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Differential Promotion of Glutamate Transporter Expression and Function by Glucocorticoids in Astrocytes from Various Brain Regions*

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Steroids that activate glucocorticoid receptors (GRs) and mineralocorticoid receptors have important regulatory effects on neural development, plasticity, and the body's stress response. Here, we investigated the role of corticosteroids in regulating the expression of the glial glutamate transporters glial glutamate transporter-1 (GLT-1) and glutamate-aspartate transporter (GLAST) in rat primary astrocytes. The synthetic glucocorticoid dexamethasone provoked a marked increase of GLT-1 transcription and protein levels in cortical astrocytes, whereas GLAST expression remained unaffected. Up-regulation of GLT-1 expression was accompanied by an enhanced glutamate uptake, which could be blocked by the specific GLT-1 inhibitor dihydrokainate. The promoting effect of dexamethasone on GLT-1 gene expression and function was abolished by the GR antagonist mifepristone. A predominant role of the GR was further supported by the observation that corticosterone could elevate GLT-1 expression in a dose-dependent manner, whereas aldosterone, the physiological ligand of the mineralocorticoid receptor, exerted only weak effects even when applied at high concentrations. Moreover, we monitored brain region-specific differences, since all corticosteroids used in this study failed to alter the expression of GLT-1 in midbrain and cerebellar glia, although expression levels of both corticosteroid receptor subtypes were similar in all brain regions analyzed. Dexamethasone, however, modestly enhanced GLT-1 expression in cerebellar glia in combination with the DNA methyltransferase inhibitor 5-aza-2-deoxycytidine, suggesting that suppression of GLT-1 expression in cerebellar cultures may at least in part be epigenetically mediated by a DNA methylation-dependent process. Taken together, our data highlight a potential role for glucocorticoids in regulating GLT-1 gene expression during central nervous system development or pathophysiogical processes including stress.

Steroid hormones possessing glucocorticoid (GC)[2] or mineralocorticoid (MC) activity exert profound regulatory effects in the central nervous system (1). Being synthesized in the adrenal gland and ultimately secreted into circulation, they penetrate the blood brain barrier and influence neuronal development and plasticity. Moreover, GCs are the main effectors of the hypothalamic-pituitary-adrenal axis and regulate the body's stress response by inducing a variety of physiological changes including mobilization of energy from storage sites and the suppression of parts of the immune system (2). Due to their anti-inflammatory properties, they are administered in the treatment of central nervous system diseases such as edema arising from brain tumors, viral encephalitis, bacterial meningitis, and multiple sclerosis (3).

The effects of adrenal steroid hormones (GCs and MCs) are classically mediated through the activation of mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) (4, 5), which display a wide distribution in the brain. Corticosterone, cortisol, and aldosterone bind with high affinity to the MR subtype. On the other hand, corticosterone and aldosterone exhibit a lower affinity to the GR (6), whereas the synthetic glucocorticoid dexamethasone shows a high affinity for the latter receptor subtype (7). Upon binding of the cognate ligands, the cytoplasmatic receptors are released from an oligomeric heat shock protein complex, homo- or heterodimerize (8), and translocate into the nucleus. Subsequently, the activated receptors associate with hormone response elements of target gene promoters, thereby enhancing or repressing gene transcription (9). However, some of the genomic effects of the receptors are mediated without direct binding to DNA but rather through modulation of the activity of other transcription factors or systems (10).

In the brain, a wide expression of GRs is contrasted by a relatively restricted expression of MRs (11). GRs are distributed throughout the brain, including high densities in the cerebral cortex, olfactory bulb, the CA1, CA2 layers, and dentate gyrus of the hippocampus, and Purkinje and granule cell layers of the cerebellum. In contrast, MRs have been localized to the limbic system and hypothalamus and colocalize with GRs in the CA1 and CA2 pyramidal neurons of the hippocampus (12, 13). In addition to neurons, cells of glial origin also express GRs and MRs (14, 15). Glucocorticoid treatment of astrocytes results in inhibition of DNA synthesis and proliferation (16, 17), activation of intracellular signaling pathways that lead to the induction of long range calcium wave propagation (18), and alterations of gene transcription patterns. *In vivo* application of corticosterone reduces adrenalectomy-mediated increases in glial fibrillary acid protein (GFAP) expression while promoting expression of glutamine synthetase (19). Glutamine synthetase is involved in recycling of the neurotransmitter glutamate, catalyzing its conversion into the amino acid glutamine.

Glutamate is the major excitatory neurotransmitter in the mammalian nervous system. The primary task of glia at glutamatergic synapses is the rapid uptake of glutamate and its conversion to glutamine in order

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[2] The abbreviations used are: GC, glucocorticoid; MC, mineralocorticoid; MR, mineralocorticoid receptor; GR, glucocorticoid receptor; GFAP, glial fibrillary acid protein; PBS, phosphate-buffered saline; MEM, minimal essential medium; TBS, Tris-buffered saline; qPCR, quantitative real time PCR; MMTV, mouse mammary tumor virus long terminal repeat; 5-AZA, 5-aza-2-deoxycytidine; GLAST, glutamate-aspartate transporter; GLT-1, glial glutamate transporter.

to avoid excitotoxic damage. Glutamate uptake is carried out by a family of high affinity sodium-dependent glutamate transporters. Currently, this family comprises five members, two of which, glutamate-aspartate transporter (GLAST) (20) and glial glutamate transporter-1 (GLT-1) (21), are highly expressed by astrocytes (22). GLT-1 and GLAST achieve the glutamate uptake against a concentration gradient by coupling the transport of Na^+ and K^+ ions down their respective gradients (23). This process occurs at an energetic cost greater than 1 ATP per glutamate molecule transported.

Cellular glutamate transporter levels are modulated by altered gene transcription as well as mRNA translation (24). The search for regulatory factors that positively regulate transporter expression is important, considering that glial glutamate transporter expression transiently decreases after brain injury, leading to secondary neuronal cell death (25, 26), and that reduced expression of GLT-1 and/or GLAST occurs in patients suffering from amyotrophic lateral sclerosis as well as Alzheimer's and Huntington's disease (25–27). Intriguingly, although the glucocorticoid-mediated regulation of glutamine synthetase is well investigated (28), the effects of glucocorticoids on glutamate transporter expression have not yet been analyzed in detail.

Therefore, the aim of the study was to analyze glucocorticoid-mediated effects on expression of GLT-1 and GLAST in type I astrocytes at the transcriptional and translational level, focusing also on brain region-specific differences. Using pharmacological antagonists of the steroid receptor subtypes, we elucidated the participation of GR/MRs in this regulatory process. In addition, we investigated changes in glutamate uptake efficiency in response to corticosteroid treatment.

EXPERIMENTAL PROCEDURES

Glia Cell Culture—Highly enriched astroglial cultures were initiated from postnatal day 1 rat pups (Sprague-Dawley, local animal facility, University of Mainz, Germany) as described previously (29). In these cultures, 90–95% of all cells represent type-1 astrocytes, and small populations of oligodendrocytes, type-2 astrocytes, and O-2A progenitors make up the remainder. Briefly, cortical hemispheres, cerebellum, or midbrain were dissected under sterile conditions and incubated for 20 min in Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS; Invitrogen) containing 0.1% trypsin and 0.02% EDTA. Trypsin action was ended by transferring tissue pieces to Hanks' balanced salt solution (Invitrogen) supplemented with 10% fetal calf serum. The tissue was repeatedly passed through a serological plastic pipette until dissociated by trituration. Cells were pelleted at 400 × *g* for 5 min, resuspended in modified Eagle's medium (MEM; Invitrogen) supplemented with 10% horse serum (Invitrogen). Cells were plated into 100-mm culture dishes that had been coated with poly-L-ornithine (0.1 mg/ml; molecular mass 100–200 kDa; Sigma). Cell cultures were maintained at 37 °C and 5% CO_2. Upon reaching confluence, cultures were trypsinized and replated at lower cell density. After the third passage, cells were either seeded onto glass coverslips (Marienfeld) for immunocytochemistry or into 60-mm culture dishes for immunoblot analysis and were maintained for the indicated time with serum-free (MEM/Ham's F-12, 1:1) N2-supplemented medium. Medium was supplemented with forskolin (Calbiochem), dexamethasone, corticosterone, aldosterone, mifepristone, spironolactone, 5-aza-2-deoxycytidine, or trichostatin A (all from Sigma) at concentrations specified throughout.

Detergent-free Isolation of Lipid Rafts/Caveolae—Detergent-free extracts of primary astrocytes grown to subconfluence on 100-mm diameter dishes were used for sucrose density gradient fractionation as described previously with some modifications (30). Briefly, cultured cells were washed once with ice-cold PBS and scraped off in 1 ml of Tris-buffered saline (TBS; 150 mM NaCl, 50 mM Tris, pH 8, 5 mM EDTA) supplemented with 5 mg/ml phenylmethylsulfonyl fluoride (Merck) and 1 mM sodium orthovanadate (Sigma). Homogenization was performed by using a loose fitting Dounce homogenizer and a sonicator (two 15-s bursts). The homogenized cell sample was spun at 800 × *g* for 10 min at 4 °C. The postnuclear supernatant was spun for an additional 1 h at 4 °C and 100,000 × *g*. The pellet, representing the membrane fraction, was dissolved in 250 μl of TBS, mixed with an equal volume of 80% (w/v) sucrose solution in TBS, and placed at the bottom of an ultracentrifugation tube. After overlaying 1450 μl of 30% (w/v) sucrose and 250 μl 5% (w/v) sucrose solution, the gradient was centrifuged at 55,000 rpm for 2 h at 4 °C with a TLS 55 Beckmann swing-out rotor. 200–400-μl fractions were collected from the top to the bottom of the gradient and were directly used for Western blot analysis.

Western Blot Analysis—Subconfluent astroglial cells were washed with ice-cold PBS and lysed in a buffer containing 62.5 mM Tris, pH 6.8, 2% SDS, 10% sucrose supplemented with phenylmethylsulfonyl fluoride (5 mg/ml) and aprotinin (1 μg/ml). Extracts were sonicated briefly and denatured for 5 min at 95 °C. Protein concentrations of the samples were determined by the BCA method (Pierce) using bovine serum albumin as a standard. Equal amounts of total protein (5–8 μg) were adjusted to similar volumes with loading buffer (10% SDS, 20% glycerin, 125 mM Tris, 1 mM EDTA, 0.002% bromphenol blue, 10% β-mercaptoethanol), denatured by heating at 95 °C for 5 min, and subsequently separated on 10 or 12% SDS-polyacrylamide gels. Semidry transfer of the proteins onto nitrocellulose membranes was followed by blocking of nonspecific binding sites in Tris-buffered saline, 0.05% Tween 20 (TBS/Tween) containing 5% nonfat milk for 1 h at room temperature. The blots were then incubated with either anti-GLT-1 (1:5000; Chemicon), anti-GLAST (1:1000, Chemicon), anti-GFAP (1:500; Dako), anti-actin (1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-caveolin-1 (1:500; BD Transduction Laboratories), or anti-GSK3β (1:500; BD Transduction Laboratories) antibodies diluted in TBS/Tween, overnight at 4 °C. The blots were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies (Jackson Laboratory) for 1.5 h at room temperature. Membrane-bound secondary antibodies were detected using the chemiluminescence Super Signal procedure (Pierce) and visualized on Eastman Kodak Co. Biomax films. After scanning the films, optical densities of bands were measured using the AIDA Image Analyzer software version 3.28 as ratios of GLT-1/GLAST to actin bands.

Reverse Transcription-PCR Analysis and Quantitative Real Time PCR (qPCR)—Total RNA from astrocytes grown to subconfluence was prepared using the Absolutely RNA reverse transcription-PCR Miniprep Kit (Stratagene) according to the manufacturer's instructions. Reverse transcription was performed on 400–1200 ng of total RNA in a reaction volume of 20 μl containing 2 μl of reverse transcriptase buffer (Qiagen), 2 μl of 5 mM dNTPs (Qiagen), 2 μl of 10 μM oligo$(dT)_{23}$ primer (Sigma), 10 units of RNasin (Promega), and 4 units of Omniscript Reverse Transcriptase (Qiagen). Synthesis of cDNA was carried out for 60 min at 37 °C. PCR was performed in a 25-μl reaction volume that contained 2 μl of cDNA, 2.5 μl of 10× PCR buffer (Invitrogen), 0.75 μl of 50 mM $MgCl_2$ (Invitrogen), 0.5 μl of 10 mM dNTPs (peqLAB), 0.5 μl of 15 pmol of sense and antisense primers, and 0.5 units of DNA *Taq* polymerase (Invitrogen). The PCR primers used for the detection of MR, GR, and actin mRNA transcripts are listed in TABLE ONE.

Primers were purchased from Thermo Hybaid (Ulm, Germany). PCR was carried out at 94 °C for 30 s, annealing of the primers was at the indicated temperatures for 40 s, and elongation was at 72 °C for 50 s for a total of 25–35 cycles. An initial denaturation at 94 °C for 5 min and a

final extension at 72 °C for 5 min were also included. PCR products were separated by size on ethidium bromide-supplemented agarose gels and visualized by UV illumination.

In the case of quantification of GLT-1, GLAST, GR, and MR transcripts, qPCR was additionally performed where indicated. Sequences of primers are listed in TABLE ONE. The PCR was carried out in duplicate and contained 2 μl of undiluted cDNA or 2 μl of 1:10 diluted cDNA for the amplification of GLT-1/GLAST/GR/MR or actin, respectively. The PCR was composed of 0.5 μl of 100 pmol of sense and antisense primers and 12.5 μl of 2× SYBR Green Supermix (Bio-Rad) to make a final volume of 25 μl. Subsequently, PCR was performed by using the iCycler (Bio-Rad) under the following conditions: initial denaturation at 95 °C for 15 min and 37 cycles of 95 °C for 30 s, 60/56 °C for 30 s, and 72 °C for 20–40 s. The generation of specific PCR products was confirmed by melting curve analysis. In order to measure relative gene expression changes, the comparative C_T method, $X = 2_T^{-\Delta\Delta C}$ was applied (31).

Fluorescent Immunocytochemistry—Astrocytes grown on polyornithine-coated glass coverslips were briefly washed two times with PBS and fixed at room temperature for 20 min with 4% formaldehyde in PBS. Cells were permeabilized with PBS containing 0.01% Saponin (Sigma) two times for 10 min each. After permeabilization, cells were rinsed in blocking solution (5% goat serum in PBS) for 60 min. The coverslips were incubated overnight at 4 °C with either anti-GLT-1 (a kind gift of Dr. J. D. Rothstein, Johns Hopkins University, Baltimore, MD) or anti-GFAP (Dako) primary antibodies (all 1:100 in PBS plus 0.2% Triton and 10% goat serum). Fluorescent staining of cells was achieved after washing three times with PBS and incubating with secondary antibodies for 1 h (anti-rabbit IgG conjugated with Cy3, 1:200 dilution, Jackson Laboratory). Cells were washed three times again in PBS and rinsed once with 1 μg/ml 4′,6-diamidino-2-phenylindole in PBS. Finally, coverslips were mounted with Prolong Antifade (Molecular Probes, Inc., Eugene, OR), and staining was visualized with a Zeiss Axiovert 200 fluorescence microscope. Images were captured digitally (SPOT RT-SE; Diagnostic Instruments Inc.) and partly processed by deconvolution to reduce out-of-focus information (AutoDeblur Software, Auto Quant Imaging Inc.).

Glutamate Uptake and Dihydrokainate Treatment—Glutamate uptake studies were performed in Tris buffer containing 5 mM Tris base (pH 7.2), 10 mM HEPES, 140 mM NaCl, 2.5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM K_2HPO_4, 10 mM dextrose, and the glutamine synthetase inhibitor methionine sulfoximine (1 mM; Sigma). Radiolabeled glutamate (1 μCi/ml; specific activity, 63 Ci/mmol; Amersham Biosciences) and unlabeled glutamate were mixed to obtain a total glutamate concentration of 960 μM, which was subsequently added to third passage glial cultures (250 μl/well). Uptake was terminated after 10 min by removing the radioactive solution and rinsing the cultures three times with ice-cold lithium-containing Tris buffer (pH 7.2). Cells were lysed in 0.1 M NaOH, and the amount of incorporated glutamate was determined by liquid scintillation counting of the cell lysate. Sodium-dependent glutamate uptake was defined to be the difference of the amount of radioactivity incorporated by glia in the presence of a sodium-containing buffer and was compared with the amount of protein determined in sister cultures. In some experiments, astrocyte cultures were treated 10 min before and during the glutamate uptake studies with 100 μM dihydrokainate diluted in uptake buffer.

Transient Transfection of Astrocytes and Luciferase Assay—Transfection of primary astrocytes with the reporter plasmid mouse mammary tumor virus long terminal repeat (MMTV)-luciferase (a kind gift of Dr. Rein, Max Planck Institute of Psychiatry, Munich, Germany) was performed using the Nucleofactor transfection reagent and electroporator according to the manufacturer's protocol (Amaxa, Cologne, Germany). Briefly, astrocytes of the third passage were trypsinized, and subsequently 3.6×10^6 cells were applied to electroporation with 4.5 μg of reporter plasmid DNA. Cells were seeded in nine wells of a 24-well plate and allowed to recover for 24 h in MEM plus 10% horse serum. After 24 h, cell culture medium was changed to MEM/HAM F-12 (1:1) N2-supplemented medium. Cells were stimulated with the indicated concentration of dexamethasone and mifepristone for 48 h. Ultimately, astroglial cells were lysed in 100 μl of lysis buffer, and a luciferase assay was carried out using the Promega luciferase detection kit according to the manufacturer's instructions. Luminescence readings were performed with an automatic counter (Fluoroskan Ascent FL, Thermolabs).

Statistical Analysis—For the evaluation of Western blots, qPCRs and luciferase assays mean values of data from three independent experiments were calculated and plotted as -fold change as compared with controls, which have been set to 1. Significance between groups was further analyzed using the post *hoc* Tukey test, and data were depicted with S.D. In the case of glutamate uptake experiments, Student's two-tailed *t* test has been applied.

RESULTS

Primary astrocytes initiated from brain tissue of postnatal day 1 rats express low basal levels of the glutamate transporter subtypes GLAST and GLT-1 (32, 33). In this study, we investigated the potential role of synthetic and natural gluco- and mineralocorticoid hormones in regulating GLT-1 and GLAST expression as well as function in early postnatal astroglial cells. In this context, we also elucidated the differential regulation of GLT-1 expression that occurred in a brain region-specific fashion.

Treatment of third passage rat cortical astrocytes with 100 nM dexamethasone for 72 h resulted in an robust increase of GLT-1 protein expression as assessed by Western blot analysis. In contrast, levels of GLAST proteins remained essentially unaffected (Fig. 1*A*). Forskolin, which has been demonstrated previously to induce increases in glutamate transporter expression as an activator of adenylate cyclase (32), enhanced the expression of both glutamate transporter subtypes and served as a positive control. Moreover, the extent of GLT-1 induction was similar when comparing the regulatory effects of forskolin and dexamethasone (Fig. 1*A*).

During development, GLT-1 protein as well as mRNA levels are reported to increase with maturation, and *in vitro* differentiation of astrocytes occurs through common signaling pathways also involved in the up-regulation of GLT-1 gene expression (32, 34). Therefore, it has been postulated that astrocyte differentiation and up-regulation of GLT-1 are tightly correlated. In order to verify whether dexamethasone exerts an effect on astrocyte maturation *in vitro*, we examined changes in astrocyte morphology using fluorescent immunocytochemistry. Immunostaining against the cytoskeleton constituent GFAP confirmed the astroglial identity of the analyzed cells and gave a good representation of the cell morphology. Untreated control cultures exhibited a polygonal morphology, whereas the addition of forskolin to the media for 72 h induced prominent process extension and stellation, morphologically more characteristic of astrocytes *in vivo*. The stellate phenotype was not observed in the case of dexamethasone treatment (Fig. 1*B*). Therefore, dexamethasone atypically induced an increase in glutamate transporter expression in cortical astrocytes without altering cell morphology.

We further characterized the regulatory effects of dexamethasone on GLT-1 expression with respect to concentration and time dependence as well as putative brain region-specific differences. Dexamethasone

induced a dose-dependent up-regulation of GLT-1 expression in cortical astrocytes at concentrations varying from 1 to 1000 nM (Fig. 2, *A* and *B*). We next treated cortical astrocytes with 100 nM dexamethasone in a time course ranging from 24 to 72 h. Interestingly, GLT-1 protein levels were steadily increasing over the analyzed time frame as determined by Western blot analysis (Fig. 2, *C* and *D*). Astrocytes from various brain regions often display differing responsiveness to factors that regulate GLT-1 expression (35). We therefore elucidated the effects of dexamethasone on GLT-1 expression in astrocytes derived from other brain regions such as the cerebellum and midbrain. We found that 100 nM dexamethasone failed to enhance GLT-1 protein expression when applied for the indicated times to the cell culture medium of cerebellar astrocytes. Only very slight alterations in GLT-1 levels in midbrain glia were observed (Fig. 2*C*).

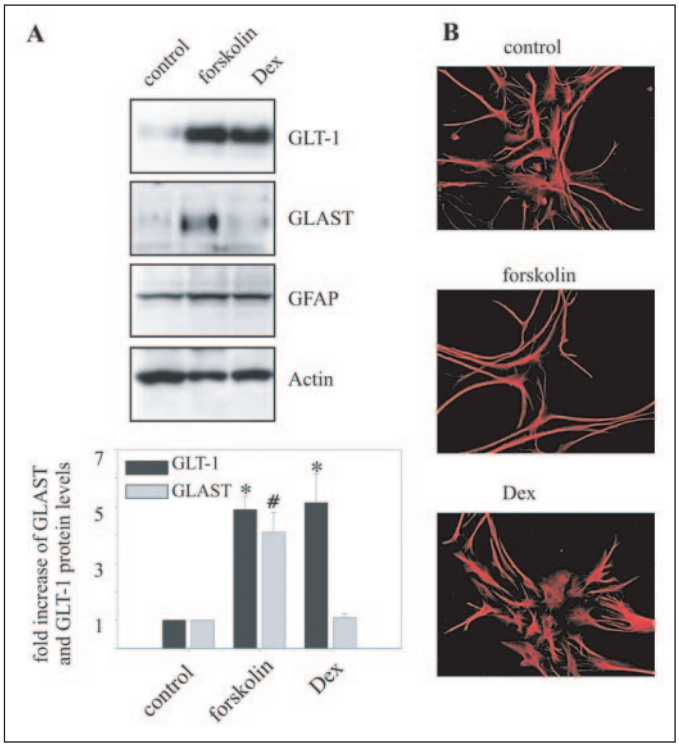

FIGURE 1. **GLT-1 expression is up-regulated by dexamethasone (*Dex*) in primary cortical astrocytes.** *A*, cell lysates of forskolin-treated (10 μM, 72 h) or dexamethasone-treated (100 nM, 72 h) cortical astrocytes were prepared and subjected to Western blot analysis using specific antibodies directed against GLT-1, GLAST, and GFAP. To ensure equal protein loading, blots were reprobed with actin-specific antibodies. Protein levels of GLT-1 and GLAST were normalized to actin and depicted as -fold increase ± S.D. of untreated control cells (considered as 1); #, *, $p < 0.05$ *versus* control ($n = 3$). *B*, morphological alterations of primary astrocytes exposed to either forskolin (10 μM) or dexamethasone (100 nM) for 72 h were microscopically examined. Immunostaining of low density cultures against the cytoskeleton protein GFAP confirmed the astroglial identity of the analyzed cells and revealed pronounced astrocytic stellation in the case of forskolin treatment, but not after dexamethasone treatment.

Since expression of glutamate transporters is regulated both at the transcriptional and the translational level, we examined whether dexamethasone affects GLT-1 transcription in cortical astrocytes. RNA was extracted from cortical astrocytes that were exposed to 100 nM dexamethasone for 24, 48, and 72 h, and total RNA was subjected to cDNA synthesis and subsequent qPCR analysis using GLT-1-, GLAST-, and actin-specific primers (TABLE ONE). qPCR analysis revealed that the amount of GLT-1 transcripts continuously increased during 72 h of dexamethasone treatment as compared with the untreated control (Fig. 3). In contrast, transcription of the GLAST gene was not altered by dexamethasone. Therefore, dexamethasone not only elevated GLT-1 protein levels in cortical astrocytes but also augmented transcription of the GLT-1 gene, whereas GLAST transcript levels remained unchanged.

Previous studies from our group (36) and others (37) have identified lipid rafts as organizational platforms for GLT-1 molecules in astrocytes. Localization of GLT-1 within cholesterol-enriched lipid rafts might be an important prerequisite for efficient glutamate uptake from the extracellular space. In order to verify whether GLT-1 associates with this membrane microdomain after exposure to dexamethasone, we performed sucrose gradient ultracentrifugation of detergent-free cell extracts of dexamethasone-treated (10 nM, 72 h) cortical astrocytes. Due to their light buoyant density properties, lipid rafts are concentrated between 5 and 30% sucrose, which represents the second fraction of the gradient. Indeed, GLT-1 cofractionated with caveolin-1, a marker protein of a subtype of lipid rafts, at the border between 5 and 30% sucrose (Fig. 4*A*). In general, GLT-1 and caveolin-1 were similarly distributed over the sucrose gradient, indicating an association of GLT-1 with raft microdomains. To exclude a possible contamination from the high density fractions, membranes were reprobed with GSK3β antibodies, a con-

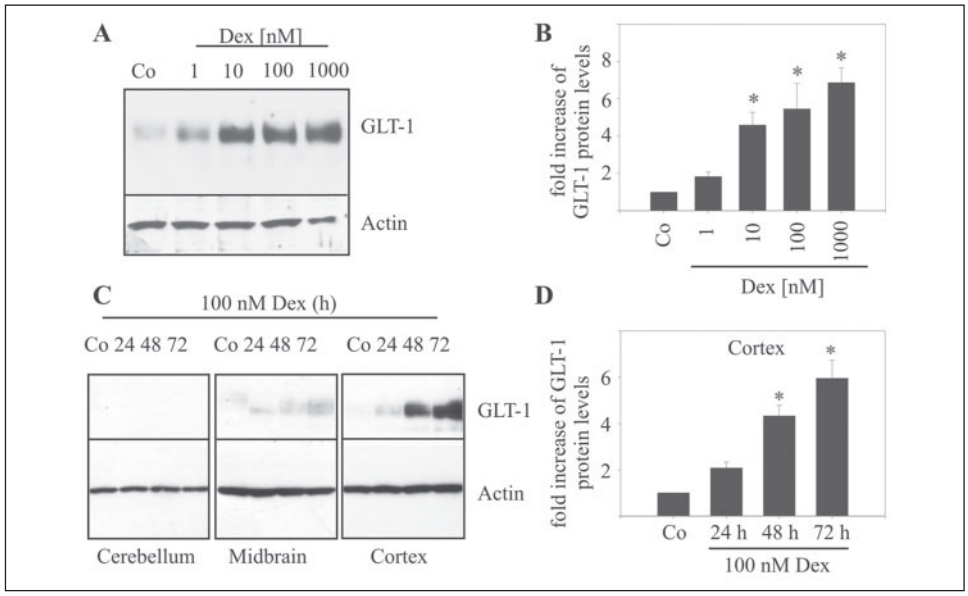

FIGURE 2. **Concentration and time dependence as well as differential regulation of GLT-1 by dexamethasone (*Dex*).** *A*, cortical astrocytes were treated with various concentrations of dexamethasone (1–1000 nM) for a period of 72 h. After pharmacological stimulation, cell lysates were used for Western blot analysis with GLT-1- and actin-specific antibodies to illustrate equal loading. *B*, optical densities of GLT-1-specific bands were determined and normalized to actin. GLT-1 levels of untreated controls were set to 1. Data shown represent the mean ± S.D. of the -fold increase (*, $p < 0.05$ *versus* control, $n = 3$). *C*, primary astrocytes initiated from cerebellum, midbrain, and cortex were exposed to 100 nM dexamethasone in a time course for up to 72 h (*Co*, untreated control). Equal amounts of proteins were subjected to Western blot analysis using GLT-1- and actin-specific antibodies. *D*, results were calculated as ratios of OD of the GLT-1 protein *versus* actin protein and expressed as the mean ± S.D. Ratios from untreated cells were considered as 1 (*, $p < 0.05$ *versus* control, $n = 3$).

TABLE ONE

Primer sequences

Primer sequences for qPCR and semiquantitative PCR (sRT) are listed for the corresponding genes. Primer annealing temperature (T_A) as well as annealing sites (as reference, the PubMed accession number is supplied in parentheses) are indicated.

Primer	Primer sequence	T_A	Position
		℃	
GLT-fwd (qPCR)	CCGAGCTGGACACCATTGA	60	1591→1609(X67857)
GLT-rev (qPCR)	CGGACTGCGTCTTGGTCAT	60	1640→1659(X67857)
GLAST-fwd (qPCR)	AATGAAGCCATCATGAGATTGGT	60	806→ 828(AF265360)
GLAST-rev (qPCR)	CCCTGCGATCAAGAAGAGGAT	60	859→ 880(AF265360)
MR-s (qPCR + sRT)	AGGCTACCACAGTCTCCCTG	56	485→ 504(M36074)
MR-as (qPCR + sRT)	GACTGGAGATTTTACACTGC	56	1309→1328(M36074)
GR-fwd (qPCR)	GGGACCACCTCCCAAGCT	60	1304→1321(AY066016)
GR-rev (qPCR)	GCACCCCGTAATGACATCCT	60	1349→1368(AY066016)
GR-s (sRT)	AGTTCCTGCAGCATTACCAC	53	1619→1638(AY066016)
GR-as (sRT)	ACTCTTCATAGGATACCTGC	53	2027→2046(AY066016)
Actin-s (qPCR + sRT)	CTACAATGAGCTGCGTGTGGC	60	Ref. 62
Actin-as (qPCR + sRT)	CAGGTCCAGACGCAGGATGGC	60	Ref. 62

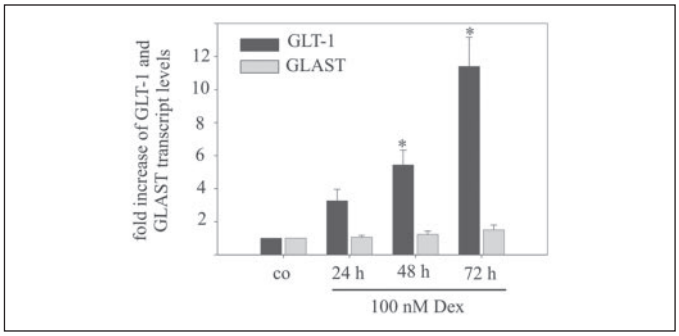

FIGURE 3. **Dexamethasone (*Dex*) up-regulates GLT-1 transcription in cortical astrocytes.** Real time PCR analysis was carried out with total RNA derived from dexamethasone-treated (100 nM; 24, 48, and 72 h) cortical astrocytes and with GLT-1-, GLAST-, and actin-specific primers. PCR cycle numbers that generated the first fluorescence signal above threshold (C_T) were determined, and subsequently C_T values were applied to the $X = 2_T^{-\Delta\Delta C}$ formula, where X represents the factor by which the amount of GLT-1 transcripts changed in dexamethasone-treated astrocytes compared with the untreated control (considered as 1). Results depict the mean ± S.D. of the -fold increase. *, $p < 0.05$ *versus* control ($n = 3$).

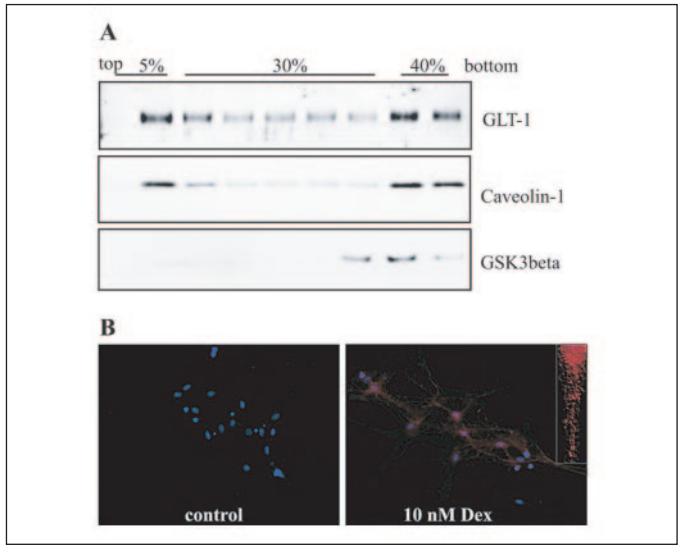

FIGURE 4. **Localization of GLT-1 molecules in lipid raft microdomains of the cell membrane after dexamethasone (*Dex*) treatment.** *A*, cofractionation of GLT-1 with the lipid raft marker caveolin-1 after sucrose density ultracentrifugation. Cortical astrocytes treated with dexamethasone (10 nM; 72 h) were homogenized, and homogenates were subsequently loaded on a discontinuous sucrose density gradient. After ultracentrifugation, fractions were recovered from the top of the gradient and directly used for Western blot analysis with GLT-1-, caveolin-1-, and GSK3β-specific antibodies. *B*, cortical astrocytes were exposed to 10 nM dexamethasone for 72 h and subsequently immunostained with primary GLT-1 antibodies and Cy3-conjugated (*red*) secondary antibodies. In order to visualize cell nuclei, 4′,6-diamidino-2-phenylindole counterstaining (*blue*) was performed (×40 magnification). The *inset* shows the proximal process of an astrocyte at higher magnification (×63 magnification).

trol protein that is thought to be absent from lipid rafts (38). We further analyzed the effects of dexamethasone (10 nM, 72 h) on GLT-1 expression at the cellular level using immunofluorescent microscopy with antibodies directed against GLT-1. Specific staining revealed that GLT-1 expression was increased as compared with untreated controls (Fig. 4*B*). The primarily dotted appearance of the staining is indicative of the presence of GLT-1 in raft microdomains of the plasma membrane (Fig. 4*B*, *inset*). Taken together, these data show that dexamethasone treatment raises cellular GLT-1 levels, and GLT-1 proteins are localized in certain membrane subdomains termed lipid rafts of dexamethasone-treated astrocytes.

Next, analysis of the role of natural gluco- and mineralocorticoids in regulating GLT-1 expression was carried out. Primary astrocytes derived from cerebellum, midbrain, and cortex were maintained with different concentrations of aldosterone and corticosterone for a time period of 72 h. In cerebellar and midbrain astroglial cells, both steroid hormones failed to induce GLT-1 expression at each concentration used (10 and 100 nM) as determined by Western blot analysis (Fig. 5*A*). In striking contrast, glial cells derived from the rat cortex responded with a robust induction of GLT-1 expression when exposed to 100 nM corticosterone. Application of 10 nM corticosterone or 10 and 100 nM aldosterone also resulted in elevated levels of GLT-1, although the extent of induction was less pronounced as compared with the regulatory effects of 100 nM corticosterone (Fig. 5, *A* and *B*). Therefore, natural gluco- and mineralocorticoids are capable of positively modulating GLT-1 expression, and this regulation occurs in a brain region-specific manner.

The genomic effects of synthetic and natural corticosteroid hormones are generally mediated through the activation of both MRs and GRs. We investigated the participation of these receptor subtypes in the regulation of GLT-1 gene expression by using two different pharmacological receptor antagonists. Mifepristone and spironolactone are widely utilized antagonists suppressing GR and MR activity, respectively. However, spironolactone has also been reported to bind GRs (39) and inhibit dexamethasone-mediated effects on gene expression (40). Primary cortical astrocytes were cotreated with each inhibitor (10 μM)

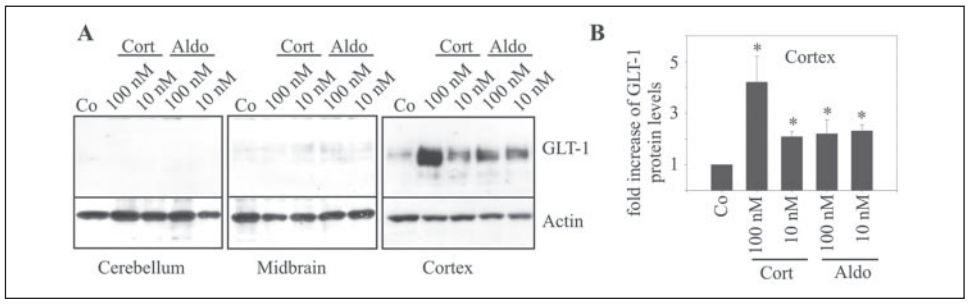

FIGURE 5. **Natural gluco- and mineralocorticoids enhance GLT-1 expression in cortical astrocytes.** *A*, cerebellar, midbrain, and cortical astrocytes were stimulated for 72 h with either corticosterone (*Cort*) or aldosterone (*Aldo*), each at concentrations of 10 and 100 nM. The expression of GLT-1 was monitored by Western blot analysis using GLT-1-specific antibodies. Actin immunoreactivity served as a control for protein loading. *B*, optical densities of GLT-1-specific bands were normalized to actin, and GLT-1 protein levels in untreated cortical astrocytes were set to 1. Data represent the mean ± S.D. of the -fold increase from three independent experiments. *, $p < 0.05$ *versus* control ($n = 3$).

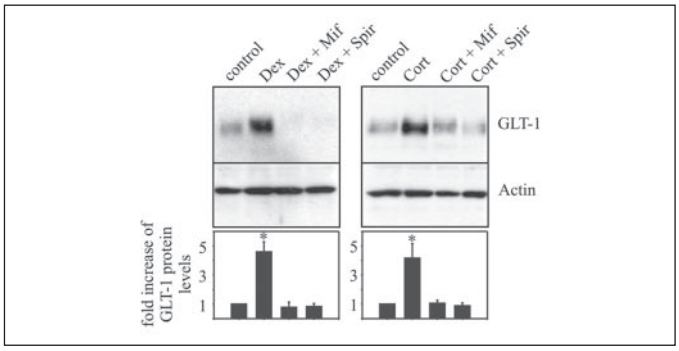

FIGURE 6. **Glucocorticoid induced up-regulation of GLT-1 expression is receptor-mediated.** Astrocytes derived from the rat cortex were treated with either 10 nM dexamethasone (*Dex*) or 100 nM corticosterone (*Cort*) in combination with the GR antagonist mifepristone (*Mif*; 10 μM) or the MR/GR antagonist spironolactone (*Spir*; 10 μM). Equal amounts of proteins were subjected to Western blot analysis, and immunodetection has been carried out with GLT-1- and actin-specific antibodies. Results were calculated as ratios of OD of the GLT-1 band *versus* actin band and expressed as the mean ± S.D. of the -fold increase compared with the untreated control (set to 1; *, $p < 0.05$ *versus* control ($n = 3$)).

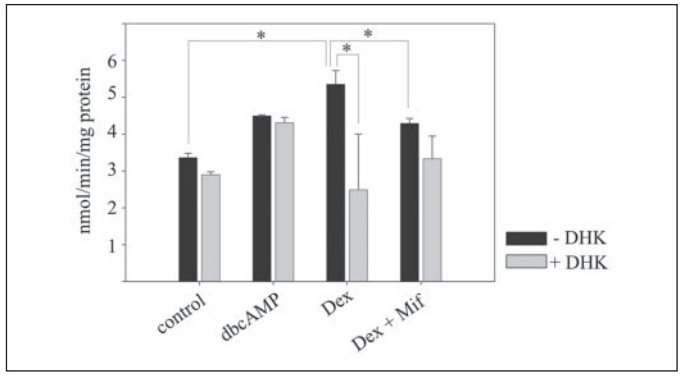

FIGURE 7. **Dexamethasone enhances glutamate uptake in primary cortical astrocytes via GLT-1.** After exposure to 10 nM dexamethasone (*Dex*) alone or in combination with 10 μM mifepristone (*Mif*) for 72 h or with 10 μM dibutyryl cyclic AMP (*dbcAMP*) as a control, glutamate uptake experiments have been performed by incubating cortical astrocytes with radiolabeled glutamate. In addition, some cultures were treated with 100 μM dihydrokainate (*DHK*), a specific GLT-1 blocker, 10 min prior to the glutamate uptake studies. Subsequently, cells were lysed, and incorporated radioactivity was determined by liquid scintillation counting. Results depict glutamate uptake in nmol/min normalized to the amount of proteins in control wells. Data represent the mean ± S.D. (*, $p < 0.05$, $n = 3$).

in combination with either dexamethasone (10 nM) or corticosterone (100 nM) for 72 h. As shown in Fig. 6, both antagonists completely abolished the induction of GLT-1 expression by dexamethasone or corticosterone. These findings strongly suggest a receptor-mediated effect of glucocorticoids in regulating the expression of the glutamate transporter subtype GLT-1 in primary cortical astrocytes.

Since exposure of glia to dexamethasone resulted in an enhanced GLT-1 expression via activation of corticosteroid receptors, we further investigated whether glutamate uptake in cortical astrocytes is altered after dexamethasone exposure and whether mifepristone would reverse these effects. Thus, cortical astrocytes were stimulated for 72 h with 10 nM dexamethasone alone or in combination with 10 μM mifepristone. Treatment with dibutyryl cyclic AMP has been carried out as a positive control. Employing a sodium-dependent uptake experiment using radiolabeled glutamate, we detected a significant increase in glutamate uptake after dexamethasone treatment (Fig. 7). The GR antagonist mifepristone dramatically reduced the stimulatory effects of dexamethasone. In order to prove that GLT-1 and not GLAST accounts for enhanced glutamate uptake, we used dihydrokainate as a specific inhibitor of the GLT-1 subtype. Indeed, dihydrokainate (100 μM) significantly reduced glutamate uptake when applied to dexamethasone-treated astrocyte cultures (Fig. 7). Hence, increases in GLT-1 levels are paralleled by an enhanced inward glutamate transport in dexamethasone-exposed cortical astrocytes.

The observation that natural ligands of MRs and GRs failed to activate GLT-1 expression in cerebellar and midbrain astrocytes prompted us to examine possible brain region-specific differences in either the receptor status or receptor function upon ligand binding. In order to characterize the receptor status and extent of receptor function of astrocyte cultures from cortex, cerebellum, and midbrain, we performed semiquantitative as well as quantitative PCR analysis and luciferase reporter assays, respectively. Using primers specifically targeting either GR or MR cDNA (TABLE ONE), we detected similar amounts of transcripts by semiquantitative and quantitative real time PCR (Fig. 8*A*). Nevertheless, in order to obtain a specific PCR product representing the MR cDNA, 3-fold more total RNA had to be initially used for cDNA synthesis. Furthermore, we determined whether differences in the extent of receptor function might account for the observed brain region-specific effects of dexamethasone on GLT expression. We therefore employed a reporter assay using a hormone-dependent MMTV promoter-driven luciferase reporter plasmid, which has been shown to be under glucocorticoid regulation (41). After transfection of primary astrocytes from cortex, cerebellum, and midbrain and subsequent stimulation with 100 nM dexamethasone for 48 h, we observed a 3–4-fold increase in luciferase activity in all brain regions analyzed (Fig. 8*B*). Moreover, this effect was blocked by simultaneous treatment with the GR antagonist mifepristone, proving the specificity of the dexamethasone-induced reporter gene expression. In conclusion, we did not observe any marked differences in receptor expression levels or extent of receptor function in cortical, cerebellar, and midbrain astrocytes.

The lack of GLT-1 induction in primary astrocytes derived from cerebellum has also been reported for other regulatory factors such as tumor growth factor α or cAMP (35). This observation raises the question of whether global epigenetic processes including DNA methylation or histone deacetylation ensure the maintenance of the GLT-1 gene in

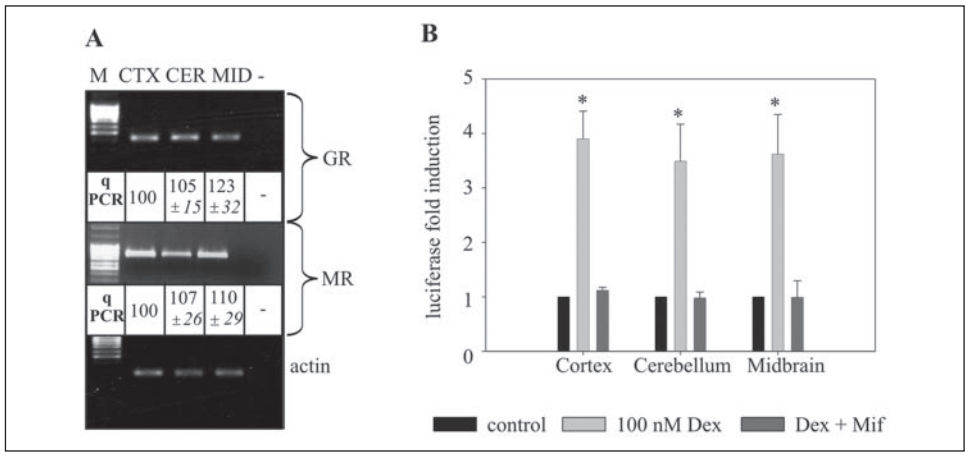

FIGURE 8. **Analysis of MR and GR status in cerebellar, midbrain, and cortical astrocytes.** *A*, semiquantitative reverse transcription-PCR and qPCR analysis was carried out with total RNA of unstimulated cerebellar (*CER*), midbrain (*MID*), and cortical (*CTX*) astrocytes. Amplification with MR-, GR-, and actin-specific primers (35, 30, and 25 PCR cycles, respectively) revealed PCR products possessing the expected size. *M*, 100-bp DNA size marker; −, negative control. Results of the qPCR are expressed as the mean ± S.D. of the percentage of cortical astrocytes (considered as 100%, $n = 3$). *B*, extent of receptor function was tested by employing an MMTV-luciferase reporter assay. Primary astrocytes of the indicated brain regions were transiently transfected with the reporter plasmid and subsequently stimulated with dexamethasone (100 nM) alone or in combination with mifepristone (10 μM) for 48 h. After harvesting, cell extracts were processed for measuring luciferase activity. Results represent the mean ± S.D. of the -fold induction (*, $p < 0.05$, $n = 3$).

TABLE TWO

Statistical analysis of the 5′-untranslated region of the rat GLT-1 gene (620 bp upstream of the ATG start codon; GenBank™ accession number AY643513)

Parameter	Value
Total number of analyzed nucleotides	620
Total number of cytosine residues	234
Total number of guanine residues	200
Number of CpG dinucleotides	72
GC content	70% (>50%)
Ratio[a] observed CpG/expected CpG	0.95 (>0.6)

[a] Ratio = (no. of CpG dinucleotides/(no. of cytosins × no. of guanines)) × no. of analyzed nucleotides.

an inactive state as observed in primary cerebellar astrocytes. The concerted action of DNA methylation and histone deacetylation at promoters or exonic/intronic sequences that are embedded within CpG islands often results in the repression of the associated genes (42). Interestingly, the 5′-leader sequence of a transcript variant (V0) of the rat GLT-1 gene (43) displays a high content of CpG dinucleotides. When statistically analyzing 620 nucleotides upstream of the AUG start codon, we calculated a GC content of 70% and a ratio of observed CpG to expected CpG of 0.95 (TABLE TWO). Hence, the 5′-primed region of the rat GLT-1 gene constitutes a CpG island by the definition of Gardiner-Garden and Frommer (44). In order to obtain an indication for the involvement of a DNA methylation-dependent mechanism of GLT-1 repression, we cultivated cerebellar astrocytes grown at low density in the presence of 80 μM 5-aza-2-deoxycytidine (5-AZA), a potent inhibitor of DNA methyltransferases (45), for 96 h. After 48 h, we simultaneously added 100 nM dexamethasone and 5-AZA to the cell culture medium. In addition, we treated cerebellar astrocytes with 100 nM trichostatin A (46), an inhibitor of histone deacetylases, for 48 h alone or in combination with dexamethasone. As shown in Fig. 9*A*, solely combined 5-AZA and dexamethasone exposure led to a slight reactivation of GLT-1 protein expression in cerebellar astrocytes. In addition, transcription of the GLT-1 gene was more than 2-fold increased in 5-AZA/dexamethasone-exposed cerebellar astrocytes as compared with untreated or dexamethasone-treated cells (Fig. 9*B*). Furthermore, 5-AZA treatment alone also reactivated GLT-1 transcription, whereby the effect was less prominent compared with the combined treatment. These findings suggest that DNA methylation may possibly contribute to preventing dexamethasone-induced GLT-1 expression in astrocytes of this particular brain region.

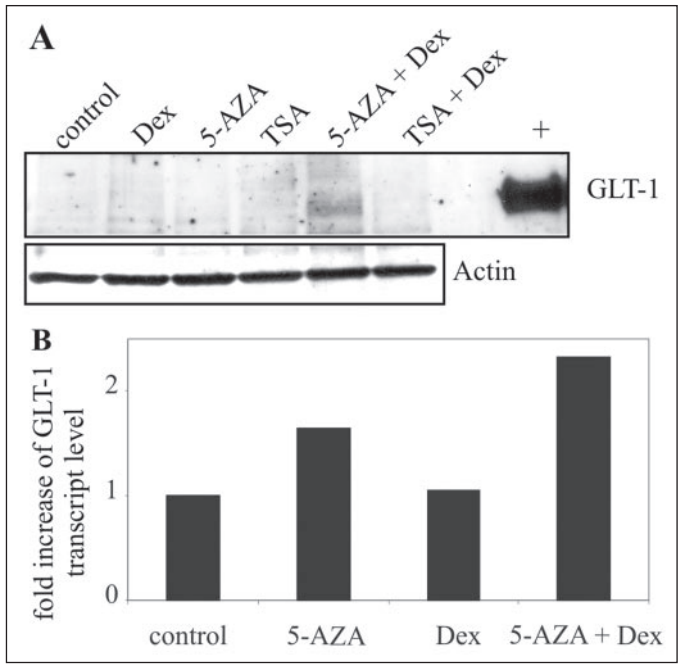

FIGURE 9. **Combined treatment of cerebellar astrocytes with dexamethasone and 5-aza-2-deoxycytidine partially reactivates GLT-1 expression.** *A*, cerebellar astrocytes were treated with 5-aza-2-deoxycytidine (80 μM) for 96 h or trichostatin A (100 ng/ml) for 48 h alone or in combination with dexamethasone (100 nM, 48 h). After extraction of proteins, Western blot analysis was performed with GLT-1- and actin-specific antibodies. GLT-1-enriched fraction 2 (see Fig. 4*A*) served as a positive control for GLT-1 immunoreactivity. A representative blot of two experiments is shown. *B*, cerebellar astrocytes were treated with 5-AZA (80 μM) alone or in combination with dexamethasone (100 nM). Subsequently, RNA was extracted and subjected to real time PCR analysis with specific primers amplifying either GLT-1 or actin cDNAs. Alterations of GLT-1 transcript levels are expressed as -fold change after normalization to actin as compared with the untreated control (set to 1; representative results of two independent experiments are shown).

DISCUSSION

Despite the well established effects on glutamine synthetase expression, actions of gluco- and mineralocorticoid hormones on glutamate transporter expression in astrocytes have yet not been studied in detail. Here, we demonstrated for the first time that activation of glucocorticoid receptors by synthetic and natural ligands results in an increase of GLT-1 expression associated with an enhanced glutamate uptake in primary cortical astrocytes.

GLT-1 is the predominant astroglial glutamate transporter in the rodent forebrain, brainstem, and spinal cord. During development, expression of GLT-1 parallels maturation of the central nervous system

(47). Around birth, GLT-1 expression is highest in the spinal cord; this is followed by a marked up-regulation by about postnatal day 10 in the forebrain and subsequently in the cerebellum by about postnatal day 24. Regulatory factors including cAMP or forskolin augment GLT-1 gene expression in primary cortical astrocytes *in vitro*, which is accompanied by changes in astrocyte morphology and antigenic properties similar to those observed in the mature brain (32, 33). Apart from the developmental regulation of GLT-1 expression, alterations in GLT-1 levels are also observed in the adult organism, notably under pathophysiological conditions. For instance, a recent study by Reagan *et al.* (48) described that GLT-1 levels are elevated in certain brain regions of adult rats that have been subjected beforehand to chronic restraint stress for 21 days (48).

Corticosteroids are crucial modulators of the stress response by controlling the activity of the hypothalamic-pituitary-adrenal (HPA) axis. In addition, they influence biochemical and morphological processes in the developing brain. The genomic actions of glucocorticoid and mineralocorticoid hormones in terms of transcriptional regulation are classically mediated through glucocorticoid and mineralocorticoid receptors (GR and MR, respectively). In agreement with Chou *et al.* (15), we found both receptor subtypes to be co-expressed in primary astrocytes derived from cortex (Fig. 8*A*). The functional integrity of the receptors with respect to ligand binding and transactivation of gene transcription was confirmed (Fig. 8*B*) by an MMTV-luciferase reporter assay (41). Primary cortical astrocytes responded with a time- and dose-dependent up-regulation of GLT-1 gene expression upon stimulation with the synthetic GC dexamethasone (Fig. 2). Moreover, the natural adrenal steroid hormones corticosterone and aldosterone were also capable of enhancing GLT-1 expression in cortical astrocytes, although the extent of induction was partly dependent on the concentrations used (Fig. 5). As opposed to GLT-1, basal GLAST expression levels were not altered by corticosteroids in cortical astrocytes at either the transcriptional or translational level (Figs. 1*A* and 3).

Pharmacological analysis enabled us to characterize which receptor subtype might be primarily involved in the up-regulation of GLT-1 expression in cortical astrocytes. In general, aldosterone at a physiological dose is an MR-specific agonist (K_d = 0.5–1.0 nM) with very low affinity to the GR subtype (K_d > 15 nM). Corticosterone exhibits a 10-fold higher affinity to the MR (K_d = 0.5–1.0 nM), whereas dexamethasone has a 3–4-fold higher affinity to the GR (K_d = 0.5–1.0 nM) (7). Since 10 nM dexamethasone caused a marked up-regulation of GLT-1 expression, and aldosterone only induced a slight activation of GLT-1 expression at concentrations of 10 nM and 100 nM, we conclude that the GR might predominantly mediate the genomic effects with regard to GLT-1 regulation. This hypothesis is strengthened by the observation that corticosterone provoked a strong induction of GLT-1 expression only when applied at higher concentrations (100 nM), whereas a less prominent induction was detected at a concentration of 10 nM (Fig. 5*B*). Moreover, the participation of GR was confirmed by examining the effect of the selective GR antagonist mifepristone (49). Mifepristone completely abolished the stimulatory effects of dexamethasone and corticosterone on GLT-1 expression (Fig. 6), indicating that the up-regulation of GLT-1 gene expression in cortical astrocytes is indeed triggered by the genomic actions of GC hormones and the respective GRs. Given that the induction of GLT-1 expression after exposure of glia to dexamethasone or corticosterone was also blocked by the commonly used GR/MR antagonist spironolactone, a more complex regulation of the GLT-1 gene activity by receptor heterodimers cannot be excluded.

The increase of GLT-1 levels induced by corticosteroids resulted in the localization of a certain population of GLT-1 proteins in lipid rafts of cortical astrocytes (Fig. 4*A*). Localization of GLT-1 in this cholesterol-enriched membrane microdomain might be an important prerequisite for efficient removal of glutamate from the extracellular space (37). Indeed, dexamethasone treatment led to an advanced glutamate uptake in cortical astrocytes, which was reduced when antagonizing GR activity with mifepristone (Fig. 7). The enhanced glutamate uptake was mainly facilitated by the glutamate transporter subtype GLT-1, since dexamethasone did not affect basal GLAST protein levels in cortical astrocytes, and dihydrokainate as a specific inhibitor of GLT-1 reversed the promoting effect of dexamethasone. A study by Zhu *et al.* (50) substantiated a positive impact of short term GC exposure on glutamate uptake. When stimulating synaptosomes of the rat cerebral cortex or human neuroblastoma cells with dexamethasone, glutamate uptake was increased by ~20–40%. The enhanced glutamate uptake occurred rapidly within minutes, suggesting a nongenomic effect of dexamethasone involving post-translational modification of the GLT-1 protein (50). Consistently, in the current study, mifepristone treatment did not completely abrogate the effects of dexamethasone, suggesting that nongenomic actions of the GC hormone might additionally and partially account for enhanced glutamate uptake in our experimental model. Taken together, it seems conceivable that transcriptional regulation of the GLT-1 gene by the genomic action of corticosteroids represents an alternative long term mechanism of facilitating glutamate uptake in astroglial cells.

Corticosteroid regulated target genes in astroglial cells include the glial fibrillary acidic protein (51), nerve growth factor (52), interleukin-1 receptor (53), myelin basic protein (54), and glutamine synthetase (28). Glutamine synthetase plays a crucial role in recycling of the neurotransmitter glutamate and is a well characterized example of a GC-regulated gene. Similarly to GLT-1, glutamine synthetase expression is developmentally regulated. An increase of glutamine synthetase expression during development correlates with maturation of the adrenal gland and with elevated blood levels of corticosteroids in a manner thought to be causative (55, 56). At the molecular level, corticosteroids activate the transcription of the glutamine synthetase gene (57, 58). Thus, together with our findings, corticosteroids might play an important part in promoting the recycling of glutamate both by induction of glutamine synthetase and by GLT-1 expression in specific subpopulations of astrocytes and in a distinct temporal manner.

In contrast to cortical astrocytes, corticosteroids did not exert any stimulatory effects on GLT-1 expression in cerebellar and midbrain astrocytes (Figs. 2*C* and 5). Nevertheless, astrocytes of both brain regions expressed GRs and MRs at similar levels compared with cortical astrocytes. In addition, we found these steroid receptors to be transcriptionally active as assessed by an MMTV-luciferase reporter assay (Fig. 8). Similarly, other regulatory factors of GLT-1 gene expression, including cAMP, pituitary adenylate cyclase-activating polypeptide, epidermal growth factor, or transforming growth factor α, also do not significantly promote GLT-1 expression in cerebellar astrocytes (35). Thus, a more global mechanism could be involved in keeping the GLT-1 gene of cerebellar astrocytes in an inactive state. DNA methylation of promoter sequences or exons/introns represents an efficient mechanism of constitutive gene repression. Herein, the methylation status of CpG islands often determines the transcriptional activity of the corresponding genes (42). Per definition, CpG islands display a high content of guanine and cytosine residues and are enriched in CpG dinucleotides. We have identified a classical CpG island upstream of the start codon of the GLT-1 transcript variant V0 (43) (TABLE TWO). When we induced partial demethylation of genomic DNA by inhibiting DNA methyltransferases

in cerebellar astrocytes, dexamethasone was capable of slightly reactivating GLT-1 mRNA and protein expression (Fig. 9). This finding is a first indication that DNA methylation might indeed contribute to the silencing of GLT-1 expression in cerebellar astrocytes. The methylation status of certain CpG dinucleotides within the rat GLT-1 gene now awaits further analysis. In this context, the expression of another astroglial specific gene, namely glial fibrillary acidic protein, has also been shown to be regulated in an epigenetic fashion (59).

Of note, a study by Liang *et al.* implicates the female steroid hormone estrogen to elevate GLT-1 and GLAST expression levels in astrocytes derived from Alzheimer disease patients (60), indicating that steroid hormones in general exert promoting effects on glutamate transporters. Interestingly, Rothstein *et al.* (61) recently published the first evidence of pharmaceutical modulation of GLT-1 expression by β-lactam antibiotics. Since these antibiotics exerted neuroprotective effects by increasing GLT-1 expression, they could represent new potential therapeutic targets for treatment of neurodegenerative disorders such as amyotrophic lateral sclerosis.

In summary, we demonstrated that synthetic and natural glucocorticoids promote glutamate uptake in cortical astrocytes by specifically activating the transcription and subsequent translation of the GLT-1 gene. We hypothesize that the differential regulation of GLT-1 expression in various brain regions might at least in part be due to epigenetic alterations of the GLT-1 gene. These results might have important implications for understanding the modes of GLT-1 regulation during central nervous system development and during pathophysiological processes in the adult. Further studies should also concentrate on the effects of glucocorticoids, especially dexamethasone on animal disease models, specifically in those pathological states with suggested excitotoxic as well as autoimmune components.

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