40× objective, Zeiss Axiophot) from three independent experiments for each culture condition. The total number of cells counted ranged from 407 to 653 cells for each culture condition. Under all culture conditions, AMPA and kainate significantly decreased while DNQX increased BrdU index in O-2A cells (*$p < 0.001$; **$p < 0.05$ vs respective control Student's t test).

immunostaining 2 hr after seeding. The newly plated fractions were comprised of 78.1 ± 3.8% ($n = 2624$) of GD3$^+$/nestin$^+$ and 4.1 ± 0.3% ($n = 4052$) of O4$^+$/nestin cells (averages ± SEM, 3 experiments, 20 microscopic fields/experiment were counted). A majority of remaining cells were nestin$^+$/GD3$^-$ (see also Gallo and Armstrong, 1995), and only <2% were O1$^+$ or GFAP$^+$.

PDGF, bFGF, and conditioned medium from B104 neuroblastoma cells (B104-CM) are mitogens known to stimulate O-2A proliferation (Raff et al., 1988; Bogler et al., 1990; Hunter and Bottenstein, 1990; McKinnon et al., 1990; Louis et al., 1992; Gard and Pfeiffer, 1993; Gallo and Armstrong, 1995). Rat cerebral cortex O-2A cells cultured with these mitogens for 24 hr displayed a three- to fourfold increase in [^{3}H]thymidine incorporation compared with cells cultured without mitogens in N1 medium (Fig. 1). Under all culture conditions, micromolar concentrations of GluR agonists (glutamate, kainate, and AMPA) significantly inhibited [^{3}H]thymidine incorporation into O-2A cells (Fig. 1A–D). As distinguished by the selective agonist AMPA, this inhibition was mediated by the AMPA GluR subtype. Similar results were also obtained in O-2A cells cultured with 30% conditioned medium from the B104 neuroblastoma cell line (B104-CM) or in O-2A cells cultured with growth factors for 24 hr, and then with GluR agonists and [^{3}H]thymidine for the next 24 hr. The finding that GluR agonists inhibited, and DNQX stimulated, O-2A cell proliferation even in cells cultured without growth factors for 24 hr (N1) is likely to be attributable to a significant percentage of cells undergoing a final round of division under these culture conditions (see also Table 1). The IC$_{50}$ values for each agonist were measured from dose–response curves obtained in cells cultured with each GluR agonists and PDGF (10 ng/ml) or PDGF + bFGF (10 ng/ml each; 2 experiments in triplicate for each condition; $n = 6$ for each agonist concentration). In cells cultured in PDGF, IC$_{50}$ values for kainate and AMPA were 32.8 ± 8.4 and 28.1 ± 3.4 μM, respectively. In cells cultured in PDGF + bFGF, IC$_{50}$ values for kainate and AMPA were 23.3 ± 2.8 and 10.3 ± 2.3 μM, respectively. The non-NMDA antagonist DNQX (30 μM) not only prevented the antiproliferative effects of kainate, but significantly stimulated O-2A cell proliferation under all culture conditions tested (Fig. 1). The stimulatory effect was very likely attributable to a blockage by DNQX of the inhibitory effects of endogenous glutamate present in the culture medium, which ranged from 8.9 ± 0.1 μM (in bFGF) to 10.2 ± 0.4 μM (in PDGF), as measured by HPLC. These concentrations of the amino acid are, in fact, within
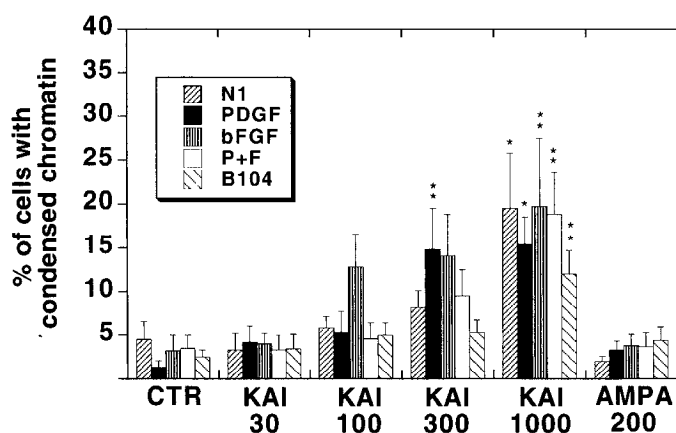
Figure 2. Glutamate receptor activation sufficient to inhibit proliferation does not cause apoptotic death of O-2A progenitor cells. Apoptotic cell death was determined by fluorescence microscopy with the nuclear dye Hoechst 33258. O-2A cells were cultured in DME-N1 + 0.5% FBS with PDGF (10 ng/ml), bFGF (10 ng/ml), PDGF I bFGF (*P I F*), or 30% B104-conditioned medium (*B104*), as well as kainate (*KAI*; 30–1000 μM) or AMPA (200 μM) for 22 hr. Cells were stained with Hoechst 33258 and examined by fluorescence microscopy. Histograms represent percentages of total O-2A cells, expressed as averages ± SEM (*n* = 20 microscopic fields). The total number of cells counted for each culture condition ranged from 221 to 406. **p* < 0.01; ***p* < 0.05 compared with their respective controls (Student's *t* test). *CTR*, Control.

the range of activation of AMPA receptors in different types of neural cells (Wyllie et al., 1991; Patneau et al., 1992, 1994).

The antiproliferative effects of GluR agonists were also analyzed at the single-cell level by pulse-labeling O-2A cells with BrdU for 20 hr to maximize the number of cells labeled during S-phase. The results summarized in Table 1 are in agreement with the [³H]thymidine incorporation assay findings. Under all culture conditions, both kainate and AMPA strongly decreased, whereas DNQX significantly increased, the percent of BrdU⁺ cells. Similar to the results shown in Figure 1, in these experiments kainate was more efficacious than AMPA.

To demonstrate that GluR agonists did not induce neurotoxicity, O-2A cell viability was evaluated with fluorescein diacetate (FDA) and propidium iodide (PI). Under all conditions tested (same as in Fig. 1 and Table 1), ~90% of O-2A cells were viable (FDA⁺/PI⁻) after a 24 hr exposure to GluR agonists (20–300 μM; data not shown). Staining with Hoechst 33258 dye demonstrated that only concentrations of kainate above 300 μM induced apoptosis in a small percentage of O-2A cells (Fig. 2). These results are in agreement with previous studies (Oka et al., 1993; Yoshioka et al., 1995), which showed that millimolar concentrations of glutamate and GluR agonists are necessary to induce neurotoxic damage in cells of the oligodendrocyte lineage.

Molecular and functional studies have demonstrated previously that O-2A cells also express GABA and muscarinic acetylcholine receptors (von Blankenfeld et al., 1991; Cohen and Almazan, 1994). To determine whether the effects of GluR agonists on O-2A cell proliferation were selective, we tested the effects of long-term incubation of O-2A cells with agonists selective for GABA and muscarinic receptors. Neither GABA, nor muscimol or carbachol modified [³H]thymidine incorporation in O-2A cells (Fig. 3). Moreover, carbachol (Fig. 3*A*) or GABA (data not shown) did not alter AMPA-induced inhibition of O-2A cell proliferation.
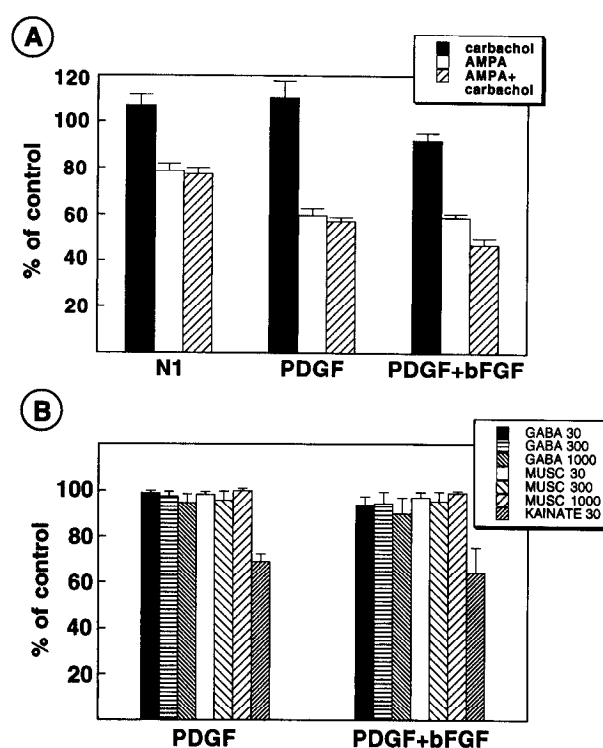

Figure 3. GABA and muscarinic receptor activation does not affect O-2A cell proliferation: [³H]thymidine incorporation assays. *A*, Carbachol does not alter O-2A cell proliferation. *B*, GABA and muscimol (*MUSC*) do not alter O-2A cell proliferation. O-2A progenitor cells were cultured in DME-N1 + 0.5% FBS with PDGF or PDGF + bFGF (both 10 ng/ml). Cells were plated in 24-well plates at a density of 30,000 cells/well. After 2 hr, GABA (30–1000 μM), muscimol (30–1000 μM), carbachol (100 μM), kainate (30 μM), AMPA (200 μM), or AMPA + carbachol was added to the cultures along with [³H]thymidine (0.5 μCi/ml). After 22 hr, [³H]thymidine incorporation was measured by trichloroacetic acid precipitation and scintillation counting. Averages of three experiments in triplicate ± SEM are shown. No significant difference was detected between controls and any of the GABA and muscarinic receptor agonists.

A time course of the antiproliferative effects of GluR agonists showed that ~12 hr of incubation are required to detect a significant inhibition on [³H]thymidine incorporation in O-2A cells cultured with PDGF + bFGF (Fig. 4*A*). Also, O-2A cells cultured with PDGF and GluR agonists for 24 hr, and then [³H]thymidine-pulsed in a GluR agonist-free medium containing PDGF, reentered S-phase with a temporal pattern similar to cells that were never exposed to GluR agonists (Fig. 4*B*). It can be concluded, therefore, that the inhibitory effects of kainate and AMPA on O-2A cell proliferation were reversible.

Activation of GluRs and subsequent opening of GluR membrane cationic channels cause a depolarization of the O-2A cell membrane (Borges et al., 1994; Patneau et al., 1994). This event may trigger the release of intracellular molecules, as shown previously for newly accumulated [³H]GABA (Gallo et al., 1989, 1991). A series of experiments was performed, therefore, to exclude the possibility that the inhibitory effects of GluR agonists on O-2A cell proliferation were attributable to depolarization-induced release of an autocrine factor that in turn inhibited O-2A cell division. Cells were cultured at a density 3- or 10-fold lower than that routinely used, to dilute out a putative autocrine factor. Cell counts were performed after a 24 hr incubation with GluR agonists/antagonist. Figure 5, *A* and *B*, shows that, at both cell
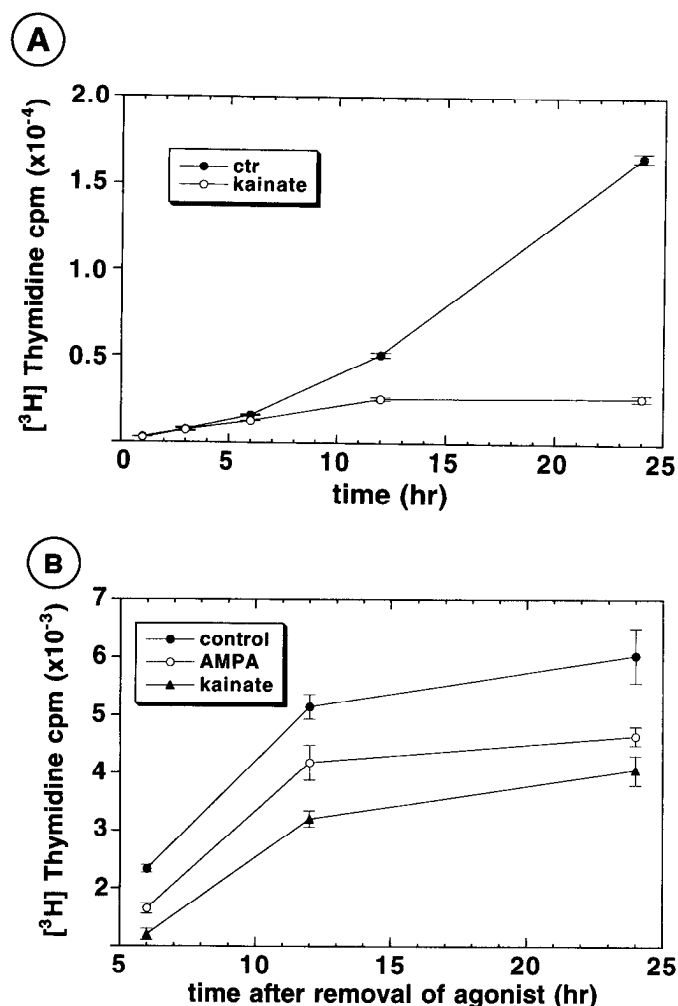
Figure 4. Time course and reversibility of the effects of glutamate receptor agonists on O-2A cell proliferation: [^{3}H]thymidine incorporation assays. *A*, Time course. O-2A progenitor cells were plated in DME-N1 + 0.5% FBS in 24-well plates. After 2 hr, PDGF (10 ng/ml), kainic acid (100 μM), and [^{3}H]thymidine (0.5 μCi/ml) were added to the culture medium. Cells were harvested at 1, 3, 6, 12, and 24 hr after plating, and [^{3}H]thymidine incorporation was measured by trichloroacetic acid precipitation and scintillation counting. Averages of a triplicate experiment ± SEM are shown. *ctr*, Control. *B*, Time course after removal of agonist. Progenitor cells were cultured in PDGF (10 ng/ml) in the absence (control condition) or in the presence of AMPA (100 μM) or kainate (100 μM). After 22 hr, all cells were shifted to fresh culture medium without GluR agonists, but containing PDGF (10 ng/ml) and [^{3}H]thymidine (0.5 μCi/ml). At 22 hr, before the shift to agonist-free medium, kainate and AMPA inhibited [^{3}H]thymidine incorporation by 60 and 43%, respectively. Cells were harvested after 6, 12, and 24 hr after shift to agonist-free medium, and [^{3}H]thymidine incorporation was determined by trichloroacetic acid precipitation and scintillation counting. Averages ± SEM (*n* = 3) are shown.

densities, kainate and AMPA decreased, and DNQX increased, the total number of O-2A cells/dish. These findings further confirmed that the antiproliferative effects observed are not *indirectly* mediated by GluR activation through the release of an inhibitory autocrine factor.

GluR agonists prevent O-2A lineage progression

Because O-2A cells proliferate much more vigorously than cells at later stages of the oligodendrocyte lineage, inhibition of cell proliferation could also result from promoting O-2A cell differ-
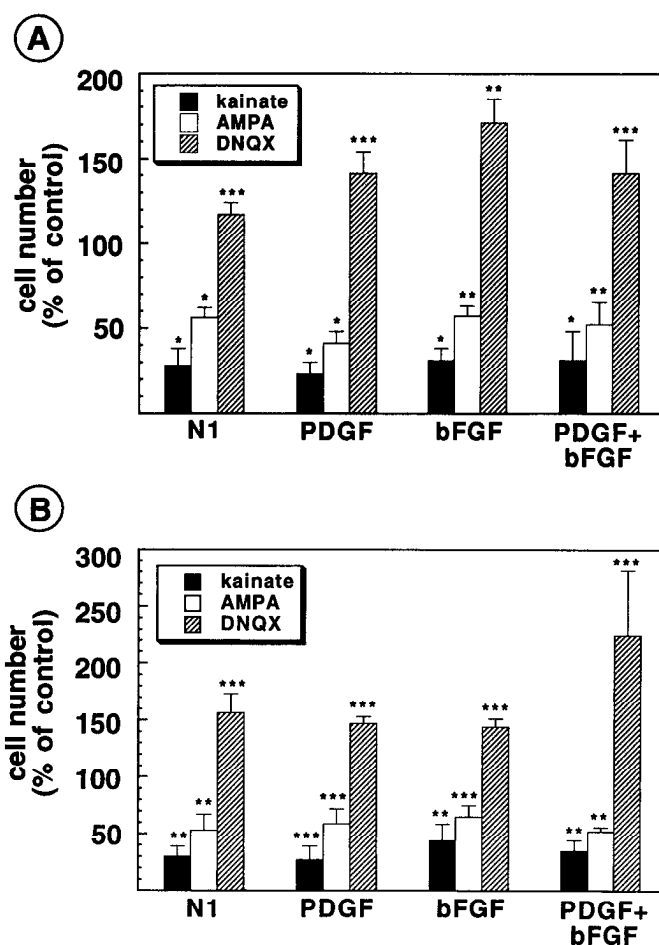
Figure 5. The inhibitory effects of glutamate receptor agonists on O-2A cell proliferation are not influenced by cell density. O-2A progenitor cells were cultured in DME-N1 + 0.5% FBS with PDGF, bFGF, or PDGF + bFGF. Cells were plated in 35 mm plates at a density of 10 (*A*) or 3 (*B*) cells/mm². After 2 hr, PDGF and/or bFGF (both 10 ng/ml), and AMPA (200 μM) or DNQX (30 μM) were added to the cultures. After 22 hr, cells were fixed and counted using an inverted microscope with a 25× objective. Ten fields/plate were counted in three independent experiments. Averages ± SEM (*n* = 30) are shown. Total number of cells counted in controls (without GluR agonists or antagonist) ranged from 5701 (*N1*) to 17,476 (*PDGF*) in *A*, and from 1557 (*N1*) to 3348 (*PDGF*) in *B*. **p* < 0.001; ***p* < 0.01; ****p* < 0.05 compared with their respective controls (Student's *t* test).

entiation. Previous studies have elegantly demonstrated that the growth factors PDGF and bFGF, as well as the factors contained in B104-CM, not only promote O-2A cell proliferation, but also differentially affect lineage progression (Raff et al., 1988; Bogler et al., 1990; Hunter and Bottenstein, 1990; McKinnon et al., 1990; Louis et al., 1992; Gard and Pfeiffer, 1993; Gallo and Armstrong, 1995). We investigated the effects of GluR activation on O-2A lineage progression by using antibodies to identify pre-O-2A cells (GD3$^-$/nestin$^+$), O-2A cells (GD3$^+$/nestin$^+$), pro-oligodendroblasts (O4$^+$), and oligodendrocytes (O1$^+$) (Gallo and Armstrong, 1995).

Cells cultured with PDGF + bFGF for 1 d (Fig. 6*A,B*) maintained the pre-O-2A or O-2A phenotype, which was not altered after a 22 hr exposure to kainate (Fig. 6*A,C*) or to AMPA (Fig. 6*A,D*). Similar results were obtained in cells cultured with PDGF or bFGF alone for 1 d (data not shown). Under conditions that prevented O-2A cell differentiation for 2 d (i.e., PDGF + bFGF
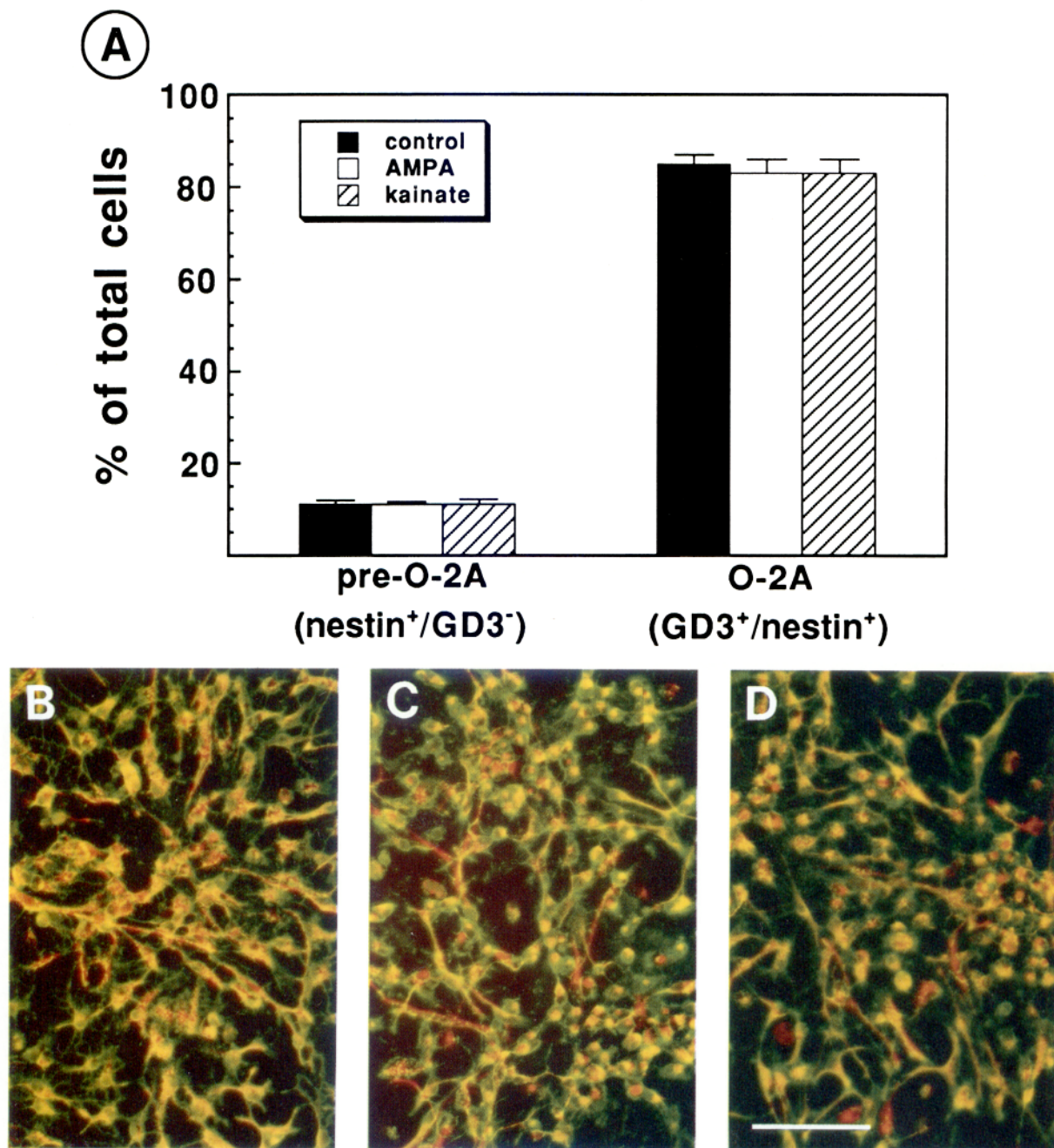
Figure 6. Activation of glutamate receptors does not affect the nestin$^+$/GD3$^+$ phenotype. O-2A cells were cultured in DME-N1 + 0.5% FBS with PDGF + bFGF (both 10 ng/ml), kainate (200 μM), or AMPA (200 μM). After 22 hr, cells were stained by double immunofluorescence with LB1 (anti-GD3; fluorescein) and anti-nestin (rhodamine) antibodies and counted. *A*, Quantitative analysis. A total of three coverslips (10 fields/coverslip) were counted from three independent experiments for each culture condition. The total number of cells counted ranged from 2029 to 2648 cells for each culture condition. Averages ± SEM are shown (*n* = 30). *B–D*, Immunofluorescence microscopy. Double exposures of the same representative microscopic fields. In each condition (*B*, control; *C*, kainate; *D*, AMPA), the O-2A cells maintained the nestin$^+$/GD3$^+$ phenotype. Scale bar, 100 μM.

or B104-CM), neither AMPA nor DNQX affected O-2A cell lineage progression (Table 2).

In contrast, under culture conditions that permitted O-2A lineage progression (i.e., N1, PDGF, or bFGF), AMPA inhibited acquisition of the O4 phenotype, whereas DNQX significantly increased the percentage of O4$^+$ pro-oligodendroblasts (Table 2). Importantly, in cultures maintained in bFGF, the small percent-

age of dividing O4$^+$ pro-oligodendroblasts was not altered by AMPA or DNQX (Table 2).

Also, the small percentage (<1%) of O1$^+$ oligodendrocytes or GFAP$^+$ astrocytes was not altered by AMPA or DNQX (data not shown). These data indicate that long-term activation of AMPA receptors selectively prevents O-2A lineage progression to the O4$^+$ pro-oligodendroblast stage.

GluR agonists do not alter O-2A cell migration

O-2A cells are highly motile; we therefore explored whether GluR agonists could act as chemoattractants or alter migration to PDGF, which is a known chemoattractant for O-2A cells (Armstrong et al., 1990b). In our microchemotaxis chamber assay, O-2A cells migrated toward PDGF-containing medium in a dose-dependent manner (Fig. 7A). GluR agonists (kainate, AMPA, or glutamate), a GluR antagonist (DNQX), and a blocker of voltage-dependent K^+ channels (TEA; data not shown, see also below) did not act as chemoattractants. Importantly, O-2A progenitor migration toward PDGF was not altered when the cells were exposed to these test compounds (kainate, AMPA, glutamate, DNQX, or TEA) during the migration (Fig. 7B). The fact that migration was not altered by GluR agonists emphasizes the viability and migratory response of the O-2A cells in concentrations of GluR agonists that strongly inhibit their proliferation. Finally, these findings are also consistent with previous observations demonstrating that proliferation is not required for O-2A cell migration (Armstrong et al., 1990b).

O-2A responses to GluR activation are mediated by delayed rectifier K^+ channels

A possible mechanism by which GluR activation inhibits O-2A proliferation and lineage progression could involve membrane ionic channels, which are differentially expressed during O-2A development (Sontheimer et al., 1989; Barres et al., 1990; Borges and Kettenmann, 1995). Delayed rectifier K^+ channels are highly expressed at the O-2A stage, but downregulated in differentiated oligodendrocytes (Fig. 8A,C) (see also Sontheimer et al., 1989). In O-2A cells, a slowly inactivating delayed rectifier current was activated at potentials positive to -40 mV ($V_{hold} = -70$ mV; Fig. 8A). Plots of the voltage dependence of activation revealed currents that were half-activated at a potential of -15 ± 0.5 mV ($n = 4$). The selective K^+ channel blocker TEA dose-dependently blocked the delayed rectifier. At a test potential of $+45$ mV, TEA (10 mM) attenuated the current by 75% and was reversible on return to drug-free saline (Fig. 8A,B). The block by TEA was
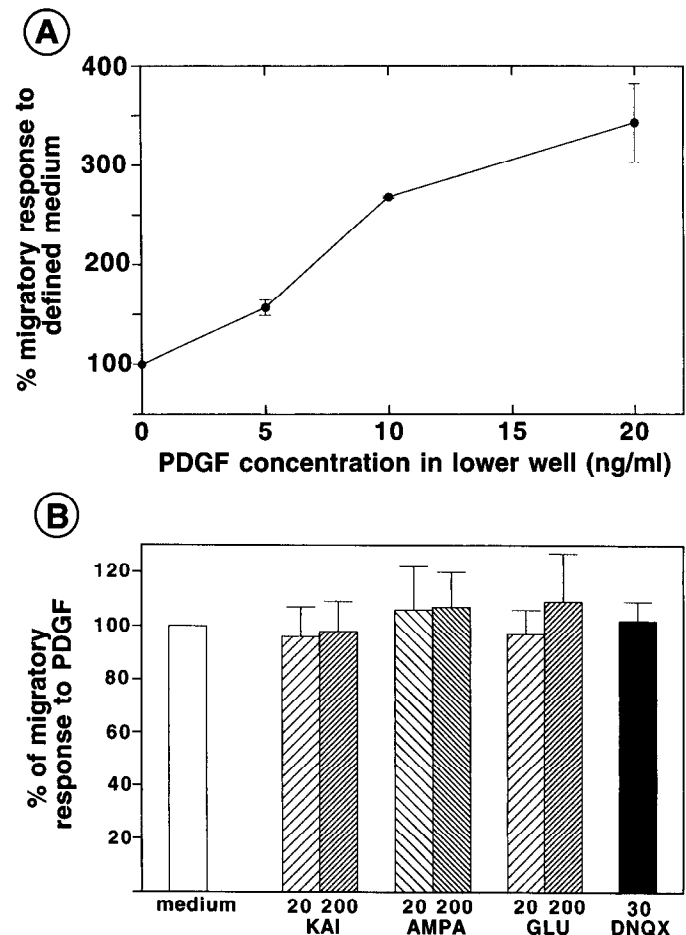
Figure 7. Glutamate receptor agonists and antagonist do not modify PDGF-induced migration of O-2A progenitor cells. Migration of purified cortical O-2A cells was assayed in microchemotaxis chambers, as described in Armstrong et al. (1990b). The chamber consists of upper and lower wells with an intervening polycarbonate membrane containing 8 µm pores. A, O-2A cells placed in the upper wells exhibit directed migration toward human recombinant PDGF diffusing from the lower wells during a 16 hr migration period. The total number of migrated cells counted per well in five fields (25× objective) was 149 ± 20 (average ± SEM, $n = 3$) in defined medium. B, O-2A cells placed in the upper well continue to migrate toward PDGF (20 ng/ml), diffusing from the lower well, when GluR agonists (20 or 200 µM) or DNQX (30 µM) is added to the upper wells with the cells. The total number of migrated cells counted per well in five fields (25× objective) was 513 ± 91 (average ± SEM, $n = 3$) in PDGF alone (shown as 100%). Neither the GluR agonists nor DNQX acted as chemoattractants when placed only in the lower wells instead of PDGF (data not shown). Similar results were obtained with TEA (10 mM) added to either the upper or the lower wells (data not shown). *KAI*, Kainate; *GLU*, glutamate.

Table 2. Glutamate receptor activation prevents oligodendrocyte progenitor lineage progression

	O4$^+$ cells (% of total ± SEM)				
	N1	PDGF	bFGF	PDGF + bFGF	B104
Control	58 ± 2	32 ± 2	48 ± 2 (12 ± 1)	15 ± 1	16 ± 1
AMPA	31 ± 4*	11 ± 1*	21 ± 1* (16 ± 2)	12 ± 1**	20 ± 2
DNQX	78 ± 2*	47 ± 3*	72 ± 2* (13 ± 3)	19 ± 2	16 ± 2

O-2A progenitor cells were purified and cultured on coverslips at a density of 3 × 10⁴ cells/cm² (Gallo and Armstrong, 1995). AMPA (200 µM) and DNQX (30 µM) were added to the culture medium 2 hr after plating. After 48 hr, cells were immunostained with O4 antibody (Gallo and Armstrong, 1995). Cells cultured in bFGF were pulsed with BrdU for 20 hr before staining with O4 and anti-BrdU antibodies. A substantial population of O4$^+$ cells was found to proliferate only in cultures maintained with bFGF. Data in parenthesis represent the percentage of O4$^+$/BrdU$^+$ cells ± SEM. Results were obtained from three independent sets of cultures ($n = 30$). A total of three coverslips (10 fields/coverslip) were counted (40× objective, Zeiss Axiophot) for each culture condition. The total number of cells counted ranged from 2000 to 4500 cells for each culture condition. A decrease in the percent of O4$^+$ cells was also found in cells cultured with 200 µM kainate (1 experiment; not shown). Note that in cells cultured in N1, PDGF, or bFGF, O4$^+$ cells were present even in the presence of AMPA. This could be attributable either to the cell cycle asynchrony or to differential sensitivity of distinct subpopulations of O-2A cells to AMPA. *$p <$ 0.001; **$p <$ 0.01 vs respective control (Student's t test).

weakly dependent on the external calcium concentration ($[Ca^{2+}]_o$; Fig. 8B). A concentration of kainate (200 µM) that inhibited O-2A cell proliferation by 70%, reduced the outward current at a test potential of $+45$ mV by 49% (Fig. 8A,B). The block by kainate was independent of $[Ca^{2+}]_o$ (Fig. 8B) and was fully reversible on washing with drug-free saline. In all cells, addition of kainate resulted in an inward ligand-gated current ranging from 200 to 600 pA. The current–voltage relationship of the kainate response was linear and reversed polarity at -3 mV ($n = 3$).

In contrast, in only 5 of 10 recordings from pro-oligodendroblasts were we able to observe any appreciable out-
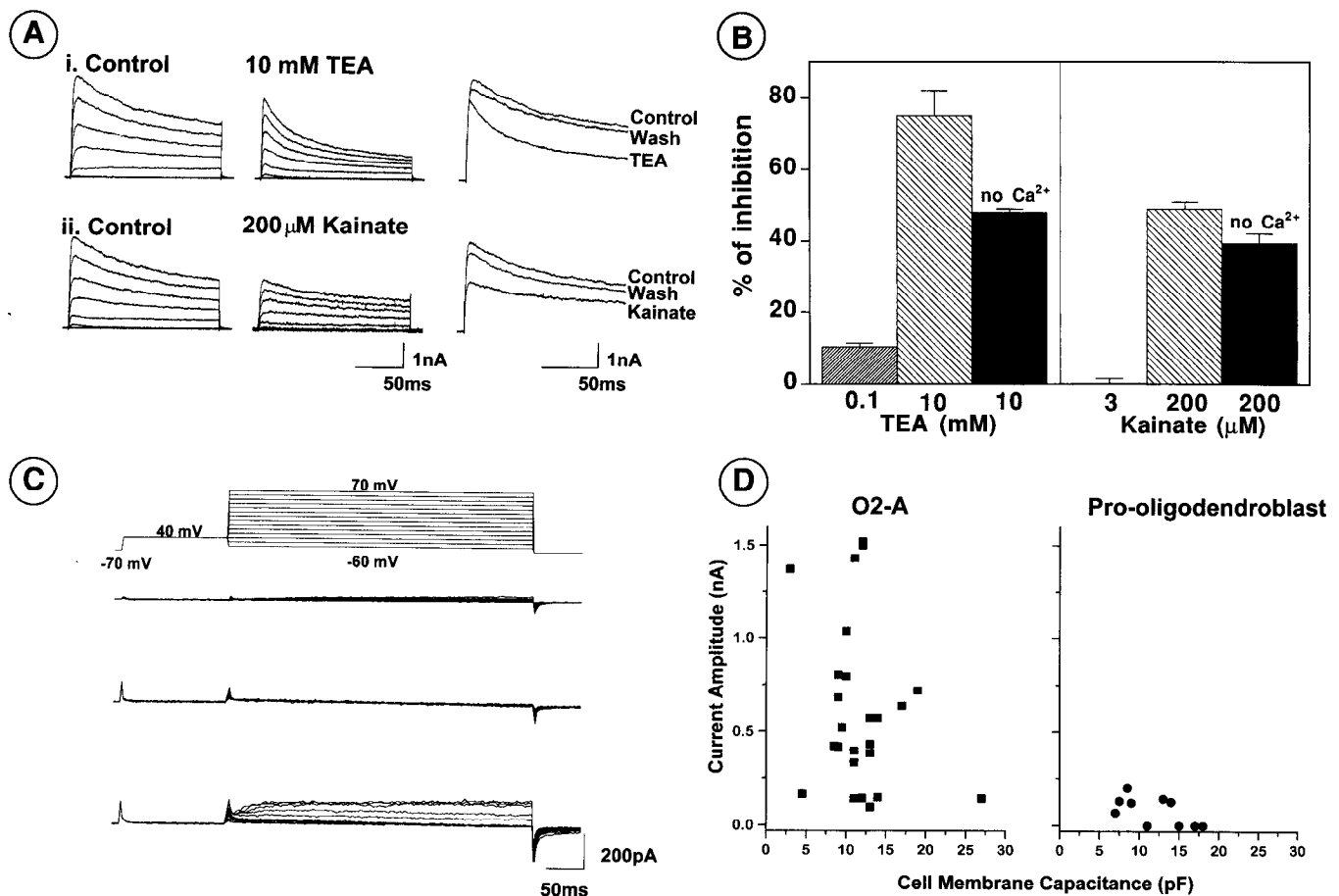
Figure 8. Glutamate receptor activation blocks delayed rectifier K^+ channels in O-2A cells. *A*, Patch-clamp recordings of O-2A cells demonstrate that TEA (*i*) and kainate (*ii*) both block delayed rectifier K^+ channels. *B*, TEA and kainate dose-dependently block Ca^{2+}-independent K^+ conductances. Each histogram is the average ± SEM obtained from 3–4 cells. *C*, Similar recordings made from O4$^+$ pro-oligodendroblasts reveal a complete lack of outward currents (*top two traces*) or only small amplitude currents (*bottom trace*) in response to test pulses up to +70 mV (V_{hold} = 40 mV). *D*, Plots of cell membrane capacitance versus current amplitude data, obtained from recordings in either O-2A cells or pro-oligodendroblasts, illustrate that despite possessing similar membrane capacitance properties, pro-oligodendroblasts do not share the wide range of current amplitudes seen in O-2A cell recordings. Data points were taken from outward currents elicited at a test potential of +70 mV.

ward current even at test potentials of up to +70 mV. In the remaining five recordings, no appreciable outward currents were detected at any test potential (Fig. 8*C*, *top two traces*). In cells in which outward currents were observed, these currents were typically of small amplitude (<250 pA) even at the most extreme test potentials (Fig. 8*C*, *bottom trace*). Plots of cell membrane capacitance against outward current amplitude at a test potential of +70 mV, for both the O-2A and pro-oligodendroblast data (Fig. 8*D*), illustrate that despite possessing a similar range of membrane capacitance, pro-oligodendroblasts do not possess currents in the amplitude range displayed by O-2A cells. These data indicate that the outward currents observed in O-2A cells are downregulated at the pro-oligodendroblast stage. The small current amplitudes observed in pro-oligodendroblasts prevented the testing of kainate on these outward currents.

Proliferation of O-2A cells was inhibited by millimolar concentrations of TEA that also suppressed K^+ currents (Fig. 9*A*), but not by concentrations of TEA (100 μM) or kainate (3 μM) that did not significantly affect K^+ channel activity (Fig. 8*B* and data not shown). Similar to AMPA and kainate, the antiproliferative effect of TEA was also reversible. O-2A cells cultured with

PDGF + bFGF and TEA for 24 hr and then [^{3}H]thymidine-pulsed in a TEA-free medium containing PDGF + bFGF reentered S-phase with a temporal pattern similar to cells that were never exposed to the K^+ channel blocker (Fig. 9*B*). Finally, TEA also delayed lineage progression to the O4$^+$ stage in cells cultured in N1, PDGF, or bFGF, but not in cells maintained in PDGF + bFGF or in B104-CM (Fig. 9*C*).

DISCUSSION

Glial cells express a multitude of neurotransmitter receptors the physiological role of which is unknown. Astrocytes and oligodendrocytes are not directly involved in synaptic transmission; therefore, transmitter receptors are likely to mediate distinct processes in these cells. In the present study, we have tested the hypothesis that neurotransmitter receptors, in particular GluRs, might regulate glial cell development, and we have used purified O-2A progenitor cells as an experimental model system. O-2A cells can be purified and cultured to near homogeneity, and the effects of neurotransmitter receptor activation on their development can be studied without the interference of other neural cell types. O-2A cells express glutamate, GABA, and muscarinic receptors (Gallo
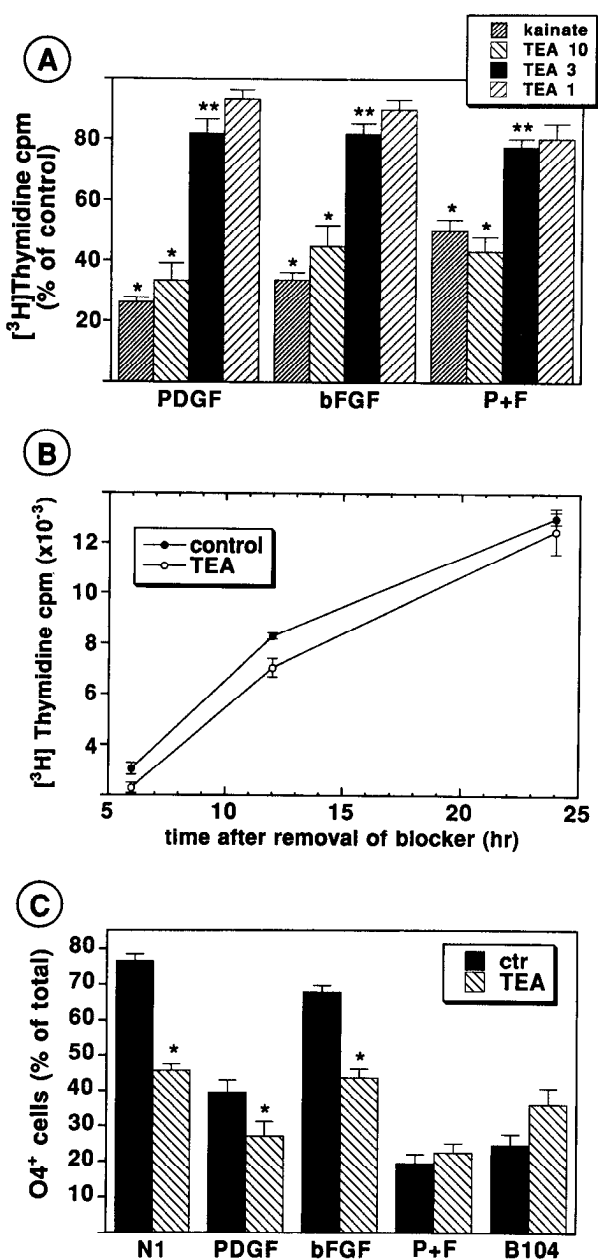
Figure 9. Blockage of delayed rectifier K^+ channels inhibits O-2A cell proliferation and lineage progression. *A,* At concentrations that block delayed rectifier K^+ channels, TEA and kainate each inhibit O-2A cell proliferation. Cells were plated in 24-well plates. After 2 hr, PDGF and/or bFGF (both 10 ng/ml), as well as TEA (1–10 mM) or kainate (200 μM), were added to the cultures along with [^{3}H]thymidine (0.5 μCi/ml). After 22 hr, [^{3}H]thymidine incorporation was measured by trichloroacetic acid precipitation and scintillation counting. Averages ± SEM obtained from three to six experiments run in triplicate are shown. *$p < 0.001$; **$p <$ 0.005 (Student's t test). *B,* The antiproliferative effects of TEA are reversible. Progenitor cells were cultured in PDGF + bFGF (both 10 ng/ml) in the absence or presence of TEA (3 mM). After 22 hr, all cells were shifted to fresh culture medium, without TEA, containing PDGF + bFGF (*P+F*) and [^{3}H]thymidine (0.5 μCi/ml). Cells were harvested after 6, 12, and 24 hr, and [^{3}H]thymidine incorporation was determined by trichloroacetic acid precipitation and scintillation counting. Averages ± SEM ($n = 3$) are shown. At 22 hr, before the shift to TEA-free medium, TEA inhibited [^{3}H]thymidine incorporation by 35%. *C,* Blockage of delayed rectifier K^+ channels with TEA prevents O-2A lineage progression, as detected by staining with O4 antibody. O-2A progenitor cells were purified and cultured on coverslips in DME-N1 medium with 0.5% FBS with PDGF (10 ng/ml), bFGF (10 ng/ml), PDGF + bFGF, or 30% B104-

et al., 1989; Barres et al., 1990; Wyllie et al., 1990; von Blankenfeld, 1991; Borges et al., 1994; Cohen and Almazan, 1994; Patneau et al., 1994) and can be cultured under specified conditions that promote either their proliferation or their differentiation. O-2A progenitor development progresses through several stages, which can be identified by the sequential expression of developmentally regulated antigens. Distinct developmental stages in the O-2A lineage each exhibit a specific complement of membrane ionic channel types (Sontheimer et al., 1989; Barres et al., 1990; von Blankenfeld et al., 1991). Our *in vitro* analysis delineates the biological effects of GluR activation on O-2A development and identifies an important step in the mechanism of GluR-mediated inhibition of cell proliferation and lineage progression, which involves a distinct set of membrane ionic channels.

The primary biological effect associated with GluR activation in cells of the oligodendrocyte lineage is inhibition of their proliferation at the O-2A developmental stage, identified by expression of the GD3 ganglioside. The pharmacological profile of the receptors involved, i.e., agonist/antagonist selectivity and efficacy, clearly points to an involvement of the AMPA receptor subtype. The range of concentration at which the GluR agonists are effective and the IC_{50} values derived from dose–response curves are consistent with their affinity for AMPA receptors. The finding that low micromolar concentrations of glutamate present in the culture medium tonically inhibit O-2A cell proliferation, as shown by the stimulatory effects of DNQX by itself, demonstrates that the endogenous receptor ligand can exert a biological effect at physiological concentrations (see below).

Proliferating O-2A cells cultured in the presence of mitogenic factors abundantly express delayed rectifier K^+ channels, which are strongly downregulated in postmitotic O4$^+$ pro-oligodendroblasts. These findings confirm previous results obtained by Sontheimer et al. (1989) in mouse O-2A cells cultured with horse serum, and by Barres et al. (1990) in rat optic nerve O-2A cells cultured with PDGF, showing that in the oligodendrocyte lineage the membrane channel phenotype is developmentally regulated. Expression of delayed rectifier K^+ channels in O-2A cells is not an *in vitro* artifact, because oligodendrocyte precursor cells express delayed rectifier K^+ channels *in vivo* (Berger, 1995). The depolarized resting membrane potential of O-2A cells suggests that the delayed rectifier will be activated at potentials close to rest and that small polarizing stimuli will strongly influence currents through these channels.

The density and activity of delayed rectifier K^+ channels correlate with the proliferation of cell types as diverse as T lymphocytes and Schwann cells, and blockage of these channels with selective compounds strongly inhibits proliferation of both cell types (DeCoursey et al., 1984; Chiu and Wilson, 1989). Our findings demonstrate not only that blockage of delayed rectifier K^+ channels in O-2A cells by TEA strongly inhibits their proliferation, but also that the antiproliferative effects associated with GluR activation are mediated through blockage of the same channels. Glutamate receptor agonists and TEA suppress K^+

←

conditioned medium (*B104*). TEA (3 mM) was added to the culture medium 2 hr after plating. After 48 hr, cells were immunostained with O4 antibody and counted. Averages ± SEM obtained from two experiments ($n = 20$) are shown. The total number of cells counted for each culture condition ranged between 679 and 2184. *$p < 0.001$; **$p < 0.005$ (Student's t test).

channel activity and O-2A cell proliferation in the same concentration range. This indicates that blockage of these cationic channels is sufficient, possibly by a simple shift in the membrane resting potential, to cause arrest in O-2A division.

The block of delayed rectifier K^+ channels by kainate is likely to be a consequence of the increase in $[Na^+]_i$ triggered by the opening of Na^+/Ca^{2+}-permeable AMPA channels in the O-2A membrane. In fact, preliminary data show that replacement of Na^+ with N-methyl-D-glucamine in the external solution eliminates the effects of kainate on the delayed rectifier current (P. Knutson and C. McBain, unpublished data). An increase in $[Na^+]_i$ has been shown to block delayed rectifier K^+ channels in invertebrate cells and, more recently, in mouse O-2A progenitor cells (Bergman, 1970; Bezanilla and Armstrong, 1972; Borges and Kettenmann, 1995). The participation of Ca^{2+} ions in the GluR-mediated blockage of delayed rectifier K^+ channels can be excluded based on the following: (1) the independence of such effect on extracellular Ca^{2+}, and (2) the findings that carbachol (Fig. 3) and the Ca^{2+} ionophore A23187 (data not shown), which trigger $[Ca^{2+}]_i$ transients in O-2A cells (Cohen and Almazan, 1994) (V. Gallo, unpublished data), did not modify [^{3}H]thymidine incorporation over a 24 hr time period.

GluR activity and suppression of delayed rectifier K^+ currents also block lineage progression at the O-2A stage. Both activation of AMPA receptors and TEA-induced block of K^+ channels significantly decreased the percentage of O-2A cells that progressed to the more differentiated stage identified by the monoclonal antibody O4. In the context of a "developmental clock" model (Raff et al., 1985; Temple and Raff, 1986), O-2A cells must undergo a certain number of cell divisions before progressing to the $O4^+$ pro-oligodendroblast stage. Therefore, GluR activation and blockage of delayed rectifier K^+ channels could have a unique effect on the O-2A cell cycle that perturbs lineage progression as well, possibly causing an arrest in proliferation before the cell can undergo the number of divisions required to differentiate. The finding that the antagonist DNQX increased the percentage of $O4^+$ cells after 48 hr is consistent with the prediction that, when the endogenous level of GluR-mediated block is relieved, O-2A cells can differentiate more rapidly.

Inhibition of O-2A differentiation also occurs in the presence of a different antiproliferative agent, retinoic acid (Laeng et al., 1994; Noll and Miller, 1994) (V. Gallo, unpublished observations). Interestingly, the biological effects of retinoic acid on spinal cord (Noll and Miller, 1994) or brain O-2A cells (Laeng et al., 1994) (V. Gallo, unpublished observations) are similar to those elicited by GluR agonists. In fact, retinoic acid not only inhibits O-2A cell proliferation, but also prevents the transition from the $GD3^+$ O-2A to the $O4^+$ pro-oligodendroblast stage (Laeng et al., 1994; Noll and Miller, 1994) (V. Gallo, unpublished observations). It can be concluded, therefore, that inhibition of O-2A cell proliferation does not necessarily promote their differentiation.

We hypothesize that the neurotransmitter glutamate may cooperate with growth factors to regulate oligodendrocyte development at critical stages *in vivo*. Glutamate concentrations in the cerebrospinal and extracellular fluid range from 1 to 50 μM (Johnson, 1978; Lerma et al., 1986), well within the range of activation of AMPA receptors in O-2A cells. Glutamate may be released from neuronal cell bodies, from axons at nodes of Ranvier, or from astrocytes (Wheeler et al., 1966; Weinreich and Hammerschlag, 1975; Dan et al., 1994; Parpura et al., 1994). During development, oligodendrocyte precursors proliferate in

CNS germinal zones, migrate to future sites of myelination, and proliferate further at these sites. O-2A cells, but not pro-oligodendroblasts, are capable of extensive proliferation and migration *in vivo* (Warrington and Pfeiffer, 1992). At the O-2A developmental stage, glutamate may counteract the mitogenic effects of growth factors, such as PDGF, without interfering with their chemotactic action, and could also prevent premature differentiation. Accordingly, GluR agonists depolarize oligodendrocyte precursors and block K^+ conductances *in vivo* (Berger, 1995). Furthermore, long-term blockage of K^+ channels in E13 spinal cord microexplant with TEA entirely eliminated myelination without affecting axonal growth and conduction (Shrager and Novakovic, 1995), indicating that K^+ channels are necessary for oligodendrocyte development.

Neurotransmitter receptors and their associated channels may play a role in the development of different neural cell lineages, as shown by the modulatory effects of GABA, glutamate, and acetylcholine receptor agonists on neuroblast and astrocyte proliferation (Ashkenazi et al., 1989; Nicoletti et al., 1990; LoTurco et al., 1995). Our analysis of oligodendrocyte development defines a physiological role not only for GluRs in glia, but also for a subtype of K^+ channels. The finding that the activity of delayed rectifier K^+ channels is closely linked to mitosis in progenitor cells of the nervous system, as in the immune system (Lewis and Cahalan, 1988), further strengthens the concept that cell proliferation and plasticity can be controlled through the modulation of preexisting receptor and channel proteins.

Note added in proof: While this manuscript was under review, a report by Liu and Almazan (1995) also showed inhibition of O-2A progenitor cell proliferation by GluR agonists.

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