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Molecular Characterization of a New Metabotropic Glutamate Receptor mGluR7 Coupled to Inhibitory Cyclic AMP Signal Transduction*

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A cDNA clone for a new rat metabotropic glutamate receptor termed mGluR7 was isolated through polymerase chain reaction-mediated DNA amplification by using primer sequences conserved among the metabotropic receptor (mGluR) family and by the subsequent screening of a rat forebrain cDNA library. The cloned mGluR7 subtype consists of 915 amino acid residues and exhibits a structural architecture common to the mGluR family with a large extracellular domain preceding the seven putative membrane-spanning domains. mGluR7 shows the highest sequence similarity to mGluR4 and mGluR6 among the members of the mGluR family. Similar to mGluR4 and mGluR6, mGluR7 inhibits forskolin-stimulated cyclic AMP accumulation in response to agonist interaction and potently reacts with L-2-amino-4-phosphonobutyrate and L-serine-*O*-phosphate in Chinese hamster ovary cells transfected with the cloned cDNA. RNA blot and *in situ* hybridization analyses of mGluR7 mRNA indicated that it is widely expressed in many neuronal cells of the central nervous system and is thus different from the more limitedly expressed mGluR4 or mGluR6 mRNA. mGluR7 together with mGluR4 thus corresponds to the putative L-2-amino-4-phosphonobutyrate receptor which plays an important role in modulation of glutamate transmission in the central nervous system.

Glutamate receptors play an important role in neuronal plasticity and neurotoxicity in the central nervous system (1–3). They are classified into two distinct groups termed ionotropic receptors and metabotropic receptors (mGluRs)[1] on the basis of physiological, pharmacological, and molecular studies (3). Recent molecular cloning studies have identified six different subtypes of mGluRs (mGluR1 to mGluR6) which possess seven putative membrane-spanning domains preceded by a large extracellular domain (3–8). The six mGluR subtypes can be subdivided into three subgroups according to their sequence similarities, signal transduction mechanisms, and agonist selectivities (3). mGluR1 and mGluR5 are coupled to the stimulation of the phosphatidylinositol hydrolysis/Ca^{2+} signal transduction and show a strong agonist selectivity to quisqualate (4, 5, 7, 9). The other four are linked to the inhibition of the cyclic AMP cascade, but mGluR2/mGluR3 and mGluR4/mGluR6 are totally different in their agonist selectivities (6, 8, 10). mGluR2 and mGluR3 effectively interact with *trans*-1-aminocyclopentane-1,3-dicarboxylate (6, 10), whereas mGluR4 and mGluR6 potently react with L-2-amino-4-phosphonobutyrate (L-AP4) (8, 10).

Electrophysiological studies indicated that there are two different actions of L-AP4 in glutamate transmission effectively responding to L-AP4 through the activation of certain mGluR subtypes (11–18). The L-AP4-sensitive action is observed in neuronal transmission between retinal photoreceptor cells and ON-bipolar cells (11–14). In this neuronal transmission, L-AP4 activates an L-AP4-sensitive mGluR and hyperpolarizes ON-bipolar cells through its coupling to the cyclic GMP cascade (11–14). We recently reported that mGluR6 is highly reactive with L-AP4 and is restrictedly expressed in the retinal inner nuclear layer where ON-bipolar cells are localized (8). Thus, mGluR6 very likely corresponds to the L-AP4 receptor in ON-bipolar cells (8). Another L-AP4-sensitive action is seen in many excitatory amino acid synapses in the central nervous system where glutamate transmission is inhibited by the addition of L-AP4 (15–18). This inhibition seems to be caused by the reduction of glutamate release as a result of the activation of an L-AP4-sensitive mGluR subtype at the presynaptic site (15–18). It has also been reported that the L-AP4-sensitive presynaptic receptors may be mGluRs negatively coupled to cyclic AMP synthesis (19, 20). In our previous molecular study, we reported that there is a good correlation between the properties of the L-AP4-sensitive mGluR4 and those reported for the putative L-AP4 receptor and thus suggested that mGluR4 represents the L-AP4 receptor at least at some neuronal synapses of the central nervous system (10). However, we also found that the expression of mGluR4 mRNA is not always high or may even be absent in some L-AP4-sensitive neuronal pathways, suggesting the existence of an additional L-AP4-sensitive mGluR in the central nervous system (10).

In this investigation, we extended our molecular screening of mGluR subtypes from a rat forebrain cDNA library. We here report the molecular cloning and characterization of a new mGluR subtype termed mGluR7. This receptor shows a high degree of similarity with mGluR4/mGluR6 in both amino acid sequence and agonist selectivity. In addition, the expression of its mRNA is widely distributed in neuronal cells of the central nervous system.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) D16817.

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[1] The abbreviations used are: mGluR, metabotropic glutamate receptor; L-AP4, L-2-amino-4-phosphonobutyrate; PTX, pertussis toxin; CHO cells, Chinese hamster ovary cells; PCR, polymerase chain reaction; bp, base pair; kb, kilobase pair.

EXPERIMENTAL PROCEDURES

Materials—Superscript™ RNase H⁻ reverse transcriptase was purchased from Bethesda Research Laboratories; GeneAmp™ DNA amplification reagent kit from Perkin-Elmer Cetus; pBluescript and λZAPII from Stratagene; L-AP4 and D-AP4 from Tocris Neuramin; D-AP4 was more than 98% enantiomerically pure but may have contained a trace amount of L-AP4 (less than 2%); L-serine-*O*-phosphate from Sigma; pertussis toxin (PTX) from Kaken Pharmaceutical Inc.; cyclic AMP radioimmunoassay kit from Amersham. All other chemicals were of reagent grade.

Polymerase Chain Reaction (PCR)—A mixture of oligo(dT)-primed cDNAs was synthesized from the adult brain poly(A)⁺ RNA by using Superscript™ RNase H⁻ reverse transcriptase (21). The primers used for PCR were two degenerate oligonucleotides made according to the amino acid sequences of transmembrane segments III and VI of the mGluR family, both of which are highly conserved among the six mGluRs reported previously (6–8); the regions used corresponded to amino acid residues 669–675 (5′ primer) and 789–796 (3′ primer) of mGluR4. The sequences of the 5′ primer and the 3′ primer were 5′-GCCCTN(G/C/T)TNACNAAPuACNAA-3′ and 5′-ATPuCANGTNGTPuTACATNGTPuAA-3′ (N = G, A, C or T; Pu = G or A), respectively. PCR amplification was performed by using the GeneAmp™ DNA amplification reagent kit according to the following schedule: in each cycle, denaturation was carried out at 94 °C for 1 min, and annealing was performed at 42 °C for 2 min (the 1st and 2nd cycles), 46 °C for 2 min (the 3rd to 6th cycles), and 55 °C for 1 min (the 7th to 31st cycles). The enzymatic reaction was carried out at 72 °C for 3 min and 10 min (for the last cycle) after denaturation and annealing in each cycle. An aliquot of the PCR reaction was electrophoresed on a polyacrylamide gel, and the amplified DNA (~380 base pairs (bp)) was excised from the gel and subcloned into pBluescript SK(+). Nine of the 47 clones were found to be a new type of the mGluR cDNA clone (psMGR7).

cDNA Cloning and Sequence Determination—The mGluR7 cDNA clone was isolated from a rat forebrain cDNA library constructed from fractions containing cDNAs more than 4 kilobase pairs (kb) by using a λZAPII vector as described previously (21). Phage clones (1.2×10^6) were screened by hybridization with the 383-bp cDNA fragment of psMGR7. Hybridization was carried out in 1 M NaCl at 60 °C, and filter washing was performed in a solution containing 15 mM NaCl, 1.5 mM sodium citrate, and 0.1% sodium dodecyl sulfate at 60 °C. Thirty-six hybridization-positive clones were isolated and rescued into pBluescript SK(−). One clone containing the largest cDNA insert (pmGR7) was chosen for further sequence analysis. The amino acid sequence was deduced from the longest open reading frame in the nucleotide sequence determined for the pmGR7 clone. Determination of nucleotide sequences was carried out by the chain termination method (22).

mGluR7 Expression in Chinese Hamster Ovary (CHO) Cells and Cyclic AMP Measurements—The 4.0-kb *Sac*I-*Not*I fragment of pmGR7 was inserted into a unique *Eco*RI site of a eucaryotic expression vector (pdKCR-dhfr) containing the mouse dihydrofolate reductase gene as a selection marker (6). This plasmid was transfected into CHO cells deficient in dihydrofolate reductase activity (CHO-dhfr⁻) by the calcium phosphate method as described previously (6). Expression levels of mGluR7 mRNA of dhfr⁺-transformant cells were determined by RNA blot hybridization analysis. A clonal cell line expressing the highest level of mGluR7 mRNA was selected and used for subsequent experiments of signal transduction mechanisms of mGluR7. Dose-response curves of various agonists for inhibition of the forskolin-stimulated cyclic AMP formation were determined by incubating mGluR7-expressing cells with test reagents in the presence of 10 μM forskolin for 10 min as described previously (6). For PTX treatment, cells were preincubated with various concentrations of PTX for 13 h at 37 °C. Measurements of phosphatidylinositol hydrolysis were conducted as described (9). Each experiment was carried out at least twice in triplicate.

RNA Blot and in Situ Hybridization Analyses—RNA blot hybridization analysis was carried out by using 10 μg of total RNA from various regions of the brain, the spinal cord, and the dorsal root ganglion. The cDNA probe used was the 804-bp *Hin*cII fragment of pmGR7. *In situ* hybridization was performed by using ^{35}S-labeled antisense riboprobes corresponding to nucleotide residues 945–2902 (*Pvu*II fragment), 177–1250 (*Eco*T14I fragment) or 1251–2760 (*Eco*T14I fragment) of pmGR7 (4). Control hybridization experiments were carried out with the same probe in the presence of excess unlabeled probe.

RESULTS

cDNA Cloning and Structure of mGluR7—The mGluR7 cDNA clone was isolated through PCR-mediated DNA amplification by using two degenerate oligonucleotide primers corresponding to the conserved amino acid sequences of transmembrane segments III and VI of the mGluR family and by the subsequent screening of a rat forebrain cDNA library. Forty-seven clones isolated by PCR amplification were sequenced, and the sequence of nine of them (psMGR7) showed an overall similarity to, but was obviously different from, those of the corresponding regions of any of the previously isolated mGluR1-mGluR6 cDNAs. A rat forebrain cDNA library was screened by hybridization with the psMGR7 cDNA probe under high stringency conditions. Thirty-six hybridization-positive clones were isolated and rescued into pBluescript SK(−). The cDNA inserts of these clones were found to share an identical restriction map except for some size difference in the 5′ ends of the cDNA inserts. The nucleotide sequence of a representative clone containing the largest cDNA insert (pmGR7) was determined by the chain termination method (22).

The mGluR7 cDNA is composed of approximately 4.3 kb. Fig. 1 shows the nucleotide sequence of a large open reading frame and its surrounding 5′ and 3′ non-coding regions of pmGR7 and the predicted amino acid sequence of mGluR7. The assigned initiation codon is the first in-frame methionine codon downstream of a stop codon, and the sequence surrounding it conforms to the consensus sequence for the translation initiation (23). The deduced amino acid sequence of mGluR7 consists of 915 amino acid residues (a calculated molecular weight of 102,227) with eight hydrophobic segments similar to a structural profile of other mGluR subtypes (3). The amino-terminal hydrophobic sequence probably serves as a signal peptide (24), while the other seven present at the carboxyl-terminal side would represent seven membrane-spanning domains (3). Thus, a large hydrophilic amino-terminal sequence composed of approximately 560 amino acid residues is very likely to form an extracellular domain preceding the seven membrane-spanning domains (3). There are potential *N*-glycosylation sites (25) in the putative extracellular region and many serine and threonine residues for possible regulatory phosphorylation (26, 27) in the cytoplasmic regions.

Fig. 2 shows an alignment of amino acid sequences of the seven mGluR subtypes. The amino acid sequence of mGluR7 is highly homologous to those of the other members of the mGluR family. The degree of amino acid identity is 38–44% between the sequence of mGluR7 and those of mGluR1, mGluR2, mGluR3, and mGluR5, and the sequence identity is much higher between mGluR7 and mGluR4 (67.4%) and between mGluR7 and mGluR6 (64.3%), as summarized in Fig. 3. Thus, among the members of the mGluR family, mGluR7 is most closely related to mGluR4 and mGluR6. In addition, many cysteine residues are conserved between mGluR7 and the other mGluR subtypes. Thus, mGluR7 has a structural architecture common to the members of the mGluR family with a large extracellular domain preceding the seven transmembrane segments.

Characterization of Properties of mGluR7—We investigated the signal transduction and pharmacological properties of mGluR7 by establishing CHO cells stably expressing mGluR7 by DNA transfection. Because mGluR7 and mGluR4/mGluR6 are highly conserved in their amino acid sequences, we first examined the coupling of mGluR7 to the inhibition of the cyclic AMP cascade. L-Glutamate added to mGluR7-expressing cells resulted in significant inhibition of forskolin-stimulated cyclic AMP accumulation (Fig. 4*a*). In this experiment, the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine was added to the medium to avoid the influence of alterations in cyclic AMP degradation. No such inhibition of the forskolin-stimulated cyclic AMP formation was observed in untransfected control cells or those transfected with the vector DNA alone (Fig. 4*a*). Fur-

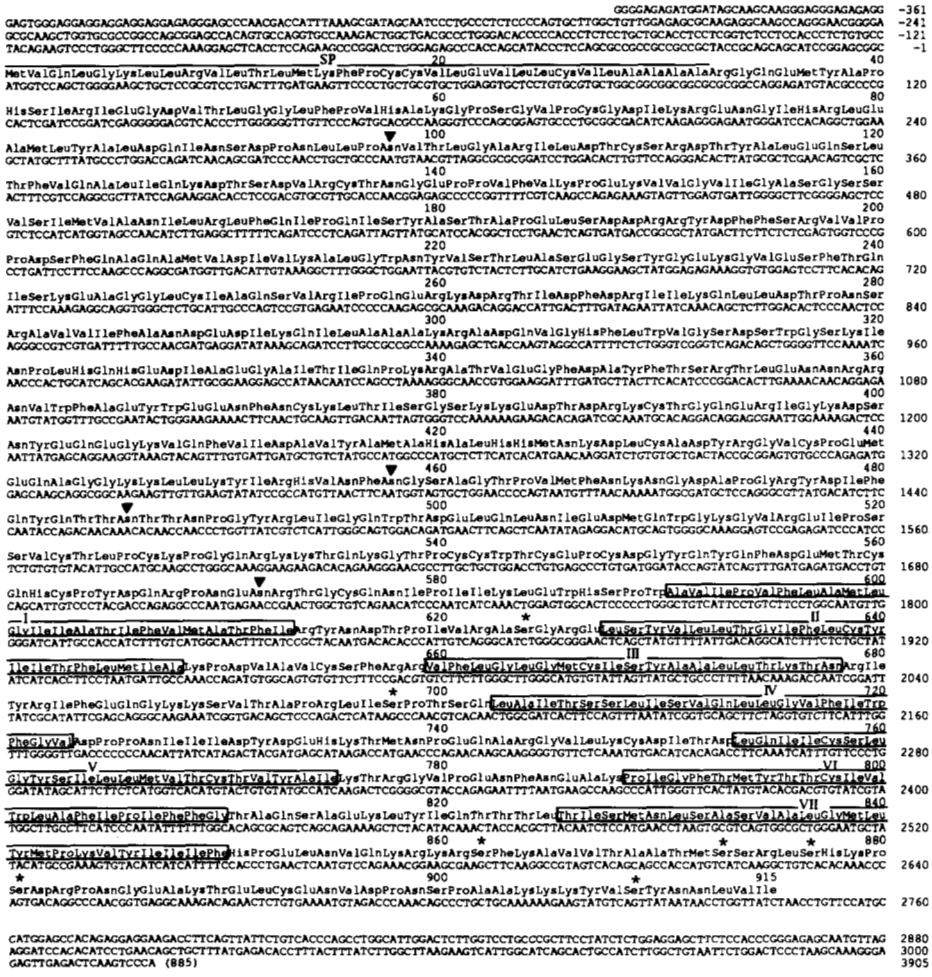

FIG. 1. **Nucleotide and deduced amino acid sequence for mGluR7.** The seven putative transmembrane segments (*I-VII*) were assigned on the basis of hydrophobicity analysis and the sequence comparison with the other six mGluRs and are indicated by enclosures with *solid lines*; the termini of these segments were tentatively assigned. The *solid line* labeled SP indicates the predicted signal peptide. *Triangles* and *stars* indicate possible *N*-glycosylation sites and phosphorylation sites, respectively.

thermore, mGluR7 showed no obvious stimulation of either cyclic AMP formation or phosphatidylinositol hydrolysis by the addition of L-glutamate up to the concentrations of 10 mM (data not shown). Thus, mGluR7, like mGluR4 and mGluR6, is linked to the inhibitory cyclic AMP cascade in CHO cells expressing mGluR7.

Dose-response curves of various agonists were determined by examining their inhibitory effects on forskolin-stimulated cyclic AMP accumulation in mGluR7-expressing cells (Fig. 4*a*). L-AP4 effectively inhibited forskolin-stimulated cyclic AMP accumulation. Similarly, L-serine-*O*-phosphate was very effective, and L-AP4 and L-serine-*O*-phosphate were more potent than L-glutamate in inhibiting the forskolin-stimulated cyclic AMP formation. The half-maximal effective concentrations (EC_{50}) of L-AP4, L-serine-*O*-phosphate, and L-glutamate were calculated to be 160 μM, 160 μM, and 1 mM, respectively. D-AP4 showed only a weak activity, but because of the possible enantiomeric impurity of the available D-AP4, it could not be ascertained whether D-AP4 indeed reacts with mGluR7. It is also to be noted that the reactivities of mGluR7 with the different agonists are all weaker than that of either mGluR4 or mGluR6 (8, 10). A similarly weak agonist reactivity was observed in a different cell line expressing mGluR7 (data not shown). This difference, however, may not reflect the nature of mGluR7 in neuronal cells because the possibility of a low expression of mGluR7 or a weak coupling of this receptor to a G protein in a heterologous CHO expression system cannot be excluded. The pharmacological characterization of mGluR7 indicates that mGluR7, like mGluR4 and mGluR6, corresponds to the L-AP4 receptor, although these three subtypes are slightly different in their relative responsiveness to different agonists.

ADP-ribosylation of some G proteins is catalyzed by PTX, and this ADP-ribosylation prevents a G protein from coupling to its linked receptor (28, 29). PTX treatment of mGluR7-expressing cells prior to the addition of forskolin and L-glutamate reduced the agonist-mediated inhibition of cyclic AMP formation in a dose-dependent manner and abolished this inhibition at 10 ng of PTX/ml of medium (Fig. 4*b*). Thus, the agonist responses of mGluR7 are mediated by a PTX-sensitive G protein.

Expression of mGluR7 mRNA—RNA blot hybridization and *in situ* hybridization analyses were performed to examine the expression of mGluR7 mRNA. The former analysis gave rise to a hybridization band with an estimated mRNA size of approximately 4.5 kilonucleotides under high stringency conditions (Fig. 5). This size agreed well with that determined for the cloned cDNA. The blot analysis showed that mGluR7 mRNA is widely distributed in many brain regions of the adult rat. Relatively high expression of mGluR7 mRNA is seen in the cerebral cortex, olfactory bulb, hippocampus, striatum, thalamus/hypothalamus, midbrain, and dorsal root ganglion.

In situ hybridization with specific riboprobes prepared from the different regions of the mGluR7 cDNA gave rise to a mutually consistent result of the expression pattern of mGluR7 mRNA and indicated that mGluR7 mRNA is widely distributed in neuronal cells of the adult brain (Figs. 6 and 7). No such hybridization was observed in parallel experiments using the same probe in the presence of an excess of unlabeled probe (Fig. 6*b*). The high expression of this mRNA is seen in neuronal cells throughout the cerebral cortex (Fig. 7*a*) and those of the thalamus, superior and inferior colliculi, striatum, olfactory tubercle, thalamus, and olfactory bulb (Fig. 6*a*). In the hippocam-

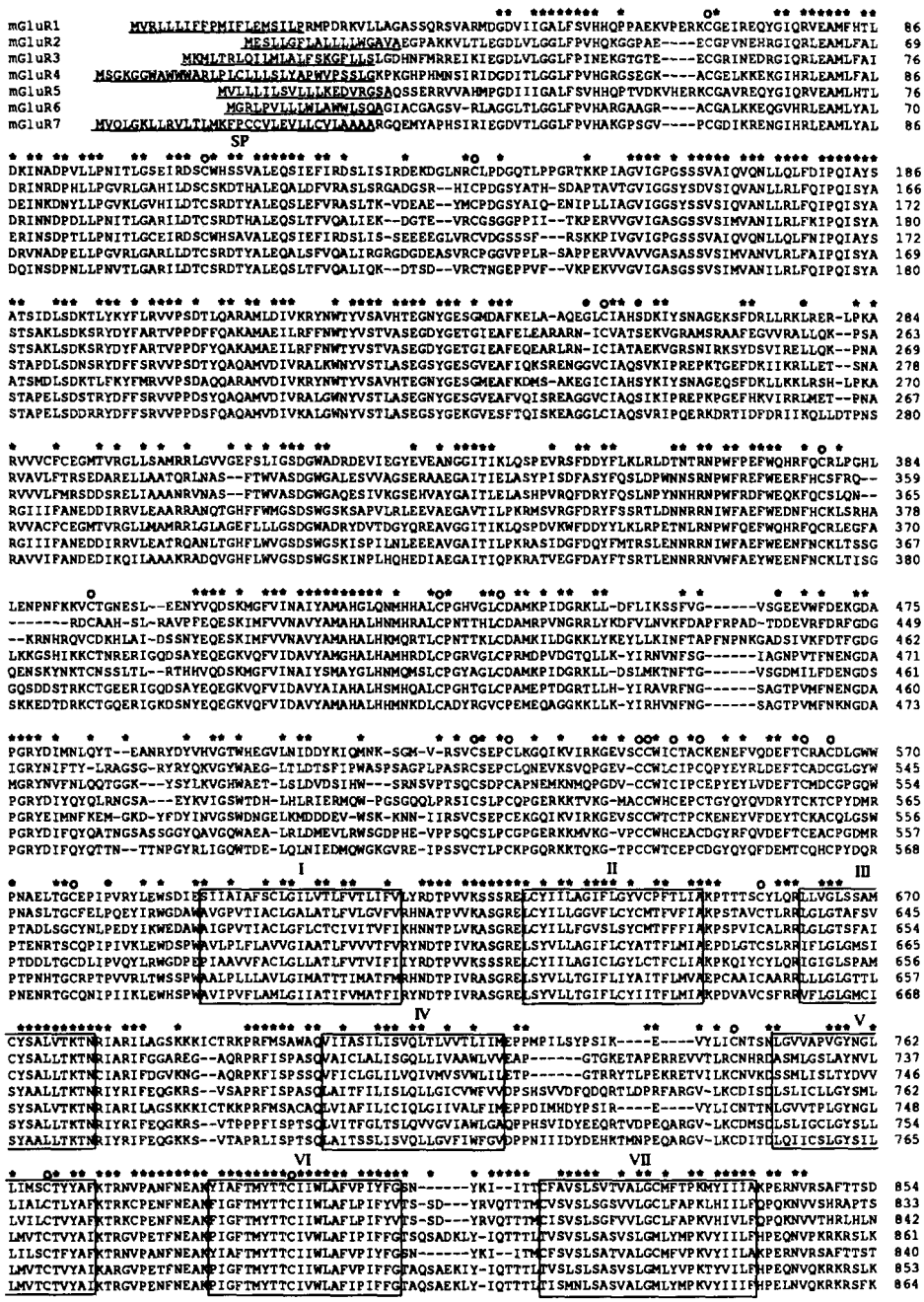

FIG. 2. **Amino acid sequence alignment of the seven mGluR subtypes.** The sequences of the seven mGluR subtypes are aligned with *single-letter* notation by inserting gaps (–) to achieve maximum homology. The amino acids identical at at least five of the seven sequences are marked by *stars* above the amino acid sequences. Cysteine residues conserved among the mGluR family are indicated by *circles*. The seven putative transmembrane segments are enclosed. *Solid lines* labeled SP indicate the signal peptides.

pus, hybridization signals are seen in pyramidal cells throughout CA1-CA4 regions as well as in granule cells of the dentate gyrus (Fig. 6*a*). Purkinje cells in the cerebellum (Fig. 7*c*) and mitral cells and tufted cells in the olfactory bulb (Fig. 7*b*) are also labeled with the mGluR7 probe. The prominent expression of mGluR7 mRNA is seen in neuronal cells of the dorsal root ganglion (Fig. 7*d*). Thus, mGluR7 mRNA is widely distributed throughout the brain, and this expression pattern of mGluR7 mRNA is in marked contrast to either the exclusively restricted expression of mGluR6 mRNA in the retina (8) or the relatively limited distribution of mGluR4 mRNA in certain brain regions (10).

DISCUSSION

Recent molecular studies have demonstrated that mGluRs consist of multiple receptor subtypes that exhibit varied agonist selectivities, signal transduction mechanisms, and expression patterns (3). Beyond these findings, studies of brain slices and primary cultures suggest that mGluRs are coupled to many signal transduction pathways in different neuronal and glial cells. These include activation of phosphatidylinositol hydrolysis, stimulation of arachidonic acid release, increased and decreased cyclic AMP formation, increased cyclic GMP degradation and modulation of ion channels (reviewed in Refs. 3 and

	mGluR1	mGluR2	mGluR3	mGluR4	mGluR5	mGluR6	mGluR7
mGluR1	100.0	43.1	41.2	39.0	60.8	40.3	38.1
mGluR2		100.0	66.9	45.3	42.5	46.0	44.0
mGluR3			100.0	44.1	41.5	44.8	43.1
mGluR4				100.0	39.8	68.6	67.4
mGluR5					100.0	39.3	37.8
mGluR6						100.0	64.3
mGluR7							100.0

FIG. 3. **Homology matrix for the seven subtypes of the rat mGluR family.** The calculations are based on the alignment shown in Fig. 2. The degree of the sequence identity indicated (%) was calculated by counting a continuous stretch of gaps as one substitution regardless of its length.

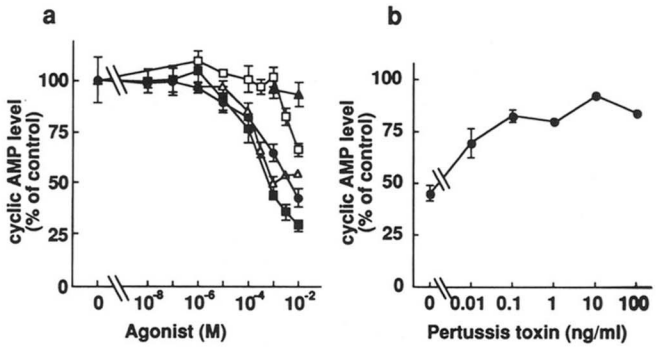

FIG. 4. **Dose-response curves of various agonists (*a*) and effects of PTX on glutamate responses (*b*) in inhibiting the forskolin-stimulated cyclic AMP accumulation of mGluR7-expressing cells.** In *a*, indicated concentrations of L-glutamate (●), L-AP4 (■), D-AP4 (□), and L-serine-*O*-phosphate (△), together with 10 μM forskolin, were added to mGluR7-expressing cells and incubated for 10 min. Cyclic AMP levels were determined as described under "Experimental Procedures." Intracellular cyclic AMP levels in cells treated and untreated with 10 μM forskolin were 74.9 ± 1.6 and 2.6 ± 0.1 pmol/well, respectively. As a control, the effects of L-glutamate on the forskolin-stimulated cyclic AMP formation in cells transfected with the vector DNA alone (▲) were determined and are indicated. Each point shows the mean ± S.D. of a representative experiment done in triplicate. In *b*, forskolin-stimulated cyclic AMP levels in mGluR7-expressing cells treated with indicated concentrations of PTX for 13 h were determined with or without addition of 10 mM L-glutamate; the levels obtained without addition of L-glutamate are taken as 100% at each concentration of PTX.

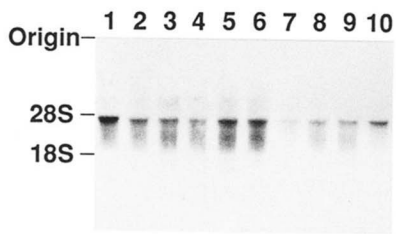

FIG. 5. **RNA blot hybridization analysis of mGluR7 mRNA.** Total RNAs analyzed were as follows: *lane 1*, cerebral cortex; *lane 2*, olfactory bulb; *lane 3*, hippocampus; *lane 4*, striatum; *lane 5*, thalamus/hypothalamus; *lane 6*, midbrain; *lane 7*, cerebellum; *lane 8*, pons/medulla oblongata; *lane 9*, spinal cord; *lane 10*, dorsal root ganglion. The size marker was rat ribosomal RNA.

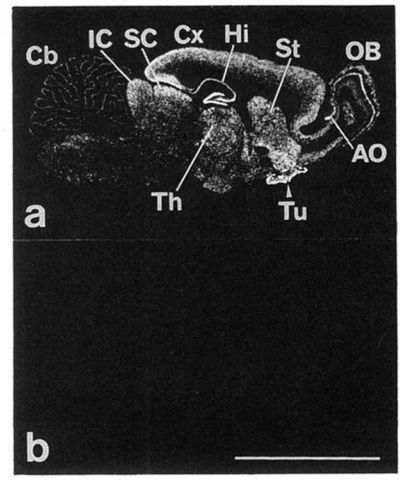

FIG. 6. **Distribution of mGluR7 mRNA in the adult rat brain by *in situ* hybridization.** Negative film images of parasagittal sections of the adult rat brain are indicated after hybridization with the labeled riboprobe (nucleotide residues of the mGluR7 cDNA, 177–1250) in the absence (*a*) and presence (*b*) of 100-fold excess unlabeled probe. *OB*, main olfactory bulb; *AO*, accessory olfactory bulb; *St*, striatum; *Hi*, hippocampus; *Cx*, cerebral cortex; *SC*, superior colliculus; *IC*, inferior colliculus; *Cb*, cerebellum; *Th*, thalamus; *Tu*, olfactory tubercle. *Scale bar*, 10 mm.

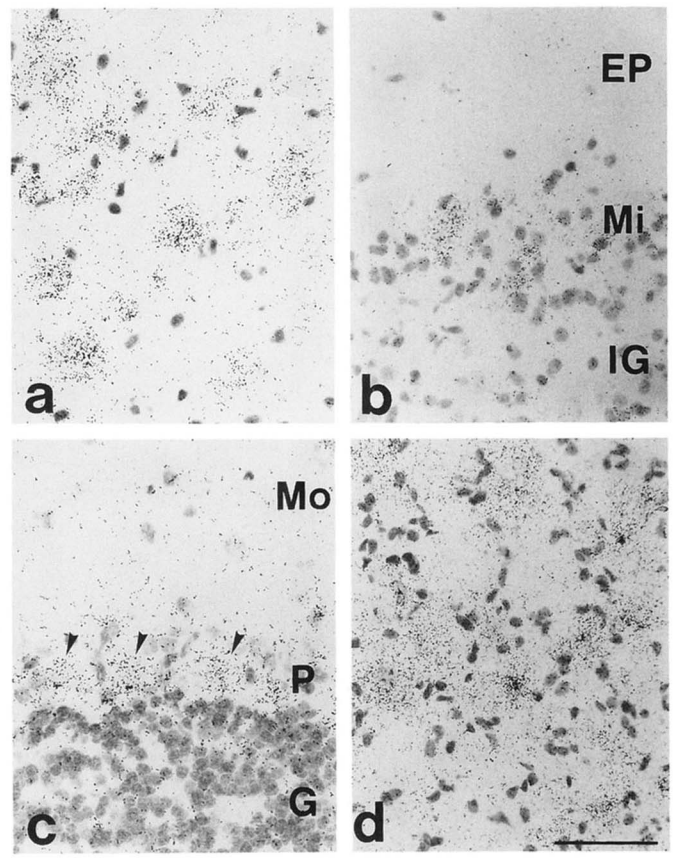

FIG. 7. **Cellular localization of mGluR7 mRNA by *in situ* hybridization.** Bright-field photomicrographs of emulsion-dipped sections through the retrosplenial cortex (*a*), olfactory bulb (*b*), cerebellar cortex (*c*), and dorsal root ganglion (*d*) are shown. The *arrowheads* in *c* indicate labeled Purkinje cells. *EP*, external plexiform layer; *Mi*, mitral cell layer; *IG*, internal granular layer; *Mo*, molecular layer; *P*, Purkinje cell layer; *G*, granule cell layer. *Scale bar*, 50 μm.

20). It is thus important to explore the molecular entities of the diverse members of the mGluR subtypes associated with functional variability of glutamate responses in the central nervous system. In this investigation, we extended our molecular screening of the mGluR family and identified a cDNA clone encoding a new mGluR subtype. The cloned mGluR7 receptor bears a structural architecture common to other mGluR subtypes and possesses a large extracellular domain preceding the seven transmembrane segments. mGluR7 shows the highest sequence similarity with mGluR4 and mGluR6 among the mGluR family, and consistent with this sequence conservation,

mGluR7, like mGluR4 and mGluR6, is linked to the inhibition of the cyclic AMP cascade and reacts potently with L-AP4 and L-serine-*O*-phosphate. Thus, this investigation explicitly demonstrates that there is an additional mGluR subtype that belongs to the subgroup of the mGluR family comprising mGluR4 and mGluR6.

Among the L-AP4-sensitive mGluR subtypes, mGluR6 mRNA is restrictedly expressed in the retinal inner nuclear layer where ON-bipolar cells are distributed (8). This unique expression pattern of mGluR6 as well as its potent reactivity with L-AP4 strongly suggests that it corresponds to the putative L-AP4-sensitive mGluR that is involved in synaptic transmission between photoreceptor cells and ON-bipolar cells (8). Similarly, the expression of mGluR4 mRNA is limitedly distributed in neuronal cells of certain regions of the central nervous system (10). This mRNA expression is localized in the cerebellum, main olfactory bulb, thalamus, lateral septum, and pontine nucleus (10). In contrast to mGluR4 and mGluR6 mRNAs, mGluR7 mRNA is more widely distributed throughout the brain regions. Furthermore, the expressions of mGluR4 and mGluR7 mRNAs are clearly differentiated in neuronal cells of some brain regions. In the cerebellum, mGluR4 mRNA is predominantly expressed in the granule cells (10), whereas mGluR7 mRNA is located in Purkinje cells. In the main olfactory bulb, mGluR4 and mGluR7 mRNAs are preferentially observed in the granule cells and the mitral/tufted cells, respectively (10). Thus, the three L-AP4-sensitive mGluRs, although similar in their signal transduction mechanisms and agonist selectivities, are distinctly expressed in neuronal cells of the different brain regions.

The electrophysiological effect of L-AP4 commonly observed in many synapses of the central nervous system is a reduction of glutamate transmission at excitatory amino acid synapses. For example, this L-AP4-mediated inhibition in glutamate transmission has been reported in the lateral entorhinal cortex-dentate gyrus pathway, the lateral olfactory tract-prepiriform cortex pathway, the mossy fiber-CA3 synapses, the dorsal root-spinal motoneuron synapses, and cultured mitral cells of the olfactory bulb (15–18, 30–37). The L-AP4-sensitive inhibition of excitatory synaptic transmission is thought to result from the reduction of glutamate release at the presynaptic site (15–18). Thus, the L-AP4 receptor appears to represent a presynaptic autoreceptor. The mechanisms by which L-AP4 decreases glutamate release have largely remained elusive. It has recently been reported that L-AP4 elicits an inhibitory effect on both forskolin-stimulated and A_{2b} adenosine receptor-mediated cyclic AMP accumulation in guinea pig cerebral cortical slices (19). Many receptors that are negatively linked to adenylate cyclase including $GABA_B$, A1-adenosine, and α_2-adrenergic receptors mediate presynaptic inhibition of transmitter release (38–40). It has also been reported that inhibition of Ca^{2+} channels are responsible for the L-AP4 receptor-mediated reduction of glutamate release in cultured mitral cells (18). This inhibition of Ca^{2+} channels is sensitive to PTX (18). It is thus possible that the L-AP4-sensitive mGluR modulates Ca^{2+} channels by membrane-delimited actions of G proteins (41) or by a G protein-coupled second messenger system. Both mGluR4 and mGluR7 expressed in the CHO are linked to a PTX-sensitive G protein (10). In our previous study, however, it was also noted that the expression of mGluR4 mRNA is not always high in neuronal cells projecting the L-AP4-sensitive neuronal pathways (10). In contrast, mGluR7 is more widely distributed in many brain regions. It is thus tempting to speculate that the widely distributed mGluR7 may be involved in a common mechanism of glutamate transmission such as autoregulation observed in many excitatory synapses, whereas mGluR4 may have a more specialized function in neuronal cells of particular brain regions.

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