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Cloning and Expression of Rat Metabotropic Glutamate Receptor 8 Reveals a Distinct Pharmacological Profile

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SUMMARY

The metabotropic glutamate receptor (mGluR) cDNAs were originally cloned from rat, except for the mouse cDNA clone encoding mGluR8. Mouse mGluR8 couples weakly to the inhibition of adenylate cyclase, thus hindering the characterization of its pharmacological properties. We isolated a rat mGluR8 cDNA that encodes a protein of 908 amino acids. *In situ* hybridization revealed prominent mGluR8 mRNA expression in olfactory bulb, pontine gray, lateral reticular nucleus of the thalamus, and piriform cortex. Less abundant expression was detected in cerebral cortex, hippocampus, cerebellum, and mammillary body. Glutamate evoked pertussis toxin-sensitive potassium currents in *Xenopus laevis* oocytes coexpressing mGluR8 and G protein-coupled inwardly rectifying potassium channels. mGluR8 was also activated by the group III-specific agonist L-2-amino-4-phosphonobutyric acid; (2(*S*),1′(*S*),2′(*S*)]-2-(carboxycyclopropyl)glycine, which has been frequently used as a selective group II agonist; and the nonselective agonist (1(*S*),3(*R*)]-1-aminocyclopentane-1,3-dicarboxylic acid but not by the group I-specific agonist 3,5-dihydroxyphenylglycine or the group II-specific agonist [2(*S*),1′(*R*),2′(*R*),3′(*R*)]-2-(2,3-dicarboxycyclopropyl)glycine. The agonist profile in order of potency was [2(*S*),1′(*S*),2′(*S*)]-2-(carboxycyclopropyl)glycine ≈ L-2-amino-4-phosphonobutyric acid > glutamate ≫ [1(*S*),3(*R*)]-1-aminocyclopentane-1,3-dicarboxylic acid, with EC_{50} values of 0.63, 0.67, 2.5, and 47 μM, respectively. Both the group I/II-specific antagonist (*R*,*S*)-α-methyl-4-carboxyphenylglycine and the group III-specific antagonist α-methyl-amino-phosphonobutyrate inhibited mGluR8. The pharmacological profile of mGluR8 is distinct among mGluRs but closely matches that of presynaptic inhibition in some central nervous system pathways. Thus, cellular responses mediated by both group II and III agonists may in some cases reflect activation of mGluR8 rather than multiple mGluR subtypes.

The role of mGluRs in synaptic transmission has become increasingly recognized, in large part due to their molecular characterization. Molecular cloning has identified a family of eight receptors that forms three subgroups based principally on their sequence identity but also on their agonist profile and primary signal transduction pathway (1, 2). Most neurons express multiple mGluR subtypes; thus, identification of the subtype responsible for particular *in vivo* responses has relied heavily on the distribution and pharmacological profile of cloned mGluRs. In some cases, such as mGluR2 in the accessory olfactory bulb (3) and mGluR6 in the retina (4), the distribution of mGluR RNA and protein is consistent with the responses evoked by group-specific agonists. However, in other cases, cellular responses to mGluR agonists do not match those of any of the cloned mGluRs. For example, responses evoked by mGluR agonists in spinal, cortical, and hippocampal neurons can display a mixed group II/III pharmacology. Specifically, MCPG antagonizes the L-AP4-induced depression of monosynaptic excitation in neonatal rat motoneurons and of forskolin-stimulated cAMP synthesis in guinea pig cerebrocortical slices (5, 6). MCPG also antagonizes the presynaptic inhibition mediated by L-AP4 and L-CCG-1 in guinea pig hippocampal neurons (7). This is surprising because L-AP4 selectively activates the cloned group III receptors (mGluRs 4 and 6–8), whereas MCPG is reported to selectively inhibit group I/II receptors (mGluRs 1–3 and 5) but not group III receptors (8–10).

In vitro pharmacological studies of the cloned group II/III mGluRs rely on assays of forskolin-stimulated cAMP inhibi-

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ABBREVIATIONS: mGluR, metabotropic glutamate receptor; GIRK, G protein-coupled inwardly rectifying potassium channel; L-AP4, L-2-amino-4-phosphonobutyric acid; L-CCG-1, [2(*S*),1′(*S*),2′(*S*)]-2-(carboxycyclopropyl)glycine; ACPD, [1(*S*),3(*R*)]-1-aminocyclopentane-1,3-dicarboxylic acid; MCPG, (*R*,*S*)-α-methyl-4-carboxyphenylglycine; MAP4, α-methyl-amino-phosphonobutyrate; DCG-IV, [2(*S*),1′(*R*),2′(*R*),3′(*R*)]-2-(2,3-dicarboxycyclopropyl)glycine; DHPG, 3,5-dihydroxyphenylglycine; GABA, γ-aminobutyric acid; SSC, standard saline citrate; PTX, pertussis toxin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

tion. This assay has presented problems for the analysis of mGluR7 and mGluR8 because these mGluRs cause only partial cAMP inhibition (2, 9, 11). Coexpression of mGluRs with GIRKs in *Xenopus laevis* oocytes provides an electrophysiological assay that is particularly useful for group II/III mGluRs (12). We have used the GIRK assay to examine the pharmacological properties of rat mGluR8. As expected for a group III mGluR, mGluR8 was activated by L-AP4. However, the pharmacological profile is unique in that mGluR8 also was antagonized by MCPG, reportedly a group I/II antagonist (8), and was activated by low concentrations of L-CCG-1, which is frequently used as a group II-specific agonist (13).

Experimental Procedures

Materials. L-AP4, L-CCG-1, ACPD, DHPG, MAP4, and MCPG were purchased from Tocris Cookson (Bristol, UK). Glutamate and PTX were obtained from Sigma Chemical (St. Louis, MO). RNA transcription kits were obtained from Stratagene (La Jolla, CA), [^{32}P]dCTP was obtained from Amersham (Arlington Heights, IL). DCG-IV was generously supplied by Dr. J. Conn (Emory University, Atlanta, GA) and Dr. H. Shinozaki (Tokyo Metropolitan Institute of Medical Science, Toyko, Japan). The pharmacological reagents were prepared as 50 or 100 mM stocks in water or equivalent NaOH and stored frozen. The cDNA clones encoding the rat GIRK1 (GIRK Kir3.1) and the rat CIR (cardiac inwardly rectifying potassium channel Kir3.4) were generously supplied by Dr. H. Lester (California Institute of Technology, Pasadena, CA) and Dr. J. Adelman (Vollum Institute, Oregon Health Sciences University, Portland, OR).

Isolation and sequence analysis of the rat mGluR8 cDNA. The cDNA encoding mGluR8 was isolated using previously described methods (9). In brief, oligonucleotides were designed to amplify sequences between transmembrane II and intracellular loop III of mGluRs using the polymerase chain reaction. Three polymerase chain reaction products (Olf 1, 2, and 8) were used as templates for the synthesis of ^{32}P-labeled *in vitro* RNA transcripts that were subsequently used to probe a rat olfactory bulb cDNA library (Stratagene). Fourteen duplicate positive bacteriophage clones were coinfected with helper phage into *Escherichia coli* for analysis of their cDNA inserts into pBluescript SK(−). Southern blot analysis identified three distinct groups of clones: Olf 8 labeled the cDNA encoding rat mGluR8, whereas Olf 2 labeled mGluR7 (9, 11), and Olf 1 labeled mGluR5 (14). The cDNA was sequenced using an Applied Biosystems 373 DNA automated sequencer (Norwalk, CT). A minimum of two sequence reactions were performed for each region of the cDNA, and at least three reactions were performed for regions that encoded amino acid sequence differences between the rat and mouse homologues. The sequence information was managed and analyzed using the University of Wisconsin Genetics Computer group software.

Expression of mGluR8 mRNA. *In situ* hybridization experiments were performed using adult female Sprague-Dawley rats that were deeply anesthetized with pentobarbital and perfused transcardially with ice-cold saline, followed by ice-cold 4% paraformaldehyde in 0.1 M sodium borate, pH 9.5. The brains were removed quickly and postfixed overnight at 4° in 4% paraformaldehyde in borate buffer, pH 9.5, containing 10% sucrose. Cryostat microtome sections (25 μm) were mounted onto gelatin- and poly-L-lysine-coated glass slides, incubated for 15 min in 4% paraformaldehyde in 0.1 M phosphate-buffered saline, washed twice in 0.1 M phosphate-buffered saline, and treated for 30 min at 37° in 10 mg/ml proteinase K in 100 mM Tris/50 mM EDTA, pH 8, followed by 0.0025% acetic anhydride in 0.1 M triethanolamine at room temperature. The sections were then washed in 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M Na citrate), dehydrated in increasing concentrations of ethanol, vacuum-dried at room temperature, and stored at −80° until use. The probe for mGluR8 mRNA was generated using an *Sma*I/*Xho*I cDNA fragment, encoding amino acids 1–522, subcloned into pBluescript KS. Using linearized template DNA, ^{35}S-labeled antisense mGluR8 cRNA probe was transcribed, heated to 65° for 5 min, and diluted to 10^7 cpm/ml in hybridization buffer (66% formamide, 260 mM NaCl, 1.3× Denhardt's solution, 13 mM Tris, 1.3 mM EDTA, 13% dextran sulfate). The hybridization mixture was applied to the sections, covered with siliconized glass coverslips, and sealed using DPX mountant. After incubation at 58° for 20–24 hr, the slides were soaked in 4× SSC to remove coverslips and then rinsed in 4× SSC (four times for 5 min each) before ribonuclease A treatment (20 mg/ml for 30 min at 37°). The slides were then rinsed in decreasing concentrations of SSC containing 1 mM dithiothreitol to a final stringency of 0.1× SSC/1 mM dithiothreitol for 30 min at 65°. After dehydrating the sections in increasing concentrations of ethanol, they were vacuum-dried and exposed to DuPont Cronex-4 X-ray film for 7 days (DuPont-New England Nuclear, Boston, MA). The film was then scanned by with an eight-bit Nikon LS3500 scanner, and the images were processed using Adobe Photoshop. Anatomical sites were identified using the *Brain Maps* atlas (15).

Functional expression of mGluR8. mGluR8 was functionally expressed in *X. laevis* oocytes by coexpression with GIRKs. cRNA transcripts encoding mGluR8, Kir3.1, and Kir3.4 were prepared by standard *in vitro* transcription reactions. Oocytes were harvested from anesthetized *X. laevis* (*Xenopus* One, Ann Arbor, MI) and enzymatically defolliculated as previously described (16). Stage V–VI oocytes were injected with 50–100 ng of a cRNA mixture containing mGluR8, Kir3.1, and Kir3.4 and then maintained at 18° in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5, mOsM 214) supplemented with 2.5 mM Na pyruvate, 0.5 mM theophylline (Sigma), and 50 μg/ml gentamicin (GIBCO, Grand Island, NY). For the PTX experiments, oocytes were incubated in 500 ng/ml PTX in ND96 for 48 hr before recording.

Electrophysiology. Electrophysiological recordings were performed 24–72 hr after injection. Patch pipettes with tip diameters of ≈1-2 μm were used as electrodes and filled with 3 M KCl. Oocytes were voltage-clamped at −80 mV in two-electrode voltage-clamp mode (Axoclamp 2A, Axon Instruments, Burlingame, CA). Currents were acquired at 1.6 Hz with MacLab 3.3 (AD Instruments, Milford, MA). The oocytes were placed in a 2-ml chamber and bathed continuously in ND96 at ≈3.5 ml/min. Solutions were changed using a solenoid valve controller with an exchange time of 15–30 sec. Oocytes were initially bathed in ND96 and then switched to a high potassium solution (2 mM NaCl, 96 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.5–7.6, mOsM 214). Agonists (diluted in high potassium solution) were applied when the basal potassium current (in high potassium solution) had reached equilibrium. The high potassium solution current was subtracted from the total current to obtain the agonist-induced current. Current amplitudes were measured off-line and analyzed using Student's *t* test when appropriate. Mean current amplitudes were normalized to the amplitude evoked by the maximal concentration of agonist and are expressed as mean ± standard error. A value of $p < 0.05$ was considered significant. Agonist dose-response curves were fitted using a least-squares algorithm to the Hill equation: $I = I_{max}\{1 - 1/[1 + ([A]/EC_{50})^n]\}$, where I_{max} is maximum response amplitude, [A] is agonist concentration, EC_{50} is half-maximal concentration of agonist, and n is the Hill coefficient. Antagonist dose-inhibition curves were fitted to the equation: $I = I_{max}\{1/[1 + ([A]/IC_{50})]\}$, where I_{max} is the response amplitude in the absence of antagonist, [A] is antagonist concentration, and IC_{50} is half-maximal concentration of antagonist.

Results

Sequence and expression of rat mGluR8. The rat mGluR8 cDNA sequence contains an open reading frame of 2724 bp and an initiation codon consensus sequence surrounding the presumed initiator methionine. The cDNA sequence predicts a protein of 908 amino acids with an esti-

A

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RAT     1  MVCEGKRLAS CPCFFLLTAK FYWILTMMQR THSQEYAHSI RVDGDIILGG LFPVHAKGER
MOUSE         ST                          *       L
       61  GVPCGELKKE KGIHRLEAML YAIDQINKDP DLLSNITLGV RILDTCSRDT YALEQSLTFV
             D                       T
      121  QALIEKDASD VKCANGDPPI FTKPDKISGV IGAAASSVSI MVANILRLFK IPQISYASTA
      181  PELSDNTRYD FFSRVVPPDS YQAQAMVDIV TALGWNYVST LASEGNYGES GVEAFTQISR
                                                                  *
      241  EIGGVCIAQS QKIPREPRPG EFEKIIKRLL ETPNARAVIM FANEDDIRRI LEAAKKLNQS
                                                      G
      301  GHFLWIGSDS WGSKIAPVYQ QEEIAEGAVT ILPKRASIDG FDRYFRSRTL ANNRRNVWFA
      361  EFWEENFGCK LGSHGKRNSH IKKCTGLERI ARDSSYEQEG KVQFVIDAVY SMAYALHNMH
             S G       S                  *                          *
      421  KERCPGYIGL CPRMVTIDGK ELLGYIRAVN FNGSAGTPVT FNENGDAPGR YDIFQYQINN
             L
      481  KSTEYKIIGH WTNQLHLKVE DMQWANREHT HPASVCSLPC KPGERKKTVK GVPCCWHCER
                                    *                                 I
      541  CEGYNYQVDE LSCELCPLDQ RPNINRTGCQ RIPIIKLEWH SPWAVVPVFI AILGIIATTF
                                                 II                 L
      601  VIVTFVRYND TPIVRASGRE LSYVLLTGIF LCYSITFLMI AAPDTIICSF RRIFLGLGMC
                III                                          IV
      661  FSYAALLTKT NRIHRIFEQG KKSVTAPKFI SPASQLVITF SLISVQLLGV FVWFVVDPPH
                                                      V
      721  TIIDYGEQRT LDPENARGVL KCDISDLSLI CSLGYSILLM VTCTVYAIKT RGVPETFNEA
                          VI                                  VII
      781  KPIGFTMYTT CIIWLAFIPI FFGTAQSAEK MYIQTTTLTV SMSLSASVSL GMLYMPKVYI
                                  o
      841  IIFHPEQNVQ KRKRSFKAVV TAATMQSKLI QKGNDRPNGE VKSELCESLE TNTSSTKTTY
      901  ISYSNHSI
             D
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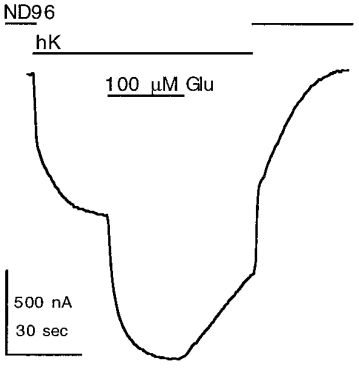
B



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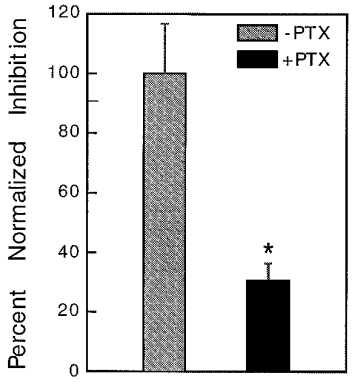

Fig. 1. Sequence and functional expression of rat mGluR8. A, The nucleotide sequence of the mGluR8 cDNA predicts a receptor comprising 908 amino acids. Alignment of rat mGluR8 with the mouse homologue shows 98.6% identity with the amino acid differences depicted. The putative leader sequence and the seven transmembrane segments were assigned based on hydrophobicity analysis. ∗, Putative *N*-glycosylation sites. ○, Potential phosphorylation sites.[2] B, An oocyte coexpressing mGluR8 and GIRK was recorded under two-electrode voltage-clamp at V_H = −80 mV. Application of high potassium solution (*hK*) resulted in inward current reflecting basally active potassium channels. The subsequent application of 100 μM glutamate diluted into the high potassium solution resulted in an agonist-induced inward current that was significantly larger than the high potassium solution current. C, Oocytes coexpressing mGluR8 and GIRK were treated with PTX (500 ng/ml) for 48 hr before recording. The application of 100 μM glutamate to PTX-treated oocytes resulted in significantly reduced current amplitudes compared with those evoked in untreated sister oocytes. The results are presented as normalized current amplitude.

mated molecular mass of 101,866 Da (Fig. 1A). Rat mGluR8 has 98.6% identity with the mouse homologue with the differences depicted in Fig. 1A. Hydrophobicity analysis predicts a signal peptide sequence at the amino terminus of the receptor and seven transmembrane domains within the receptor, characteristic of G protein-coupled receptors. Characteristic of mGluRs, mGluR8 has a large amino-terminal domain (583 amino acids) and a prominent second intracellular loop, a domain of mGluRs involved in G protein coupling (17). There are five putative *N*-glycosylation sites in the amino terminus and three putative serine/threonine phosphorylation sites in intracellular domains. Amino acid sequence comparison of mGluR8 to other mGluRs reveals that it shares the most identity to the group III receptors (mGluR4, 75.0%; mGluR6, 69.8%; mGluR7, 74.5%) and less identity to the group I and II receptors (mGluR1, 42.9%; mGluR2, 44.5%; mGluR3, 47.2%; mGluR5, 42.4%). It also shares 29.1% identity with the bovine parathyroid calcium binding protein and 28.5% identity with the human calcium binding protein (18).

To test the function of mGluR8, we injected mGluR8 cRNA into *X. laevis* oocytes. Glutamate evoked no response in normal extracellular saline (four oocytes), indicating that mGluR8 does not activate phospholipase C via G_q in oocytes. We then coexpressed mGluR8 with the GIRK subunits Kir3.1 and Kir3.4 (19, 20). Glutamate (100 μM) now evoked large inward currents (1010.1 ± 51.7 nA, eight oocytes), as shown for one oocyte in Fig. 1B, demonstrating that rat mGluR8 is a functional glutamate receptor. Oocytes coexpressing mGluR8 and GIRK were incubated in 500 ng/ml PTX for 48 hr before recording. Currents evoked by 100 μM glutamate had a mean current amplitude of 296.6 ± 49.3 nA (seven oocytes) in control oocytes, whereas currents were significantly smaller (90.4 ± 17.3 nA, 11 oocytes) in PTX-treated oocytes (Fig. 1C). Thus, mGluR8 activates GIRK via a PTX-sensitive G_i/G_o in oocytes, which is similar to other group II/III mGluRs (12).

[2] GenBank accession no. U63288.

Distribution of mGluR8 RNA. *In situ* hybridization studies show that rat mGluR8 mRNA has a highly restricted expression pattern compared with that of mGluR4 and mGluR7 (21, 22). The most prominent expression was detected in the olfactory bulb, piriform cortex, pontine gray, and lateral reticular nucleus of the thalamus (Fig. 2). Lower levels of expression were also detected in the cerebral cortex, hippocampus, cerebellum, and mammillary body. In the accessory olfactory bulb, the granule cell layer had a more intense signal than the mitral cell layer. However, in the main olfactory bulb, the mitral cell layer had a more intense signal, although there was also abundant expression in the granule cell layer (Fig. 2B). Pyramidal cells of the piriform cortex also showed high levels of expression, whereas the olfactory tubercle was virtually lacking mGluR8 mRNA (Fig. 2C). The septum and layers V/VI of the isocortex expressed lower levels of mRNA, as well as the hippocampus. The mammillary body also expressed low levels of mRNA, which was in contrast to the abundant expression detected in the mouse homologue (2). However, the rat homologue showed prominent expression in the pontine gray (Fig. 2D) and the lateral reticular nucleus of the thalamus (Fig. 2E).

Pharmacological profile of mGluR8. In *X. laevis* oocytes coexpressing mGluR8 and GIRK, glutamate, L-AP4, L-CCG-1, and ACPD all evoked inward potassium currents in high potassium solution. Currents evoked by L-CCG-1 were dose dependent, as shown for one oocyte (Fig. 3A). The concentration-response curves were plotted by normalizing the current amplitudes to the response evoked by a saturating agonist concentration for each oocyte (Fig. 3B). The pharmacological profile for mGluR8 was L-CCG-1 $\approx$ L-AP4 $>$ glutamate $\gg$ ACPD with EC_{50} values of 0.63, 0.67, 2.5, and 47 μM, respectively. ACPD was a partial agonist because saturating concentrations of glutamate evoked currents that were 2-fold larger than those evoked by saturating concentrations of ACPD (six oocytes, data not shown). The Hill coefficients for the high affinity ligands were much lower

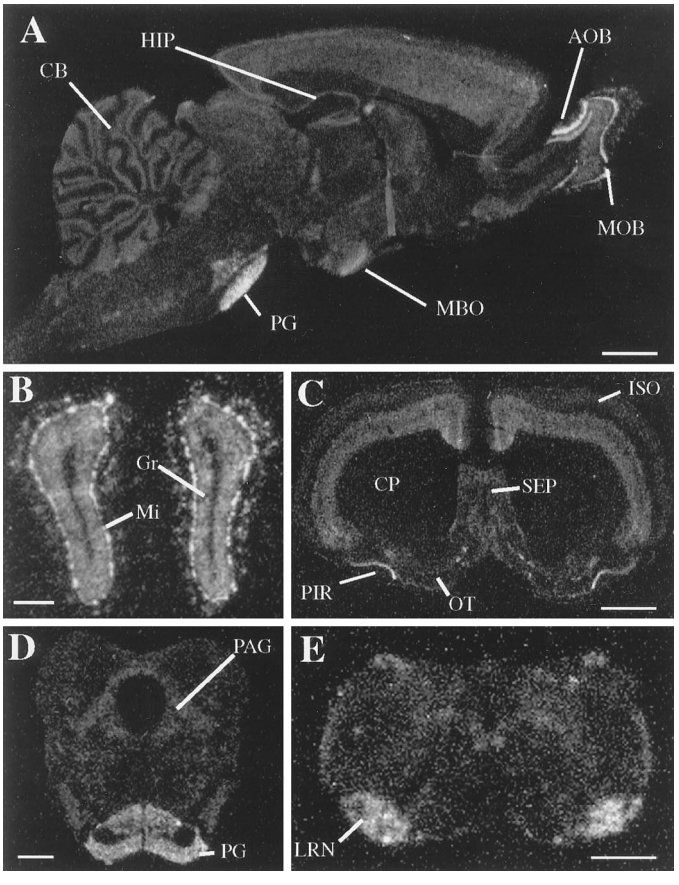

Fig. 2. Distribution of mGluR8 RNA. *In situ* hybridization of adult rat brain sections hybridized with an mGluR8 antisense cRNA probe was performed as described in the text. A, Parasagittal section revealing hybridization in the main (*MOB*) and accessory (*AOB*) olfactory bulbs and the pontine gray (*PG*). Relatively less hybridization was seen in the mammillary body (*MBO*), the cerebellum (*CB*), and the hippocampus (*HIP*). *Scale bar*, 2 mm. B, Coronal section of the MOB. *Mi*, mitral cell layer; *Gr,* granule cell layer. *Scale bar*, 0.5 mm. C, Coronal section showing hybridization in piriform cortex (*PIR*), septum (*SEP*), and deep layers of the isocortex (*ISO*). Far less staining was seen in the olfactory tubercle (*OT*) and the caudoputamen (*CP*). *Scale bar*, 2 mm. D, Coronal section at the level of the PG. *PAG*, periaqueductal gray. *Scale bar*, 1 mm. E, Coronal section of the lateral reticular nucleus (*LRN*). *Scale bar*, 1 mm.

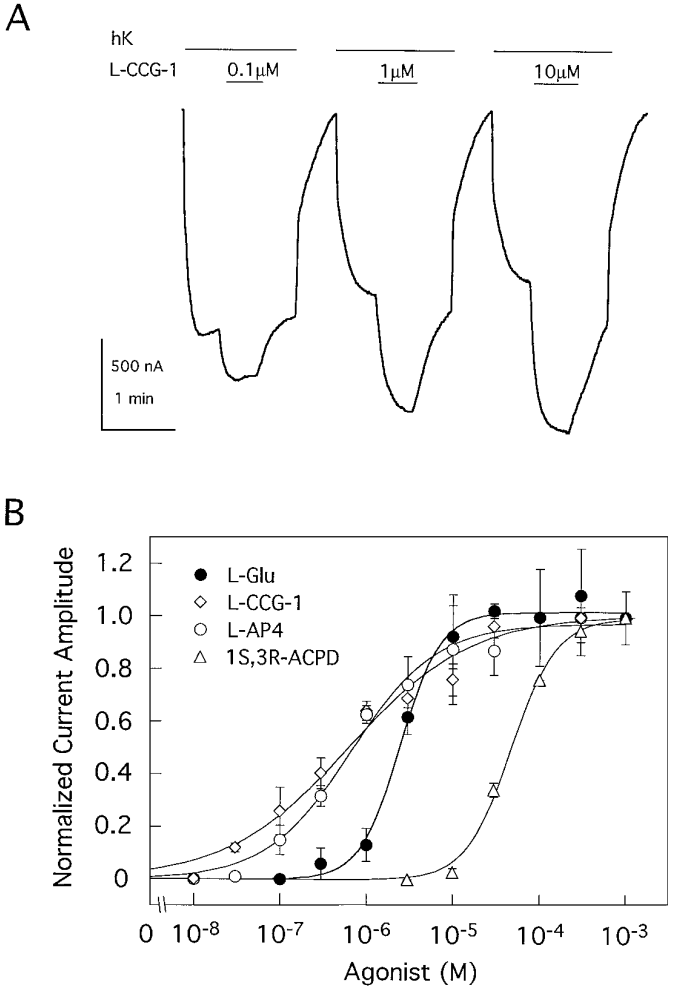

Fig. 3. Agonist profile of mGluR8. *X. laevis* oocytes were coinjected with mGluR8, Kir3.1, and Kir3.4 cRNAs and then assayed by two-electrode voltage-clamp 2–4 days after injection at $V_H = -80$ mV. A, Currents were evoked by 0.1, 1.0, and 10 μM L-CCG-1 in isometric solution (one oocyte). B, Dose-response curves were calculated as the current amplitude evoked by each concentration of agonist normalized to the amplitude evoked by the maximal concentration of agonist. This method allows for variation in the maximal current between individual oocytes. *Data points,* minimum of three responses. *hK*, high potassium solution.

(L-CCG-1 = 0.6;, L-AP4 = 0.9) than for the two lower affinity ligands (ACPD = 1.7; glutamate = 1.9).

The group I-specific agonist (*R*,*S*)-DHPG (100 μM) did not evoke currents in mGluR8-expressing oocytes (0 nA, five oocytes), although glutamate (100 μM) evoked currents in the same oocytes (282.8 ± 38.5 nA, five oocytes). However, as expected, DHPG did evoke responses in mGluR1a-expressing oocytes (1537.4 ± 162.5 nA, five oocytes), demonstrating that the reagent was active. In contrast to L-CCG-1, the group II-specific agonist DCG-IV (23) did not evoke responses in mGluR8-expressing oocytes at either 1 or 5 μM (6.4 ± 6.4 nA, five oocytes; 38.7 ± 15.3 nA, three oocytes, respectively) while glutamate (100 μM) did evoke currents in the same oocytes (290.6 ± 26.3 nA, five oocytes; 515.7 ± 68.8 nA, three oocytes, respectively). DCG-IV was active because it evoked responses in mGluR2-expressing oocytes (1 μM: 1030.8 ± 79.5 nA, four oocytes; 5 μM: 997.7 ± 125.1 nA, three oocytes).

MCPG is reported to be specific antagonist for group I/II receptors because it does not antagonize mGluR4 or mGluR7 responses (8, 9). However, MCPG did antagonize mGluR8 responses. As shown in Fig. 4A for one oocyte, MCPG (1 mM) completely blocked the mGluR8 responses evoked by 5 μM glutamate (92.6% block, seven oocytes). At 300 μM, MCPG partially blocked glutamate-evoked responses (33.6% block, six oocytes), although 100 μM had no effect (six oocytes; data not shown). The estimated IC_{50} of value of 0.32 mM was similar to that calculated for mGluR2 expressed in sister oocytes (0.22 mM; data not shown).

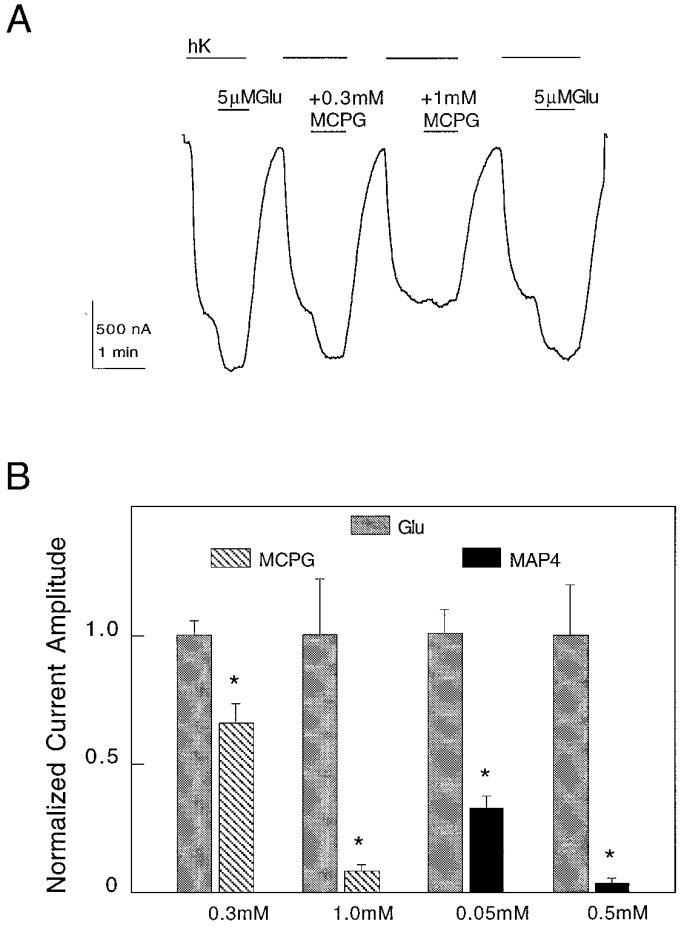

Fig. 4. Antagonism of mGluR8 by MCPG and MAP4. A, Glutamate application (5 μM) to one oocyte coexpressing mGluR8 and GIRK evoked inward currents under two-electrode voltage-clamp. The application of glutamate plus 0.3 mM or 1.0 mM MCPG resulted in current inhibition, with 1 mM completely blocking the response. This oocyte showed less inhibition at 0.3 mM than that calculated for the mean. B, The mean inhibition caused by MCPG (0.3 and 1.0 mM) and MAP4 (0.05 and 0.5 mM) was significant and resulted in approximate IC_{50} values of 0.32 and 0.025 mM, respectively. The data are presented as normalized current amplitudes. *hK*, high potassium solution.

MAP4 is reported to be a selective group III antagonist because it blocks L-AP4 effects in spinal cord, thalamus, and lateral perforant path (24–26), yet MAP4 was recently reported to block mGluR2 responses (10). MAP4 also inhibited mGluR8 responses evoked by glutamate (10 μM) in a dose-dependent manner (Fig. 4B). Normalized current amplitudes show that 50 μM MAP4 inhibited responses by 65.4% (five oocytes), whereas 500 μM MAP4 inhibited responses by 96.8% (five oocytes), suggesting that the IC_{50} value was <50 μM. In sister oocytes, MAP4 (0.5 mM) alone had no agonist activity (five oocytes).

Discussion

We isolated and expressed the cDNA encoding rat mGluR8 that shows a restricted expression pattern in the adult rat brain. mGluR8 is a group III mGluR based on amino acid homology and activation by L-AP4. However, the pharmacology of mGluR8 was distinct. MCPG, considered to a group I/II antagonist, inhibited mGluR8. Likewise, mGluR8 was activated by low concentrations of L-CCG-1, suggesting similarities in the binding domains of group II and III mGluRs. Thus, cellular responses with mixed group II/III behavior may in some cases reflect activation of mGluR8 rather than the additive effects of two mGluRs.

The pharmacological profile of mGluR8 does not fit the structural classification of receptor subtypes. In general, the reported selectivity of newer generation mGluR ligands has been consistent with the group classification that was based on structural homology. The similar high affinity of L-CCG-1 for mGluR8 as well as the group II receptors is unusual in that regard, perhaps reflecting the somewhat higher homology of group II and III receptors in the gene family (2). In addition, one recent report found that low concentrations of L-CCG-I can activate another group III receptor, mGluR4a (27). The inhibition of mGluR2 by MAP4, previously regarded as group III specific, is also consistent with similarities in the pharmacological properties between groups II and III mGluRs (10).

Such pharmacological overlap is not entirely surprising because the ligand binding pocket of mGluRs in the extracellular amino terminus is among the most conserved domains, being homologous to bacterial periplasmic binding proteins (28). For example, Ser165 and Thr188, which are critical to glutamate binding in mGluR1, are conserved in all mGluRs. The "universal" action of glutamate at ionotropic and metabotropic receptors is presumably due to its molecular flexibility and the length of its carbon backbone. In contrast, the conformationally restricted analogs ACPD and DCG-IV seem to be selective for all mGluRs and group II mGluRs, respectively. However, the degree of conformational restriction is not sufficient to account for the selectivity of various mGluR agonists. For example, the flexible analog of glutamate, L-AP4, is selective for group III receptors, presumably based on the bioisosteric replacement of a carboxyl group by

a phosphono group. MCPG and MAP4 also show limited selectivity between group I and III receptors in that they antagonize both mGluR2 (10) as well as mGluR8. Thus, more-selective drugs will be necessary to distinguish between group II and group III receptors.

The features of mGluR8 coupling to GIRK in oocytes. We used the ability of mGluRs to couple to GIRK as a convenient assay to test the pharmacological properties of mGluR8. The coupling of mGluR8 to GIRK was PTX sensitive, which is consistent with other group II/III mGluRs. Likewise, mGluR8 did not activate phospholipase C, the effector for group I receptors. It is not yet clear whether GIRK activation by mGluRs occurs in neurons (12). However, the GIRK assay revealed some interesting differences between agonists that activated mGluR8. In particular, the Hill slope of the mGluR/GIRK dose-response curves was strikingly different for the high affinity agonists (L-CCG-1, 0.6; L-AP4, 0.9) than for the lower affinity agonists (ACPD, 1.7; glutamate, 1.9). Hill coefficients reflect multiple steps from G protein-coupled receptor activation to the effector response. In our experiments, the final step is activation of GIRK by $G\beta\gamma$ subunits (29). Receptors when coupled to G proteins often show a higher affinity for agonists, accounting for the GTP dependence of agonist binding for many G protein-coupled receptors. Thus, binding of agonists to the high and low affinity states of a receptor can create dose-responses curves with a shallow slope (30). This effect is more apparent for agonists with higher affinities, which is consistent with the shallower slopes for L-CCG-I and L-AP4. The supralinearity of the Hill coefficients for glutamate and ACPD also implies cooperativity at some stage in the pathway other than receptor activation. Because there presumably is only one agonist binding site per mGluR (28), other steps in the cascade must be responsible for the supralinear Hill coefficients. This step could be due to $G\beta\gamma$ subunit activation of GIRK channels because Hill coefficients for this interaction range from 1.5 to 3 (31, 32). Similar to our results, $GABA_B$ receptor responses in hippocampal neurons also show a supralinear relationship for activation of G protein-coupled potassium conductances (Hill coefficient = 1.7; Ref. 33).

Functional roles for mGluR8. The distribution patterns of mGluRs vary widely, with some receptors showing extremely restricted patterns (e.g., mGluR6), whereas others are very broadly expressed (e.g., mGluR1). The tissue distribution of mGluR8 mRNA was most prominent in three regions of the central nervous system. The pontine gray that projects to the cerebellum shows high levels of mGluR8 mRNA, whereas mGluR4 is highly expressed in cerebellar granule cells (21). This distribution suggests that group III receptors may influence motor control. The lateral reticular nucleus of the thalamus also shows prominent mGluR8 RNA expression. The GABAergic input of lateral reticular nucleus neurons onto thalamic relay neurons is critical in the synchronization of thalamocortical circuits in sleep and in primary generalized seizures. ACPD can postsynaptically enhance excitability of thalamic relay neurons, presumably via a group I mGluR. However, blockade of GIRK-mediated $GABA_B$ responses in relay neurons has been shown to abolish these synchronized oscillations (34). Thus, it will be interesting to examine whether mGluR8 modifies the activity of this circuit via GIRK or other effectors.

In the olfactory bulb, mGluR8 mRNA is prominent in both the mitral and granule cell layer, regions that also express other group III receptors. For example, L-AP4 inhibits transmitter release at mitral cells axons (35, 36). This effect initially seemed to be due to mGluR7, the first L-AP4-sensitive mGluR to be identified in mitral cells (9, 11). The seemingly redundant colocalization of mGluR7 and mGluR8 conceivably could be explained by selective targeting. However, subcellular targeting is not the complete explanation because mitral cell nerve terminals also seem to contain both mGluR7 and mGluR8 (38).[3] However, these two group III receptors could serve complementary functions at nerve terminals. mGluR7 requires high levels of glutamate for activation ($EC_{50} \approx 1$ mM) compared with mGluR8 ($EC_{50} \approx 2.5$ μM). On interneurons expressing mGluR1a, hippocampal presynaptic active zones have a ≥10-fold higher level of mGluR7 than other terminals (39). Thus, at least at these synapses, mGluR7 receptors must be transiently exposed to millimolar levels of glutamate in the cleft (40), suggesting a role for mGluR7 in homosynaptic modulation. Because mGluR8 is also located presynaptically (38), its higher sensitivity may allow activation by spillover of glutamate onto neighboring synapses, thus providing a mechanism for heterosynaptic modulation.

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