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Glutamate regulates intracellular calcium and gene expression in oligodendrocyte progenitors through the activation of DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors

(immediate early genes/NGFI-A/O-2A progenitors/kainate receptors)

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ABSTRACT **Oligodendrocytes and their progenitors (O-2A) express functional kainate- and DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-preferring glutamate receptors. The physiological consequences of activation of these receptors were studied in purified rat cortical O-2A progenitors and in the primary oligodendrocyte cell line CG-4. Changes in the mRNA levels of a set of immediate early genes were studied and were correlated to intracellular Ca^{2+} concentration, as measured by fura-2 Ca^{2+} imaging. Both in CG-4 and in cortical O-2A progenitors, basal mRNA levels of NGFI-A were much higher than c-*fos*, c-*jun*, or *jun-b*. Glutamate, kainate, and AMPA greatly increased NGFI-A mRNA and protein by activation of membrane receptors in a Ca^{2+}-dependent fashion. Agonists at non-*N*-methyl-D-aspartate receptors promoted transmembrane Ca^{2+} influx through voltage-dependent channels as well as kainate and/or AMPA channels. The influx of Ca^{2+} ions occurring through glutamate-gated channels was sufficient by itself to increase the expression of NGFI-A mRNA. AMPA receptors were found to be directly involved in intracellular Ca^{2+} and NGFI-A mRNA regulation, because the effects of kainate were greatly enhanced by cyclothiazide, an allosteric modulator that selectively suppresses desensitization of AMPA but not kainate receptors. Our results indicate that glutamate acting at AMPA receptors regulates immediate early gene expression in cells of the oligodendrocyte lineage by increasing intracellular calcium. Consequently, modulation of these receptor channels may have immediate effects at the genomic level and regulate oligodendrocyte development at critical stages.**

In the forebrain and cerebellum, oligodendrocytes develop from progenitor cells that originate in the subventricular zone and migrate into gray and white matter in early postnatal life (1, 2). The developmental potential of oligodendrocyte progenitors (O-2A) is maintained when these cells are isolated, purified, and cultured in the presence of appropriate growth factors (3–5). O-2A development depends on homologous and heterologous interactions with other cells in the central nervous system (6), including neurons, which may synthesize and release factors that are essential for O-2A progenitor cell division and differentiation (7). Recently, a primary oligodendrocyte cell line (CG-4) has been described (5). CG-4 cells can be fully induced to differentiate into myelinating oligodendrocytes with the same time schedule as O-2A progenitors purified and cultured from different parts of the brain (5). CG-4 progenitors sequentially express oligodendrocyte-specific markers throughout their development (5, 8) and respond to the same growth factors that regulate O-2A proliferation and differentiation (5).

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Cells of the oligodendrocyte lineage have been shown to express both ligand- and voltage-gated ionic channels (9–11). We have demonstrated, by electrophysiological and molecular analyses in CG-4 cells and cortical glia, that functional kainate- and DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-preferring glutamate receptors are coexpressed in cells of the oligodendrocyte lineage (8, 12). In O-2A progenitor cells and mature oligodendrocytes, glutamate and structurally related compounds generated large depolarizing currents due to the opening of membrane cationic channels (8, 12). Similar responses to glutamate have also been obtained previously in acutely dissociated O-2A progenitors (10), indicating that glutamate-gated channels are also expressed in these cells *in vivo*. The physiological function of these receptor channels, however, is still unknown. One possibility is that glutamate may act on O-2A progenitors as a developmental signal and that glutamate receptors are expressed in oligodendrocytes early in development to mediate neuron–O-2A progenitor cell interactions. In the present study, we have begun to analyze the physiological consequences of glutamate receptor activation in cells of the oligodendrocyte lineage. We tested the hypothesis that glutamate may regulate intracellular calcium concentration ($[Ca^{2+}]_i$) and gene expression in O-2A progenitor cells. In particular, we analyzed a set of immediate early genes (IEGs) in view of their rapid receptor-dependent regulation previously described in other neural cells (13). These IEGs are known to encode transcription factors that can in turn regulate the expression of other genes necessary for cell growth and differentiation (14, 15).

MATERIALS AND METHODS

Cell Culture and Immunocytochemistry. CG-4 cells were cultured as described by Louis *et al.* (5). Primary cortical glial cultures and purified O-2A progenitors were prepared from embryonic day 20 to postnatal day 1 Sprague–Dawley rats as described by McCarthy and de Vellis (16). Both CG-4 and primary progenitors were grown in Dulbecco's modified Eagle's medium (DME)-N1, supplemented with 30% B-104 conditioned medium. Cultures enriched in $O1^+$ oligodendrocytes were obtained by replacing the B-104 conditioned medium after 3 days with DME-N1 plus 0.5% fetal bovine

Abbreviations: AMPA, DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; 1*S*,3*R*-ACPD, 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid; $[Ca^{2+}]_i$, intracellular calcium concentration; IEG, immediate early gene; NMDA, *N*-methyl-D-aspartate.

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serum (FBS) for 1 day, followed by DME-N1 plus 2% FBS for 4–5 days. Cultures were characterized immunocytochemically using the monoclonal antibodies LB1 (17), O4 (18), and O1 (18) as described (19). More than 95% of the CG-4 and cortical O-2A progenitors were LB1$^+$ (5, 8). In oligodendrocyte-enriched cultures, 80–85% of the cells were O1$^+$ and the remaining cells were O4$^+$ (5, 8).

For immunocytochemical detection of NGFI-A protein, cells were incubated with kainate for 1 hr in culture medium, followed by a 3- to 5-hr incubation without agonist. Cells were then fixed in 4% paraformaldehyde/0.2% glutaraldehyde in 1× PBS (phosphate-buffered saline, pH 7.4) for 15 min. After permeabilization in ethanol/acetic acid (95/5%), the cultures were preincubated with 3% normal goat serum (NGS) for 20 min, followed by incubation with anti-NGFI-A antibodies [egr-1 (588), 1:500 in 3% NGS; Santa Cruz Biotechnology, Santa Cruz, CA] for 1 hr and biotinylated goat anti-rabbit IgG (1:200 in 1.5% NGS; Vector Laboratories) for 1 hr. After extensive washes in PBS, the antigen was visualized by using a Vectastain ABC peroxidase kit (Vector Laboratories). Nonspecific staining for NGFI-A was determined in a set of parallel cultures by omitting the primary antibody from the staining procedure. No staining was detected under these conditions.

Incubation with Excitatory Amino Acids and Northern Blot Analysis. Glutamate receptor agonists and/or antagonists were added directly to the cell culture medium. To depolarize the cells with high extracellular K^+, the culture medium was removed and replaced with the isotonic balanced salt solution containing 45 mM KCl used in the calcium imaging experiments (see below). After incubation with the designated treatment for 1 hr at 37°C, cells were directly solubilized in RNAzol (Tel-Test, Friendswood, TX) to isolate RNA using a simple single-step procedure (20). RNA samples (10–15 μg per lane) were electrophoresed through a 1.5% agarose-formaldehyde denaturing gel, electrotransferred onto Nytran (Schleicher & Schuell) membranes, covalently cross-linked to the membranes by UV irradiation (Stratalinker, Stratagene), and hybridized with cDNA probes. The following cDNAs were used for random priming with [α-^{32}P]dCTP: *Eco*RI fragments for NGFI-A (3.1 kb), c-*fos* (2.1 kb), c-*jun* (2.6 kb), and *jun-b* (1.9 kb); *Bam*HI internal fragment for cyclophilin (0.8 kb). The probes were labeled to ≈10^8 cpm per μg of DNA. Blots were hybridized in 50% formamide at 42°C, washed with 0.1× SSC at 60°C, and exposed to film for 2–4 days. NGFI-A and cyclophilin transcripts were quantified by using a PhosphorImager system (Molecular Dynamics).

Neurotoxicity Assay. Cells were incubated with kainate or glutamate (100–1000 μM) for 1 or 24 hr at 37°C and 10% CO_2 in cell culture medium. Cell death was then assessed by fluorescein diacetate/propidium iodide staining as described (21).

Calcium Imaging. CG-4 cells grown on coverslips were loaded with fura-2 as described (22). The microscopy buffer had the following composition: 130 mM NaCl/5.4 mM KCl/1.5 mM $CaCl_2$/0.8 mM $MgSO_4$/1 mM Na_2HPO_4/2.5 mM $NaHCO_3$/20 mM Hepes/25 mM glucose/1 mM sodium pyruvate/1 mM ascorbic acid/1.5 mg of bovine serum albumin per ml. In the high K^+ medium, NaCl was partially replaced to give a final KCl concentration of 45 mM. Cells were imaged at 37°C in 1% CO_2 and data were analyzed as described (22).

Materials. Fura-2 was purchased from Molecular Probes. Kainic acid and nimodipine were obtained from Sigma. AMPA, 6,7-dinitroquinoxaline-2,3-dione (DNQX), and 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) were obtained from Tocris Neuramin (Bristol, U.K.). Cyclothiazide was the generous gift of Eli Lilly.

RESULTS

Maintained bath application of glutamate or kainate to CG-4 progenitor cells caused a large increase in $[Ca^{2+}]_i$ (20- to 30-fold with kainate and 2- to 4-fold with glutamate; Fig. 1*A*). The resting $[Ca^{2+}]_i$ in CG-4 cells ranged between 30 and 60 nM. The effect of kainate was dose dependent (Fig. 1 *A* and *D*). At the concentration tested (200 μM), the response to glutamate was consistently slower in onset and smaller in amplitude than that of kainate in all the cells analyzed (Fig. 1*A*). Application of 1*S*,3*R*-ACPD (300–1000 μM), a selective agonist for metabotropic glutamate receptors, never increased $[Ca^{2+}]_i$ in any of the cells tested (data not shown). The non-*N*-methyl-D-aspartate (NMDA) receptor antagonist DNQX (30 μM) inhibited kainate and glutamate responses by 85% and 77%, respectively (Fig. 1*B*). Depolarization of CG-4 progenitor cells induced by 45 mM KCl also increased $[Ca^{2+}]_i$, although to a lower extent than kainate (Fig. 1*C*). The responses to kainate, glutamate, and 45 mM K^+ were completely prevented by the absence of extracellular Ca^{2+} ions (Fig. 1*B*). The voltage-dependent Ca^{2+}-channel blocker nimodipine (10 μM) only partially inhibited the effects of kainate or glutamate but completely blocked the increases in $[Ca^{2+}]_i$ triggered by 45 mM KCl (Fig. 1*B*). The direct involvement of AMPA-preferring receptors in the modulation of $[Ca^{2+}]_i$ in CG-4 cells was analyzed by using the allosteric modulator cyclothiazide, which selectively suppresses desensitization at AMPA- but not at kainate-preferring receptors in different cell types, including O-2A progenitors (8, 23–25). Cyclothiazide greatly potentiated the kainate-induced $[Ca^{2+}]_i$ increase (Fig. 1*D*). The effects of the allosteric modulator were particularly evident at concentrations of kainate (30 μM) that were weakly effective on their own. AMPA responses were also similarly potentiated by cyclothiazide (data not shown).

We have previously demonstrated that incubation of CG-4 cells with kainate produced a time-dependent elevation of the mRNA for the IEG NGFI-A, which reached a peak at 1 hr (12). Fig. 2*A* shows that treatment of purified cortical O-2A progenitors with kainate (3–1000 μM) for 1 hr also caused a dose-dependent increase in the mRNA of the IEG NGFI-A and reached a plateau at 300 μM kainate, as determined by Northern blot analyses (Fig. 2*A*). The mRNAs for c-*fos*, c-*jun*, and *jun-b* were also increased by kainate treatment, although their basal mRNA levels were much lower than NGFI-A (Fig. 2*A*). In both cortical and CG-4 progenitors, treatment with 300 μM kainate also increased NGFI-A protein levels, as detected by immunostaining (Fig. 2*B*). The NGFI-A immunostaining (both basal and kainate induced) was stronger in cortical O-2A progenitors than in CG-4 cells, in agreement with Northern blot analysis of NGFI-A mRNA (Figs. 2 and 3). Neurotoxicity assays showed that in CG-4 and cortical progenitors >95% of the cells were still viable after incubation with 1 mM kainate for up to 24 hr (data not shown). In cortical (Fig. 2*A*) and CG-4 (Fig. 3*A*) progenitors, the effects of kainate on NGFI-A mRNA were mimicked by glutamate and AMPA. Glutamate appeared to be less effective than kainate, whereas AMPA caused an increase in NGFI-A mRNA similar to kainate. In CG-4 cells, the effects of all the agonists were inhibited by the non-NMDA receptor antagonist DNQX (Fig. 3*A*). The metabotropic glutamate receptor agonist 1*S*,3*R*-ACPD (300–1000 μM) did not increase NGFI-A mRNA (data not shown). Chelation of extracellular Ca^{2+} by the addition of EGTA to the medium prevented stimulation of NGFI-A expression by kainate (Fig. 3*A*). Finally, treatment with nimodipine did not modify the effect of kainate but completely antagonized the increase in expression of NGFI-A mRNA caused by K^+ depolarization (Fig. 3*A*).

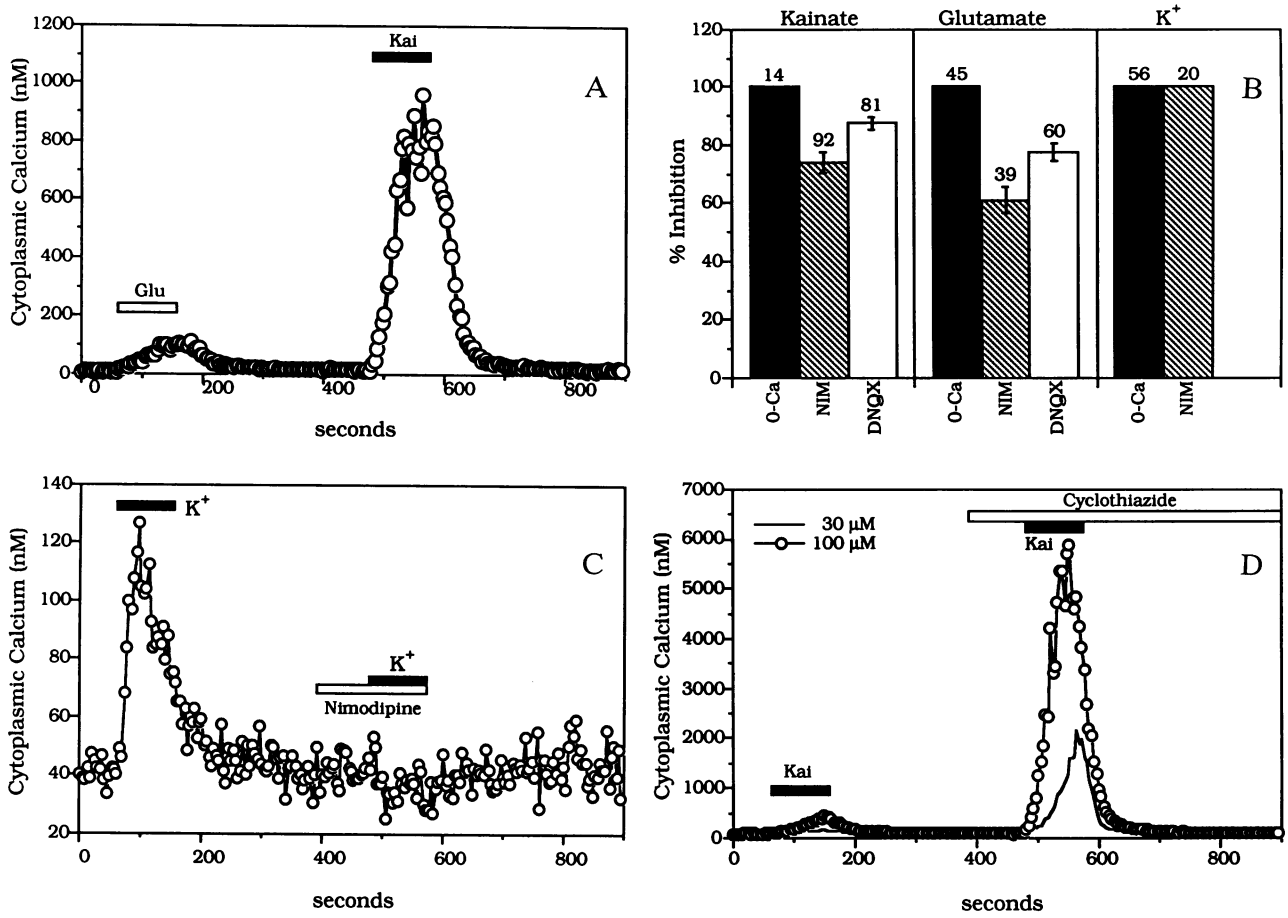

FIG. 1. Effects of glutamate receptor agonists and K^+-induced depolarization on $[Ca^{2+}]_i$ in CG-4 cells. (*A*) Typical response produced by maintained bath application of 200 μM glutamate (open bar) and 200 μM kainate (solid bar). (*B*) Effects of Ca^{2+}-free (0 mM Ca^{2+}/0.1 mM EGTA) nimodipine (NIM; 10 μM), and DNQX (30 μM) on kainate (200 μM)-, glutamate (200 μM)-, and K^+ (45 mM)-induced increase in $[Ca^{2+}]_i$. DNQX and nimodipine were applied 1.5 min before and Ca^{2+}-free medium was applied 5 min before stimulation. For each experimental condition, cells were stimulated with kainate, glutamate, or K^+, followed by the same stimulation in the presence of 0 mM Ca^{2+}, 10 μM nimodipine, or 30 μM DNQX. Histograms represent percentage of inhibition obtained from the ratios of the integrated responses under the two conditions for each cell in the field (two or three independent experiments for each category). Bars represent SEM. Numbers above bars represent number of cells. (*C*) Effects of nimodipine on 45 mM K^+-induced Ca^{2+} responses. Solid bars, K^+ application; open bar, nimodipine. (*D*) Effects of cyclothiazide (30 μM) on kainate-induced increase in $[Ca^{2+}]_i$. Kainate (Kai) was 30 or 100 μM (solid bars). Cells were pretreated with cyclothiazide (open bar) for 1.5 min before stimulation.

In a previous study, we demonstrated that the expression of kainate and AMPA receptors is maintained in differentiated oligodendrocytes (8). Fig. 3*B* shows a direct comparison between cultures enriched either in rapidly dividing, $LB1^+$ progenitor cells or in postmitotic $O1^+$ oligodendrocytes. Interestingly, the basal NGFI-A mRNA levels were higher in progenitor cells than in oligodendrocytes. In both types of cells, kainate greatly increased the expression of NGFI-A mRNA, although the relative increase over basal levels was much higher in differentiated oligodendrocytes than in progenitors (40- and 4-fold increase, respectively; Fig. 3*B*).

Cyclothiazide strongly potentiated not only the effects of kainate on $[Ca^{2+}]_i$ in CG-4 cells but also its effects on NGFI-A mRNA. Fig. 4*A* shows that the allosteric modulator potentiated the effects of 30 and 100 μM kainate by 11- and 5-fold, respectively (Fig. 4*B*). Previous electrophysiological studies have demonstrated a similar range of potentiation of kainate and AMPA responses by cyclothiazide in CG-4 cells and cortical O-2A progenitors (8). The effect of cyclothiazide with 30 μM kainate was particularly striking, because the agonist by itself was unable to increase the expression of NGFI-A mRNA (Fig. 4). AMPA-induced expression of NGFI-A mRNA was also strongly potentiated by cyclothiazide (data not shown).

DISCUSSION

The protein products of IEGs act as nuclear messengers and interact with specific DNA sequences in the promoter of other target genes to regulate their expression (13). In neurons and in astrocytes, glutamate receptors and changes in $[Ca^{2+}]_i$ regulate the expression of mRNAs for various IEGs (26–30). We have studied IEG induction in oligodendrocyte progenitor cells at an early phase of their development, which corresponds to their proliferative and migratory phase *in vivo* (2). We have previously demonstrated coexpression of functional kainate- and AMPA-preferring receptors in CG-4 and O-2A progenitors at this developmental stage (8, 12). In the present study, we show that glutamate receptor activation promotes changes in $[Ca^{2+}]_i$ and in the mRNA levels of IEGs. In addition, we provide evidence that these two events are causally related.

Agonists acting at non-NMDA glutamate receptors increased the mRNA levels of NGFI-A, c-*fos*, c-*jun*, and *jun-b* in purified cortical O-2A progenitors. The basal mRNA levels of c-*fos*, c-*jun*, and *jun-b*, however, were much lower (5- to 10-fold) than those of NGFI-A. This was also observed in CG-4 cells (data not shown). We focused our studies on the relationship between $[Ca^{2+}]_i$ and NGFI-A expression. All our data are consistent with the idea that Ca^{2+} ions play a pivotal

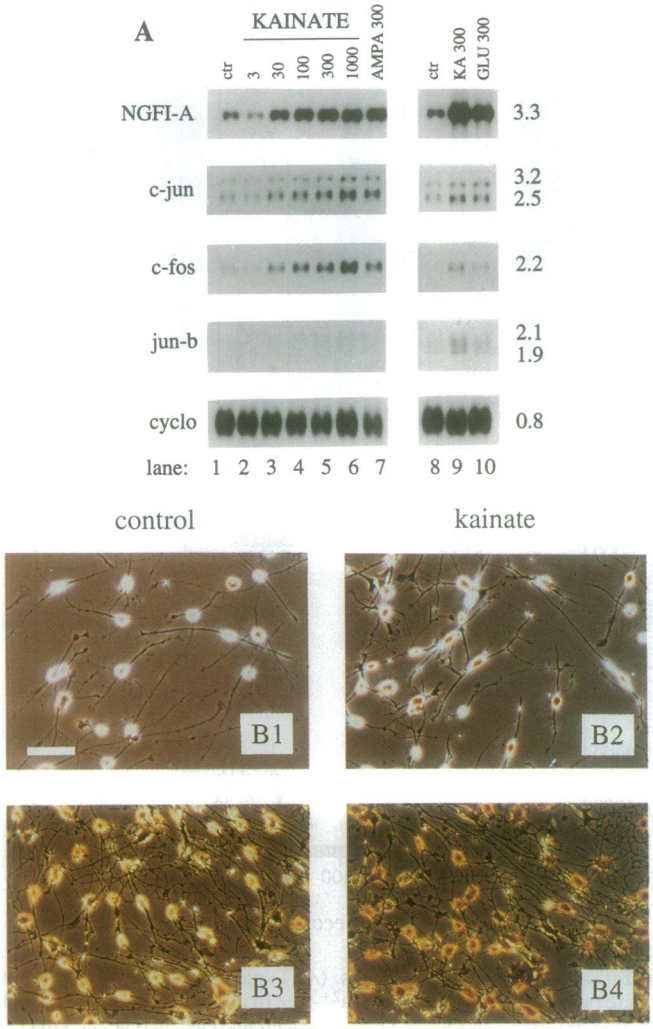

FIG. 2. Effects of glutamate receptor agonists on expression of IEGs in cortical O-2A progenitors and CG-4 cells. Northern blot analyses of NGFI-A, c-*fos*, c-*jun*, and *jun-b* mRNAs in cortical O-2A progenitors (*A*) and immunocytochemistry of NGFI-A protein in CG-4 cells (*B1* and *B2*) and cortical O-2A progenitors (*B3* and *B4*). (*A*) Dose–response curve with kainate (3–1000 μM; lanes 2–6) and effects of AMPA (300 μM; lane 7) and glutamate (300 μM; lane 10). ctr, Cells treated with DMEM. Total RNA (10 μg) was hybridized with cDNA probes for the different IEGs and for constitutively expressed cyclophilin mRNA (cyclo), the latter to control for RNA loading. Exposure times of the blots to x-rays were 12 hr for NGFI-A and 48 hr for c-*fos*, c-*jun*, and *jun-b*. The NGFI-A signal corresponding to treatment with 3 μM kainate (lane 2) was the same as its control (lane 1) when normalized with the internal standard cyclophilin. Molecular sizes (kb) for NGFI-A and cyclophilin are indicated on the right. (*B2* and *B4*) Cells were incubated with kainate (300 μM) for 1 hr and stained for NGFI-A after 5 hr. Representative experiments were replicated two or three times. (Bar = 100 μm.)

role in coupling glutamate receptor channels to changes in NGFI-A mRNA. Furthermore, our immunostaining experiments, performed with a specific anti-NGFI-A antibody, clearly indicate that the receptor-induced stimulation of NGFI-A mRNA leads also to an increase in the protein levels (Fig. 1*B*).

The efficacy of kainate and glutamate on NGFI-A mRNA levels corresponds to their ability to increase $[Ca^{2+}]_i$ in CG-4 cells, with glutamate being less effective than kainate (compare Figs. 1 and 2). The effects of glutamate agonists on $[Ca^{2+}]_i$ and NGFI-A mRNA are due to the exclusive activation of ionotropic kainate and/or AMPA receptors, as shown by inhibition with the non-NMDA antagonist DNQX and total dependence on extracellular Ca^{2+} ions. The lack of

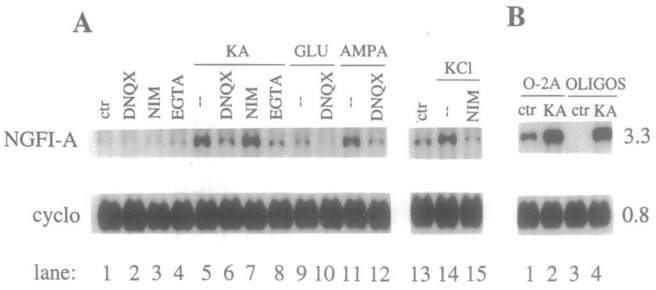

FIG. 3. Induction of NGFI-A expression in CG-4 cells by glutamate receptor agonists and K^+ depolarization. (*A*) Lanes 1–12, Northern blot analyses of total RNA (15 μg per lane) from CG-4 cells treated with dimethyl sulfoxide (lane ctr; final concentration, 0.3%), 200 μM kainate (lanes KA), 200 μM glutamate (lanes GLU), 200 μM AMPA in the presence or in the absence of DNQX (30 μM), nimodipine (lanes NIM; 10 μM), and EGTA (3 mM); lanes 13–15, effects of 45 mM K^+ on NGFI-A mRNA. CG-4 cells were treated with DNQX, nimodipine, and EGTA 10 min before stimulation. (*B*) Northern blot analysis of total RNA (15 μg per lane) from CG-4 progenitor cells (O-2A; lanes 1 and 2) or oligodendrocytes (OLIGOS; lanes 3 and 4) treated with DMEM (lane ctr) or with 300 μM kainate (lanes KA). Total RNA was hybridized with cDNA probes for NGFI-A and for constitutively expressed cyclophilin mRNA (cyclo), the latter to control for RNA loading. Molecular sizes (kb) of the NGFI-A and cyclophilin transcripts are indicated on the right.

effect of the metabotropic glutamate receptor agonist 1*S*,3*R*-ACPD on $[Ca^{2+}]_i$ and on NGFI-A mRNA levels (M.P., L.A.H., J.T.R., and V.G., unpublished data) is also consistent with this interpretation.

In our experiments, we could not determine the relative contribution of AMPA- vs. kainate-preferring receptors to regulation of $[Ca^{2+}]_i$ and to induction of NGFI-A mRNA. The saturating concentration for kainate (300 μM) derived from the dose–response curve obtained in cortical glia (Fig. 2*A*) is similar to that previously reported for AMPA receptors in CG-4 cells and in hippocampal neurons (8, 23). The similar effects of kainate and AMPA (Fig. 2*A*) and their strong potentiation by cyclothiazide (Figs. 1 and 4) conclusively demonstrate that AMPA receptors regulate $[Ca^{2+}]_i$ and NGFI-A mRNA in cells of the oligodendrocyte lineage. Importantly, the experiments with cyclothiazide show that allosteric modulation of AMPA ionic channels can have a direct impact on genomic responses. The findings, however, that the non-NMDA antagonist DNQX does not completely prevent the effects of kainate and glutamate on $[Ca^{2+}]_i$ (Fig. 1*B*) and NGFI-A mRNA (Fig. 2*A*) suggest also that high-affinity kainate-preferring receptors are involved. In fact, electrophysiological studies have shown that membrane currents generated by the activation of kainate-preferring receptors in CG-4 cells (12) and in hippocampal neurons (31) are less sensitive to the related non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione than AMPA-preferring receptors.

Our Ca^{2+}-imaging experiments and Northern blot analysis of NGFI-A mRNA clearly indicate that voltage-dependent Ca^{2+} channels and Ca^{2+}-permeable glutamate-gated channels coparticipate in transmembrane Ca^{2+} influx and in regulation of NGFI-A gene expression. The Ca^{2+}-channel blocker nimodipine completely abolished the K^+-induced increase in $[Ca^{2+}]_i$ but only partially inhibited the effects of kainate and glutamate (70% and 60%, respectively; Fig. 1). Nimodipine also blocked depolarization-induced NGFI-A mRNA expression but did not alter the effects of kainate on the IEG (Fig. 3*A*). These findings indicate that (*i*) a significant proportion of glutamate receptor channels in oligodendrocyte progenitors are permeable to Ca^{2+} ions; and (*ii*) the influx of Ca^{2+} that occurs through the AMPA and/or kainate channels themselves is sufficient to reach the threshold necessary for NGFI-A mRNA stimulation. Our previous molecular analysis

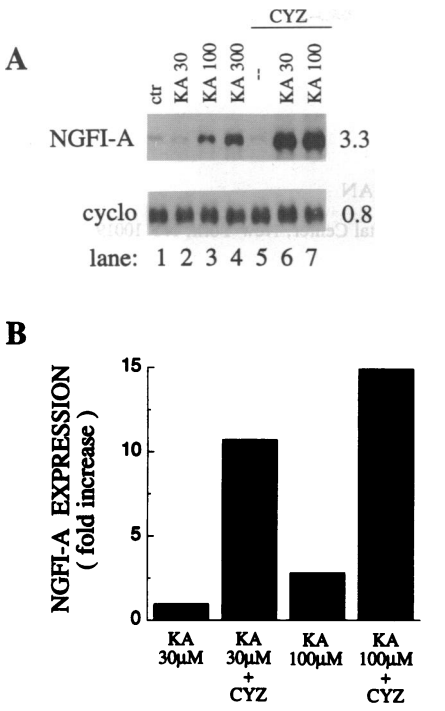

FIG. 4. Effects of cyclothiazide on kainate-induced expression of NGFI-A in CG-4 cells. (*A*) Northern blot analyses of total RNA (15 μg per lane) from CG-4 cells treated with dimethyl sulfoxide (lane ctr; final concentration, 0.3%), cyclothiazide (lanes CYZ; 30 μM), kainate (lanes KA; 30, 100, or 300 μM) or kainate and cyclothiazide. Cyclothiazide was added 5 min before stimulation. Total RNA was hybridized with cDNA probes for NGFI-A and for constitutively expressed cyclophilin mRNA (cyclo), the latter to control for RNA loading. Molecular sizes (kb) of the NGFI-A and cyclophilin transcripts are indicated on the right. (*B*) Histograms obtained from PhosphorImager quantitation of the Northern blot shown in *A*. NGFI-A transcript data were normalized for cyclophilin in the corresponding lanes. Cyclothiazide caused an 11- and a 15-fold increase with 30 and 100 μM kainate, respectively. No increase in NGFI-A mRNA was observed with 30 μM kainate alone (ratio KA 30/control = 1).

of glutamate receptor subunits expressed in CG-4 cells and in purified O-2A progenitors has demonstrated that the AMPA receptor subunit GluR-2 is present in both cell types throughout their development (8, 12). This subunit is known to confer Ca^{2+} impermeability to AMPA channels (32, 33). Our data indicate, therefore, that a significant proportion of the heteromeric glutamate receptor channel assemblies in CG-4 cells do not contain GluR-2 or are assembled from Ca^{2+}-permeable kainate-preferring subunits (34). Independent studies by Fulton *et al.* (35) in optic nerve cells and by Holzwarth *et al.* (36) in cortical cells have also demonstrated that oligodendrocyte progenitors express Ca^{2+}-permeable kainate and/or AMPA ionic channels at early developmental stages.

The transcription of IEGs, including c-*fos* and NGFI-A, is known to be elevated by various mitogenic signals in different cell types (14, 15). In O-2A progenitors, agents that stimulate cell proliferation increase the mRNA levels for c-*fos* and c-*jun* (37). We have also observed that platelet-derived growth factor and basic fibroblast growth factor increase the expression of NGFI-A mRNA both in CG-4 and in cortical O-2A progenitors in parallel with their stimulatory effects on DNA synthesis (M.P., P. W. Wright, and V.G., unpublished data). These data suggest that in oligodendrocyte progenitors as well, NGFI-A may be involved in cell proliferation. We hypothesize that glutamate may serve as a developmental signal for oligodendrocytes, because these cells divide and differentiate postnatally in a neuronal environment (7). O-2A progenitors are likely, therefore, to be exposed to significant levels of extracellular glutamate *in vivo*. The neurotransmitter, through the activation of multiple receptor subtypes, initiates a cascade of intracellular events that regulate the expression of genes that may be crucial to the developmental progression of O-2A progenitors.

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