Homology Modeling of Metabotropic Glutamate Receptors. (mGluRs) Structural Motifs Affecting Binding Modes and Pharmacological Profile of mGluR1 Agonists and Competitive Antagonists

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A three-dimensional model of the amino terminal domain (ATD) of the mGluR1 receptor subtype was constructed on the basis of the previously reported sequence homology with bacterial periplasmic proteins. The model was utilized for revealing structural motifs affecting the interaction with mGluR1 agonists and competitive antagonists. The agonist binding site region, identified on the basis of published site-directed mutagenesis experiments, is located on the surface of one of the two lobes constituting the mGluR1 ATD. A number of electrostatic and hydrogen-bonding interactions can be detected between mGluR1 agonists such as L-Glu (**1**), Quis (**2**), and (1*S*,3*R*)-ACPD (**4**) and binding site residues. A different binding mode was proposed for mGluR1 competitive antagonists such as 4CPG (**5**), 4C3HPG (**6**), and UPF523 (**10**). Interactions with both lobes of the ATD of mGluR1 and the lack of a specific role for the phenyl moiety of mGluR1 antagonists are important features of the proposed antagonist binding mode. The correspondence of the molecular modeling results with the pharmacological data of mGluR1 agonists and competitive antagonists is a confirmation of the plausibility of the model.

Introduction

Physiological functions of glutamic acid (L-Glu, **1**), the major neurotransmitter in the central nervous system (CNS),[1] are mediated by two main receptor families, namely ionotropic glutamate receptors (iGluRs), classified as NMDA, AMPA, and KA receptors which contain integral, cation specific, ion channels and metabotropic glutamate receptors (mGluRs) which are coupled through GTP-binding proteins to effector systems such as the inhibition of adenylyl cyclase (AC), the activation of phosphoinositide-specific phospholipase C (PLC), and the regulation of ion channel currents. At least eight mGluR subtypes (plus several splice variants) have so far been identified and divided into three groups according to sequence homology, transduction mechanism, and pharmacology, as reported in Chart 1.[2]

Since their first discovery and subsequent characterization, mGluRs have exhibited significant structural differences with respect to other members of the G-protein-coupled receptor (GPCR) superfamily[3] and, indeed, mGluRs are supposed to form together with the newly reported bovine parathyroid Ca^{2+}-sensing receptor (PCaR1),[4] a distinct class of the GPCR superfamily. Characteristic features of the mGluRs can be deduced from the analysis of the aligned primary sequences of members of the family. Thus, all the mGluRs are characterized by a putative signal peptide, an unusually large (470−510 amino acids) extracellular amino terminal domain (ATD), seven hydrophobic fragments corresponding to the seven membrane-spanning regions characteristic of the GPCR superfamily, and an intracellular carboxy terminal domain variable in size and amino acid composition among the various members of the family. Sequence homology is in the range 65−70% between mGluRs belonging to the same group but falls to 40−45% among members of different groups. More specifically, conserved regions are found in the membrane-spanning regions and in a hydrophobic region located in the extracellular ATD.

Chart 1. Classification of Cloned mGluR Receptors

Group	Receptor Subtype	Splice Variants	Effector System
		a	↑PLC / IP3; ↑AC / cAMP
		b	↑PLC / IP3
	mGluR1	c	↑PLC / IP3
		d	↑PLC / IP3
		e	?
I		a	↑PLC / IP3
	mGluR5	b	↑PLC / IP3
	mGluR2		↓AC / cAMP
II			
	mGluR3		↓AC / cAMP
	mGluR4		↓AC / cAMP
	mGluR6		↓AC / cAMP
III	mGluR7		↓AC / cAMP
	mGluR8		↓AC / cAMP

While some progress in the understanding of the pathophysiological functions mediated by mGluRs have been achieved over the last few years,[2a] the molecular basis and the three-dimensional features of ligand recognition and signal transduction processes are still to be elucidated. Certainly, increased knowledge of the structural motifs governing mGluR binding affinity and selectivity could be of great help in the development of new ligands endowed with increased potency and selectivity. In turn, an increased knowledge of the molecular mechanism of signal transduction processes of mGluRs with respect to other members of the GPCR superfamily can shed light on their evolutionary divergence.[5] In this respect, two different binding domains have so far been identified on members of the GPCR superfamily. The first one is located within the transmembrane domain and is used by neurotransmitters

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(such as acetylcholine, dopamine, serotonine, adrenaline);[6] the second one, up to now known to be utilized by large glycohormones, is located in the extracellular ATD.[7] There is now enough evidence supporting the hypothesis that the small neurotransmitter L-glutamate also interacts with the G-protein-coupled mGluRs in a binding domain localized in the extracellular ATD. In this connection, a number of key experiments have been carried out over the last few years providing informative clues about the localization of the L-Glu (**1**) binding domain in mGluR1. Thus, on the basis of the different pharmacological profile of mGluR1 and mGluR2 subtypes, Takahashi et al. have produced a series of chimeric receptors by exchanging parts of the extracellular domain of mGluR1 and mGluR2.[8] The subsequent pharmacological characterization of these chimeric receptors allowed them to conclude that substitution of up to half of the mGluR2 ATD in the homologous region of mGluR1 ATD is sufficient to convert the pharmacological profile of mGluR1 into that of mGluR2, in agreement with an extracellular localization of the L-Glu binding region. In another approach,[9] polyclonal antibodies raised by Shigemoto et al. against two different portions of the ATD of rat mGluR1 and tested on CHO cells stably expressing mGluR1 were shown to inhibit the action of L-Glu (**1**) on the stimulation of PI hydrolysis, an effect shared by known competitive mGluR1 antagonists, thus confirming the extracellular localization of the binding site. Finally, because of the sequence homology between mGluRs and PCaR1, the observation that mutations in human PCaR1 are responsible for familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism is of interest[10] since two of these mutations occurs in the putative extracellular domain, in regions that align with those predicted to affect the ligand binding in mGluRs.

Within this experimental framework, the discovery by O'Hara et al.[11] in 1993 that the ATD of mGluR1 shares significant, albeit limited, sequence homology with some bacterial periplasmic binding proteins (PBPs) acquires considerable importance.

Although there is at present no evidence for an evolutionary relationship between mGluRs and PBPs, the biological meaning of the similarity between the ATD of mGluR1 and PBPs was established through a sophisticated approach, including sequence database searching, homology modeling, and three-dimensional compatibility search techniques. At the same time, a three-dimensional model of the ATD was constructed and a prediction of the L-Glu (**1**) binding mode was attempted. Basic features of the O'Hara model are reported briefly. The mGluR1 ATD is characterized by an ellipsoidal shape, formed by two globular lobes consisting of β-sheets flanked by α-helices and by three interdomain cross-over segments (hinge regions). By assuming an analogous ligand recognition mechanism for PBPs and mGluR1, it could be hypothesized that the L-Glu (**1**) binding region lies on the surface of the cleft between the two lobes.[12] Two conserved residues, Ser_{165} and Thr_{188} (mGluR1 sequence numbering) are predicted to be directly involved in the interaction with L-Glu (**1**), as confirmed by a number of observations. In particular, the amino acids predicted to be involved in L-Glu binding are situated in (or close to) the regions against which antibodies were raised[9] and are entirely comprised within the first two-thirds of the amino terminal domain.[8] Moreover, the binding affinities for L-Glu (**1**) and quisqualate (Quis, **2**) were abolished when Ala→Ser_{165} and Ala→Thr_{188} mutants were produced. Since mutations of the neighboring Ser_{164} and Ser_{186} had no effect on the affinity, the abolition of binding caused by Ala→Ser_{165} and Ala→Thr_{188} mutations is likely not to be attributable to a global receptor misfolding. Finally, a mechanism was proposed for the mGluR1 signal transduction process, analogous to that already reported for PBPs.[13] According to this mechanism, once L-Glu (**1**) has bound to the receptor, the two ATD lobes collapse thus trapping the neurotransmitter into the cleft. The conformational change thus originated is thereby propagated through interactions with the covalently attached transmembrane domain to the intracellular loops, allowing them to interact with G-proteins.

Within this experimental and theoretical framework and in connection with ongoing work on the design and synthesis of new mGluRs ligands,[14] we have undertaken a molecular modeling study directed toward ascertaining the different binding modes of mGluR1 agonists and competitive antagonists, analyzed on the basis of their interactions with the putative binding site on the three-dimensional model of the amino terminal domain of mGluR1; the results are reported below.

Methods

Following the guidelines reported in the work of O'Hara et al.,[11] the sequence of the amino terminal domain of mGluR1 was aligned with the sequences of leucine/isoleucine/valine binding protein (LIVBP)[13] and leucine binding protein (LBP).[15] Sequence alignment was performed using the Needleman–Wunsch algorithm[16] as implemented in the COMPOSER module of the Sybyl 6.2 molecular modeling package.[17] The Swiss2 homology matrix was used for evaluating amino acid similarity.[18] In agreement with the O'Hara's results,[11] 18.3% and 17.9% sequence homologies were found with LBP and LIVBP, respectively. The alignment significance was established by the value of the alignment score and by jumbling the sequence alignments. Eleven structurally conserved regions connected by 12 loops, three of which were added after removing the steric-bump check filter, were found (Figure 1).

The model of the ATD of mGluR1 thus resulting was carefully checked for the correct chirality of constituent amino acids, and regions of bad steric contacts were locally annealed. The ATD model was then subjected to a full minimization by means of the AMBER-united force field (conjugate gradient minimizer) until convergence (0.05 kcal/mol Å) was reached. A cutoff of 8 Å and a dielectric constant of 4.5 were used.[19] The inspection of the minimized structure revealed that disruption of the secondary structure of structurally conserved regions did not occur during the minimization procedure. The docking experiments were carried out by manually fitting the chosen ligands into the predicted binding site and by annealing the resulting complexes until convergence was reached. The Tripos force field and MOPAC-derived charges on ligands were used in this step. Where different orientations of ligands (or different conformations of ligands) were possible, they were examined and compared in term of more or less favorable states as recognized by the overall interaction energies. However, due to the impossibility of estimating entropic contributions, no correlation between docking energies and binding affinities were attempted.

The interactive modeling was carried out by means of the Sybyl 6.2 molecular modeling package running on SG Indy R4400 or ESV R3000 workstations.

Results

i. Three-Dimensional Model of mGluR1 ATD. The AMBER-minimized mGluR1 ATD exhibits an elon-

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                10        20        30        40        50        60        70        80
mGluR1   MVRLLLIFFPMIFLEMSILPRMPDRKVLLAGASSQRSVARMDGDVIIGALFSVHHQPPAEKVPERKCGEIREQYGIQRVE

LeuBP                                              DDIKVAVV                AMSGPIAQWGIMEFN
LivBP                                              EDIKVAVV                AMSGPVAQYGDQEFT
                                                   :   ::::                  :| :
                  90        100       110       120       130       140       150       160
mGluR1   AMFHTLDKINADPVLLPNITLGSEIRDSCWHSSVALEQSIEFIRDSLISIRDEKDGLNRCLPDGQTLPPGRTKKPIAGVI
LBP      GAEQAIKDI       KGGIKGDKLVGVEY                          DACDPKQAVAVANKVNDGIKYVI
LivBP    GAEQAVADI       KGGIKGNKLQIAKY                          DACDPKQAVAVANKVNDGIKYVI
          ::   : |           |  : : :                              | |    :   ::   | ||
                170       180       190       200       210       220       230       240
mGluR1   GPGSSSVAIQVQNLLQLFDIPQIAYSATSIDLSDKTLYKYFLRVVPSDTLQARAMLDIVKRYNWTYVSAVHTEGNYGESG
LBP      GHLCSSSTQPASDIYEDEGILMISPGATAPELTQRGYQHIMRTAGLDSSQGPTAAKYILETVKPQRIAIIHDKQQYGEGL
LivBP    GHLCSSSTQPASDIYEDEGILMITPAATAPELTARGYQLILRTTGLDSDQGPTAAKYILEKVKPQRIAIVHDKQQYGEGL
         |:::||    :        |: |   || ::|  :        :        |   |:       ::::|   :|||  :
               250       260       270       280       290       300       310       320
mGluR1   MDAFKELAAQEGLCIAHSDKIYSNAGEKSFDRLLRKLRERLPKARVVVCFCEGMTVRGLLSAMRRLGVVGEFSLIGSDGW
LBP      ARSVQDGLKAANANVVFFDGITAGEKDFSIARLKKEYGGYYPEMGQMLRQARSVGLKTQFMGPEGVGNASLSNIAGDAAE
LivBP    ARAVQDGLKKGNANVVFFDGITAGEKDFSVARLKKEYGGYHPEMGQILRQARAAGLKTQFMGPEGVANVSLSNIAGESAE
          : |  :::    : ::: | | :   : |: || :       | :  ::    :: ::    :    : :    ::|  :
               330       340       350       360       370       380       390       400
mGluR1   ADRDEVIEGYEVEANGGITIKLQSPEVRSFDDYFLKLRLDTNTRNPWFPEFWQHRFQCRLPGHLLENPNFKKVCTGNESL
LBP      GMLVTMPKRYD            DPANQGIVDALKADKKDPSGPYVWITYAAVQSLA                      AL
LivBP    GLLVTKPKNYD            VPANKPIVDAIKAKKQDPSGAFVWTTYAALQSLQ                      AG
         :    :  |:              |  :   |     : |     :|        :                       ::
               410       420       430       440       450       460       470       480
mGluR1   EENYVQDSKMGFVINAIYAMAHGLQNMHHALCPGHVGLCDAMKPIDGRKLLDFLIKSSFVGVSGEEVWFDEKGDAPGRYD
LBP      ERT                                          SDEPLALVKDLKANGANTVIGPLNWDEKGDLKGDFG
LivBP    LNQ                                          SDDPAEIAKYLKANSVDTVMGPLTWDEKGDLKGEFG
                                                      |    :   :|:  :      : :|||||: | :
                490       500       510       520       530       540       550
mGluR1   IMNLQYTEANRYDYVHVGTWHE
LBP      VFQWHADGSSTKAK
LivBP    VFDWHANGTATDAK
           :       :
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Figure 1. Multiple sequence alignment of mGluR1 ATD and two PBPs, LIVPB and LBP. Aligned positions in which a residue in the mGluR is identical to or similar to a residue in the PBPs are indicated with a vertical line or a colon.

Figure 2. View of the C-α trace of the ATD of mGluR1. Secondary structure elements are highlighted. The two lobes constituting the ATD are clearly visible, as well as the deep cleft in which the binding site is located. The key residues Ser_{165} and Thr_{188} are labeled.

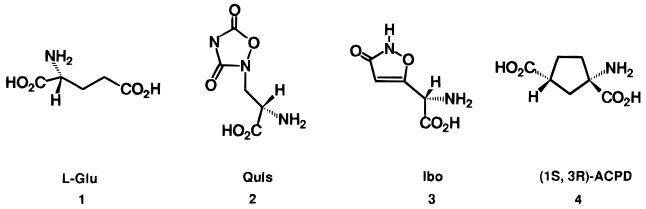
gated ellipsoidal shape with two lobes linked by three hinge fragments and maintains a secondary structure analogous to that of PBPs, characterized by a β-sheet structure flanked by as many as 11 α-helices. The proposed binding site (Ser_{165} and Thr_{188}) appears to be confined in one of the lobes and to be directly facing the cleft formed by the two lobes. While the structural homology between the ATD of mGluR1 and PBPs is stringent in the surroundings of the binding area, secondary structure predictions in the regions of large insertion domains are certainly less accurate (Figure 2). Geometrical details of the model are given in Table 1.

ii. Binding Mode of mGluR1 Agonists. The most potent agonist at the mGluR1 subtype is quisqualate,

Table 1. Geometrical Details of the mGluR1 ATD Model

accessible surface area, Å^2	21116.0
hydrophilic accessible surface area, Å^2	4555.5
hydrophobic accessible surface area, Å^2	13488.1
secondary structure	
% α helix	21.7
% strands	8.9
mean torsional angle, deg	
α-helix	
ϕ	-60.80 ± 10.99
ψ	-42.68 ± 12.47
β-sheet	
ϕ	-130.62 ± 5.94
ψ	137.80 ± 14.69
backbone	
ω	-179.62 ± 19.27

Chart 2. mGluR1 Agonists



(Quis, **2** (Chart 2)) followed by L-Glu (**1**), ibotenic acid (IBO, **3**), and (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, **4**) with potencies ranging from submicromolar to micromolar values depending on the pharmacological system used for their characterization.[20]

In analogy with the other ionotropic glutamate receptors, an α-amino acidic as well as an ω-acidic moiety are required for mGluR activation. It has been our first task, therefore, to perform docking experiments in order

to locate appropriate complementary areas for these crucial moieties in those ATD regions known to affect the agonist binding.[8,9,11] According to the directed site mutagenesis experiments,[11] and in analogy with PBPs,[13] it can be assumed that the zwitterionic moiety of agonists interacts with the side chain of Ser_{165} and Thr_{188}. A close inspection of the postulated mGluR1 binding site reveals several possible ways in which agonists can be accommodated. In particular, besides the Ser_{165} and Thr_{188} amino acids, there is also a number of polar residues (including Ser_{164}, Ser_{166}, Tyr_{185}, Ser_{186}, Ser_{189}, and Arg_{358}) able to form hydrogen bonds with the ω-acidic group. Since the ligands that we have utilized in this study are flexible, special attention has been devoted to the definition of their bioactive conformations. In this respect, although a number of studies have appeared in the literature describing structure activity relationship of mGluRs ligands, there is still a lack of three-dimensional pharmacophore models of mGluR1 agonists that could have been used as a guide for docking experiments.[6b] Thus, in order to take into account simultaneously the two features characterizing the ligand, *i.e.* its conformation and its orientation within the binding site, we adopted the followed procedure. The ligands were manually fitted in the binding site in a way allowing the α-amino acidic moiety to be hydrogen bonded with the hydroxyl groups of Ser_{165} and Thr_{188}. A conformational analysis was then performed on each ligand docked in the binding site. In this way, the number of conformations accessible for each ligand was greatly reduced due to the steric bump with the receptor residues. The sterically accessible conformations thus obtained were then minimized inside the complex in order to detect the energetically favorable disposition of the ligand in the binding site. Since (1*S*,3*R*)-ACPD (**4**), albeit less potent than L-Glu (**1**) or Quis (**2**), is endowed with a reduced conformational freedom, it was investigated first.

Several conformations of (1*S*,3*R*)-ACPD (**4**) can be generated by rotation around the pseudotorsional angles of the cyclopentane ring. Among these, the one with the 3-carboxy group in a pseudoequatorial orientation and the one with the 3-carboxy group in a pseudoaxial orientation are characterized by a nearly equal strain energy and can provide sufficient information. When docked in the binding site, it becomes apparent that the 3-pseudoequatorial conformation of (1*S*,3*R*)-ACPD (**4**) is unable to find appropriate complementary residues in the binding area. Conversely, when the 3-pseudoaxial conformation was analyzed, it showed a good accommodation, due to the following features (Figure 3). The amino acidic carboxy group interacts with the side chain oxygen as well as with the backbone nitrogen of Ser_{165}; the α amino group interacts with the side chain oxygen of Thr_{188} and is intramolecularly hydrogen bonded with the 3-carboxy group. Interestingly, the 3-carboxy group was also shown to interact with the side chain of Arg_{358} and with the side chain hydroxy group of Thr_{188}. These findings seem to be particularly important for the following reasons. First, the intramolecular hydrogen bond between the 3-carboxy group and the 1-amino group stabilizes the conformation of (1*S*,3*R*)-ACPD (**4**) in a folded disposition. In this respect, we have previously reported that the bioactive conformation of (1*S*,3*R*)-ACPD (**4**) acting at the adenylcyclase coupled group II mGluRs is an extended one,[21] characterized by having the 3-carboxy group in a pseudoequatorial disposition. According to the present result, the relative unselectivity of (1*S*,3*R*)-ACPD (**4**) toward group I and group II mGluRs can be qualitatively explained in terms of different conformational requirements. Secondly, the interaction of (1*S*,3*R*)-ACPD (**4**) with Arg_{358} is of note since it indicates that this residue, the only charged one partially buried in the binding site region, may have a functional role in the signal transduction process.[22]

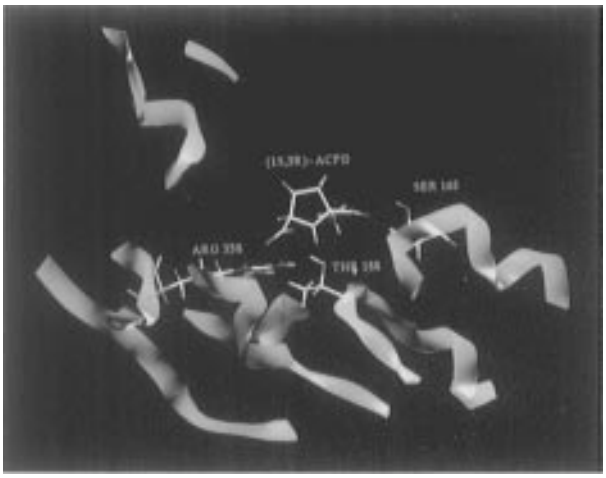


Figure 3. Docking of (1*S*,3*R*)-ACPD (**4**) in the proposed binding site of mGluR1 ATD. Hydrogen bonds to Ser_{165}, Thr_{188}, and Arg_{358} are depicted as green dotted lines.

L-Glu (**1**) and Quis (**2**) were submitted to constrained conformational analysis by using the proposed bioactive conformation of (1*S*,3*R*)-ACPD (**4**) as the template and were shown to adopt a similarly folded conformation. When docked in the binding site, moreover, L-Glu (**1**) and Quis (**2**) were shown to adopt the same interaction features already described for (1*S*,3*R*)-ACPD (**4**) (Figures 4 and 5).

iii. Binding Mode of mGluR1 Competitive Antagonists. A major contribution to the development of mGluR ligands has come from the discovery that members of the (carboxyphenyl)glycine (CPGs, Chart 3) class are endowed with an array of functional activity (from antagonism to full agonism through partial agonism) according to the substitution pattern.[23] In particular, (*S*)-(4-carboxyphenyl)glycine (4CPG, **5**), (*S*)-(4-carboxy-3-hydroxyphenyl)glycine (4C3HPG, **6**), (*S*)-(4-hydroxy-3-carboxyphenyl)glycine (4H3CPG, **7**), and (+)-α-methyl(4-carboxyphenyl)glycine (M4CPG, **8**) are full antagonist of the L-Glu-stimulated PI hydrolysis and have potencies in the order of the micromolar (Table 2). Conversely, (*S*)-(3-hydroxyphenyl)glycine (3HPG, **9**) is an mGluR1 agonist.[24] More recently, we have reported the synthesis and biological evaluation of (±)-1-aminoindan-1,5-dicarboxylic acid (UPF523, **10**) as a conformationally constrained CPG analog endowed with good potency and selectivity as an mGluR1 antagonist.[14b]

The following structures were selected for the docking experiments: UPF 523 (**10**), which can give useful information on the conformation of the amino acidic side chain; 4CPG (**5**); and 4C3HPG (**6**). Furthermore, 3HPG (**9**) was also investigated due to its peculiar behavior as an mGluR1 agonist. Since all these derivatives are reported to act competitively with L-Glu (**1**), it was assumed that the α-amino acidic moiety interacts with

Figure 4. Docking of L-Glu (**1**) in the proposed binding site of mGluR1 ATD. Hydrogen bonds to Ser_{165}, Thr_{188}, and Arg_{358} are depicted as green dotted lines.

Figure 5. Docking of Quis (**2**) in the proposed binding site of mGluR1 ATD. Hydrogen bonds to Ser_{165}, Thr_{188}, and Arg_{358} are depicted as green dotted lines.

the key Ser_{165} and Thr_{188} residues. However, the bulky phenyl group of CPGs clearly prevents an accommodation on the surface of the cleft due to steric overlapping with neighboring residues. Accordingly, when **10** is docked into the binding site, it becomes apparent that the ω-carboxylate can point toward the second lobe of the ATD through the solvent-exposed cleft. In this disposition, the ω-carboxy group becomes hydrogen bonded with the side chain atoms of Tyr_{236} and Asn_{335}. Obviously, the same orientation is effective for the

Chart 3. Representative Phenylglycine Derivatives

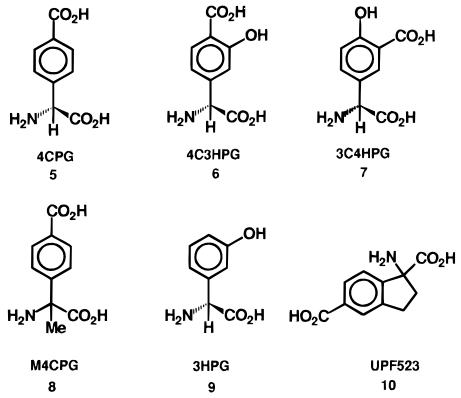


Table 2. Summary of the Potencies of the Carboxyphenylglycine Derivatives (**5**−**10**)[a]

	mGluR1	
compound	IC_{50} (μM)	EC_{50} (μM)
QUIS (**2**)[b]		0.2
Ibo (**3**)[b]		6
(1*S*,3*R*)-ACPD (**4**)[b]		22
S-4CPG (**5**)[c]	65 ± 5	
S-4C3HPG (**6**)[c]	40 ± 3	
S-4H3CPG (**7**)[c]	290 ± 47	
M4CPG (**8**)[c]	155 ± 38	
S-3HPG (**9**)[c]		68 ± 7
UPF523 (**10**)[d]	214 ± 20	

[a] Concentrations (μM) for half-maximal inhibition (IC_{50}) or stimulation (EC_{50}) of PI hydrolysis (mGluR1a). [b] Data are from the following: Nakanishi, S.; *et al. Nature* **1991**, *349*, 760. Nakanishi, S.; *et al. Neuron* **1992**, *8*, 757. [c] Data from ref 24. [d] Moromi, F.; et al. Manuscript in preparation.

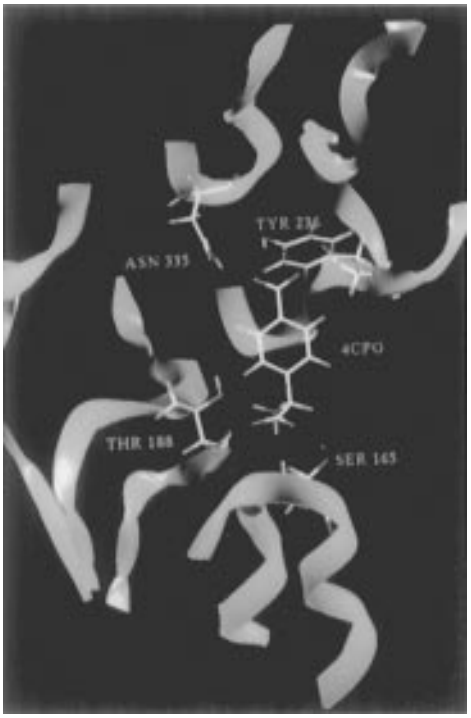


Figure 6. Docking of 4CPG (**6**) in the proposed binding site of mGluR1 ATD. Hydrogen bonds to Ser_{165}, Thr_{188}, Tyr_{236}, and Arg_{358} are depicted as green dotted lines. The phenyl moiety is exposed to the cleft and does not show any specific interaction with binding site residues.

parent derivative 4CPG (**5**) (Figure 6) and for 4C3HPG (**6**). This proposed disposition reveals a different binding mode between agonists and antagonists which can

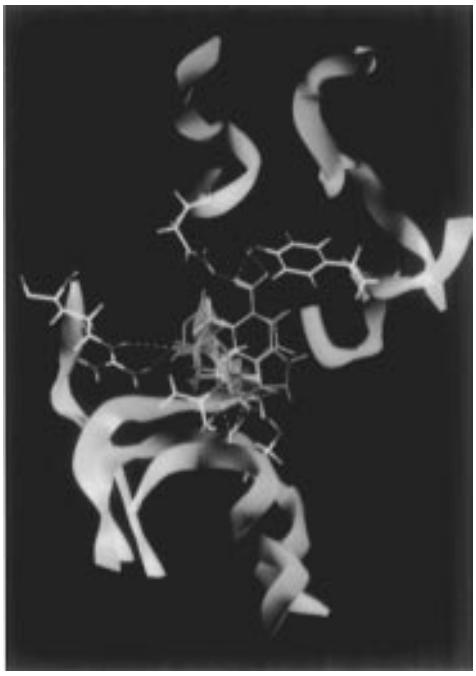


Figure 7. Comparison of the different binding mode of mGluR1 agonists (purple) and mGluR1 antagonists (green). Both agonists and antagonists bind to Ser_{165} and Thr_{188}. Antagonists are projected through the cleft and also bind to Asn_{335} and Tyr_{236}.

be instrumental for speculating on the process of signal transduction.

Domain closing in periplasmic binding proteins was proposed to initiate transmembrane signal transduction through the interaction of the two lobes with the membrane.[13] A similar process may occur in the ATD of mGluR1, where domain closing may propagate conformational changes through the transmembrane helices.[11] Although our model does not provide plausible explanations about the molecular mechanism that can eventually lead to domain closing upon agonist binding, the proposed binding mode of antagonists might confirm the hypothesis. Thus, antagonists are disposed through the cleft, interacting with both domains, and this peculiar dispositions may prevent the lobes from collapsing, thus precluding the starting of the signal transduction process (Figure 7).

This proposal for the antagonist binding mode applies to all the CPGs endowed with antagonistic activity but is apparently unable to explain the agonist mGluR1 profile of 3HPG (**9**), which differs from CPGs only by the lack of the distal carboxy moiety. An explanation for the pharmacological profile of 3HPG (**9**) comes from close inspection of its binding mode (Figure 8). While the lack of the 4-carboxylate group prevents 3HPG (**9**) from interacting with either Tyr_{236} or Asn_{335}, the presence of the 3-hydroxy group allows the ligand to bend toward the hinge region and form a hydrogen bond with Arg_{358}, the same residue that is involved in Quis (**2**), L-Glu (**1**), and (1*S*,3*R*)-ACPD (**4**) binding. It can therefore be concluded that, despite its similarity with CPGs, 3HPG (**9**) is endowed with a binding mode closer to that of full agonists such as Quis (**2**), L-Glu (**1**), and (1*S*,3*R*)-ACPD (**4**).

Discussion

In order to be reliable, three-dimensional models of proteins built according to homology rules should fulfill a number of physicochemical criteria[25] and be in agreement with all the available experimental data. In particular, the model must take into account pharmacological data, be able to predict the stereoselectivity of ligand−receptor interaction, provide reasonable explanation for the inactivity of compounds structurally related to active ones, and, finally, be in agreement with mutagenesis experiments, where available.

On the basis of the sequence homology between the ATD of mGluR1 and PBPs first reported by O'Hara and further supported by site directed mutagenesis experiments, we have built a three-dimensional model of the mGluR1 ATD consistent with the pharmacological profile of known potent mGluR1 agonists and antagonists. Our docking experiments suggest that agonists such as **1**−**4** are placed in a well on the surface of one lobe of the ATD. The zwitterionic α-amino acidic moiety and the distal carboxylate group are neutralized by a hydrogen bond network which mainly involves side chain atoms of Ser_{165}, Thr_{188} and Arg_{358}, this latter being ion-paired with the distal *ω*-acidic group. Further, a role for the macrodipole generated by the α-helix ending with Ser_{165} may be assumed to be operative in ligand stabilization. A peculiar aspect of our model is the role played by Arg_{358}. This residue, only partially exposed to the solvent (37% of the side chain surface area), is directly involved in the interaction with the *ω*-distal acidic group of all the agonists investigated and should be considered as a good target for further site-directed mutagenesis experiments. As far as the structural features of ligands are considered, all the agonists are shown to bind in their folded conformations, as exemplified by the 3-pseudoaxial conformation of the bound (1*S*,3*R*)-ACPD (**4**). Since our approach does not take into consideration entropic and solvation effects, it is not possible to correlate experimental binding affinities with docking energies. Our model can only be interpreted in terms of the more or less favorable disposition of ligands within the binding site, and in this respect, there are no apparent explanations for the higher affinity of Quis (**2**) with respect to (1*S*,3*R*)-ACPD (**4**) or L-Glu (**1**) and we can only propose that a common binding mode is operative among agonists.

A particularly interesting feature which emerges from our study is the peculiar binding mode of competitive antagonists. In this case, too, the α-amino acidic moiety of competitive antagonists can be positioned between Ser_{165} and Thr_{188}. Different from agonists, however, the bulky phenyl ring cannot be accommodated within the well in the surface of one of the mGluR1 ATD lobes, and the distal *ω*-carboxylate is forced to be projected through the cleft toward the second lobe of the ATD, where it can form effective hydrogen bonds with Asn_{335} and Thr_{236} residues. Conversely, 3HPG (**9**), structurally related to CPGs but endowed with an agonist profile, only interacts with Arg_{358}. It is important to note that all the antagonists that we have taken into consideration can adopt the proposed disposition without inducing distortions of the α-carbon backbone of the mGluR1 ATD. Since the 3D structure of the ATD of mGluR1 has been modeled on the open form of PBPs, this proposed binding mode of competitive antagonists brings additional support to the idea that the ATD of mGluR1 and PBPs share a common mechanism of signal transduction. In fact, it may be speculated that the resting

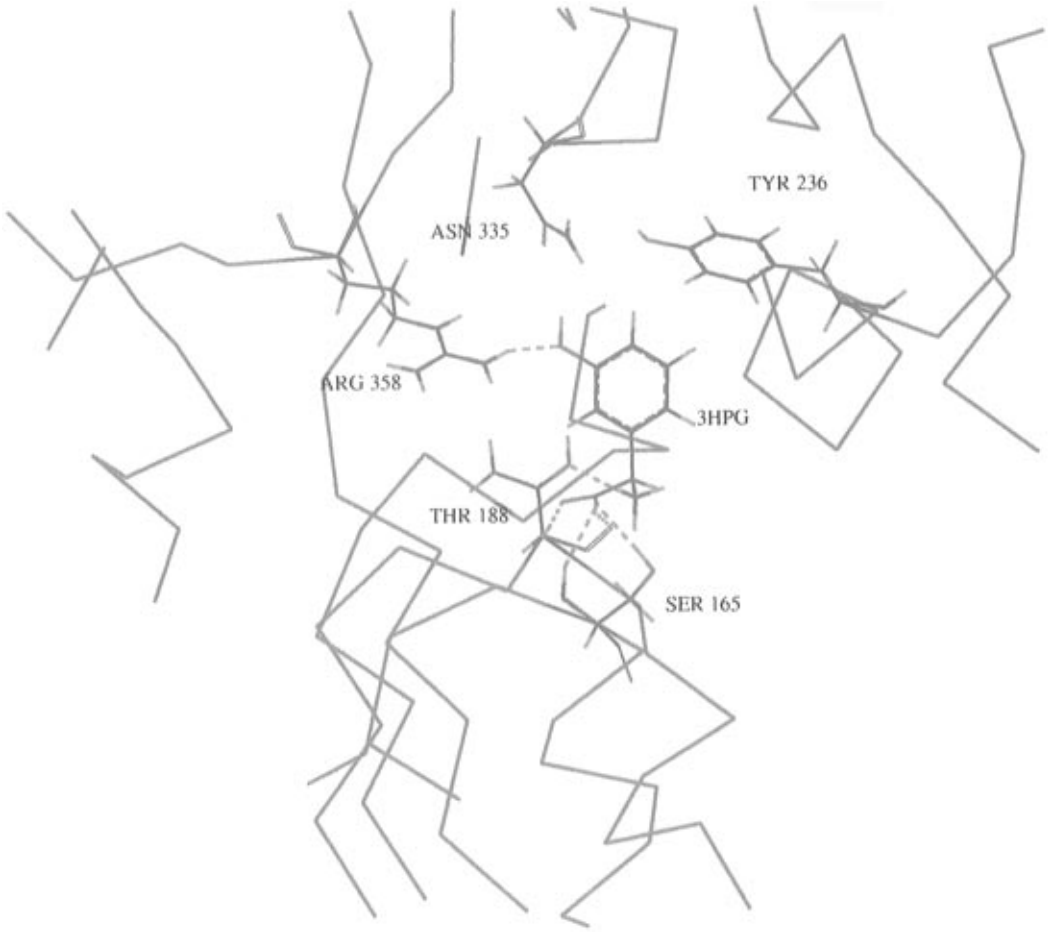
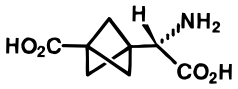


Figure 8. Docking of 3HPG (**9**) in the proposed binding site of mGluR1 ATD. The 3-hydroxy group interacts with the Arg_{358} residue, while no interactions are detected with Tyr_{236} and Asn_{335}.

form of the ATD of mGluR1 is similar to the open form of PBPs and that a collapse of the two lobes takes place upon agonist binding. Since there is an apparent complementarity between the open form-like structure of the mGluR1 ATD and that of mGluR1 antagonists, it is conceivable that antagonists recognize and stabilize the open form of the receptor, thus precluding the conformational movement of the hinge segments. This mechanism of binding makes the effective role played by the phenyl ring of CPGs debatable. According to our model, in fact, the phenyl moiety of the CPG antagonists seems not to be involved in interactions with the binding site although a positive entropic effect may be envisaged. In this connection it should be mentioned that the X-ray structure of the open forms of LBP and LIVPB contain as many as 16 water molecules in the cleft[13] and, hence, the phenyl moiety of CPGs might be able to displace ordered water molecules from the solvent-exposed crevice of the mGluR1 ATD. According to this hypothesis, derivatives structurally different from CPGs can be as effective as mGluR1 antagonists, provided that they are endowed with suitable spacers between the α-amino acidic moiety and the ω-distal acidic group. In this respect, we have recently reported that 3-carboxybicyclo[1.1.1]pentane-1-α-aminoacetic acid (UPF596, **11**) is endowed with activity as a mGluR1 antagonist with an IC_{50} of 25 μM.[26]

A characteristic feature of **11** is the [1.1.1]propellane ring which confers on the molecule a coplanar disposition of the α-amino acidic moiety and the ω-distal carboxylate, similar to that possessed by CPGs.



UPF 596
11

Indeed, our model shows that **11**, although significantly shorter than (*S*)-(4-carboxyphenyl)glycine (**5**) (about 0.8 Å), can be neatly accommodated into the mGluR1 binding site (Figure 9) without any apparent expenditure of energy, thus confirming that the presence of a phenyl ring, because of the lack of specific interactions, is not strictly required for mGluR1 antagonism.

Our model also accounts for the enantioselectivity of mGluR1 toward antagonists. Indeed, the inactivity as mGluR1 antagonists of CPGs (*R*)-enantiomers[23] can be explained by the lack of productive hydrogen bonds between their α-amino acidic moiety and the Ser_{165}–Thr_{188} couple, which forces the α-carboxylate to point toward the cleft, thus remaining directly exposed to the solvent. Recently, a number of bioisosterically substituted analogs of CPGs including α-methyl(4-phosphonophenyl)(4-sulfonylphenyl) and (4-tetrazoylphenyl)-glycines have been described[27] and shown to be either inactive as mGluR1 antagonists or to display high micromolar to millimolar activities. In agreement with our model, the low activity exhibited by these compounds can be explained with the bulkier size of the

Figure 9. Docking of the structurally novel mGluR1 antagonist UPF596 (**11**) in the proposed binding site of mGluR1 ATD. Despite the shortness of the distance between the α-aminoacidic moiety and the distal carboxy group, hydrogen bonds with Asn_{335} and Tyr_{236} are found.

phosphonyl, the sulfonyl or the tetrazoyl groups which results in a steric compression with the Tyr_{236} residue.

Conclusions

On the basis of a known sequence homology between the ATD of mGluR1 and PBPs we have been able to build a 3D model of the mGluR1 ATD which accounts for the pharmacological behavior exhibited by agonists and competitive antagonists and gives useful insights into structural features affecting receptor binding affinity. Important aspects which emerge from our model are summarized here. (a) Agonists such as Quis (**2**), L-Glu (**1**), and (1*S*,3*R*)-ACPD (**4**) can be accommodated into a crevice in one of the two ATD lobes, where ligand−receptor electrostatic and hydrogen-bonding interaction can be detected. (b) Antagonists such as 4CPG (**5**), 4C3HPG (**6**), and UPF523 (**10**) hae been shown to adopt a different binding mode which involves interaction with both lobes of mGluR1 ATD. This binding mode allowed us to hypothesize a molecular mechanism for mGluR1 antagonism. (c) The agonist profile of a phenylglycine derivative such as 3HPG (**9**) is explained in terms of its interaction with Arg_{358}, a residue that we propose as playing a key role in the signal transduction process. (d) The phenyl moiety common to all the mGluR1 antagonists reported up to now is not involved in any specific interactions with the receptor and could be substituted by suitable spacers.

Similar studies on other members of the mGluR family would be of great interest and will allow us to address the extremely important problem of ligand selectivity. However, a FASTA database search utilizing the sequence of ATD of mGluR2 or mGluR4 (as representative of group II and group III of mGluRs, respectively) as a query did not find any structurally known protein in the first 1000 matches. It is certainly possible to speculate that, despite the low homology, members of group II or group III will share structural similarities with mGluR1, at least in the region of the binding site, however, further proof of the present mGluR1 ATD model is necessary before engaging in the problem of subtype selectivity.

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