Ligand Binding to the Amino-terminal Domain of the mGluR4 Subtype of Metabotropic Glutamate Receptor*

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Guangming Han and David R. Hampson‡
From the Faculty of Pharmacy and Department of Pharmacology University of Toronto, Toronto, Ontario M5S 2S2, Canada

The metabotropic glutamate receptor (mGluR) 4 subtype of metabotropic glutamate receptor is a presynaptic receptor that modulates neurotransmitter release. We have characterized the properties of a truncated, epitope-tagged construct containing part of the extracellular amino-terminal domain of mGluR4. The truncated receptor was secreted into the cell culture medium of transfected human embryonic kidney cells. The oligomeric structure of the soluble truncated receptor was assessed by gel electrophoresis. In the presence of high concentrations of a reducing agent, the truncated receptor migrated as a monomer; at lower concentrations of the reducing agent, only higher molecular weight oligomers were observed. Competition binding experiments using the radiolabeled agonist \[^{3}H\]L-2-amino-4-phosphonobutyric acid revealed that the rank order of potency of metabotropic ligands at the truncated receptor was similar to that of the full-length membrane-bound receptor. However, the truncated receptor displayed higher affinities for agonists and lower affinities for antagonists compared with the full-length receptor. Deglycosylation produced a shift in the relative molecular weight of the soluble protein from \(M_r = 71,000\) to \(M_r = 63,000\); deglycosylation had no effect on the binding of \[^{3}H\]L-2-amino-4-phosphonobutyric acid, indicating that the asparagine-linked carbohydrates are not necessary for agonist binding. These results demonstrate that although the primary determinants of ligand binding to mGluR4 are contained within the first 548 amino acids of the receptor, additional amino acids located downstream of this region may influence the affinity of ligands for the binding site.

The metabotropic glutamate receptors (mGluRs)\(^7\) are a family of neurotransmitter receptors that mediate a variety of physiological functions in the central nervous system including the modulation of synaptic transmission. The mGluR family of receptors has been divided into three subgroups based on sequence homology, signal transduction properties, and pharmacological profiles (1, 2). Group I mGluRs include mGluR1 and mGluR5 and are coupled to the stimulation of phosphoinositide turnover. Group II receptors include mGluR2 and mGluR3, and group III receptors include mGluR4, mGluR6, mGluR7, and mGluR8. In cell lines, group II and III mGluRs couple to the inhibition of cAMP formation. The mGluRs are homologous with the calcium-sensing receptors, the GABAB\(_R\) receptors, and a class of mammalian pheromone receptors (2). Within the family of mGluRs, the amino acid sequence identity among members of a subgroup is approximately 70%, whereas the homology between the different groups is about 45%.

The basic structural domains of mGluRs include a large extracellular amino-terminal domain (ATD), a hydrophobic region containing seven putative transmembrane domains, and an intracellular carboxyl terminus. A molecular model of the tertiary structure of the ATD of the mGluR1 subtype of mGluR has been formulated based on the sequence similarity between the mGluRs and the periplasmic-binding proteins in prokaryotes (3). The periplasmic-binding proteins are a family of proteins that transport nutrients into bacteria. Within the family of bacterial proteins, the leucine, isoleucine, valine-binding protein is the most homologous with a region within the ATD of mGluRs. The leucine, isoleucine, valine-binding protein, like other periplasmic-binding proteins in bacteria, is a soluble protein that forms the recognition unit of a multimeric transport complex. In addition to the binding proteins, the bacterial transport complexes also include membrane-bound transport modules responsible for the translocation of amino acids and other nutrients across the membrane (see Ref. 4 for a review).

Previous studies using site-directed mutagenesis and chimeric receptors have suggested that the ligand binding pockets of mGluRs are located in the ATDs (3, 5, 6). It remains unclear, however, whether all of the determinants for ligand binding to mGluRs are contained within the ATD, or whether there are additional amino acids in other regions of receptor protein located extracellularly that may contribute directly or indirectly to ligand binding affinity in mGluRs. Recently, Okamoto et al. (7) have shown that a construct containing the entire ATD of the mGluR1 receptor forms a soluble protein that retains the ability to bind the group I mGluR agonist \[^{3}H\]quisqualic acid. In the present study, we demonstrate that a fragment of the ATD of mGluR4 forms a soluble protein that is secreted from cells. This truncated receptor retains the ability to bind the group III mGluR agonist \[^{3}H\]L-AP4 and displays the basic pharmacological profile of the full-length receptor. However, the truncated receptor has a higher affinity for agonists and a lower affinity for antagonists compared to the membrane-bound receptor, indicating that additional structural determinants contribute to ligand binding affinity in mGluR4.

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‡ To whom correspondence should be addressed: Faculty of Pharmacy, University of Toronto, 19 Russell St., Toronto, Ontario, Canada M5S 2S2. Tel.: 416-978-4494; Fax: 416-978-8511; E-mail: d.hampson@utoronto.ca.

\(^1\) The abbreviations used are: mGluR, metabotropic glutamate receptor; ATD, amino-terminal domain; CPPG, (R,S)-1-cyclopropyl-4-phosphonophenyglycine; DTT, dithiothreitol; HEK, human embryonic kidney; \(\text{L-AP4, L-2-amino-4-phosphonobutyric acid; L-SOP, serine-O-phosphate; MAP4, (S)-2-amino-2-methyl-4-phosphonobutyric acid; MPPG, (R, S)-a-methyl-4-phosphonophenylglycine.}\)

\(^2\) This paper is available online at http://www.jbc.org
**EXPERIMENTAL PROCEDURES**

**Expression Vectors**—For the expression of wild-type mGluR4a in human embryonic kidney (HEK) 293 cells, the BglII-EcoRI fragment of rat mGluR4a cDNA in the pBluescript SK− phagemid (8) was subcloned into the pcDNA3 mammalian expression vector (Invitrogen Corp.) at the BamHI site. For the construction of c-myc-tagged mGluR4a, the mGluR4-pcDNA3 plasmid was cut with XhoI, and the largest fragment containing the pcDNA3 backbone was ligated to itself (the 5′-mGluR4-pcDNA3 plasmid). The oligonucleotides encoding the c-myc epitope s-BeSII-e-c-myc (5′-GTCGACCAAAAACTGTCTTGAGAAGACTTGGATCCAG) and rev-BeSII-e-c-myc (5′-GTCGACCTGGATCACAAGCTTCCATGAAATAAAGCTTTGTC) were phosphorylated, annealed to each other, and inserted into the 5′-mGluR4-pcDNA3 plasmid at the BamHI site to produce 5′-mGluR4-e-c-myc-pcDNA3. The 931-bp fragment of the full-length receptor from 5′-NdeI-XhoI-c-myc-pcDNA3 and a 3353-bp XhoI-NcoI fragment of mGluR4-pcDNA3 were subcloned into pcDNA3 at the NdeI-NcoI sites using a three-piece ligation to produce c-myc-mGluR4-pcDNA3. The construction of an expression vector containing a segment of the ATD of mGluR4 was made by digesting c-myc-mGluR4 with KpnI. The 1686-bp KpnI fragment contained a segment of the mGluR4 signal peptide, the c-myc epitope, and the amino-terminal domain of mGluR4 truncated immediately after Tyr-548 (numbering is based on the untagged rat receptor as indicated by the terminal domain of mGluR4 truncated immediately after Tyr-548 containing a segment of the ATD of mGluR4) was made by digesting c-myc-mGluR4 with KpnI immediately after XhoI (8). The KpnI fragment from the c-myc-mGluR4 construct was subcloned into the pcDNA3 vector.

**Plasmid Preparations and Transfections**—cDNA constructs were transformed into XL-1 blue competent cells (Stratagene, La Jolla, CA). Ampicillin-resistant colonies were inoculated in 3 ml of Luria-Bertani medium containing 100 μg/ml ampicillin and grown at 37 °C for 16 h. The colonies were subcloned into the pcDNA3 vector and then transformed into XL-1 blue competent cells (Stratagene, La Jolla, CA). The plasmid DNA was prepared using the QIAprep Miniprep Kit (Qiagen). The DNA from the plasmid preparation was checked for the orientation of the insert. The plasmids were transiently expressed into HEK cells by calcium phosphate precipitation. The transfected cells were grown in 100-mm plates to 80% confluence; 10 ml of fresh medium containing 6% fetal bovine serum (Hyclone, Logan, UT) were added to the cells 3 h before transfection. For induction, the cells were grown in 100-mm plates to 80% confluence; 10 ml of fresh medium containing ampicillin and grown at 37 °C for 16 h; the cells were harvested three times over a 24-h period; the samples were pooled and stored at −75 °C.

**Immunoblotting**—SDS-polyacrylamide gel electrophoresis was conducted on samples containing 6% sodium dodecyl sulfate (SDS) and 2% 3-mercaptoethanol. The proteins were transferred onto nitrocellulose membranes (pore size, 0.45 μm; Schleicher & Schuell) by transblotting (Bio-Rad Laboratories, Hercules, CA) for 2 h, followed by three 5-min washes with 3.5% nonfat dry milk powder for 16 h, followed by three 5-min incubations with washing buffer for 5 min each. The blots were incubated with the anti-c-myc monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h, followed by three 5-min washes, and then incubated in anti-mouse IgG conjugated to horseradish peroxidase (Amersham Canada, Oakville, Ontario, Canada) for 2 h, followed by three 5-min washes. After an additional 30-min washing period, the membrane was soaked in ECL Western blotting detection reagents for 2 min and exposed to Hyperfilm ECL (Amersham).

**RESULTS**

**Expression of the Truncated Receptor**—A truncated cDNA expression construct was produced that contained a sequence encoding the mGluR4 signal peptide, a c-myc epitope tag, and a portion of the amino-terminal domain of mGluR4 up to and including Tyr-548. The carboxyl terminus of the truncated protein was 39 amino acids upstream from first putative transmembrane domain (Fig. 1). Previous experiments in our laboratory have shown that the insertion of the c-myc epitope at this position in mGluR4 does not alter the pharmacological properties of the full-length receptor (data not shown).

The full-length c-myc-tagged mGluR4a, or the truncated c-myc-tagged expression plasmids were transiently expressed in HEK cells, and aliquots of the cell culture media (the soluble fraction) and the transfected cells (the cellular fraction) were collected 48 h after transfection and examined by immunoblotting using an anti-c-myc antibody. Cells transfected with full-length c-myc-tagged mGluR4a expressed immunoreactive proteins (M1, 93,000 and M2, 109,000) that likely correspond to the unglycosylated and glycosylated mGluR4a monomers. An additional higher molecular-weight band at approximately M1 was observed in the soluble fraction. Protein expression in the transfected cells was also verified by immunoprecipitation and SDS-PAGE followed by autoradiography for [3H]L-AP4 binding (Kd of 100 pm). The autoradiograms showed a single band corresponding to the 93,000-Mr monomer of mGluR4a, with no additional bands present. The specificity of the c-myc antibody used for immunoblotting was confirmed by the absence of cross-reactivity with other proteins in HEK cell lysates. The truncated c-myc-mGluR4a expression construct was produced that contained a sequence encoding the mGluR4 signal peptide, a c-myc epitope tag, and a portion of the amino-terminal domain of mGluR4 up to and including Tyr-548. The carboxyl terminus of the truncated protein was 39 amino acids upstream from first putative transmembrane domain (Fig. 1). Previous experiments in our laboratory have shown that the insertion of the c-myc epitope at this position in mGluR4 does not alter the pharmacological properties of the full-length receptor (data not shown).

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224,000 was also observed; this protein is likely a dimer of mGluR4 (Fig. 2). The soluble fraction from cells transfected with the truncated mGluR4 construct contained a c-myc immunoreactive protein that migrated at \( M_r 71,000 \) (Fig. 2, lane 2). Samples of the soluble fraction collected from untransfected HEK cells contained low levels of a protein that cross-reacted with the c-myc antibody; this protein migrated at a slightly slower rate than the \( M_r 71,000 \) protein present in the soluble fraction from transfected cells (Fig. 2, lane 1).

**Oligomeric Structure of Truncated mGluR4**—The effects of the reducing agent DTT on the truncated receptor were examined using SDS-polyacrylamide gel electrophoresis and immunoblotting. In the presence of high concentrations of DTT (1–100 mM), the receptor migrated as a monomer with a black box indicates the position of the c-myc epitope tag. The dotted line depicts the 5′ untranslated DNA. SP, signal peptide.

**Pharmacological Profile of the Soluble Receptor**—High affinity binding of L-AP4 and L-SOP are unique pharmacological features of group III mGluRs. No specific [\( ^{3}H \)-L-AP4 binding was detected in samples of the soluble fraction from untransfected HEK cells. However, in the soluble fraction of cells transfected with the truncated mGluR4, a high level of specific binding was consistently observed. Typically, 100 \( \mu \)M of the soluble preparation in a 250-\( \mu \)l assay yielded approximately 12,770 specific d.p.m. (0.12 pmol) bound at 30 \( n \)M [\( ^{3}H \)-L-AP4], and specific binding represented \( 66 \pm 3\% \) of the total binding. The inhibitory potency of the agonists L-AP4, L-SOP, L-glutamate, and cyclobutylene AP5 and the group III mGluR antagonists MAP4, CPPG, and MPPG for the truncated soluble mGluR4 receptor were compared with the full-length membrane-bound receptor. The rank order of potency of the agonists at the soluble truncated receptor (L-AP4 > L-SOP > L-glutamate > cyclobutylene AP5) was identical to that of the full-length membrane-bound receptor. The affinities of all four agonists were higher for the truncated receptor compared with the full-length receptor. The affinity for the endogenous ligand L-glutamate at the truncated receptor was about 2-fold higher than that at the membrane-bound receptor (Fig. 4; Table I). The rank order of the antagonists at the truncated receptor (MAP4 > MPPG > CPPG) was similar to that at the membrane-bound receptor (CPPG > MAP4 > MPPG) except for CPPG, which was the most potent of the three compounds for the full-length receptor but was the least potent at the soluble receptor (Table I; Fig. 4). In contrast to the agonists, the antagonists displayed lower affinities for the soluble truncated receptor compared with the membrane-bound receptor.

The binding properties of the soluble truncated mGluR4 were also compared with the membrane-bound receptor using other glutamate receptor ligands including the nonselective mGluR agonist (1S,3R)-1-amino cyclopentane-1,3-dicarboxylic acid, the group I mGluR antagonist (R,S)-\( \alpha \)-methyl-4-carboxyphenylglycine, and the ionotropic glutamate receptor agonists kainic acid and N-methyl-D-aspartate (Fig. 5). Each drug was tested at a concentration of 100 \( \mu \)M. At the truncated receptor, kainic acid, N-methyl-D-aspartate, and (R,S)-\( \alpha \)-methyl-4-carboxyphenylglycine produced less than 10% inhibition of [\( ^{3}H \)-L-AP4 binding; a similar low level of inhibition was observed with the membrane-bound receptor. The very low potency of (R,S)-\( \alpha \)-methyl-4-carboxyphenylglycine for mGluR4 has also been reported for experiments on human mGluR4 expressed in Chinese hamster ovary cells (11). (1S,3R)-1-Amino cyclopentane-1,3-dicarboxylic acid showed a greater degree of inhibition at

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**FIG. 1.** Schematic diagram of the expression constructs of mGluR4. The upper diagram depicts the full-length wild type (WT) mGluR4a receptor, and the lower diagram depicts the truncated receptor (KpnI ATD-mGluR4). The seven black boxes represent the putative transmembrane domains (TMD) and the carboxyl-terminal domain (CTD) of the full-length receptor; the open box depicts the segment of the ATD that is homologous with the leucine, isoleucine, valine-binding protein. The vertical arrow indicates the position of the c-myc epitope tag. The dotted line depicts the 5′ untranslated DNA. SP, signal peptide.

**FIG. 2.** Immunoblotting analyses of full-length membrane-bound mGluR4a and the soluble truncated mGluR4. Samples of the soluble preparation (2 \( \mu \)g of protein) or the whole cell preparation (16 \( \mu \)g of protein) from untransfected or transfected HEK cells were separated on 8% SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and detected with an anti-c-myc monoclonal antibody. Lane 1, cell culture (soluble) fraction from mock-transfected HEK cells; lane 2, cell culture (soluble) fraction from cells transfected with truncated mGluR4; lane 3, total cell extract from mock-transfected HEK cells; lane 4, total cell extract from HEK cells transfected with c-myc-mGluR4a.

**FIG. 3.** The effects of the reducing agent DTT on the soluble truncated mGluR4. The electrophoresis samples were treated with various concentrations of DTT as indicated, separated on a 10% polyacrylamide gel, and transferred to nitrocellulose. The immunoblot was labeled with the anti-c-myc antibody.
the truncated mGluR4 receptor (27% of control binding) compared with the full-length receptor (61% of control binding), indicating that as seen with the other agonists tested, (1S,3R)-1-amino cyclopentane-1,3-dicarboxylic acid may be more potent at the truncated receptor. The relatively low potency of (1S,3R)-1-amino cyclopentane-1,3-dicarboxylic acid compared with group III mGluR ligands such as l-AP4 and l-SOP is consistent with previous binding data from mGluR4 expressed in sf9 insect cells (10).

Deglycosylation of the Truncated Receptor—The soluble truncated receptor migrated on SDS-polyacrylamide gel electrophoresis with a $M_r$ of 71,000; this is approximately $M_r$ 8,000 larger than the predicted molecular weight based on the amino acid sequence, suggesting that the truncated receptor was glycosylated. Treatment of the soluble fraction containing the truncated mGluR4 receptor with PNGase F, which cleaves all asparagine-linked carbohydrates, shifted the immunoreactive band from $M_r$ 71,000 to $M_r$ 63,000 (Fig. 6); the lower molecular weight estimate was very close to the predicted molecular mass of the nonglycosylated protein (62,700 daltons). The binding of [3H]-l-AP4 to the deglycosylated receptor was also assessed. [3H]-l-AP4 binding to samples treated with PNGase was 99% of control samples (average of two independent determinations) that were not treated with the enzyme, indicating that agonist binding to truncated mGluR4 is not dependent upon asparagine-linked carbohydrates.

**DISCUSSION**

The truncated receptor analyzed in this study included the first 548 amino acids of the 912 amino acids of mGluR4a. The expression construct contained a part of the ATD that extended from the amino terminus to Tyr-548, which is located 39 amino acids upstream from the junction of the ATD and the first putative transmembrane domain. This portion of the ATD includes the entire leucine, isoleucine, valine-binding protein homology region. The expression construct incorporated a c-myc epitope tag inserted immediately downstream of the signal peptide. The truncated receptor was secreted into the cell culture medium, indicating that the protein did not possess regions of sufficient hydrophobicity to cause retention in the endoplasmic reticulum or plasma membrane of the cell and that it was soluble in an aqueous environment.

The soluble truncated mGluR4 receptor exists as a monomer in the presence of high concentrations of a reducing reagent and as a dimer and trimer in the presence of low concentrations of reducing agents. Dimeric forms of mGluR1 (12, 13), mGluR2 and mGluR3 (14), mGluR4 (15), mGluR5 (16), mGluR6 (17), and mGluR7 and mGluR8 (18) have been observed on immunoblots of brain tissue and transfected cells. Romano et al. (16) have shown that mGluR5 migrates on SDS-polyacrylamide gel electrophoresis as a dimer under nonreducing conditions and as a monomer under reducing conditions, indicating that the dimers are formed by intermolecular disulfide bonds. Analysis
of the electrophoretic mobility of a truncated construct of mGluR5 indicated that the intermolecular disulfide bonds are formed between cysteine residues located in the ATD of mGluR5 (16). Modulation of monomer and dimeric forms by reducing agents was also seen with the soluble truncated form of the mGluR1 receptor (7).

Despite the studies outlined above providing evidence for dimers of mGlurS, no data exist demonstrating a direct link between the ability to bind ligands or activate signal transduction pathways and a particular oligomeric configuration of an mGluR. However, in the experiments described here, the \([^{3}H]\)\text{-AP4} radioligand binding assay was carried out in the absence of reducing agents, whereas the addition of reducing agents decreased binding activity in a concentration-dependent fashion.\(^2\) It is likely that in the typical oxidation/reduction environment of the extracellular space in nerve tissue, mGlurS exist as oligomeric complexes. Although the oligomeric configurations of the mGlurS as they exist in nerve cells in vivo are not known, our observations on the amino-terminal domain of mGluR4, together with the results on mGluR1 and mGluR5 noted above all suggest that intermolecular disulfide bonds are a general structural feature of the mGluR family of receptors and that the active form of the receptor is likely to be a multimeric complex. The homologous calcium-sensing receptor is also present in tissues and transfected cells in a dimeric configuration (19). The oligomeric structures of mGlurS may resemble the structures of the insulin and insulin-like growth factor receptors. The insulin receptors exist as preformed disulfide-linked dimers, and unlike other tyrosine kinase receptors, monomer-to-dimer transitions are not modulated by the presence of ligand (20).

The truncated mGluR4 receptor contains four consensus sequences for asparagine-linked glycosylation. The decrease in the molecular weight of the truncated protein by about \( \text{M}_{r} \) 8,000 after complete deglycosylation indicates that one or more of these sites are glycosylated. An analysis of the effects of deglycosylation on the binding of \([^{3}H]\)\text{-AP4} to the soluble receptor demonstrated that the bound carbohydrate is not required for agonist binding. Similarly, asparagine-linked oligosaccharides also do not appear to be obligatory for the activation of ionotropic glutamate receptors (21). Although our data show that asparagine-linked oligosaccharides are not required for ligand binding to mGluR4, it is possible that the bound carbohydrates may be important in the subcellular targeting of this receptor in the nervous system. In the case of mGluR4, the targeting is to presynaptic nerve terminals (18, 22) where the receptor acts to inhibit glutamate release and thereby modulates synaptic transmission (15).

Many G-protein-coupled receptors display biphasic binding curves indicative of the presence of high and low affinity binding sites. The commonly accepted explanation for high and low affinity binding sites is that they reflect receptors with bound and unbound G-protein, respectively. However, some G-protein-coupled receptors, such as muscarinic acetylcholine receptors, show multiple states of affinity in the absence of G-proteins (23, 24). Conversely, other G-protein-coupled receptors such as the cloned mGluR4 receptor display only a single class of sites in radioligand binding assays (9, 10); it is possible that lower affinity sites may exist in mGluR4 but cannot be detected in the binding assay because of the very low affinity. Alternatively, it is possible that the unitary nature of the binding site of the cloned membrane-bound mGluR4 receptor reflects only a low affinity site due to the absence of the appropriate G-proteins in the host cell lines (baby hamster kidney cells, Ref. 9; s9 insect cells, Ref. 10; HEK-293 cells, the present study). However, we have observed that co-expression of full-length mGluR4 with various mammalian G-protein subunits including \( G_{11}, G_{21}, G_{23}, \) and \( G_{13} \), in insect s9 cells does not significantly increase the affinity or the capacity (\( B_{\text{max}} \)) of \([^{3}H]\)\text{-AP4 for mGluR4.}\(^3\) Moreover, \([^{3}H]\)\text{-AP4 binding experiments conducted in rat (25) and mouse (26) brain have detected only a single class of sites with a \( K_{D} \) value very similar to that seen with the cloned receptor. Thus, the binding site in the full-length receptor with an affinity for \([^{3}H]\)\text{-AP4 in the 400–500 nM range may represent the G-protein-coupled form of mGluR4.}

A pharmacological analysis of the truncated receptor was conducted by assessing the ability of various mGluR4 ligands to compete with the binding of the radioiodinated agonist \([^{3}H]\)\text{-AP4. Our results demonstrate that the binding properties displayed by the soluble truncated mGluR4 reflect the unique pharmacological profile of the group III mGluRs. Except for the antagonist CPPG, the rank order of potency for a series of group III mGluR ligands at the truncated receptor was the same as at the full-length membrane-bound receptor. Moreover, ligands at group I mGluRs or ionotropic glutamate receptors showed little or no affinity for either receptor. These observations suggest that the primary determinants of \([^{3}H]\)\text{-AP4 binding are conferred by residues present in the ATD of mGluR4. However, the inhibition constants for \( L \)-glutamate, \( L \)-AP4, \( L \)-SOP, and cyclobutylene AP5 were lower than those of the full-length receptor, indicating that the truncated soluble receptor displayed higher affinities for agonists compared with the membrane-bound receptor. The differences in affinity of the ligands could have been caused by differences in assay conditions for the soluble and membrane-bound receptors. However, if differences in assay conditions were responsible for the differences in the affinities, it is likely that the direction of change would have been the same for all ligands tested. This explanation seems unlikely because the affinity of the truncated receptor for antagonists was lower compared with the full-length receptor whereas the affinity for agonists was higher.

An alternative explanation for the differences in affinity is that ligand affinity might be influenced by regions of mGluR4 that were excluded from the soluble construct that was examined in the present study; these regions include the transmembrane domains, the extracellular loops between the transmembrane domains, and the 40 amino acids immediately upstream

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\(^2\) G. Han and D. R. Hampson, unpublished observation.

\(^3\) L. Blythe and D. R. Hampson, unpublished observation.
from the first putative transmembrane domain. It is possible that the ATD may interact with one or more of these regions to induce an increase in affinity for antagonists and a decrease in affinity of the receptor for agonists. Whether or not ligand binding in other mGluRs is affected by regions outside the ATDs remains to be determined. Although the pharmacological results reported by Okamoto et al. (7) on the binding of [3H]quisqualic acid to the soluble mGluR1 receptor are difficult to compare with ours because they did not report IC50 values for l-glutamate or other compounds that were assessed in the present study, it appears that the affinity for [3H]quisqualic acid for the soluble receptor was very similar to the affinity measured for the full-length receptor. One difference between the truncated mGluR1 receptor and the truncated mGluR4 receptor is that the former contained the entire ATD of mGluR1, whereas the latter did not include the 39 amino acids immediately upstream from the first putative transmembrane domain. Our results demonstrate that this part of the receptor is not required for high affinity binding of [3H]-AP to mGluR4. This observation is consistent with mutational and chimeric studies conducted on mGluR1 that showed that several residues located in the more amino-terminal regions of the ATD of mGluR1 are required for ligand binding (3, 5). However, we cannot rule out the possibility that this 39-amino acid segment of mGluR4 may affect the affinity of ligands for the binding site.

Of interest in this regard are studies on the receptor for the glycoprotein hormones luteinizing hormone and choriongonadotropin. The luteinizing hormone/choriongonadotropin receptor is a member of the G-protein-coupled receptor family and, like mGluRs, it has a large ATD that can be separated from the seven transmembrane domains and still retain ligand binding activity. Radioligand binding studies using 125I-labeled human choriongonadotropin have shown that the exodomain (equivalent to the ATD) alone has a higher affinity for the glycoprotein hormone than the full-length receptor (27, 28). A structural analysis of this receptor using site-directed mutagenesis has shown that several amino acids in the second extracellular loop within the transmembrane domain region affect the affinity of the ligand for the receptor; it was suggested that residues within this loop constrain the affinity of the hormone for the receptor (28). Although the amino acid sequences of the mGluRs are not homologous with the glycoprotein hormone receptors, our observations suggest that residues in the extracellular loops or residues located within or immediately upstream of the membrane domains may influence the affinity of ligands for the binding site located in the ATD of mGluR4.

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