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ACCELERATED COMMUNICATION

Cloning and Expression of a New Member of the L-2-Amino-4-phosphonobutyric Acid-Sensitive Class of Metabotropic Glutamate Receptors

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SUMMARY

Despite the cloning of several metabotropic glutamate receptors (mGluR1–6), the activity and localization of the cloned mGluRs do not account for the action of L-2-amino-4-phosphonobutyric acid (L-AP4) on mitral/tufted cells in the rat olfactory bulb. Thus, we screened a rat olfactory bulb library for novel cDNA clones, using probes derived from mGluR1 and mGluR4. A full length cDNA clone encoding a metabotropic receptor (mGluR7) whose sequence was 69% identical to that of mGluR4 was isolated. Stimulation of mGluR7 with L-AP4 and glutamate (each at 1 mM) in stably transfected baby hamster kidney cells inhibited forskolin-stimulated cAMP formation, whereas ACPD (1 mM) and quisqualate (0.5 mM) were less effective. Inhibition of cAMP required high concentrations of agonist in the transfected cells, suggesting that inhibition of adenylate cyclase may not be the predominant transduction mechanism for this receptor in neurons. RNA blot analysis and *in situ* hybridization revealed that mGluR7 has an expression pattern in the central nervous system distinct from that of other L-AP4-sensitive mGluRs. Double-labeling with probes for mGluR1 and mGluR7 revealed that individual mitral/tufted neurons in the olfactory bulb expressed both mRNAs. The expression pattern and L-AP4 sensitivity of mGluR7 suggest that it mediates inhibition of transmitter release at selected glutamatergic synapses. The coexpression of multiple mGluR mRNAs in single neurons indicates that the cellular effects of mGluR activation are likely to result from the integrated action of several receptor subtypes.

Glutamate is recognized as the major excitatory neurotransmitter in the central nervous system, with an action that is primarily mediated by ligand-gated ion channels composed of subunits from the AMPA, high affinity kainate, and *N*-methyl-D-aspartic acid classes (1–3). However, the observation that glutamate also induces responses mediated by second messengers led to the discovery of a distinct group of glutamate receptors coupled to G proteins, termed mGluRs (4). The first described action of mGluRs was PI hydrolysis (5, 6), which appears to be mediated primarily by mGluR1 and mGluR5 (7–9). However, molecular cloning has revealed a large family of mGluRs with distinct transduction mechanisms, patterns of expression, and agonist sensitivities (4). Consistent with this molecular heterogeneity, electrophysiological studies have suggested diverse roles for mGluRs in synaptic plasticity, presynaptic inhibition, and regulation of cell excitability by ion channel modulation (10–14). The specific mGluR subtypes mediating these cellular functions are largely undefined.

Evidence for the physiological role of specific mGluR subtypes has been derived from studies of ACPD, which is a potent agonist at mGluR1–3 and mGluR5 (7, 9, 15, 16), and L-AP4, which activates only mGluR4 and mGluR6 (16–18). In retinal bipolar neurons, L-AP4 activates a phosphodiesterase (presumably through mGluR6) (19), whereas L-AP4 inhibits transmitter release and voltage-dependent calcium entry in selected brain and spinal cord neurons (20–22). Because L-AP4 can inhibit transmitter release in neurons that express neither mGluR4 nor mGluR6, there must be additional mGluR subtypes. By screening a rat olfactory bulb cDNA library, we identified a novel mGluR (mGluR7). The L-AP4 sensitivity and expression pattern of mGluR7 suggest that it is a presynaptic autoreceptor in selected central nervous system pathways.

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ABBREVIATIONS: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP3, 2-amino-3-phosphonopropionic acid; AP4, 2-amino-4-phosphonobutyric acid; ACPD, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid; MCPG, (*RS*)-α-methyl-4-carboxyphenylglycine; PI, inositol phospholipid; BHK, baby hamster kidney; CHO, Chinese hamster ovary; bp, base pair(s); mGluR, metabotropic glutamate receptor.

Materials and Methods

cDNA clones. Degenerate oligonucleotide primers were used to amplify a region of first-strand olfactory bulb cDNA between putative transmembrane region II and intracellular loop III of mGluR cDNAs. The sequence of the 5′ primer was GC(TCAG)GG(TCAG) AT(ACT)TT(CT)(CT)T(TCAG)(GT)G (TCAG) and the 3′ primer was AT(TCAG)(GT)(AG)(CT)TT (TCAG)GC(CT)TC(AG)TT(AG)AA. The 5′ ends of both primers also contained sequences for an *Eco*RI restriction site. The polymerase chain reaction samples were analyzed by agarose gel electrophoresis, and amplified fragments of approximately 350 bp were digested with *Eco*RI and cloned into pBluescript II KS(+). Of 65 clones initially identified, three were shown by DNA sequence analysis (23) to be related to, but distinct from, previously isolated mGluR cDNAs (7–9, 15, 16, 18). These clones, designated as Olf 1, 2, and 8, were used as templates to generate ^{32}P-labeled *in vitro* RNA transcripts. These were mixed equally and used to probe an adult rat olfactory bulb cDNA library (Stratagene, La Jolla, CA). Fourteen duplicate positive clones were rescued by coinfection with helper bacteriophage into pBluescript SK(−), and the size of their inserts was analyzed by restriction analysis. Southern blot analysis (24) with Olf 1, 2, or 8 identified three distinct groups of clones; the full length clone isolated with Olf 2 was designated as mGluR7.

Analysis of RNA. Total RNA was isolated as described (25), from freshly dissected rat brain regions representing cerebellum, hippocampus, hypothalamus, olfactory bulb, brainstem, midbrain, thalamus, and cortex. Poly(A)$^+$ RNA was purified using a Magnesphere RNA isolation kit (Promega). Two micrograms of each poly(A)$^+$ RNA were fractionated by denaturing agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with a random-primed ^{32}P-labeled 719-bp *Xho*I fragment encoding amino acids 1–197 of the mGluR7 cDNA. For *in situ* hybridization, adult Sprague-Dawley rats (200–250 g) were anesthetized with pentobarbital and perfused transcardially with ice-cold saline, followed by ice-cold fixative (4% paraformaldehyde, 0.1 M sodium borate, pH 9.5). The brains were postfixed overnight in fixative containing 10% sucrose. Sections (25 μm) were mounted on gelatin- and poly-L-lysine-coated glass, fixed for 15 min in 4% paraformaldehyde in 0.1 M phosphate-buffered saline, washed twice with 0.1 M phosphate-buffered saline, treated for 30 min at 37° with proteinase K (0.001% in 0.1 M Tris, 0.05 M EDTA, pH 8), followed by 0.0025% acetic anhydride in 0.1 M triethanolamine at room temperature, and dehydrated. A 719-bp *Xho*I fragment encoding amino acids 1–197 of mGluR7 and a 1234-bp *Xho*I/*Pst*I fragment of mGluR4 encoding amino acids 110–521 were subcloned into pBluescript KS(+). ^{35}S-labeled antisense RNA was transcribed from each template and used for hybridization (for 20 hr at 58°) at 10^7 cpm/ml in hybridization buffer (26). Slides were processed as described (27), dipped in NTB-2 liquid photographic emulsion (Kodak), exposed for 13 days, developed with D-19 developer, and counterstained with thionin.

For double-labeling, mGluR1 probes were labeled with digoxigenin-UTP and mGluR7 probes were labeled with ^{35}S-UTP by *in vitro* transcription. Digoxigenin-labeled *in vitro* RNA transcripts for mGluR1 were generated from a 1363-bp *Eco*RI/*Sac*I cDNA fragment containing nucleotides 361–1724 of mGluR1 (7). Sections were incubated with both probes and processed as described above until the dehydrations with ethanol. Before dehydration, slides were incubated overnight in 2× standard saline citrate (0.3 M NaCl, 0.03 M NaCitrate), 0.05% Triton X-100, 2% normal goat serum, followed by antidigoxigenin-alkaline phosphatase conjugate (Boehringer Mannheim), diluted 1/1000 in 0.1 M Tris·HCl, pH 7.5, 0.15 M NaCl, 0.3% Triton X-100, 1% normal goat serum, for 5 hr. Alkaline phosphatase was detected by incubation of slides overnight in the dark in 0.1 M Tris·HCl, pH 9.5, 0.1 M NaCl, 0.05 M $MgCl_2$, containing 337.5 mg/ml 4-nitro-blue tetrazolium chloride, 175 mg/ml 5-bromo-4-chloro-3-indolyl phosphate, and 300 mg/ml levamisole. The reaction was stopped with 0.01 M Tris·HCl, pH 8.1, 1 mM EDTA, followed by dehydration as rapidly as possible in increasing concentrations of ethanol containing 0.1× standard saline citrate and 1 mM dithiothreitol. After vacuum drying, slides were dipped in Ilford K.5 photographic emulsion, followed by development with D-19 developer. Sections were not counterstained.

Expression of mGluR7. BHK cells were transfected with mGluR7 subcloned into pZEM229R. The cells were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum, and transfected clones were selected for integration of the cDNA by methotrexate resistance. Resistant clones were assayed for the presence of mGluR7 RNA and protein. Confluent cells were assayed for cAMP production (Amersham) in the presence of isobutylmethylxanthine (1 mM); cAMP levels were measured after 10-min treatment with forskolin (10 μM) in the presence or absence of mGluR agonists or antagonists. cAMP was measured by displacement of [^{3}H]cAMP (cyclic AMP Assay Kit, Amersham). Assays were performed in triplicate at least two times. The average values from these experiments are expressed as the mean ± standard error of cAMP levels. Data were analyzed by analysis of variance using Dunnett's *t* test.

For oocyte expression studies, *Xenopus laevis* (Xenopus I, Ann Arbor, MI) were anesthetized with tricaine, and oocytes were harvested using sterile technique. RNA transcripts for mGluR1 and mGluR7 were injected into stage V-VI oocytes (1–100 ng of RNA/oocyte), and cells were assayed for production of a calcium-activated chloride current using a two-electrode voltage clamp. Oocyte harvesting, solutions, and recording methods were as described by Lester and colleagues (28).

Results

cDNA cloning and sequencing of mGluR7. Three polymerase chain reaction products (Olf 1, 2, and 8) were identified as unique receptors and were used to screen a rat olfactory bulb cDNA library. Olf 1 labeled 10 clones identified as mGluR5 (9). Olf 2 labeled a single clone, designated mGluR7, that is a new member of the mGluR family. Olf 8 identified three clones that also show unique DNA sequences related to the mGluR family. The nucleotide sequence and predicted amino acid sequence for mGluR7 are shown in Fig. 1. The cDNA sequence contains an open reading frame of 2745 bp and an initiation codon consensus sequence surrounding the presumed initiator methionine (29). The deduced amino acid sequence of mGluR7 contains 915 amino acids, with an estimated molecular mass of 102,304 Da. Hydrophobicity analysis (30) predicts a seven-transmembrane domain receptor with the characteristic features of the mGluRs, including a large amino-terminal domain of 590 amino acids and a large second cytoplasmic loop that appears to be involved in coupling of mGluRs to G proteins (31). There are four putative *N*-glycosylation sites in the amino terminus and one consensus calcium/calmodulin-dependent protein kinase II phosphorylation site in the second intracellular domain. Amino acid sequence comparison of mGluR7 and other members of the mGluR family showed a high degree of conservation with mGluR4 (69%) and mGluR6 (67%), with a lower degree of conservation with mGluR1 (42%), mGluR2 (45%), mGluR3 (45%), or mGluR5 (45%). These overall homologies place mGluR7 in a subset of mGluRs including mGluR4 and mGluR6.

Expression of mGluR7. To express mGluR7, we first synthesized RNA transcripts *in vitro* and injected these into *Xenopus* oocytes. In several experiments, oocytes injected with mGluR7 mRNA evoked no glutamate responses, despite large calcium-activated chloride currents in sister oocytes injected with mGluR1 mRNA (data not shown). These data suggested that mGluR7, unlike mGluR1 and mGluR5 (7–9), does not activate phospholipase C. The other mGluRs (mGluR2–4 and mGluR6) have been shown to inhibit cAMP production in CHO cells (15, 16, 18) or BHK cells (17). As shown in Fig. 2,

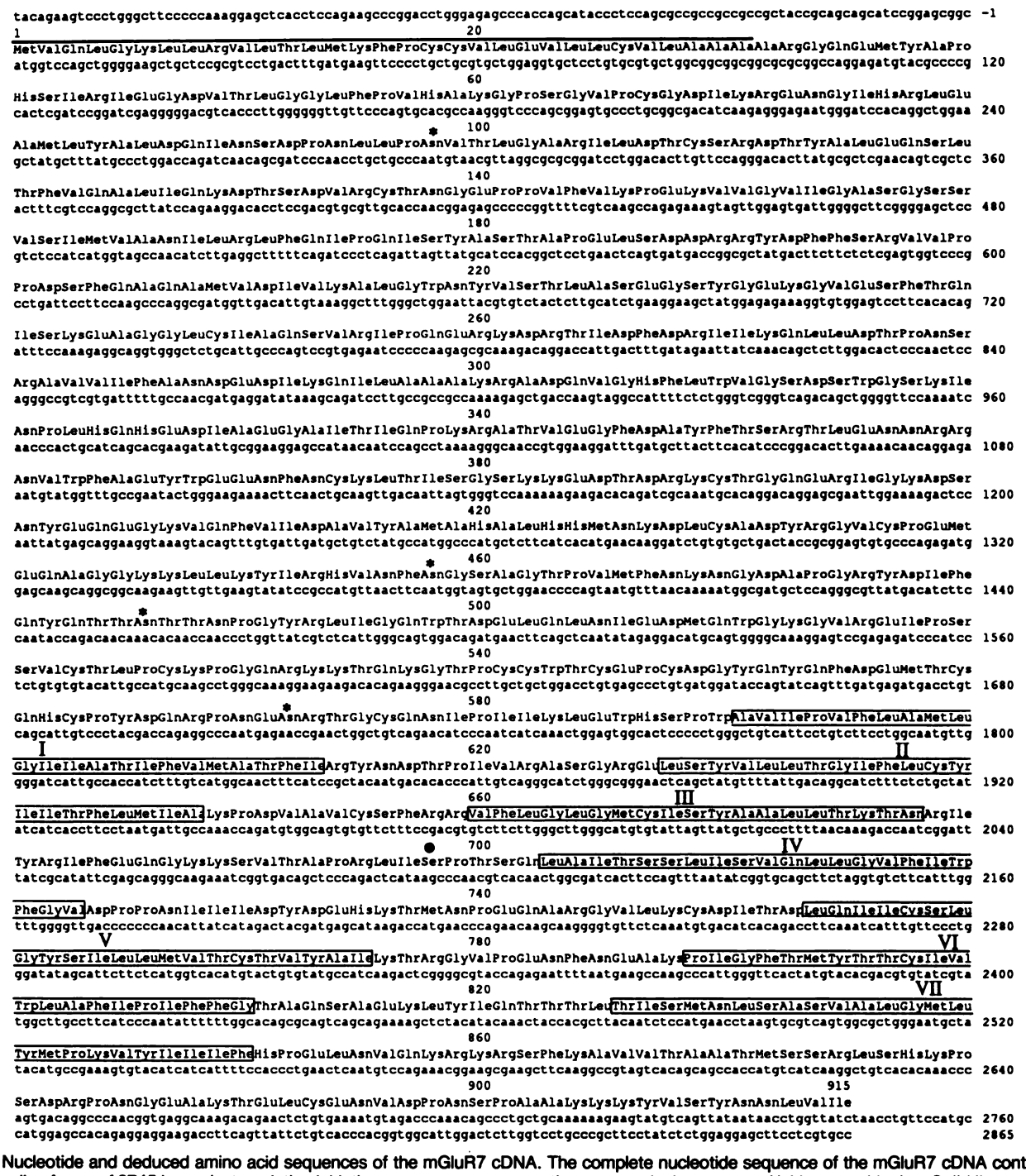

Fig. 1. Nucleotide and deduced amino acid sequences of the mGluR7 cDNA. The complete nucleotide sequence of the mGluR7 cDNA contains an open reading frame of 2745 bp and a translation initiation consensus sequence that surrounds the presumed initiator methionine. *Solid line*, predicted signal peptide. The deduced amino acid sequence contains 915 amino acids. The seven transmembrane segments (*boxes*) were assigned based on hydrophobicity analysis. *, Putative *N*-glycosylation sites; ●, consensus calcium/calmodulin-dependent protein kinase II phosphorylation site. GenBank accession no. U06832.

L-AP4 (1 mM) or glutamate (1 mM) inhibited forskolin-stimulated cAMP production in BHK cells stably expressing mGluR7 cDNA, confirming that mGluR7 is a member of the mGluR family. The presence of mGluR7 in the transfected BHK cells was confirmed by RNA blot analysis and immunoblot analysis using a polyclonal antiserum prepared against a peptide representing amino acids 896–909 of mGluR7. Immunoblots demonstrated a protein of 110 kDa that was not seen in untransfected BHK cells.

L-AP4 had no significant effect on basal cAMP levels or on forskolin-stimulated cAMP levels in untransfected BHK cells. Lower concentrations of L-AP4 or glutamate (30 μM or 100 μM) produced small decreases in cAMP that were not statistically significant. ACPD (1 mM) and quisqualate (0.5 mM) were much less effective at concentrations far in excess of those that result in maximal stimulation of mGluR1. Likewise, L-AP3 (1 mM) or MCPG (1 mM), which antagonize some mGluR responses (4, 32), did not antagonize mGluR7-mediated cAMP inhibition. L-AP4 (1 mM) stimulation of a BHK clone expressing mGluR4 (17) produced 45% inhibition, confirming the sensitivity of the

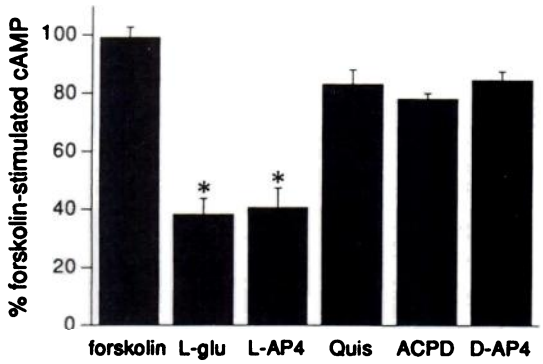

Fig. 2. Inhibition of forskolin-stimulated cAMP production by mGluR7 in BHK cells. BHK cells transfected with mGluR7 were preincubated for 20 min with isobutylmethylxanthine (1 mM) and then incubated for 10 min with forskolin (10 μM), in the presence or absence of agonists or antagonists. Basal cAMP production was subtracted from the stimulated levels and the inhibition was plotted as a percentage of the forskolin-stimulated control. L-AP4 (1 mM) and glutamate (*L-glu*) (1 mM) produced significant inhibition, whereas quisqualate (*Quis*) (0.5 mM) and ACPD (1 mM) produced small decreases that did not reach statistical significance. Incubation with L-AP3 (1 mM) or MCPG (1 mM) in the presence of L-AP4 (1 mM) did not antagonize the inhibition of cAMP production (data not shown). *, Significant ($p < 0.05$) decreases in cAMP, compared with forskolin-treated controls.

assay. L-AP4 and glutamate were much less potent agonists of mGluR7 in our experiments than has been observed for mGluR4 or mGluR6. Estimated half-maximal inhibitory concentrations were 0.5 mM for L-AP4 and 1.3 mM for glutamate. The modest inhibition at low agonist concentrations that we observed may be due to either lower levels of receptor expression or less efficient coupling to adenylate cyclase for mGluR7. The latter may indicate that adenylate cyclase inhibition is not the principal signal transduction mechanism for mGluR7. For example, mGluR6 inhibits cAMP in CHO cells, but its primary action in neurons appears to be stimulation of a phosphodiesterase (19).

Distribution of mGluR7 RNA. Hybridization of a mGluR7 cDNA probe to poly(A)$^+$ RNA isolated from several regions of adult rat brain revealed a single class of mRNA of approximately 4.4 kilobases (Fig. 3A). The previously identified L-AP4-sensitive receptors mGluR4 and mGluR6 show quite constricted patterns of RNA expression. However, mGluR7 was widely expressed in the central nervous system. The highest levels of expression were seen in the thalamus, neocortex, and hypothalamus, with significant levels of expression in the hippocampus, olfactory bulb, brainstem, and midbrain. *In situ* hybridization with a mGluR7 probe was consistent with the RNA blot analysis, showing a broad distribution of hybridization in cortex, olfactory bulb, hippocampus, thalamus, and caudate putamen but with relatively low signal in the cerebellum (Fig. 3B).

Comparison of mGluR7 with mGluR4 revealed complementary patterns of hybridization in the hippocampus and olfactory bulb, although both subtypes were highly expressed in the entorhinal cortex, subiculum, and presubiculum (Fig. 4A). There was strong hybridization of mGluR7 in the dentate gyrus, the hippocampus (CA1-CA4), and the mitral/tufted cells of the olfactory bulb (Fig. 4B). In contrast, mGluR4 was present in the glomerular and granular cell layers of the olfactory bulb (Fig. 4B). In the cerebellum, mGluR4 mRNA was present in

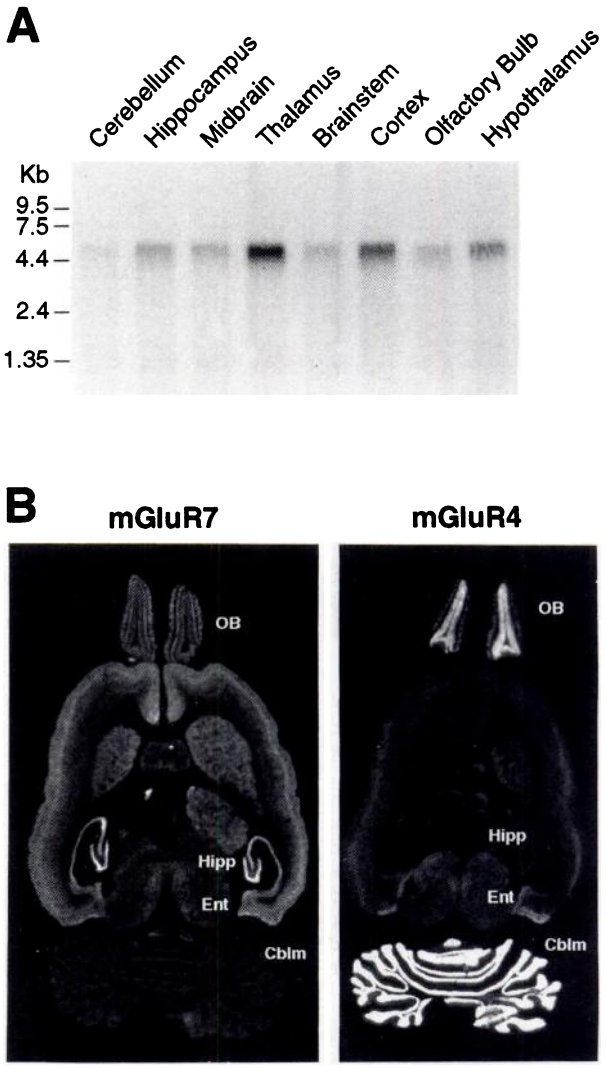

Fig. 3. Distribution of mGluR7 RNA. A, RNA blot analysis of adult rat brain hybridized with a ^{32}P-labeled mGluR7 cDNA probe, which labeled a single size class of mRNA, of approximately 4.4 kilobases. B, *In situ* hybridization of transverse sections labeled with a probe for mGluR7 mRNA, compared with a probe for mGluR4 mRNA, another member of the AP4-sensitive class of mGluRs. The distribution of mGluR7 was more widespread than that of mGluR4, and complementary patterns were seen in olfactory bulb and cerebellum. *OB*, olfactory bulb; *Hipp*, hippocampus; *Ent*, entorhinal cortex; *Cblm*, cerebellum.

the granular layers, whereas mGluR7 was restricted to the Purkinje cells (data not shown).

Expression of mGluR7 and mGluR1 mRNA in single mitral/tufted cells. The existence of multiple mGluRs with distinct but overlapping patterns of distribution suggests that single cells may contain more than one mGluR subtype. We tested this possibility in the olfactory bulb by a double-labeling method using probes for mGluR7 and mGluR1 mRNA. As shown in Fig. 5, most mitral/tufted cells were labeled with the mGluR7 RNA probe, as represented by the accumulation of silver grains over perikarya. Likewise, all of the mGluR7-labeled cells were also labeled with an mGluR1 RNA probe, using digoxigenin as the chromagen. The sections were not counterstained and thus the diffuse staining of the soma represents the digoxigenin reaction product. mGluR1 has been reported to have a primarily postsynaptic localization (33), whereas the effects of L-AP4 on mitral/tufted cells are presyn-

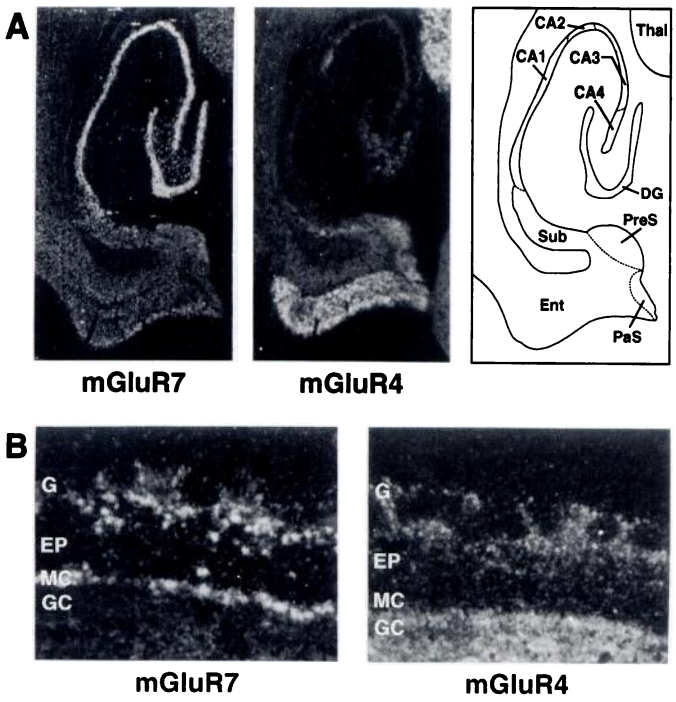

Fig. 4. Comparison of the mRNA expression patterns for two L-AP4-sensitive receptors, mGluR7 and mGluR4. A, Dark-field photomicrographs of emulsion-dipped sections of the hippocampus and entorhinal cortex hybridized to a ^{32}P-labeled RNA probe for mGluR7. Both mRNAs were highly expressed in entorhinal cortex, whereas only mGluR7 was expressed in the dentate gyrus and CA1-CA4. *Thal*, thalamus; *CA1-CA4*, subfields of hippocampal formation; *DG*, dentate gyrus; *PreS*, presubiculum; *PaS*, parasubiculum; *Sub*, subiculum; *Ent*, entorhinal cortex (magnification, 32.5 ×). B, In the olfactory bulb, mGluR7 was expressed in the mitral cell layer, whereas mGluR4 was expressed in the granule cell layer. *G*, glomerular layer; *EP*, external plexiform; *MC*, mitral cell layer; *GC*, granule cell layer (magnification, 100 ×).

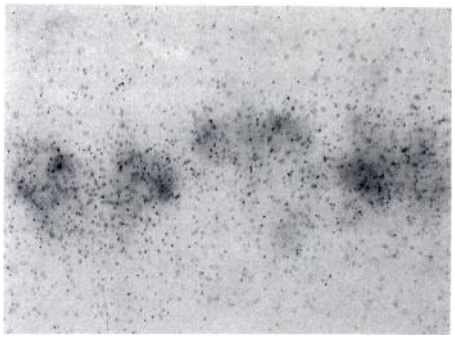

Fig. 5. Individual mitral/tufted cells of the olfactory bulb contain both mGluR7 and mGluR1 mRNAs. A rat brain olfactory bulb section through the mitral cell layer was hybridized *in situ* with a ^{35}S-labeled mGluR7 RNA probe and a digoxigenin-labeled mGluR1 RNA probe and was examined under light microscopy. The mGluR1 probe labeled mitral/tufted cells, as indicated by the diffuse cytoplasmic stain. Subsequently the sections were dipped in emulsion to identify mGluR7-labeled neurons. The speckled pattern of silver grains over digoxigenin-labeled cells demonstrates colabeling of mitral/tufted cells with the mGluR7 and mGluR1 probes (magnification, 504 ×).

aptic (21). This may suggest compartmentalization of different mGluR subtypes within the same neuron.

Discussion

We report the identification and expression of a novel mGluR. Based on sequence homology and agonist selectivity, mGluR7 is a member of a mGluR subfamily that includes mGluR4, mGluR6, and mGluR7. These receptors are sensitive to L-AP4 and inhibit cAMP production in transfected cells, although their transduction mechanism in neurons is less well defined. mGluR7 is the most widely distributed of the L-AP4-sensitive mGluRs and is likely to mediate L-glutamate inhibition of transmitter release at selected pathways in the brain and spinal cord.

AP4-sensitive mGluRs. The primary action of L-AP4 on brain and spinal neurons is the inhibition of transmitter release, consistent with the presence of a glutamate autoreceptor on presynaptic nerve terminals. A characteristic feature of this presynaptic inhibition is a marked difference in L-AP4 sensitivity in different synaptic pathways, with the lateral perforant path and the lateral olfactory tract being among the most sensitive (1). Because mGluR6 appears to be selectively localized to retinal bipolar neurons (18), either mGluR4 or mGluR7 is likely to mediate the presynaptic effects of L-AP4 in other brain regions. The pattern of mGluR4 and mGluR7 mRNA expression is consistent with this hypothesis. mGluR4 and mGluR7 are highly expressed in entorhinal cortex, from which the lateral perforant path originates (20), whereas mGluR7 is present in mitral/tufted cells of the olfactory bulb, the neurons whose axons constitute the lateral olfactory tract (21).

Although mGluR4, mGluR6, and mGluR7 appear to have similar agonist profiles, it is interesting that mGluR7 was quite insensitive to agonist stimulation in BHK cells. This seems unlikely to have resulted from a low level of receptor expression in the cells, because several BHK clones had similar sensitivities to glutamate and L-AP4. However, G protein coupling may be different or less efficient in BHK or CHO cells, compared with neurons, as has been observed for mGluR6 (18, 19). mGluRs that couple to inhibition of cAMP in CHO and BHK cells may utilize similar G proteins, because they share structural features such as two conserved proline residues in the second intracellular loop, a region known to be important in the coupling specificity of mGluR1 (31). Similarly, the proximal portion of the carboxyl terminus, another region putatively involved in G protein specifity, is highly conserved in the AP4-sensitive group of receptors, suggesting that this region may be important in determining differences in their G protein specificity, compared with the mGluR2/mGluR3 subfamily.

Coupling mechanism for presynaptic L-AP4 receptors. Inhibition of cAMP could be the mechanism for L-AP4-mediated decreases in transmitter release. For example, stimulation of adenylate cyclase increases transmitter release at many synapses in both vertebrates and invertebrates (34). However, it is equally likely that alternative coupling mechanisms are involved. Our experiments suggest that mGluR7 does not couple to phospholipase C or to G_s-induced increases in cAMP. The decrease in cAMP in BHK cells is consistent with the involvement of a G_i/G_o protein; however, many neurotransmitter receptors that utilize G_i/G_o also modulate voltage-dependent potassium and calcium channels (35). Inhibition of voltage-dependent calcium channels could underlie presynaptic inhibition by mGluR4 or mGluR7. This may involve a membrane-delimited action of a G protein, as has been described in cultured olfactory bulb neurons (21). The inhibition of the calcium current had an agonist sensitivity similar to that of inhibition of transmitter release from mitral cells. G protein-

coupled receptors can also activate more than one second messenger. For example, in CHO cells mGluR1 is coupled to PI hydrolysis but also increases cAMP levels and arachidonic acid release (36). These "secondary" coupling mechanisms may have different agonist sensitivities. For example, similar phenomena have been seen in cells expressing m2 and m4 muscarinic receptors. In these cells, low concentrations of muscarinic agonists inhibit cAMP generation, whereas higher concentrations stimulate PI hydrolysis (37). Analysis of mGluR7 action in neuronal cells will be necessary to resolve this issue.

Role of mGluR subtypes in the olfactory bulb. The olfactory bulb provides a particularly rich model system to explore the action of mGluRs, because all but mGluR6 are expressed in sets of neurons within the bulb. Multiple subtypes may also be present within the same neurons. For example, mRNAs for mGluR1 and mGluR7 are expressed in the mitral/tufted layer, whereas mGluR2, mGluR4, and mGluR5 are expressed in the granule cell layer (7, 9, 15, 16). The cellular specificity of mGluR action has been elegantly demonstrated in the accessory olfactory bulb, where mGluR2 inhibits release of γ-aminobutyric acid from granule cells at the dendrodendritic synapse onto mitral cells (38). Thus, the cellular and subcellular localization of mGluRs may be an important determinant in shaping incoming sensory information.

Acknowledgments

We thank R. Simerly and M. Mortrud for advice about *in situ* hybridization and M. Hallin, D. Beacham, and J. Volk for technical assistance.

Note Added in Proof

After this report was submitted, Okamoto *et al.* reported the cloning of a mGluR with an identical amino acid sequence. (Okamoto, N., S. Hori, C. Akazawa, Y. Hayashi, R. Shigemoto, N. Mizuno, and S. Nakanishi. Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. *J. Biol. Chem.* **269:** 1231–1236 (1994).)

References

1. Mayer, M. A., and G. L. Westbrook. The physiology of excitatory amino acids in the vertebrate central nervous system. *Prog. Neurobiol.* **28:**197–276 (1987).
2. Gasic, G. P., and M. Hollman. Molecular neurobiology of glutamate receptors. *Annu. Rev. Physiol.* **54:**507–536 (1992).
3. Sommer, B., and P. Seeburg. Glutamate receptor channels: novel properties and new clones. *Trends Pharmacol. Sci.* **13:**291–296 (1992).
4. Schoepp, D. D., and P. J. Conn. Metabotropic glutamate receptors in brain function and pathology. *Trends Pharmacol. Sci.* **14:**13–20 (1993).
5. Nicoletti, F., J. L. Meek, J. Iadarola, D. M. Chuang, B. L. Roth, and E. Costa. Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus. *J. Neurochem.* **46:**40–46 (1986).
6. Sugiyama, H., I. Ito, and C. Hirono. A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature (Lond.)* **325:**531–533 (1987).
7. Masu, M., Y. Tanabe, K. Tsuchida, R. Shigemoto, and S. Nakanishi. Sequence and expression of a metabotropic glutamate receptor. *Nature (Lond.)* **349:**760–765 (1991).
8. Houamed, K. M., J. L. Kuijper, T. L. Gilbert, B. A. Haldeman, P. J. O'Hara, E. R. Mulvihill, W. Almers, and F. S. Hagen. Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. *Science (Washington D. C.)* **252:**1318–1321 (1991).
9. Abe, T., H. Sugihara, H. Nawa, R. Shigemoto, N. Mizuno, and S. Nakanishi. Molecular characterization of a novel metabotropic glutamate receptor, mGluR5, coupled to inositol phosphate/Ca^{2+} signal transduction. *J. Biol. Chem.* **267:**13361–13368 (1992).
10. Bashir, Z. I., Z. A. Bortolotto, C. H. Davies, N. Berretta, A. J. Irving, A. J. Seal, J. M. Henley, D. E. Jane, J. C. Watkins, and G. L. Collingridge. Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature (Lond.)* **363:**347–363 (1993).
11. Linden, D. J., M. J. Dickinson, M. Smeyne, and J. A. Connor. A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. *Neuron* **7:**81–89 (1991).
12. Baskys, A., and R. C. Malenka. Agonists at metabotropic glutamate receptors presynaptically inhibit epscs in neonatal rat hippocampus. *J. Physiol. (Lond.)* **444:**687–701 (1991).
13. Charpak, S., B. H. Gähwiler, K. Q. Do, and T. Knöpfel. Potassium conductances in hippocampal neurons blocked by excitatory amino-acid transmitters. *Nature (Lond.)* **347:**765–767 (1990).
14. Lester, R. A. J., and C. E. Jahr. Quisqualate receptor-mediated depression of calcium currents in hippocampal neurons. *Neuron* **4:**741–749 (1990).
15. Tanabe, Y., M. Masu, T. Ishii, R. Shigemoto, and S. Nakanishi. A family of metabotropic receptors. *Neuron* **8:**169–179 (1992).
16. Tanabe, Y., A. Nomura, M. Masu, R. Shigemoto, N. Mizuno, and S. Nakanishi. Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. *J. Neurosci.* **13:**1372–1378 (1992).
17. Thomsen, C., P. Kristensen, E. Mulvihill, B. Haldemann, and P. D. Suzdak. L-2-Amino-4-phosphonobutyrate (L-AP4) is an agonist at the type IV metabotropic glutamate receptor which is negatively coupled to adenylate cyclase. *Eur. J. Pharmocol.* **227:**361–362 (1992).
18. Nakajima, Y., H. Iwakabe, C. Akazawa, H. Nawa, R. Shigemoto, N. Mizuno, and S. Nakanishi. Molecular characterization of a novel retinal metabotropic glutamate receptor, mGluR6, with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J. Biol. Chem.* **268:**11868–11873 (1993).
19. Nawy, S., and C. E. Jahr. Suppression by glutamate of cGMP-activated conductance in retinal bipolar cells. *Nature (Lond.)* **346:**269–271 (1990).
20. Koerner, J. F., and C. W. Cotman. Micromolar L-2-amino-4-phosphonobutyric acid selectively inhibits perforant path synapses from lateral entorhinal cortex. *Brain Res.* **216:**192–198 (1981).
21. Trombley, P. Q., and G. L. Westbrook. L-AP4 inhibits calcium currents and synaptic transmission via a G protein-coupled glutamate receptor. *J. Neurosci.* **12:**2043–2050 (1992).
22. Sahara, Y., and G. L. Westbrook. Modulation of calcium currents by a metabotropic glutamate receptor involves fast and slow kinetic components in cultured hippocampal neurons. *J. Neurosci.* **13:**3041–3050 (1993).
23. Sanger, F., S. Nicklen, and A. R. Coulson. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:**5463–5367 (1977).
24. Southern, E. M. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98:**503–517 (1975).
25. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:**156–159 (1987).
26. Mountjoy, K. G., L. S. Robbins, M. T. Mortrud, and R. D. Cone. The cloning of a family of genes that encode the melanocortin receptors. *Science (Washington D. C.)* **257:**1248–1251 (1992).
27. Simerly, R. B., and B. J. Young. Regulation of estrogen receptor messenger ribonucleic acid in rat hypothalamus by sex steroid hormones. *Mol. Endocrinol.* **5:**424–432 (1991).
28. Dascal, N., C. Ifune, R. Hopkins, T. P. Snutch, H. Lubbert, N. Davidson, M. I. Simon, and H. A. Lester. Involvement of a GTP-binding protein in mediation of serotonin and acetylcholine responses in *Xenopus* oocytes injected with rat brain messenger RNA. *Brain Res.* **387:**201–209 (1986).
29. Kozak, M. An analysis of the 5′-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* **15:**8125–8148 (1987).
30. Kyte, J., and R. F. Doolittle. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157:**105–132 (1982).
31. Pin, J. P. Functional roles of intracellular domains of metabotropic glutamate receptors. *Funct. Neurol.* (Suppl. 8) 42 (1993).
32. Jane, D. E., P. L. Jones, P. C. Pook, T. E. Salt, D. C. Sunter, and J. C. Watkins. Stereospecific antagonism by (+)-*alpha*-methyl-4-carboxyphenylglycine (MCPG) of (1*S*,3*R*)-ACPD-induced effects in neonatal rat motoneurones and rat thalamic neurones. *Neuropharmacology* **32:**725–727 (1993).
33. Martin, L. J., C. D. Blackstone, R. L. Huganir, and D. L. Price. Cellular localization of a metabotropic glutamate receptor in rat brain. *Neuron* **9:**259–270 (1992).
34. Kaczmarek, L. K., and I. B. Levitan. *Neuromodulation.* Oxford University Press, New York (1987).
35. Brown, A. M., and L. Birnbaumer. Ionic channels and their regulation by G protein subunits. *Annu. Rev. Physiol.* **52:**197–213 (1990).
36. Aramori, I., and S. Nakanishi. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. *Neuron* **8:**757–765 (1992).
37. Peralta, E. G., A. Ashkenazi, J. W. Winslow, J. Ramachandran, and D. J. Capon. Differential regulation of PI hydrolysis and adenylyl cyclase by muscarinic receptor subtypes. *Nature (Lond.)* **334:**434–437 (1988).
38. Hayashi, Y., A. Momiyama, T. Takahashi, H. Ohishi, R. Ogawa-Meguro, R. Shigemoto, N. Mizuno, and S. Nakanishi. Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. *Nature (Lond.)*, **366:**687–690.

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