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A Novel Metabotropic Glutamate Receptor Expressed in the Retina and Olfactory Bulb

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A novel metabotropic glutamate receptor, mGluR8, was identified by screening a mouse retina cDNA library. This receptor is most related to mGluR4, mGluR7, and mGluR6 (74%, 74%, and 70% identical amino acid residues, respectively). Similar to these receptors, stimulation by L-glutamate or L-2-amino-4-phosphonobutyrate (L-APB) of Chinese hamster ovary (CHO) cells stably transfected with mGluR8 result in the inhibition of forskolin-stimulated adenylyl cyclase. *In situ* hybridization studies revealed a strong expression of the mGluR8 gene in the olfactory bulb, accessory olfactory bulb, and mammillary body. A weaker expression was found in the retina, and in scattered cells in the cortex and hindbrain. During development, the distribution of mGluR8 expression was more widespread.

These results extend the diversity of metabotropic glutamate receptors in the CNS. Because at least two APB receptors are expressed in the retina, the use of this drug to block selectively the ON pathway needs to be reconsidered. The pharmacology and expression of mGluR8 in mitral/tufted cells suggest it could be a presynaptic receptor modulating glutamate release by these cells at their axon terminals in the entorhinal cortex.

[Key words: L-2-amino-4-phosphonobutyrate (APB, L-AP4), glutamate receptor, metabotropic receptor, retina, olfactory bulb, ontogenesis, ON pathway]

The actions of glutamate, the major excitatory neurotransmitter in the vertebrate CNS, are mediated through glutamate receptors (GluRs). There are two broad classes of GluRs. One class, the ionotropic receptors, are ligand-gated ion channels whose response to selective agonists define the NMDA, α-amino-3-hydroxy-5-methylisoxasole-4-propionate (AMPA) and kainate subtypes (Monaghan et al., 1989). They are assembled from an as yet undetermined number of subunits encoded by related gene families (reviewed by Nakanishi, 1992; Seeburg et al., 1993; Hollmann and Heinemann, 1994).

In contrast, metabotropic glutamate receptors (mGluRs) are coupled through G proteins to second messenger pathways. They are formed by a single polypeptide, predicted to span the plasma membrane seven times. Thus, they would share a common structural architecture with the G protein–coupled receptor superfamily, even though at the primary structure level, no homology is apparent. Molecular cloning has identified seven distinct metabotropic receptors termed mGluR1 through mGluR7 (Houamed et al., 1991; Masu et al., 1991; Tanabe et al., 1992; Abe et al., 1993; Nakajima et al., 1993; Okamoto et al., 1994; Saugstad et al., 1994). Recently, a Ca^{2+}-sensing receptor, identified from a bovine parathyroid cDNA library, has been found to be related to mGluRs, thus expanding this gene family (Brown et al., 1993). Metabotropic GluRs can be classified into three groups according to their sequence similarities, pharmacological properties, and preferred signal transduction mechanism (Nakanishi, 1992): group I receptors, mGluR1 and mGluR5, are most strongly activated by quisqualate and are coupled to phosphoinositol turnover; group II receptors, mGluR2 and mGluR3, are most sensitive to *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD); and group III receptors, mGluR4, mGluR6, and mGluR7, are selectively activated by L-2-amino-4-phosphonobutyrate (L-APB, or L-AP4). Group II and III receptors, when expressed in transfected fibroblast cell lines, are coupled to the inhibition of adenylyl cyclase.

Because of detailed anatomical, physiological, and pharmacological studies, the retina is well suited to analyze the relationship between GluR diversity and neural function. For example, an APB-activated mGluR, most likely mGluR6 (Nakajima et al., 1993; Nomura et al., 1994), plays a critical role in the retina in generating the depolarizing response of ON bipolar cells which is at the origin of the ON pathway (Slaughter and Miller, 1981). Many studies have used APB to block selectively ON bipolar cells and analyze the function of the ON pathway in vision processing (reviewed by Schiller, 1992).

To better understand the role of glutamatergic neurotransmission in the retina in particular, and in the CNS in general, ultimately all GluRs need to be identified, their distribution mapped and their pharmacological and physiological characteristics determined. By screening mouse retina cDNA libraries, we have identified a novel metabotropic receptor, sensitive to APB, which we are naming mGluR8. This is the second mGluR activated by APB found in retina. The effects of this drug in this tissue are thus likely to be more complex than previously thought, and not limited to blocking the ON pathway. Until we understand better the role of mGluR8 in retinal function, caution is needed in interpreting experiments using APB in the retina.

Materials and Methods

Degenerate primer PCR, construction and screening of cDNA libraries. Two approaches were used to identify additional mGluRs expressed in

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retina: PCR using degenerate primers and screening of retina cDNA libraries. For PCR, the following primers were synthesized on a PCR-mate (Applied Biosystems, Foster City, CA): 5′-TACCTG(C/G)TGGA(C/T)GAGTTCAC(T/C)TG, 5′-ATGCAGGTGGTGTACATGGT(A/G)AA. PolyA$^+$ RNA was isolated from dissected retina of C57BL/6 mice using the QuickPrep mRNA Purification Kit (Pharmacia, Piscataway, NY) and cDNA was synthesized using oligo(dT) as primer. Following RNA template removal with RNaseH (Boehringer Mannheim, Indianapolis, IN), the cDNA was amplified by PCR; 740 bp long PCR products were cloned into pBluescriptSK(−) (Stratagene, La Jolla, CA), and their nucleotide sequences were determined (Sequenase, United States Biochemicals, Cleveland, OH). Sequences encoding mGluR1, mGluR3, and two previously unidentified receptors were isolated.

The alternate approach was to screen two mouse retina cDNA libraries. One was a generous gift by Drs. Cathy Bowes and Debora Farber (University of California, Los Angeles; Bowes et al., 1989). The other was constructed using the λZAP-cDNA Cloning Kit (Stratagene) and polyA$^+$ RNA isolated as described above. Approximately 10^6 phages from the first library were screened using a mixed probe ^{32}P-labeled by random priming (Boehringer Mannheim). The probes were prepared from PCR fragments encoding the seven transmembrane domain of mGluR1 and mGluR3. The screening was performed under medium stringency conditions: the filters were hybridized overnight at 65°C in 5× SSC, 0.5% SDS, 0.5 mg/ml denatured sonicated herring sperm DNA and 1× Denhardt's solution with 2×10^5 cpm/ml of each probe. Washings were done at room temperature in 2× SSC and 0.1% SDS. SSC and Denhardt's solution are as defined by Sambrook et al. (1986). The cDNA insert of four positive clones was excised *in vivo* (Short et al., 1988; Duvoisin et al., 1989), plasmid DNA was prepared, and analyzed by Southern blot to identify fragments hybridizing to the mGluR probe. These fragments were subcloned into pBluescriptSK(−) and their nucleotide sequences were determined. One of the positive clones was shown to encode the mouse mGluR1 gene, the sequence of another was unrelated to metabotropic receptors, whereas the two remaining clones were identical and their sequence overlapped one of the novel sequences identified by degenerate primer PCR. The cDNA insert was not full-length and a PCR fragment corresponding to bases 1791–2236 of the final sequence (Fig. 1) was used to screen both retina cDNA libraries. Hybridization conditions were the same as above, washings were at high stringency each at 65°C for 30 min; twice in 2× SSC, 0.1% SDS; once in 0.5× SSC, 0.1% SDS; and a final wash in 0.2× SSC, 0.1% SDS. In this way three additional clones were isolated. The nucleotide sequence of segments homologous to mGluR coding regions was determined using primers synthesized according to previously established sequence.

Construction of a full-length clone and functional expression in CHO cells. The 5′-RACE-PCR kit (GIBCO/Bethesda Research Labs, Gaithersburg, MD) was used to clone the remaining portion of the coding region. Two rounds of PCR were needed. After the first round, a sample of the PCR product was used for a Southern blot with an end-labeled nested primer as probe to determine the size of the sought after DNA piece. The remainder of the PCR was size fractionated on a 1% agarose gel, eluted and a second round of PCR was done using the nested and anchor primers. The PCR product was digested with *Sal*I (a site contained in the anchor primer) and *Kpn*I (a site identified in the cDNA-derived sequence) and size-fractionated on an agarose gel. The fragment was ligated into similarly digested pBluescriptSK(−). The full-length cDNA insert was constructed from pieces of the RACE-PCR, and two overlapping cDNA clone-derived sequences and cloned into pGEM3Z (Promega, Madison, WI). To verify the final construct, at least one strand was sequenced over the full length. Between the sequences of the cDNA clones and the RACE-PCR fragment, the nucleotide sequence was determined on both strands throughout. Nucleotide sequence analysis was facilitated by the use of the GCG software package (Devereux et al., 1984).

For expression studies the G/C tail generated during the RACE-PCR and approximately 450 bases of 5′ untranslated region were removed. The *Apa*I-*Sac*I fragment was cloned into pCMV5 (Chen et al., 1991). Chinese hamster ovary (CHO) cells were transfected with this construct together with one tenth the amount of pSVneo (Southern and Berg, 1982). These cells were maintained in DMEM with 10% dialyzed fetal bovine serum and selected with 400 μg/ml G418 (all from GIBCO/Bethesda Research Labs). Northern blots were done to determine the highest expressing cell lines. Cells were plated at 1.5×10^5 cells per well in 12 well plates and grown for 2 d. Medium was replaced by 1 mM 3-isobutyl-1-methylxanthine (IBMX) in phosphate-buffered saline (PBS, pH 7.4, 0.1 M) for 30 min, and then varying concentrations of agonist in PBS, 1 mM IBMX, 10 μM forskolin were applied for 10 min at 37°C. The reaction was stopped and the cells collected and lysed in 66% ethanol, 4 mM EDTA. Cyclic AMP concentrations were measured in triplicate using a cAMP detection kit (Amersham, Arlington Heights, IL). Each experiment was done at least three times. L-APB and quisqualate were obtained from Cambridge Research Chemicals (Cambridge, UK); (1S,3R)-ACPD from Tocris Neuramin (Buckhurst Hill, UK). IBMX, forskolin, L-glutamate, and ibotenate were from Sigma (St. Louis, MO).

In situ *hybridization.* The full-length cDNA clone in pGEM3Z was linearized with *Apa*I or *Sac*I, transcribed *in vitro* using T7 or SP6 polymerase and ^{35}S-UTP (New England Nuclear, Boston, MA) to generate antisense and sense probes, respectively. Probes were hydrolyzed to about 200–400 bases in length according to Cox et al. (1984), and used at about 5×10^6 cpm/ml. Adult C57BL/6 mice (Charles River, Wilmington, MA) were sacrificed with CO_2, and their brain and eyecups were fixed in 4% paraformaldehyde (PFA) in PBS for 3–4 hr. Sixteen days following the occurrence of a vaginal plug, pregnant mice were sacrificed with CO_2 and embryos were removed and frozen on dry ice. Tissues were cryoprotected in 30% sucrose in PBS and embedded in O.C.T. Compound (Miles, Elkhart, IN); 12 μm thick sections were cut on a cryostat, collected on gelatin- and polylysine-coated slides, and fixed in 4% PFA-PBS. Following hybridizations and washings, as previously described (Hughes et al., 1992), the slides were dipped in Kodak NTB-2 nuclear emulsion diluted in water (1:1), stored in the dark for 4–8 weeks, developed in D-19, and fixed (Kodak, Rochester, NY). Sections were covered with glycerin and PBS (1:1) and coverslipped. Final washings were done also at higher stringency in 20% formamide, 0.1× SSC, 1 mM DTT at 55°C and produced similar patterns of expression.

Results

Molecular characterization of a novel metabotropic glutamate receptor

To identify additional metabotropic receptors whose expression might be restricted to the retina, two approaches were used. Mouse retina polyA$^+$ RNA was reverse transcribed and amplified (RT-PCR) using degenerate oligonucleotide primers derived from two conserved amino acid sequence stretches in the extracellular domain and in the putative sixth transmembrane segment. PCR fragments of the expected 742 bp size were cloned, and their nucleotide sequence was determined. Sequences encoding mGluR1, mGluR3, and two previously unidentified receptors were isolated.

In a separate approach, a mouse retina cDNA library (Bowes et al., 1989) was screened using two PCR fragments encoding the seven transmembrane region of mGluR1 and mGluR3 as probes (Houamed et al., 1991; Masu et al., 1991; Tanabe et al., 1992). One of the λ recombinant phages isolated contained a sequence encoding a new member of the mGluR family; it was identical to one of the two sequences identified by degenerate primer-PCR. Nucleotide sequencing revealed that this cDNA clone, although large enough to contain a complete receptor, only encoded the carboxy-terminal region of this receptor. This was most likely due to a cloning artifact. A PCR fragment derived from this partial cDNA clone was used to screen additional retina libraries, and three overlapping cDNA inserts were isolated. In addition, the RACE-PCR procedure (Frohman et al., 1988) was used to clone the remaining 5′ region.

For functional expression studies and to provide a longer probe for *in situ* hybridization experiments, a full-length clone was engineered from parts of the RACE-PCR product and two overlapping cDNA clones. The resulting construct contains the entire coding region. Its nucleotide sequence is shown in Figure 1, together with the deduced amino acid sequence.

From the assigned initiation codon, which is in conformity

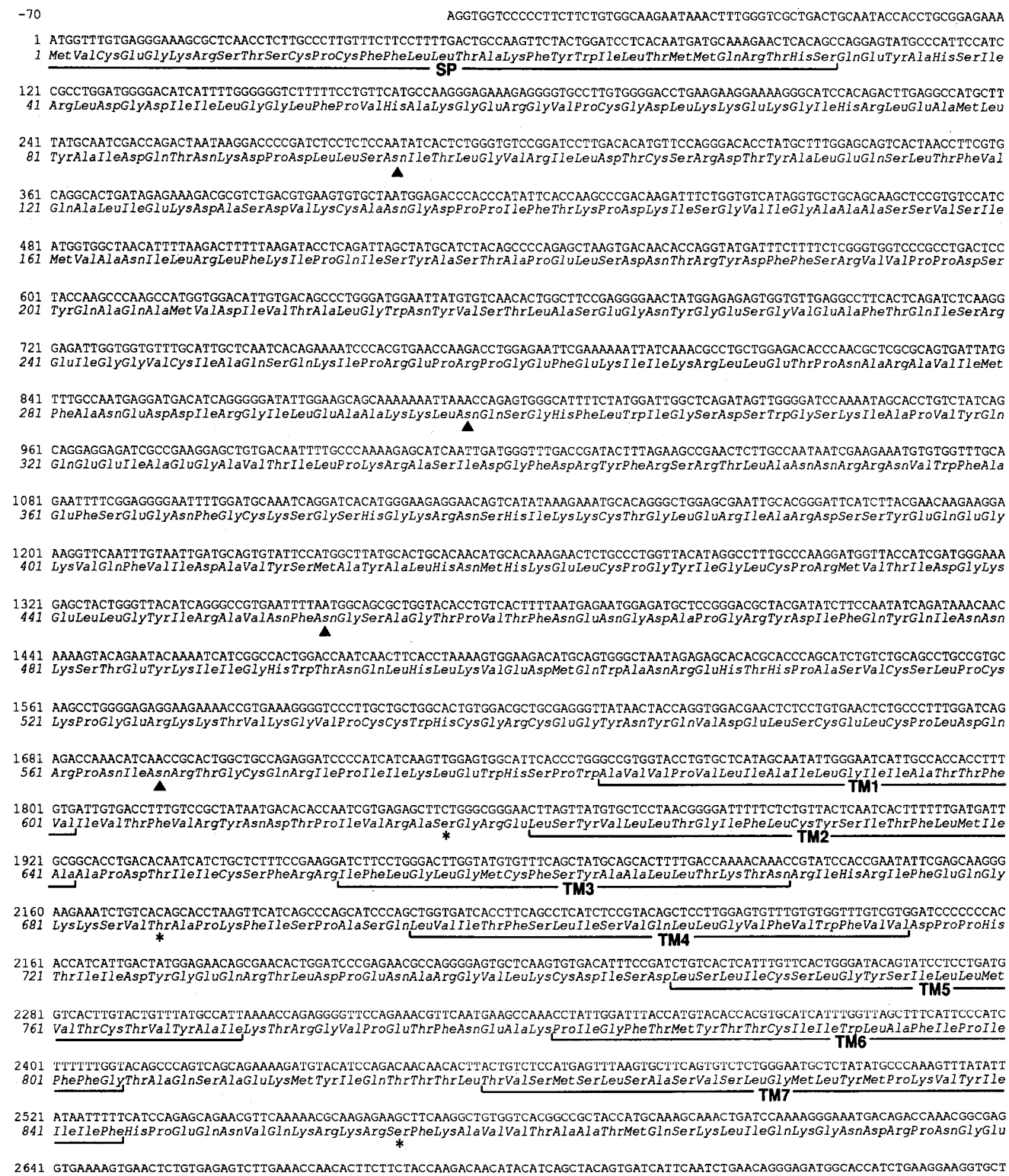

Figure 1. Nucleotide sequence and deduced amino acid sequence of mGluR8. The seven putative transmembrane segments and leader peptide indicated were assigned based on hydrophobicity analysis and alignment with the previously published seven mGluRs. Potential N-linked glycosylation sites are indicated by *solid triangles*, potential phosphorylation sites by *asterisks*. GenBank accession number U17252.

with the consensus sequence for translation initiation (Kozak, 1986) and is also the first in-frame methionine codon following a non-sense codon at position −93 (not shown), to the termination codon at position 2725, an open reading frame encodes a 908 residue protein. Analysis of the deduced amino acid sequence reveals features characteristic of a mGluR, including seven putative transmembrane segments. By analogy with other mGluRs, the amino-terminal residues are likely to form a signal

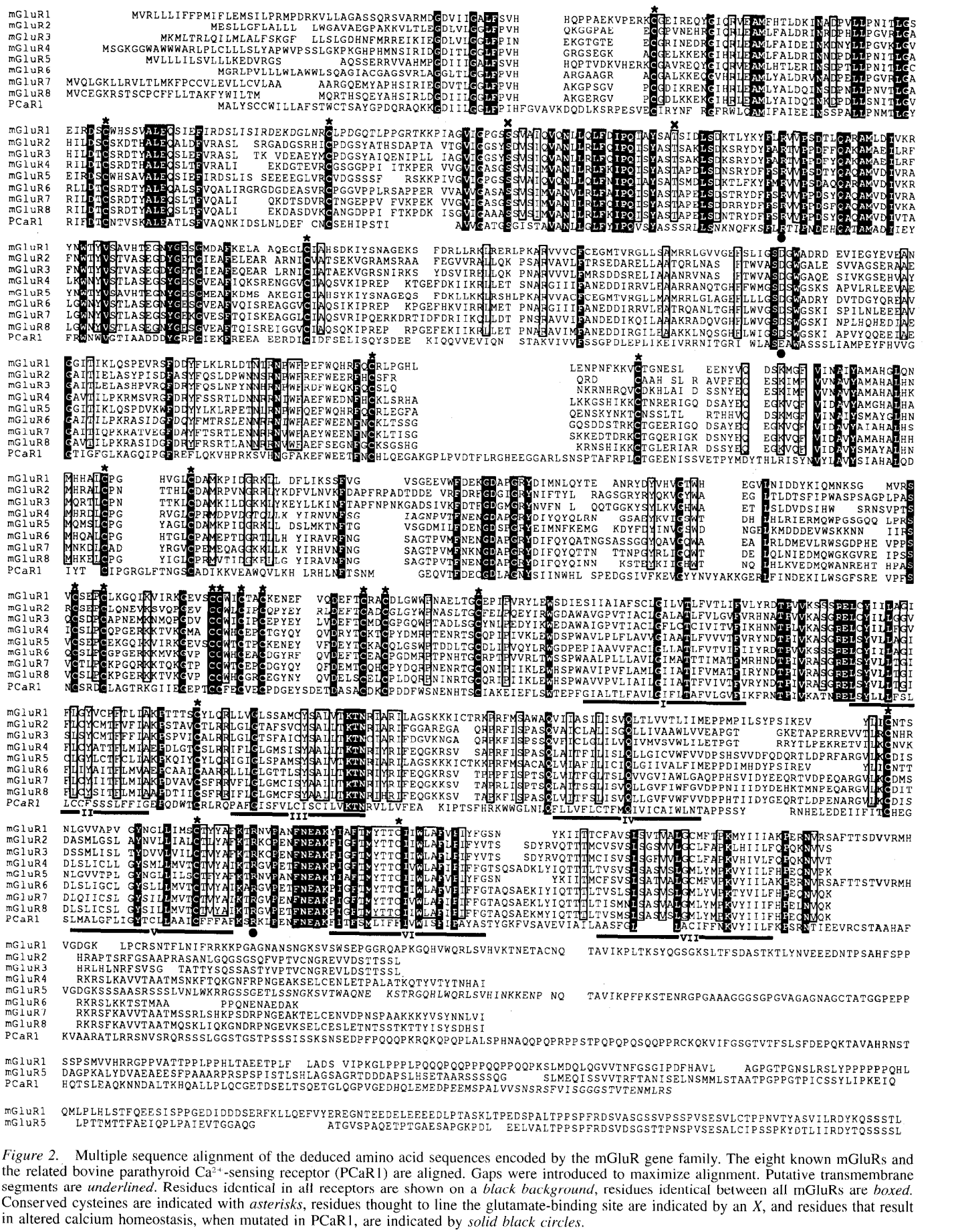

Figure 2. Multiple sequence alignment of the deduced amino acid sequences encoded by the mGluR gene family. The eight known mGluRs and the related bovine parathyroid Ca^{2+}-sensing receptor (PCaR1) are aligned. Gaps were introduced to maximize alignment. Putative transmembrane segments are *underlined*. Residues identical in all receptors are shown on a *black background*, residues identical between all mGluRs are *boxed*. Conserved cysteines are indicated with *asterisks*, residues thought to line the glutamate-binding site are indicated by an *X*, and residues that result in altered calcium homeostasis, when mutated in PCaR1, are indicated by *solid black circles*.

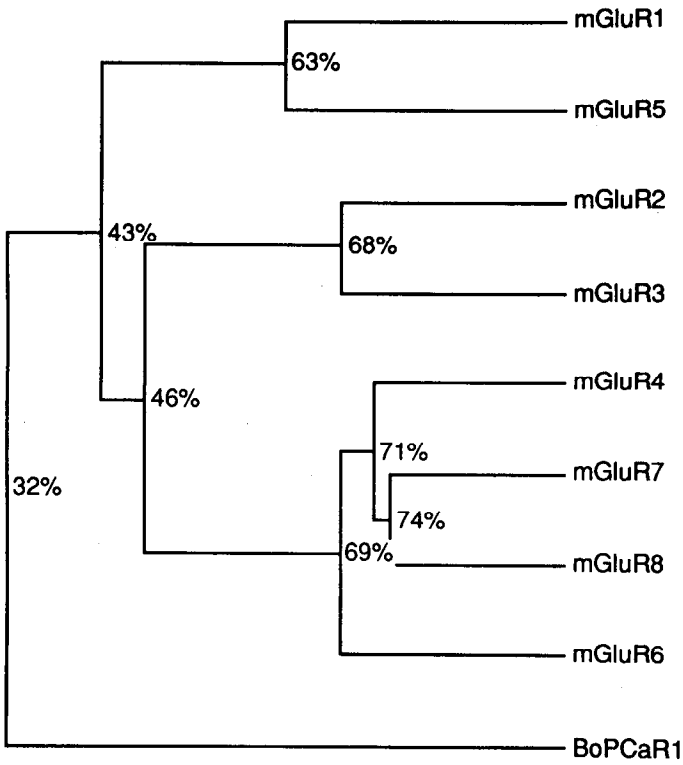

Figure 3. Schematic representation of the mGluR gene family similarity. Percent sequence identities were calculated from pairwise sequence alignments. Averages are given for comparisons between more than two sequences.

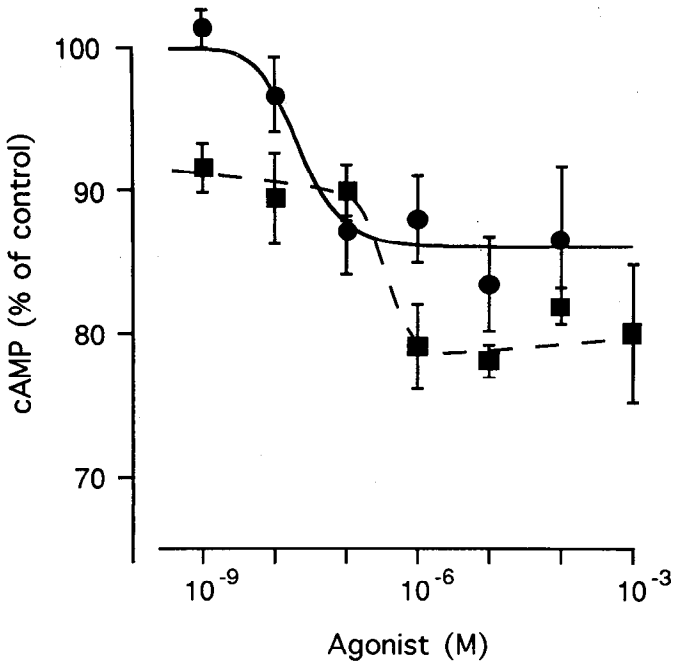

Figure 4. Dose–response curves of L-glutamate and L-APB. Inhibition of forskolin-stimulated cAMP production in mGluR8 expressing CHO cells in response to the indicated concentration of L-glutamate (●) and L-APB (■) were determined as described in Materials and Methods. Each point shows the mean ± SD of at least three experiments measured in triplicates.

peptide, but the method of von Heijne (1986) does not predict an unambiguous cleavage site. The highest score suggests a proteolytic cleavage following the Serine residue at position 33, which would yield a mature receptor with a calculated molecular weight of 97,451. The following large amino-terminal domain is predicted to be extracellular and contains four potential N-linked glycosylation sites. Three potential phosphorylation sites are available in the predicted cytoplasmic loops and carboxy-terminal tail (Fig. 1).

A multiple sequence alignment between all known mGluR sequences and the related Ca^{2+}-sensing receptor (Brown et al., 1993) is shown in Figure 2. The most conserved regions are a hydrophobic domain in the extracellular domain, postulated to form the ligand binding domain (O'Hara et al., 1993), and segments surrounding this region. The first and third intracellular loops, possibly involved in G-protein coupling, and several putative transmembrane segments, especially the sixth, are also very conserved between mGluRs, but not as much with the Ca^{2+} receptor. The 21 cysteine residues conserved in all mGluRs are also present in mGluR8.

Pairwise alignments between mGluR8 and other members of the mGluR family show that it is most related to mGluR4 (74% sequence identity) and mGluR7 (74%), although mGluR4 is only 69% identical to mGluR7 (Fig. 3). mGluR8 is also very similar to mGluR6, the proposed APB receptor of retinal ON bipolar cells (70% sequence identity). It has been found that the relative potencies of various mGluR agonists is conserved among more closely related receptors. This suggests that mGluR8 belongs to the group III mGluRs, which respond to APB by an inhibition of forskolin-stimulated adenylyl cyclase.

Functional expression of mGluR8

To test this hypothesis, we established stable cell lines by transfecting CHO cells with a plasmid in which the mGluR8 cDNA is driven by the viral CMV promoter. cAMP production in forskolin-stimulated cells in the presence and absence of several glutamate agonists was measured. Maximal inhibitions were obtained with L-APB and L-glutamate, and dose–response curves were determined for these agonists (Fig. 4). The calculated half-maximal effective concentration (EC_{50}) of L-glutamate is 22 nM. In contrast to the other group III mGluRs, it is lower than the EC_{50} of L-APB, which is 400 nM. Maximal inhibitions (about 20%) are lower than reported for previously tested mGluRs. This difference could reflect a physiological difference between mGluR8 and the other mGluRs or could be due to an experimental difference, for example in the level of mGluR expression. In our control experiments, mGluR2 expressing cells had a maximal inhibition of 57%, which was less than the 80% previously reported (Tanabe et al., 1992).

Other glutamate agonists tested gave smaller responses with 100 μM (1S-3R)-ACPD, quisqualate, and ibotenate producing 10%, 7%, and 7% inhibition of forskolin-stimulated cAMP formation, respectively. As control, no inhibition of adenylyl cyclase was detected in cells transfected with the selection plasmid vector pSVneo alone (not shown).

We also tested whether mGluR8 could be coupled to phosphoinositol turnover in the *Xenopus* oocyte expression model. It has been shown that in this system, the accumulation of inositol phosphates promotes the release of Ca^{2+} from intracellular stores and leads to the opening of Ca^{2+}-activated Cl^- channels. No Cl^- currents were recorded in response to 1 mM glutamate in oocytes injected with mGluR8 synthetic RNA, whereas large currents were measured in a parallel experiment with mGluR1 RNA-

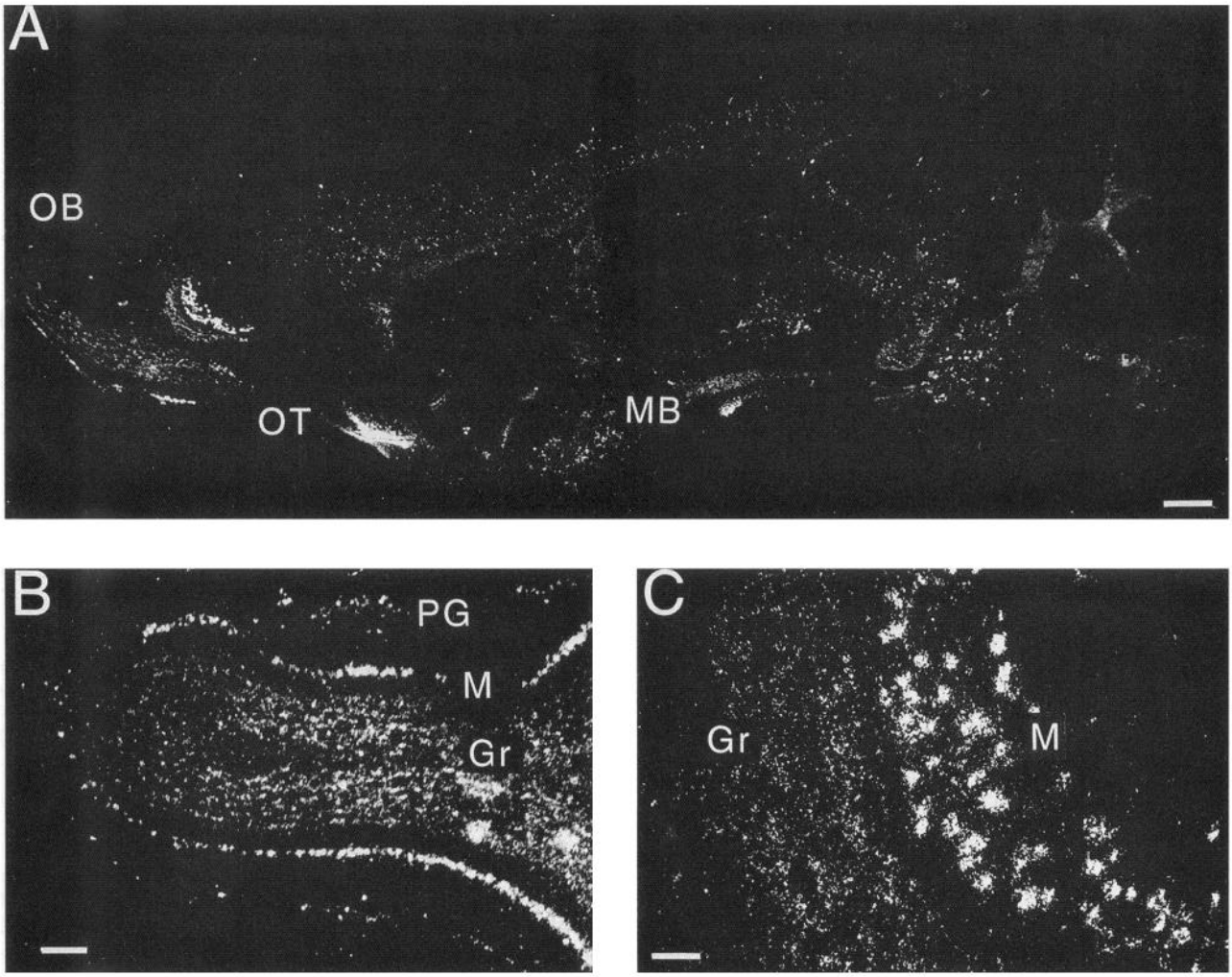

Figure 5. Distribution of mGluR8 mRNA in adult mouse CNS. *A*, parasagittal section of the adult brain. Labeling is detected in the olfactory bulb (*OB*), the olfactory tubercle (*OT*), and in the mammillary body (*MB*). *B*, Horizontal section through the olfactory bulb. Mitral (*M*), granule (*G*), and periglomerular (*PG*) cell layers are labeled. *C*, Sagittal section through the accessory olfactory bulb. Mitral (*M*) and granule (*G*) cells are labeled. Scale bars: *A*, 500 μm; *B*, 200 μm; *C*, 50 μm.

injected oocytes (not shown). From these experiments we conclude that mGluR8 does not couple to phosphoinositol turnover, at least in *Xenopus* oocytes.

Localization of expression by in situ *hybridization analysis*

To determine the pattern of mGluR8 gene expression in the adult and developing brain, *in situ* hybridization histochemistry using a ^{35}S-UTP–labeled antisense riboprobe was performed. A sense probe was used as negative control and to determine washing conditions. Horizontal and sagittal sections through the adult mouse brain, transversal retinal sections, and sagittal sections through the developing mouse head were hybridized with both probes. Strong expression of mGluR8 was found in the olfactory bulb, olfactory tubercle, and mammillary body (Fig. 5*A*). Scattered cells were also labeled in the deeper layers of the cortex and in the hindbrain. A horizontal section through the olfactory bulb reveals that cells in the granule, mitral, and periglomerular layers are expressing mGluR8 (Fig. 5*B*). It is possible that a few displaced tufted cells are also positive. In addition, mitral and granule cells of the accessory olfactory bulb are also strongly labeled (Fig. 5*C*). No expression of mGluR8 was detected in the cerebellum and in the hippocampus, two regions where APB has been shown to inhibit excitatory glutamate neurotransmission. In these regions this effect is thus likely to be mediated by mGluR4 and mGluR7 receptors.

The pattern of mGluR8 expression was also analyzed in the developing brain and in the retina (Fig. 6). A widespread expression was found in the embryonic day 16 (E16) mouse. Hybridization was visible with varying intensities in parts of the developing telencephalon, thalamus, hypothalamus, midbrain, pons, and medulla oblongata, as well as in the olfactory bulb and retina (Fig. 6*A,B*). Expression was also detected in the PNS, in the developing dorsal root and trigeminal ganglia. Overall, the hybridization signals appeared stronger than in the adult. This could reflect higher levels of expression during development than in the adult or a difference in permeability of the probe into developing tissue as compared to adult tissue. This observation was confirmed in a developmental series study of the retina (Fig. 6*C*). mGluR8 transcripts were clearly detected at E16 in the developing retina. At this stage, most cells are still actively dividing in the ventricular zone, located in the outer retina, but a few postmitotic cell have migrated towards the inner

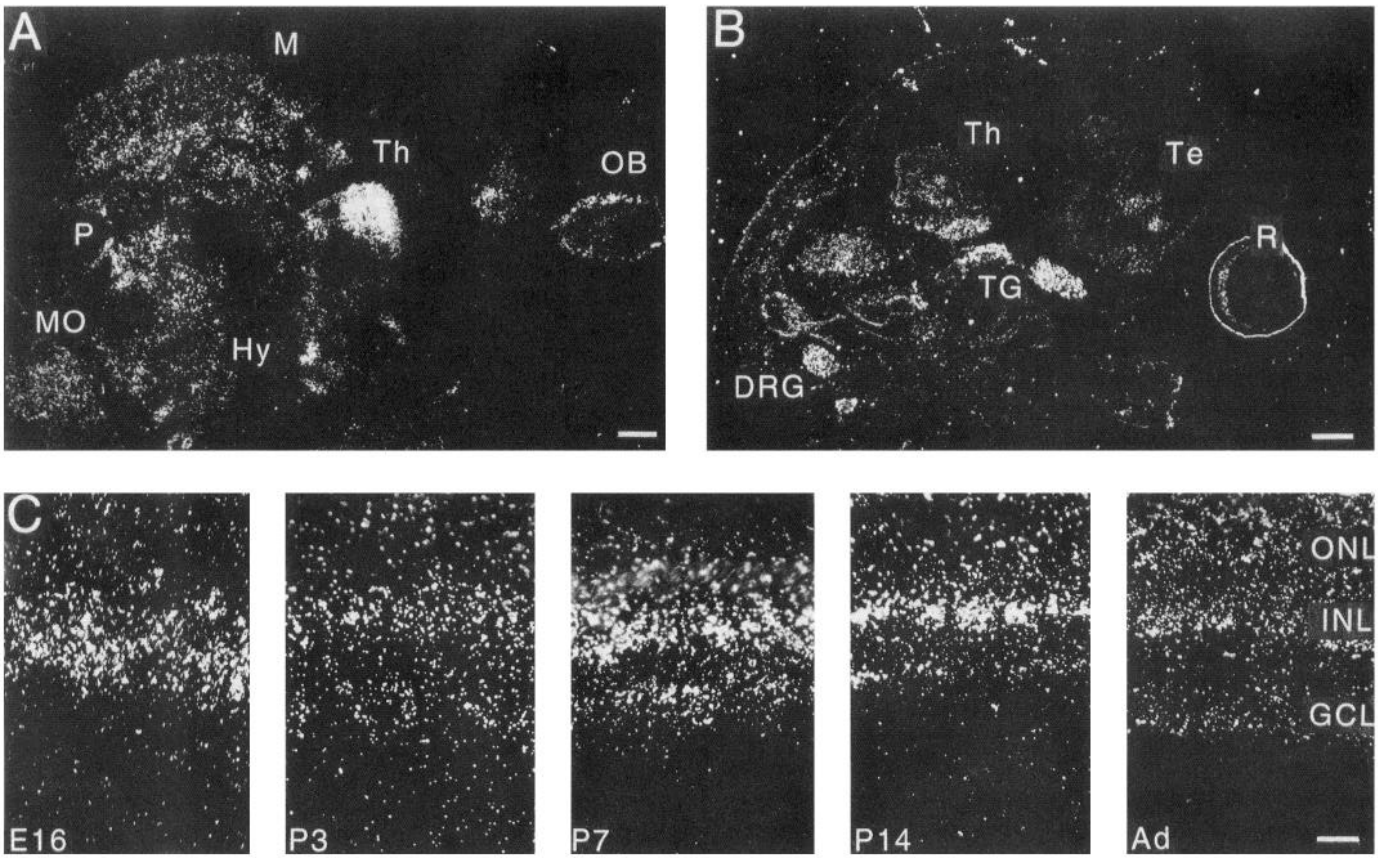

Figure 6. Distribution of mGluR8 mRNA during development. Dark-field photomicrographs of emulsion-dipped 20 μm sections that were hybridized *in situ* with ^{35}S-radiolabeled antisense full-length mGluR8 probe (see Materials and Methods). *A*, Sagittal section, and *B*, parasagittal section through an E16 mouse embryo. Labeling is visible in the telencephalon (*Te*), thalamus (*Th*), hypothalamus (*Hy*), midbrain (*M*), pons (*P*), medulla oblongata (*MO*), olfactory bulb (*OB*), retina (*R*), dorsal root and trigeminal ganglia (*DRG* and *TG*). *C*, developmental series of transverse retinal sections. Labeling is observed in the ganglion cell and inner nuclear cell layers and possibly in the outer nuclear layer, where photoreceptor cell bodies reside (GCL, INL, ONL, respectively). Scale bars: *A* and *B*, 100 μm; *C*, 25 μm.

retina and started to differentiate into ganglion and amacrine cells (Young, 1985a,b). Many of these cells express mGluR8. Differentiation of the retina proceeds from the center to the periphery, and it is apparent that at E16 more cells are mGluR8 positive in the central retina than in the periphery (Fig. 6*B*). Around birth, the ganglion cell layer (GCL) becomes separated from the inner nuclear layer (INL) by a synaptic layer, the inner plexiform layer (IPL). From then on, mGluR8 expression is visible in both the INL and GCL, at first at similar intensities. While hybridization signals stay high in the INL during differentiation of the bipolar and Müller cells, the level of expression in the GCL is reduced. In the adult, levels appear lower and about similar in the INL and GCL, but because of the poor resolution of ^{35}S-labeling it is not possible to determine in which specific cell types mGluR8 is expressed. It is possible that photoreceptor cells in the outer nuclear layer (ONL) are positive for mGluR8, but because of the low level of expression and the long exposure times necessary to detect a signal, it is difficult to determine whether the grains only reflect a higher background above these cells. When mGluR8-specific antisera become available, immunohistochemical studies might provide an answer.

Discussion

The identification of a novel metabotropic glutamate receptor, named mGluR8, is reported. This receptor is most related to the group III mGluR subfamily, which includes mGluR4, mGluR6 and mGluR7. Transfected in fibroblasts, these receptors have been shown to respond to APB stimulations by the inhibition of forskolin-stimulated adenylyl cyclase. The EC_{50} for L-APB of mGluR4, mGluR6, and mGluR8 is about 1 μM (Thomsen et al., 1992; Nakajima et al., 1993; this work), whereas for mGluR7 it is about 160 μM (Okamoto et al., 1994). For mGluR4, mGluR6 and mGluR7, the potency of L-glutamate is lower than for L-APB (EC_{50} values of 27 μM, 16 μM, and 1 mM, respectively). mGluR8 is distinctive in having a reversed relative potency order for L-glutamate and L-APB.

In our experiments with mGluR8, the average maximal inhibition of forskolin-stimulated cAMP formation was only 20%. This modest inhibition could be due to technical difficulties; different laboratories report widely different maximal inhibition in experiments expressing other mGluRs [compare Thomsen et al. (1992) with Tanabe et al. (1993) or Okamoto et al. (1994) with Saugstad et al. (1994)]. Such differences could be due to variations in the level of receptor expression, or in the precise experimental protocol. Even when care is taken not to include glutamate in the culture medium, metabolic glutamate could be released into it. This glutamate could activate the transfected mGluRs, and produce a growth disadvantage for cells that express the most receptors, eventually leading to cell lines expressing little functional receptor. When antibodies become available,

it will be possible to test this hypothesis by immunoprecipitating the receptors expressed in transfected cells after different passage numbers.

Alternatively, this low efficiency of coupling could be due to the absence of the appropriate G protein in CHO cells or reflect the possibility that mGluR8 is not normally coupled to adenylyl cyclase inhibition. Various mGluRs have been proposed to inhibit cGMP-phosphodiesterase, activate or inhibit adenylyl cyclase, activate phospholipase C or phospholipase A2, or even be directly coupled to Ca^{2+} and K^+ ion channels (Trombley and Westbrook, 1992; Schoepp and Conn, 1993). Such diversity in the effector function results from the specific combination of trimeric G proteins available in the cell and the preferred coupling of the receptor. Furthermore, the same receptor could activate multiple pathways, for example mGluR1 is coupled to phosphoinositol turnover, but also to stimulations of cAMP formation and arachidonic acid release (Aramori and Nakanishi, 1992).

Metabotropic glutamate receptors in the olfactory bulb

APB has been shown to antagonize glutamatergic neurotransmission in various parts of the CNS, such as the hippocampus, the olfactory cortex, and the spinal cord (Mayer and Westbrook, 1987). This effect is thought to result from the presynaptic activation of mGluRs, perhaps of the mGluR4 type (Tanabe et al., 1993), similar to the presynaptic depression of GABA release by olfactory granule cells following mGluR2 activation (Hayashi et al., 1993). However, the distribution of mGluR4 receptor expression is not consistent with the distribution of some of the reported physiological effects of APB, and it is likely that additional receptors could be involved. For example, the inhibitory effects of APB in the entorhinal cortex have been proposed to be mediated by mGluR7 receptors (Saugstad et al., 1994). The axons of mitral and tufted cells of the olfactory bulb form the lateral olfactory tract and project to the entorhinal cortex (Haberly and Price, 1977). The activation of presynaptic APB-sensitive mGluRs on mitral/tufted cell axons could account for the antagonist effect of APB applied to entorhinal cortex slices (Hearn et al., 1986). However, micromolar concentrations of APB applied to isolated olfactory bulb neurons were sufficient to produce an effect (Trombley and Westbrook, 1992). This is inconsistent with the low EC_{50} of APB on mGluR7. It is possible that mGluR8 receptors, which were shown here by *in situ* hybridization to be expressed in mitral/tufted cells, are responsible for the presynaptic regulation of glutamate release in the entorhinal cortex.

Metabotropic glutamate receptors in retina

In the retina, light information is encoded by processing various attributes through numerous pathways. Most prominent and basic among these pathways are the ON and OFF pathways. They signal increments and decrements of light and are established at the level of bipolar cells. Within the IPL, the ON and OFF bipolar output synapses are stratified into distinct sublaminae. This segregation, which is gradually acquired during differentiation, is thought to depend on synaptic activity. Recently, it has been shown that intraocular injections of APB during retinal differentiation in the newborn cat prevented normal stratification of the IPL (Bodnarenko and Chalupa, 1993). This finding was interpreted as resulting from the block of ON bipolar cell activity by a constant stimulation of the APB-sensitive receptor on these cells, most likely the recently cloned mGluR6 (Nakajima et al., 1993; Nomura et al., 1994). However, we have shown here that more than one APB-activated mGluR is present in the retina. It is therefore possible that the effects of APB infusions during retinal development are more complex than previously thought. The activation of metabotropic receptors coupled to second messenger pathways would be expected to affect dendritic outgrowth, synaptogenesis, and synaptic pruning. Similarly, intravitreal injections of APB have been shown to influence the emmetropization process (Smith et al., 1991). The inference was made that this effect was caused by blocking the ON pathway; for the same reasons, this conclusion could be premature.

Several mGluRs are known to be expressed in the retina. mGluR1 has been localized immunohistochemically in ganglion and amacrine cells (Peng et al., 1992) and mGluR6 to rod bipolar cells (Nomura et al., 1994). We have described briefly here the identification of mGluR3 sequences among RT-PCR products using mouse retinal mRNA as template; and mGluR8 was isolated from retinal cDNA libraries. Aside from mGluR6, the role of these receptors in retinal function remains unclear. APB has been reported to antagonize the horizontal cell response to light (Nawy et al., 1989). This action could be caused by a presynaptic depression of transmitter release by photoreceptors, an antagonist effect on horizontal cell AMPA/kainate GluRs, or a modulation of these receptors or some other conductance through a second messenger pathway activated by APB and/or ACPD (Yang and Wu, 1991; Takahashi and Copenhagen, 1992). The resolution of *in situ* hybridization histochemistry is not sufficient to determine which cell types are expressing mGluR8 in the retina and whether this receptor is mediating those effects. Clearly, to better understand the role of mGluRs in the development and function of the CNS, receptor subtype specific tools, such as specific antibodies, agonists, and antagonists, are needed.

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