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Structural Organization of the Mouse Metabotropic Glutamate Receptor Subtype 3 Gene and Its Regulation by Growth Factors in Cultured Cortical Astrocytes[1]

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Metabotropic glutamate receptors (mGluRs) belong to the class of G protein-coupled receptors and consist of eight different subtypes. We have characterized the structural organization of the mouse mGluR3 gene by genomic cloning in combination with rapid amplification of 5′- and 3′-cDNA ends and examined regulatory expression of mGluR3 mRNA in cultured cortical astrocytes. The mGluR3 gene consists of six exons and spans over 95 kb. Exon 1 and its preceding putative promoter are located distantly from the following protein-coding region. In the mGluR family, mGluR3 and mGluR5 are both expressed in neuronal and glial cells and are upregulated during the early postnatal period. They are, however, coupled to two distinct signaling cascades and have been shown to exert opposite influences on some functions of cultured astrocytes. In cultured astrocytes, mGluR3 and mGluR5 mRNA levels were significantly increased by exposure to epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or transforming growth factor-α; and EGF was more efficacious than bFGF in producing this increase. Hence, mGluR3 and mGluR5 mRNAs are concertedly upregulated in cultured astrocytes by specific growth factors. This finding suggests that the two mGluR subtypes may play an important role in maintaining the proper balance of astrocyte functions *via* two distinct signal transduction mechanisms.

Key words: gene organization, growth factor, metabotropic glutamate receptor, mRNA upregulation, transcription initiation.

Glutamate, the major excitatory neurotransmitter in the mammalian brain, plays an important role in neuronal plasticity, neural cell development, and neurotoxicity (*1-3*). Metabotropic glutamate receptors (mGluRs) belong to the class of seven transmembrane domain receptors and are coupled to the G protein-mediated signal transduction cascades (*3-6*). The mGluRs consist of eight different subtypes, which are classified into three groups (*5-7*). Group 1 receptors (mGluR1 and mGluR5) are coupled to the stimulation of inositol trisphosphate/Ca^{2+} signal transduction (*8-10*). Group 2 (mGluR2 and mGluR3) and group 3 (mGluR4, mGluR6, mGluR7, and mGluR8) receptors are both linked to the inhibitory cyclic AMP cascade but differ in their selective agonists (*7, 11-15*).

mGluR3 is widely distributed in various brain regions and is expressed in both neuronal and glial cells (*16-18*). This expression is developmentally regulated and is high during the early postnatal period (*19*). It has been reported that group 2 mGluRs (presumably mGluR3) in astrocytes contribute to protection of neighboring neuronal cells against glutamate excitotoxity *via* the transforming growth factor-β (TGF-β) pathway (*20, 21*). However, neither agonists, antagonists, nor antibodies specific for mGluR3 are currently available. The lack of selective tools for mGluR3 prevents a clear definition of the function and regulation of mGluR3 in neuronal and glial cells. In an attempt to explore regulatory mechanisms underlying the mGluR3 function, we isolated genomic clones covering the whole exon sequences of the mouse mGluR3 gene and here report the gene organization, the transcription initiation site, and the upstream 5′ genomic sequence of mGluR3. We also show that mGluR3 mRNA is upregulated in cultured astrocytes by specific growth factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and transforming growth factor-α (TGF-α).

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Abbreviations: bFGF, basic fibroblast growth factor; DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; FCS, fetal calf serum; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; HSF, heat shock factor; mGluR, metabotropic glutamate receptor; NF-1, nuclear factor 1; 5′- or 3′-RACE, rapid amplification of 5′- or 3′-cDNA ends; RT-PCR, reverse transcriptase-mediated polymerase chain reaction; SF medium, serum-free medium; TGF-α or -β, transforming growth factor-α or -β.

MATERIALS AND METHODS

Materials—Materials were obtained from the following sources: [α-^{32}P]dCTP (110 TBq/mmol), Amersham Pharmacia Biotech (Little Chalfont, Bucks.); oligonucleotide primers, ESPEC (Ibaragi); Taq polymerase, Perkin Elmer (Branchburg, NJ); bFGF, EGF, and TGF-α, Upstate Biotechnology (Lake Placid, NY); Dulbecco's modified Eagle's medium (DMEM), Nissui (Tokyo); fetal calf serum (FCS), Flow/ICN (Aurora, OH).

Genomic Library Screening and DNA Sequencing—A 129SV mouse genomic library (Stratagene, La Jolla, CA) was screened separately with five ^{32}P-labeled probes composed of nucleotide residues 2-535, 2-750, 1615-2722, and 2722-3123 of the rat mGluR3 cDNA clone, pmGR3 (*11*), and the 5′-upstream cDNA sequence isolated by rapid amplification of 5′-cDNA ends (5′-RACE) described below. Hybridization and filter washing were performed as described previously (*22, 23*). Three to nine hybridization-positive clones were isolated from each screening and representative clones were subcloned into pBluescriptII KS(+) (Stratagene). The nucleotide sequences of cloned genomic DNAs were determined by the dideoxychain termination method (*24*). All DNA sequences were confirmed by determining both strands. Transcription factor recognition sites were searched using MatInspector database in the TRANSFAC (*25*).

5′-RACE and 3′-RACE—5′-RACE and 3′-RACE were carried out using a mouse brain cDNA mixture (Mouse Brain Marathon-Ready™ cDNA, Clontech, Palo Alto, CA) according to the vendor's manual. The PCR conditions used for both 5′-RACE and 3′-RACE were as follows: for the first PCR, 5 cycles of 95°C, 30 s and 72°C, 2.5 min, 5 cycles of 95°C, 30 s and 70°C, 2.5 min, and 25 cycles of 95°C, 30 s and 68°C, 2.5 min; for the subsequent nested PCR, 20 cycles of 95°C, 30 s and 68°C, 2.5 min. For 5′-RACE, three different 3′ primers, one corresponding to a part of the exon 1 sequence (Primer C1) and the other two corresponding to different parts of the exon 2 sequence (Primers A1 and B1), were mixed with Primer AP1 (5′-CCATCCTAATACGACTCACTATAGGGC-3′; the adaptor DNA sequence attached to the cDNA mixture) and subjected to the first PCR. Three appropriate 3′ primers upstream of the above 3′ primer sequences (Primers C2, A2, B2) and the adaptor Primer AP2 (5′-ACTCACTATAGGGCTCGAGCGGC-3′) were then mixed and subjected to nested PCR; for the nucleotide sequences used as 3′ primers, see Fig. 2A. The nested PCR products were electrophoresed on a 0.9% agarose gel. The specific 5′-RACE products with sizes of approximately 100 bp (Primers C2/AP2), 300 bp (Primers B2/AP2) and 500 bp (Primers A2/AP2) were extracted from the gel, subcloned into pGEM®-T Easy Vector (Promega, Madison, WI), and sequenced. Primer 3′-1 and Primer AP1 were used for the first PCR of 3′-RACE, and Primer 3′-2 and Primer AP2 were employed for the subsequent nested PCR; for the sequences used as 5′ primers, see Fig. 2B. The nested PCR products with the size of approximately 700 bp were extracted, subcloned into pGEM®-T Easy Vector, and sequenced.

Astrocyte Culture—Astrocyte cultures were performed according to the procedures described previously (*26*) with a minor modification. In brief, mixed astrocytes were prepared from the neocortex of ICR strain mouse pups (1-2 d old) and cultured in DMEM adjusted to pH 7.4 with 25 mM HEPES and 14.3 mM $NaHCO_3$ and supplemented with 10% FCS. After 9 d of culture, the culture flask was shaken mechanically at 260 rpm overnight. Type 1 astrocytes were enriched by removing the top layer of cells comprising mostly oligodendrocytes, microglia, and process-bearing type 2 astrocytes. One d after this purification, secondary astrocyte cultures were started in DMEM supplemented with 10% FCS. After 3 d, astrocytes were rinsed and maintained in a minimally supplemented serum-free control medium (SF medium) consisting of DMEM, 25 mM HEPES, 14.3 mM $NaHCO_3$ (pH 7.4), 1 mM pyruvate, 2 mM glutamine, transferrin (50 μg/ml), D-biotin (10 ng/ml), sodium selenite (5.2 ng/ml), fibronectin (1.5 μg/ml), heparan sulfate (0.5 μg/ml), and insulin (5 μg/ml). Secondary cultures were maintained in the presence and absence of growth factors for 3 d before use in experiments.

Quantitation of mRNA Levels by Reverse Transcriptase-Mediated PCR (RT-PCR)—Total RNA was isolated from cultured astrocytes by the acid guanidinium thiocyanate-phenol-chloroform method using an RNA extraction solution (TRIzol® Reagent, GibcoBRL, Grand Island, NY) and treated with RQ1 RNase-free DNase (Promega). RT-PCR was carried out as described previously (*22, 27*). The 5′ and 3′ primer sequences used for PCRs of mGluR3, mGluR5, and glyceraldehyde 3-phosphate dehydrogenase (G3PDH) were as follows: 5′ primer of mGluR3, 5′-GAGAAGCGGGAAACAGTCATCC-3′ (residues 2525-2546 within exon 4); 3′ primer of mGluR3, 5′-ATATTCTGGAGCAGCATGTGAGC-3′ (residues 3050-3072 within exon 6); the nucleotide residues of the mouse mGluR3 mRNA are numbered from the exon sequences of the mouse genomic DNA determined in this study by counting the transcription initiation site as residue 1; 5′ primer of mGluR5, 5′-GTCTCCTGATGTCAAGTGGTT-3′ (residues 1254-1274 of the rat mGluR5 cDNA) (*10*); 3′ primer of mGluR5, 5′-GGACCACACTTCGTCATCATC-3′ (residues 1747-1767); 5′ primer of G3PDH, 5′-GACCACAGTCCATGCCATCACT-3′ (residues 590-611 of the rat G3PDH cDNA) (*28*); 3′ primer of G3PDH, 5′-TCCACCACCCTGTTGCTGTAG-3′ (residues 1022-1042). A sample of total RNA (1 μg) was used for reverse transcription in 24 μl of reaction mixture with 21 ng/μl of oligo(dT) primer and 8.3 units/μl of Superscript™II RNase H$^-$ reverse transcriptase (GibcoBRL). The single-stranded cDNA products were subjected to PCR using 0.2 mM concentrations of 5′ primer and 3′ primer. After 34 cycles (mGluR3), 36 cycles (mGluR5), and 30 cycles (G3PDH) (94°C for 30 s, 60°C for 30 s, and 72°C for 2 min) of PCR, the products were electrophoresed on a 0.9% agarose gel, stained with ethidium bromide and quantified by densitometoric analysis using an image analyzer (Printgraph, AE-6910, ATTO, Tokyo) with the Densitograph version 4.10 (ATTO). Care was taken to quantify changes in individual mRNA levels by employing appropriate RT-PCR conditions under which a linear relationship existed between amounts of RNA added and intensities of the RT-PCR products. However, comparison of relative levels of different mRNA species was difficult, due to possible differences in efficacy of primer annealing and PCR elongation, or in other conditionally undefined factors.

RESULTS

Genomic Cloning of the Mouse mGluR3 Gene—A mouse genomic library was screened with four ^{32}P-labeled probes derived from the rat mGluR3 cDNA. Fifteen independent hybridization-positive clones were isolated. Hybridization-positive, restriction enzyme-digested DNA fragments were isolated from cloned genomic DNAs, subcloned, and subjected to DNA sequencing. By comparison with the rat mGluR3 cDNA sequence, we were able to assign five exon sequences (exons 2–6) of the mouse mGluR3 gene (Fig. 1).

5′-RACE Analysis of Transcription Initiation Site—Transcription initiation sites of the mouse mGluR3 mRNA were examined by 5′-RACE analysis of a mouse brain cDNA mixture (Fig. 2A). Two sets of nested PCRs were performed using two combinations of 5′ and 3′ primers. For 5′ primers, we used the adaptor DNA sequences (Primer AP1 and Primer AP2) near the ligation site of the attached brain cDNA mixture. For 3′ primers, two paired sequences were used corresponding to the 5′-upstream portions of the rat mGluR3 cDNA (Primers A1/A2 and Primers B1/B2 in Fig. 2A). Independent clones containing different 5′-termini of mGluR3 cDNAs were isolated, and all 5′-RACE products were found to extend beyond the 5′ end of the rat mGluR3 mRNA determined by cDNA cloning (141 nucleotides upstream of the translation initiation site) (*11*) (Fig. 2A). To confirm the presence of the extended 5′-upstream sequence of mouse mGluR3 mRNA as compared to the corresponding rat mGluR3 cDNA sequence, we performed an additional nested 5′-RACE analysis. In this analysis, 3′ primers, Primer C1 and Primer C2, were designed from the newly identified 5′-upstream sequence of the above 5′-RACE products (Fig. 2A), while Primer AP1 and Primer AP2 were used as 5′ primers. Data revealed that the 5′ end of mouse mGluR3 mRNA extended 364 nucleotides upstream of the translation initiation site (Fig. 2A).

The newly identified 5′-upstream sequence of the 5′-RACE product was found not to hybridize with any of the cloned genomic DNAs described above. We therefore rescreened a mouse genomic library with the newly identified 5′-upstream sequence of 5′-RACE products as a probe and isolated three independent hybridization-positive clones (YM-1 to YM-3 in Fig. 1). Sequence analysis of hybridization-positive DNA fragments showed that the whole 5′-upstream sequence of the 5′-RACE product is present within a single exon of the mGluR3 gene. We assigned the transcription initiation site to the upmost 5′-upstream residue of the 5′-RACE products, 364 nucleotides upstream of the translation initiation site of mGluR3 mRNA (Fig. 2A).

3′-RACE Analysis of Transcription Termination Site—We next examined the termination site of the mouse mGluR3 mRNA by 3′-RACE analysis. As 5′ primers for nested PCR, two sequences were used corresponding to the carboxy-terminal portion of the mGluR3 protein to cover the whole 3′-untranslated sequence in the resultant nested PCR products (Fig. 2B). Primer AP1 and Primer AP2 in the adaptor DNA sequences were again used as 3′ primers. The reaction gave rise to a predominant band with the size of approximately 700 bp. This product was subcloned and sequenced. The sequence determined was contained within exons 5 and 6 and ended 600 bp downstream from the exon 5/6 boundary (Fig. 2B). The canonical sequence for a polyadenylation signal (AATAAA) (*29*) was found at three positions and the most downstream AATAAA sequence was located at residues 20–25 upstream from the mRNA termination site (Fig. 2B). Data indicate the 3′-untranslated region of mouse mGluR3 mRNA consists of 529 nucleotides.

Structural Organization of the Mouse mGluR3 Gene—The results of genomic cloning in combination with 5′-RACE and 3′-RACE analyses indicate that the mouse mGluR3 gene consists of six exons and spans over at least 95 kb. Intron sequences between exon 1 and exon 2 and between exon 3 and exon 4 were missing in the genomic clones isolated (Fig. 1). The sequences of the exon/intron boundaries all possess the consensus splicing signal conforming to the GT/AG rule (*30*) (Fig. 2C).

On the basis of the above exon arrangement of the mGluR3 gene, the mouse mGluR3 mRNA was calculated to consist of 3,530 nucleotide residues. Exon 1 (223 bp) encodes the 5′-untranslated region, and exon 2 (609 bp) covers the downstream 5′-untranslated region, the translation initiation site, and the amino-terminal sequence including the signal peptide of mGluR3. Exon 3 (856 bp) corresponds to most of the large extracellular domain responsible for glutamate ligand binding. Exon 4 (1,067 bp) encodes the carboxy-terminal extracellular domain and its following membrane-spanning domain up to the 6th transmembrane segment of the receptor. Exon 5 (175 bp) covers the 7th transmembrane segment, and exon 6 (600 bp) corresponds to the carboxy-terminal cytoplasmic tail and

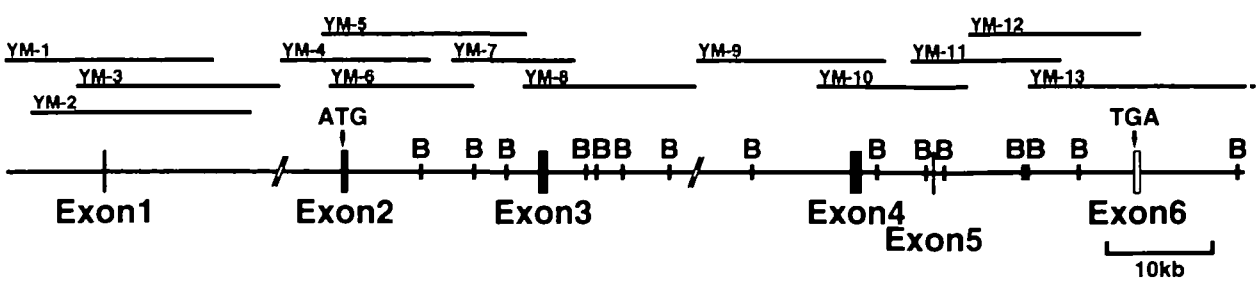

Fig. 1. **Structural organization of the mouse mGluR3 gene.** Overlapping clones (YM-1 to YM-13) containing the mouse mGluR3 gene are aligned according to the restriction mapping and sequence determination. Parts of the genomic sequences that are not overlapped by cloned genomic DNA are indicated by double slash lines. The locations of six exons are indicated by boxes; the closed and open boxes show the protein-coding region and the 5′- and 3′-untranslated regions, respectively; ATG, the translation initiation site; TGA, the translation termination site. All *Bam*HI sites (B) are shown.

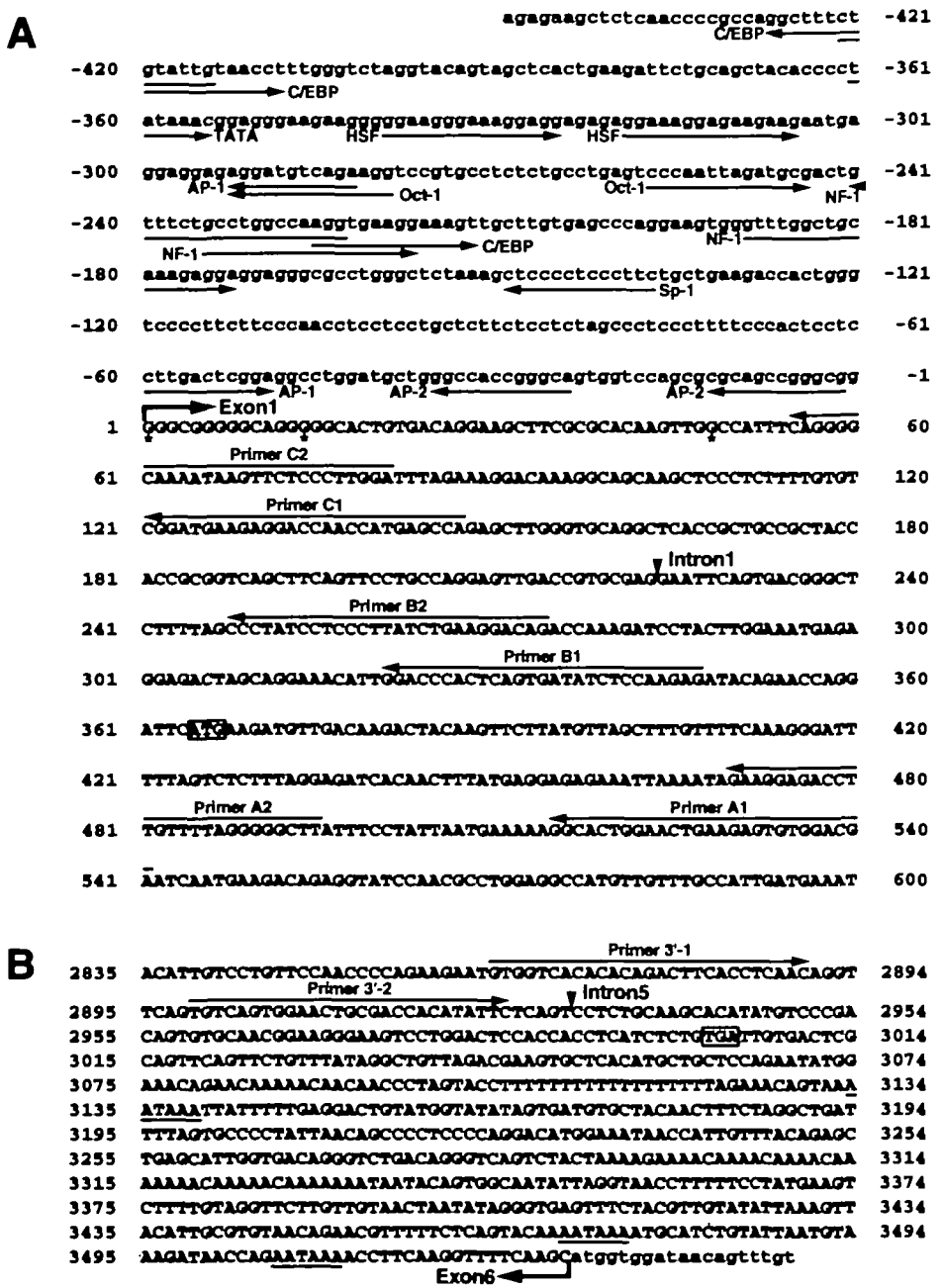

Fig. 2. **Determination of the transcription initiation and termination sites and exon/intron arrangements of the mouse mGluR3 gene.** (A) The nucleotide sequences corresponding to the 5′-untranslated and protein-coding regions (capital letters) and their 5′-flanking region (lower-case letters) of the mGluR3 gene are numbered from the transcription initiation site as +1. Three paired 3′ primers (Primers A1/A2, B1/B2, and C1/C2) complementary to the indicated sequences were used for nested 5′-RACE analysis, and their locations are shown above the nucleotide sequence. Asterisks under the nucleotide sequence indicate the upmost 5′ ends of individual 5′-RACE products generated by three different paired 3′ primers; G in residue 1, G in residue 14, and G in residue 48 were assigned using Primers C1/C2, A1/A2, and B1/B2, respectively. The translation initiation ATG codon of the mGluR3 gene is enclosed. Consensus sequences for potential promoter, enhancer, and regulatory DNA elements are shown under the nucleotide sequence. (B) The nucleotide sequences corresponding to the protein-coding and 3′-untranslated regions (capital letters) and their 3′-flanking region (lower-case letters) of the mGluR3 gene are presented. Locations of the 5′ primers (Primers 3′-1/3′-2) used for nested 3′-RACE analysis are indicated above the nucleotide sequence. Three canonical sequences for a polyadenylation signal are underlined. The translation termination TGA codon is enclosed. (C) The sequences at exon/intron boundaries and the nucleotide numbers of exons and introns are indicated. Junctions of exon/intron boundaries are positioned according to the GT/AG rule for the splice donor-acceptor site.

the 3′-untranslated region. The 5′-flanking region of the mGluR3 gene includes a TATA box, which is thought to serve polymerase selection signals (*30*), but this TATA box is located distantly at 355-361 bp from the putative transcription initiation site (Fig. 2A). There are many potential promoter, enhancer, and regulatory DNA motifs in the 5′-flanking region (Fig. 2A), including two AP-1, two AP-2, three C/EBP, two HSF (heat shock factor), three NF-1 (nuclear factor 1), two Oct-1, and one Sp-1. However, the involvement of these regulatory elements in mGluR3 gene expression remains to be determined.

The Deduced Amino Acid Sequence of Mouse mGluR3—The amino acid sequence of mouse mGluR3 was deduced from the exon sequences of the mouse genomic DNA (Fig.

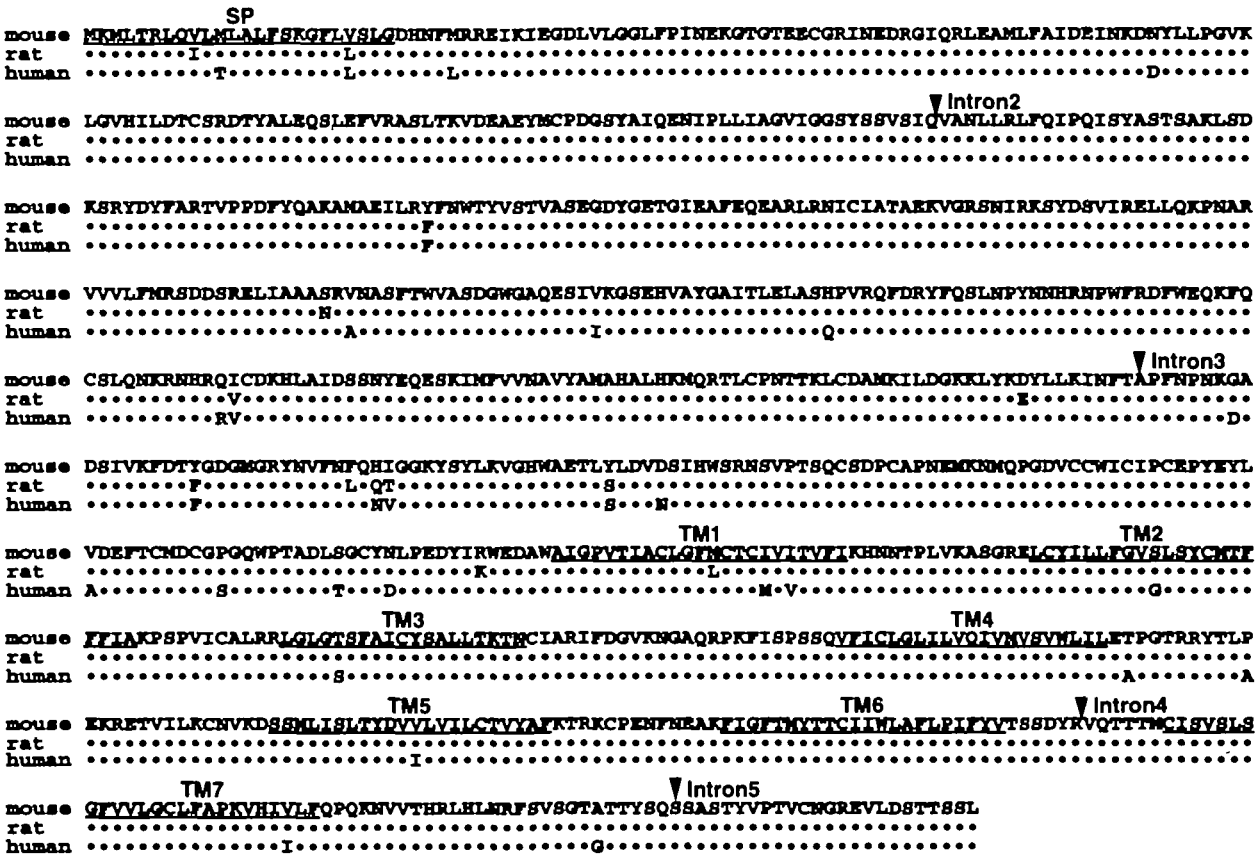

Fig. 3. **Amino acid sequence comparison of mouse, rat, and human mGluR3.** Amino acid residues are indicated by the single-letter notation, and residues in rat and human sequences that are identical to the corresponding residue in monse are indicated by dots. Positions of the seven transmembrane segments (TM1-TM7) and four introns in the protein-coding region are indicated above the amino acid sequences; SP, signal peptide sequence.

3). The mouse mGluR3 protein is composed of 879 amino acids with a calculated molecular mass of 99,109 Da. The signal peptide was predicted to be cleaved between residues 24 and 25 (*31*). A comparison of the amino acid sequences of mouse, rat, and human mGluR3 (*11, 32, 33*) indicated that the amino acid sequences of mGluR3 are highly homologous between species, with an amino acid identity of 98.5% between mouse and rat and 96.7% between mouse and human (Fig. 3).

Growth Factor Upregulation of mGluR3 mRNA in Cultured Astrocytes—Little is known about the regulatory mechanism of mGluR3 expression. Of the various mGluR subtypes, mGluR3 and mGluR5 are expressed in both neuronal and glial cells (*10, 16, 17*). mGluR5 has been shown to be upregulated in cultured cortical astrocytes by specific growth factors such as EGF, bFGF, and TGF-α (*26, 34*). Because cultured astrocytes provide a useful *in vitro* tool to investigate the regulatory mechanism of gene expression, we examined effects of specific growth factors on astrocyte mGluR3 expression. Type 1 astrocytes were enriched from the neonatal brain cortex by removing other glial cell types after mechanical shaking. In cultured astrocytes, mGluR3 mRNA levels were too low to be detected by conventional RNA blotting. To quantify low levels of mGluR3 mRNA, RT-PCR was employed using total astrocyte RNA as a template. The sequences within exon 4 and exon 6 were used as 5′ and 3′ primers, respectively, thus avoiding any concomitant inclusion of genomic PCR products in RT-PCR analysis. RT-PCR was also performed for mGluR5 mRNA, and the effects of growth factors on changes in mGluR3 and mGluR5 mRNA levels in cultured astrocytes were compared (Fig. 4A). Additionally, the mRNA encoding a glycolytic enzyme, G3PDH, was included as a control (Fig. 4A). For all three mRNAs, each reaction gave rise to a single band of the RT-PCR product (Fig. 4A), which was confirmed to represent the corresponding mRNA by subcloning and subsequent sequence determination. In control experiments, no products were detected without addition of reverse transcriptase in the reaction mixture. Importantly, appropriate RT-PCR conditions were sought to quantify changes of mRNA levels in cultured astrocytes under conditions that allowed a linear relationship between amounts of astrocyte RNA added and intensities of the RT-PCR products.

Type 1 astrocytes were cultured in a serum-free medium supplemented minimally with several components to maintain healthy cultures (SF medium). In the SF medium, both mGluR3 and mGluR5 mRNAs were expressed at low levels in cultured astrocytes. Consistent with the previous reports (*26, 34*), exposure to either EGF or bFGF for 3 d produced an appreciable increase in mGluR5 mRNA levels (Fig. 4, A and B). Similarly, addition of either EGF or bFGF to the SF medium significantly increased mGluR3 mRNA levels (Fig. 4, A and B). In addition, TGF-α, similar to

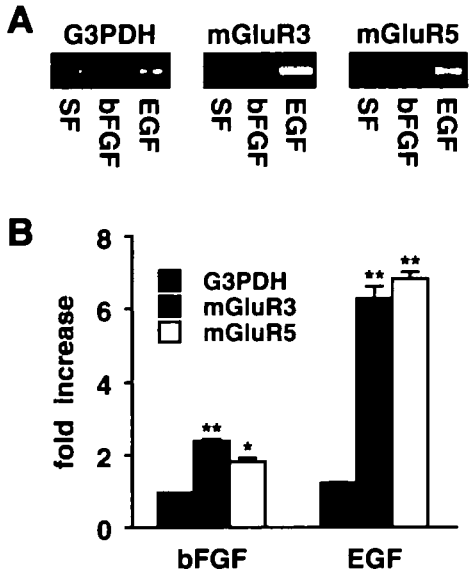

Fig. 4. **RT-PCR analysis of mGluR3, mGluR5, and G3PDH mRNA levels after exposure of cultured astrocytes to EGF and bFGF.** (A) RT-PCR products of mGluR3, mGluR5, and G3PDH mRNAs in astrocytes cultured in SF medium with or without added bFGF or EGF (10 ng/ml each) were analyzed by agarose gel electrophoresis. A single band with the expected size of the respective RT-PCR product was detected. A significant increase in the intensity of ethidium bromide-stained RT-PCR products of both mGluR3 and mGluR5 mRNAs, but not that of G3PDH mRNA, was observed in astrocytes exposed to the two growth factors. (B) Relative levels of mGluR3, mGluR5 and G3PDH mRNAs with and without addition of growth factors (10 ng/ml each) were determined; mRNA levels are expressed relative to that of astrocytes cultured in SF medium without added growth factor. At least three experiments were performed, and the values shown are the mean ± SE in duplicate gel electrophoresis in a representative experiment. Both mGluR3 and mGluR5 mRNAs were significantly increased by addition of each growth factor ($^*p<0.05$, $^{**}p<0.01$) as analyzed by two-sample *t*-test.

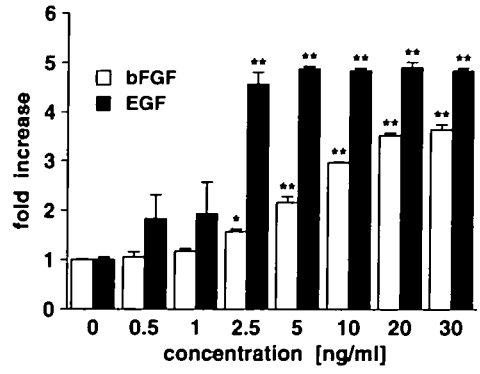

Fig. 5. **Dose-dependent effects of EGF and bFGF on mGluR3 mRNA upregulation.** Effects of EGF and bFGF on mGluR3 mRNA levels were determined by varying the concentrations of growth factors in SF medium for astrocyte cultures. mRNA levels are expressed relative to that of astrocytes cultured in SF medium without added growth factor. At least two experiments were performed, and the values shown are the mean ± SE in duplicate or triplicate gel electrophoresis in a representative experiment. Significant increase of mGluR3 mRNA levels was noted at concentrations from 2.5 ng/ml of EGF ($^{**}p<0.01$) and bFGF ($^*p<0.05$, $^{**}p<0.01$) as analyzed by one-way analysis of variance (ANOVA), followed by Sheffé's post hoc test.

EGF, produced a marked increase in both mGluR3 and mGluR5 mRNA levels (data not shown). For both mRNAs, EGF appeared more efficacious than bFGF. This was confirmed for mGluR3 mRNA by examining mGluR3 mRNA levels after culturing astrocytes with varying concentrations of either EGF or bFGF (Fig. 5). EGF produced its maximal effect at a concentration of 2.5-5.0 ng/ml, while bFGF was most effective at 20-30 ng/ml; the half-maximal concentrations of mGluR3 mRNA increase were calculated to be 1.5 ng/ml of EGF and 5.5 ng/ml of bFGF. The control G3PDH mRNA showed no change in expression level under any culture conditions. The results demonstrate that mGluR3 mRNA is upregulated in cultured astrocytes by specific growth factors.

DISCUSSION

We have investigated the exon/intron organization, the transcription initiation site, and the 5′-flanking region of the mouse mGluR3 gene, together with the regulation of mGluR3 mRNA in cultured astrocytes by specific growth factors. Of the eight subtypes of the mGluR family, which are classified into three groups according to their sequence similarities, agonist selectivities, and intracellular transduction mechanisms (*5-7*), the complete genomic sequence has been reported only for the human mGluR6 gene (*22*). This gene consists of 16,742 bp with 10 exons separated by nine introns. The number of exons and the exon/intron arrangement of the mGluR6 gene differ from those of the mGluR3 gene. However, introns 2, 3, and 4 of the mGluR3 gene are present at the corresponding regions of introns 1, 6, and 9 of the mGluR6 gene, respectively. This matched exon/intron arrangement of the two mGluR genes suggests that introns may have been either inserted into the pre-existing exons or removed from the common ancestral gene during evolution of the mGluR family.

The mouse mGluR3 gene spans at least 95 kb. Data on the human mGluR3 gene recently deposited in the DDBJ/EMBL/GenBank Data Bank (accession numbers: AC002081, AC005009, and AC004829) indicate that it possesses five introns at exactly the same positions as the mouse counterpart. The entire human mGluR3 gene is composed of 220,229 bp with the large intron sequences consisting of 120,133 bp of intron 1, 20,647 bp of intron 2, 51,722 bp of intron 3, 10,464 bp of intron 4, and 13,737 bp of intron 5. The mGluR3 gene is extremely large in both the mouse and human, the human mGluR3 gene being more than 13 times larger than the human mGluR6 gene. Interestingly, exon 1 and its 5′-flanking putative promoter region are located distantly from the following coding region of the mGluR3 gene. This large and characteristic organization of the mGluR3 gene raises interesting questions concerning its relevance to the regulatory mechanism of expression and the processes involved in its evolution.

Accumulating evidence indicates that different members of the mGluR family play distinct and key roles in synaptic modulation and plasticity (*4-7*). However, little is known about the regulatory mechanisms of gene expression of this receptor family. The present investigation, combined with the previous report (*34*), has demonstrated that specific growth factors concertedly regulate mGluR3 and mGluR5

mRNA levels in cultured cortical astrocytes. This upregulation is specific for these mGluR mRNAs, because the expression of an unrelated enzyme, G3PDH, was found not to respond to the same growth factors. The concerted upregulation of mGluR3 and mGluR5 mRNAs in cultured astrocytes may result from either transcriptional induction or mRNA stabilization, or both. The genomic sequence upstream of the transcription initiation site of the mouse mGluR3 gene (up to 450 bp) shows no particular sequence homology nor conserved DNA stretches with the rat mGluR5 gene (*23*). The mechanism underlying the coordinate regulation of the two mGluR mRNAs awaits further investigation. Among members of the mGluR family, mGluR3 and mGluR5 mRNAs exhibit a unique expression pattern in that they are expressed in glial cells as well as neuronal cells and upregulated during the early postnatal days (*10, 19, 35, 36*). Furthermore, exposure of cultured astrocytes to bFGF, EGF, and TGF-α has been shown to markedly increase the amount of mGluR5 protein in astrocyte membrane preparations (*34*). Although upregulation of mGluR3 protein in cultured astrocytes remains to be determined, specific growth factors such as TGF-α are highly expressed in the postnatal period (*37*) and may therefore have the potential to control both mGluR3 and mGluR5 expressions during development. It has recently been reported that mGluR3 and mGluR5 exert opposing influences on astrocyte proliferation, with mGluR3 suppressing proliferation and mGluR5 enhancing it (*38*). Thus, the coordinate upregulation of mGluR3 and mGluR5 may be important in maintaining a status-quo of astrocyte functions such as cell proliferation and cell growth. Recently, it has also been reported that activation of group 2 mGluRs (presumably mGluR3) increases the formation and release of TGF-β in cultured astrocytes, which in turn contributes to protecting neighboring neuronal cells from glutamate-mediated excitotoxicity (*20, 21*). This observation, combined with the present *in vitro* results, indicates that mGluR3 not only is regulated by specific growth factors but also participates in controlling growth factor generation. In closing, the interaction between mGluR3 and specific growth factors may play an important role in development, differentiation, and the neuroprotective function of astrocytes.

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