Agonist Selectivity of mGluR1 and mGluR2 Metabotropic Receptors: A Different Environment but Similar Recognition of an Extended Glutamate Conformation

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To investigate the structural requirements for selective activation or blockade of metabotropic glutamate receptors, we developed a pharmacophore model for group I (mGluR1) and group II (mGluR2) agonists. The Apex-3D program was used with a training set of known active, inactive, and/or selective compounds with a wide structural diversity. The pharmacophore models were then validated by testing a set of additional known agonists. We also used competitive antagonist superpositions in order to define more precisely the topology of the mGluR1 and mGluR2 agonists' recognition site. Both models account for the activity of most potent compounds and show that the selectivity between mGluR1 and mGluR2 subtypes may be due to excluded volumes and additional binding sites, while the relative spatial position of functional groups (NH_2, α- and γ-CO_2H) remains very similar. On both models glutamate lies in an extended form. An additional binding site is disclosed on mGluR1, while this region would be forbidden on mGluR2. This new site combines a closed and an open model for mGluR1 and accounts for the increased affinity of quisqualic acid. The models show another large hydrophobic region which is tolerated for mGluR2 and restricted for mGluR1.

Introduction

The common amino acid glutamate is the neurotransmitter of most fast excitatory synapses in the brain. As such it is supposed to play important roles in many brain functions such as learning and memory, vision, control of movements, pain sensitivity, etc. Glutamate is also involved in many neurological disorders, such as epilepsy and neuronal death observed after ischemia or several neurodegenerative diseases,[1] as well as in many psychiatric disorders such as anxiety and schizophrenia. To exert its actions, glutamate activates two major receptor types: the ionotropic receptors that are responsible for its fast excitatory effects and the metabotropic glutamate receptors (mGluRs) that mainly modulate the fast excitatory glutamatergic transmission. The mGluRs are G-protein-coupled receptors which modulate the activity of second-messenger-producing enzymes and ion channels.[2] These receptors constitute an excellent target for drugs modulating the central action of glutamate.

Eigth mGluR subtypes have been identified that can be subdivided into three groups based on their sequence similarity.[2,3] Group I is composed of mGluR1 and mGluR5 which activate PLC, whereas group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) can inhibit adenylyl cyclase activity. Group I mGluRs can increase cell excitability,[4−6] potentiate glutamate release,[7,8] facilitate the glutamate-induced neuronal toxicity,[9−11] and participate in pain sensitivity.[12−15] Accordingly, potential therapeutic applications of group I antagonists are expected.[2,16] In contrast, in many instances, group II and group III mGluRs are located on glutamatergic terminals to inhibit glutamate release.[2] Accordingly, agonists for these receptor types are expected to have many potential therapeutic applications by inhibiting the glutamatergic system.[2,16] These include, for example, protection from excitotoxicity[17−20] and, as demonstrated recently, treatment of anxiety,[21] schizophrenia,[22] and drug addiction.[23]

High-throughput screening may allow for the discovery of new mGluR antagonists. However this approach would lead to a limited number of hits when screening for agonists since such molecules have to bind to the receptor but also have to activate it. Moreover, one is not expecting a large number of activating sites in a receptor, so the rational design of new drugs seems more appropriate in the present case. It is therefore of interest to determine precisely the structural requirements of agonists for all mGluR subtypes. Thus, more data on the glutamate binding site of these receptors are needed. Although some homology between the binding site of mGluR1 and a crystallized bacterial periplasmic protein (LIVBP) has been shown,[24] we are yet unable to describe the precise topology of this domain as well as the geometry of the bound glutamate. Nevertheless pharmacophore models can afford valuable data for the rational design of new agonists.

It is generally recognized that glutamic acid binds to its various receptors via its three ionizable groups. The relative spatial position of these functions is characteristic of each receptor associated with a different protein environment. Glutamic acid (**1**) is a flexible molecule

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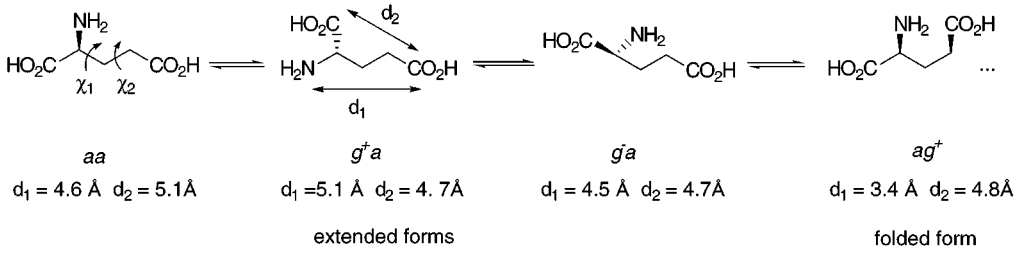


Figure 1. Conformations of glutamic acid characterized by C(2)−C(3) and C(3)−C(4) rotamers (a, g^+, g^-) and by d_1, d_2 distances.

that can adopt several folded or extended conformations (Figure 1). These can be described by the dihedral angles χ_1 and χ_2 (defined in staggered conformations as a, g^+, or g^-, see refs 25−28 and Figure 1) and two distances d_1 (α-amino−γ-carboxyl) and d_2 (α-carboxyl−γ-carboxyl) (see refs 29 and 30 and Figure 1). All extended forms are described by d_1, d_2 > 4.5 Å and represented by aa, g^+a, and g^-a conformations (Figure 1). To gain more insight on bioactive conformations of mGluR ligands, we have previously undertaken the conformational analysis of pharmacological group I- and group II-selective agonists (1*S*,3*R*)- and (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid (**(−)-4** and **(+)-5**; Chart 1).[29] These cyclic glutamate analogues were shown to be flexible, and thus bioactive conformations could not be deduced. Later we and others synthesized and evaluated rigid analogues of aminobicycloheptanedicarboxylic acids **8**−**11**[30] and aminobicyclohexanedicarboxylic acids **12**−**14**[31] that tend to demonstrate that glutamate lies in an extended conformation at both mGluR1 and mGluR2 binding sites in agreement with literature data.[25,28,32−34] However although an extended *aa* conformation of glutamate at the mGluR2 binding site is generally admitted, a folded conformation at the mGluR1 binding site has been proposed when using a homology model of the receptor site,[35] and selectivity with mGluR2 is still unclear.

The purpose of this study was to investigate in more detail the mGluR1 and mGluR2 binding site and to provide new features that account for the mGluR1/mGluR2 selectivity. We now present the pharmacological evaluation of a series of methylglutamates used as topological probes of mGluRs and the elaboration of pharmacophore models using known ligands together with this set of new probes. The construction of pharmacophore models requires the availability of several active, inactive, and/or selective ligands on each receptor. We have thus chosen to concentrate our study on mGluR1 and mGluR2 as group I and group II representatives for which pharmacology is most detailed. For validation of our pharmacophore models, we used another set of molecules with known activities. Finally, we obtained a description of mGluR1 and mGluR2 agonist binding sites, and we were able to explain the relationships between the structure and activity of most ligands.

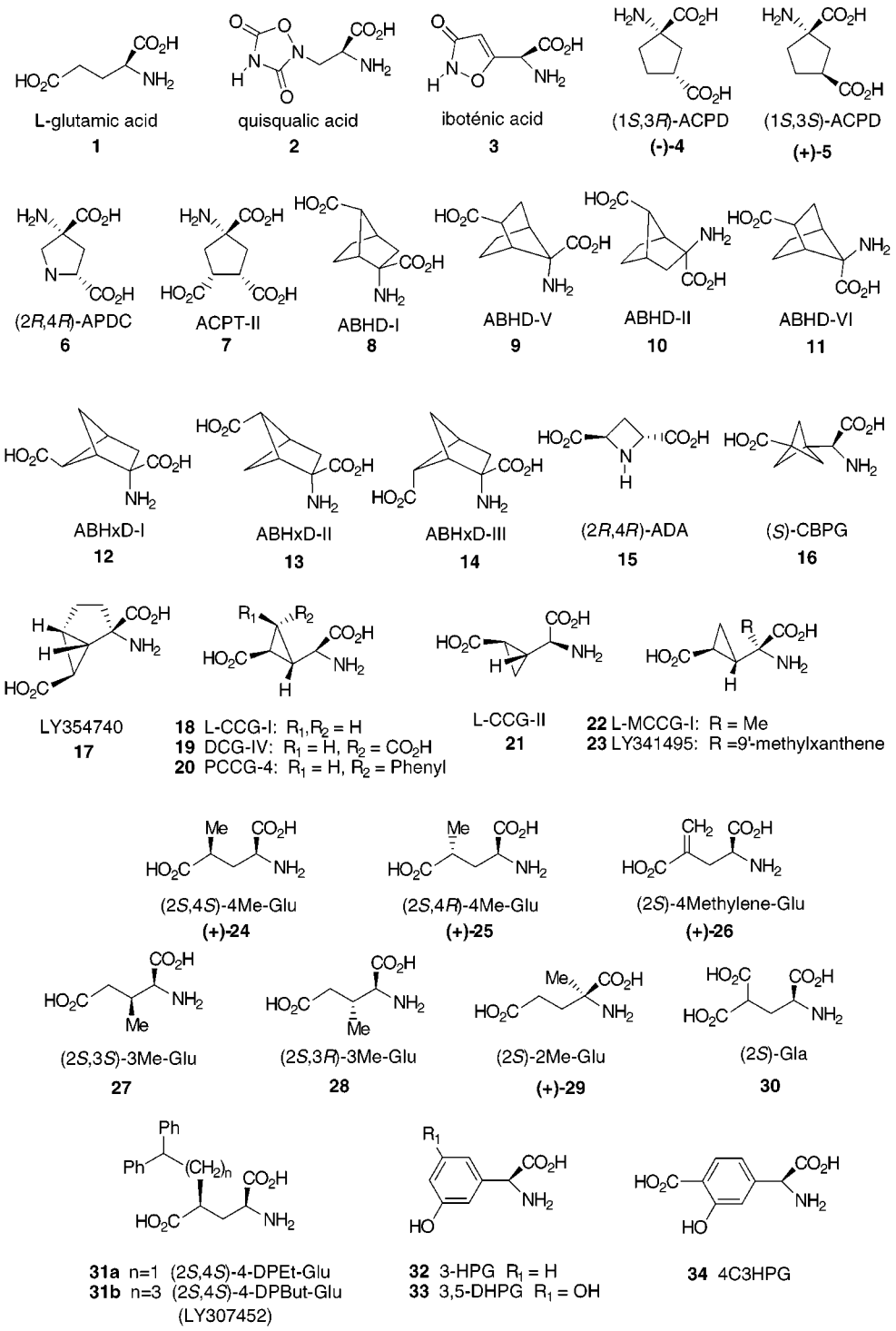
Material and Methods

Biological Assays. The activity of the various compounds was tested on cells transiently expressing the rat mGluR1a receptor (either LLC-PK1 or HEK 293 cells) or the rat mGluR2 receptor (HEK 293 cells), as previously described.[36−38] In both cases, the receptor activity was measured as the agonist-induced inositol phosphate formation as previously described.[38] The mGluR2 receptor does not activate PLC under normal conditions but rather inhibits adenylyl cyclase activity. Because this latter transduction cascade is very difficult to measure in a transient transfection assay, we cotransfected this receptor with a chimeric G-protein Gqi9 which allows mGluR2 to activate PLC. We previously reported that the pharmacological profile of mGluR2 determined under these conditions was identical to that reported measuring the inhibition of AC activity.[38] The effect of at least eight different concentrations of agonists over 3 orders of magnitudes was used to construct full dose−response curves. These were fitted according to the equation $y = ((y_{max} - y_{min})/1 + (x/EC_{50})n^H) + y_{min}$, using the kaleidagraph program in order to determine the maximal effect of the drug (y_{max}), its EC_{50} (or IC_{50}), and the Hill coefficient (n^H). To compare agonist activities from different types of assay, EC_{50}'s are displayed as the ratio between the measured EC_{50} and the glutamate EC_{50} measured in the same conditions (Table 1).

Molecular Modeling. For all molecules (from training set and validation set) in their zwitterionic form, the three-dimensional structure was generated by using InsightII and Discover (version 95.0) for minimizations.[39] The cvff force field[40] was used for all calculations, assuming a dielectric constant of 80. The conformational flexibility of each molecule was investigated through a simulated annealing protocol. After initial minimization, the temperature of the system was raised to 900 K for 1 ps and cooled to 600 and 300 K during 5 ps. The resulting conformation was minimized again, by using a combination of steepest descent (untill derivative less than 5 kcal/mol) and conjugated gradient (derivative less than 0.05 kcal/mol) methods. This procedure was repeated 100 times for each molecule, and each final minimized conformation was archived. The advantage of this procedure versus a complete conformational search by defining each torsion angle is speed: for each molecule, it takes about 30 min versus 6−8 h for an extensive conformational search (on a SGI Indigo2 R4400-200 MHz workstation). Then a clustering analysis based on root-mean-square deviation (rmsd) of all heavy atom positions was performed. For each family extracted, the lowest energy conformer was selected as a representative one. We validated this procedure with the glutamate molecule for which we were able to reproduce the eight conformers that have been widely studied and reported.

Assuming that all molecules, classified according to their activity (inactive when EC_{50} ratio > 25, Table 1), bind to the same receptor site, the Apex-3D program was used for the pharmacophore generation[39,41] with all the training set molecules. The program is based on the identification of common descriptor centers: hydrogen bond donor, hydrogen bond acceptor, partial charges, etc. A priority can be given during the calculations for an active selective compound: we used it for the quisqualate **2** for the mGluR1 calculations. No priority was given for mGluR2. Apex-3D generated a list of phamacophore models for each binding site: first a filtering operation was performed in order to select the models described with more than 80% of the active molecules. Then a further graphical analysis was necessary to select significant models. To test the validity of the proposed pharmacophores, we used a series of molecules with known activities, namely, the validation set compounds (Table 1). These molecules have been submitted to the same conformational analysis as reported for the training set molecules (see above). Then, we selected the conformer with the d_1 and d_2 distances closest to a given

Chart 1



pharmacophore model, and we superimposed each compound by using the three functional groups as anchor points (distal and proximal carboxylate carbon atoms, amino group nitrogen atom).

Results

Biological Data. Affinities of selected known agonists and antagonists at mGluR1 and mGluR2 are displayed in Table 1. Although competitive antagonists are assumed to bind to the receptors in a similar mode as agonists do,[33] they were not kept for the generation of the models but used for their validation. Glutamate was alkylated in positions 2, 3, and 4 to evaluate the bulk tolerance of each receptor. We previously reported the synthesis and conformational analysis[26,42−44] of these topological probes; their pharmacological activity is now presented on cloned receptors transiently expressed in LLC-PK1 or HEK 293 cells. On both mGluR1 and mGluR2 receptors (2*S*,4*S*)-4-methylglutamate (**(+)-24**) and (*S*)-4-methyleneglutamate (**(+)-26**) are good agonists with affinity similar to that of glutamate (Table 1) as previously reported.[45,46] The (2*S*,4*R*)-4-methylglutamate isomer (**(+)-25**) binds with a decrease of EC_{50}. When the methyl group is a β substituent of glutamate (3-methylglutamates **27** and **28**) the affinity is even

Table 1. Pharmacology of mGluR1 and mGluR2 Receptors

agonists and/or antagonists	mGluR1			mGluR2			
	EC_{50}[a] ratio	IC_{50}[a] (μM)	T/V set[b]	EC_{50}[a] ratio	IC_{50}[a] (μM)	T/V set[b]	ref
1	1		T	1		T	37, 38, 53
2	0.02		T	>90		T	37, 38
3	0.91		T	25		T	37, 38
(−)-4	0.75		T	1.6		V	37, 38
(+)-5	>25			1.2		T	37, 38
(+)-4	10			10		V	37, 38
6	>80		V	0.4		V	*c*
7	ne	115[d]	V	ne	88[d]	V	49
8		300[d]	V	>80		V	48
9	>80		V		>1000		30
10	>80[e]		V	>80		V	30
11		>1000		>80		V	30
12	0.32		V	1.1		V	31
13	25		V	186		V	31
14	47		V	131		V	31
15[e]	15		V	>80			47
16		25		>60	>300		50
17		458	V	0.004		V	*c*
18	5		T	0.04		T	38, 53
21	20		V	nd			56
19		389	T	0.03		T	53
20	ne		V		9.9	V	*c*
22	ne				77		*c*
23	nd				0.034[f]	V	54
(±)-24	0.65			0.53			*c*
(+)-24	0.5		T	0.3		T	*c*
(−)-24	4.9			1.1		V	*c*
(+)-25	4.6			23		V	*c*
(±)-26	1.1			0.4			*c*
(+)-26	0.7		T	nd		T	*c*
(−)-26	14			nd			*c*
27	13			80		V	*c*
28	23			12		V	*c*
(±)-29				>80		V	*c*
(+)-29	>80		V	nd			*c*
(−)-29	>80			nd			*c*
30	ne	ne			102	V	53
31a		ne			3−18[f]	V	55, 60
31b		ne			0.4[f]		60
32[e]	7.8		V	>80			36
33	0.53		V	ne			36
34		40	V	4.0		V	51, *c*

[a] EC_{50}, concentrations giving half of the maximal response; IC_{50}, concentrations giving half of the maximal inhibition, in mGluR-expressing cells (HEK, LLC−PK1, or CHO[56]); nd, not determined in the same conditions; ne, no effect. The EC_{50} ratio is the ratio between the measured EC_{50} and the glutamate EC_{50} measured in the same assay. [b] Compounds used in the training or validation set are respectively indicated by T or V. [c] This study. [d] K_b value. [e] Partial agonist. [f] Values obtained with human mGluRs.

lower, and no activity is detected with 2-methylglutamate **29** (α substitution) (Table 1).

Molecular Modeling. The high-temperature molecular dynamics procedure was applied to all compounds listed in Table 1. Those of the training set (T, Table 1) were clustered. The number of conformers obtained, the energy ranges, and the energy of the conformers fitting the mGluR1 and mGluR2 models are displayed in Table 2. Compounds of the validation set (V, Table 1) were classified according to their distances d_1, d_2 (Figure 1). For each receptor, Apex-3D generated around 200 pharmacophore models. Carboxylic groups are defined as 2-point pharmacophore sites, nitrogen is considered as one, and excluded volumes are not considered as a pharmacophore point. Thus a filtering procedure was applied by selecting models with at least five descriptor centers and containing more than 80% of the active molecules, with a probability score higher than 0.66 for mGluR1 and 0.90 for mGluR2. This resulted in 67 models for mGluR1 and 11 for mGluR2. The next step consisted in a graphical analysis: the pharmacophores were evaluated according to their biochemical significance. Mainly, it consisted in excluding all models with the distal carboxylate group superimposed onto the proximal one of the other molecules. Finally, we ended with two models for mGluR1 (Figure 2) and one model for mGluR2 (Figure 5). These models are characterized

Table 2. Training Set Compounds Selected by Apex-3D[a]

compd	no. of conformers	energy range (kcal/mol)	mGluR1 (kcal/mol)	mGluR2 (kcal/mol)
1	8	−66.2 to −56.2	−57.1	−59.7
2	4	−69.2 to 61.0	−64.0	x
3	2	−39.8 to −39.7	−39.7	x
4	3	−44.5 to −34.3	−44.5	−34.3
5	3	−39.0 to −38.3	x	−38.3
18	2	85.4 to 86.9	86.9	86.2
19	6	56.6 to 61.5	58.8	58.9
(+)-24	13	−47.4 to 38.4	−42.5	−42.5
(+)-26	11	−54.9 to −44.0	−45.8	x

[a] A number of conformers obtained after high-temperature molecular dynamics sampling and clusterization, along with their relative energies are shown. Energies of conformers fitting respectively mGluR1 and mGluR2 pharmacophore models are shown; compounds that were not used for the superposition are indicated by an x symbol.

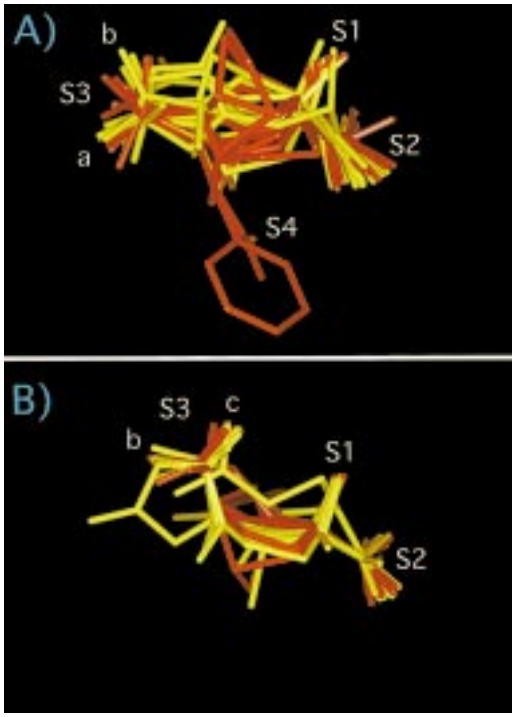


Figure 2. mGluR1 pharmacophore models A and B generated by the Apex-3D program, using compounds of the training set. Ligands of the validation set have been added by superposition of the functional groups of adequate conformers. Active molecules (EC_{50} ratio < 25) are drawn in yellow, inactive ones (EC_{50} ratio > 25) in red. Thus compounds **1**, **2**, **3**, **12**, **18**, **21**, **(+)-24**, **(+)-26**, **33** (model A) and **1**, **2**, **(−)-4**, **15**, **(+)-24**, **(+)-25**, **(+)-26**, **27**, **28** (model B) are shown in yellow, and **6**, **9**, **10**, **11**, **13**, **17**, **19**, **20**, **29** (model A) and **6**, **14**, **19**, **29** (model B) are shown in red. **(−)-4**, **(−)-24**, **27**, and **28** are not shown on model A for clarity. Interaction sites S1, S2, S3(a, b, c), and S4 are labeled.

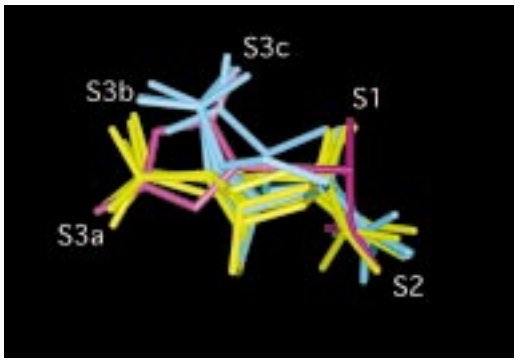


Figure 3. mGluR1 pharmacophore model C. In order to evidence the additional S3c site, selected molecules fitting features of model A (**10**, **12**, **1**, **(−)-4** in yellow) or model B (**14**, **15**, **1**, **(−)-4** in blue) or both (**2** in magenta) are shown.

by 5 points which are the nitrogen atom of the α-amino function and the four oxygen atoms or heteroatoms of the proximal and distal acidic functions. Yet to simplify the description of the models, the two oxygen or heteroatoms of the acidic functions were not specifically identified when not needed for the discussion. In those cases the central atom of the group was designated as an anchor point. Moreover a receptor model would be needed to define precisely the orientation of the carboxylate groups when they are not superposed to a rigid isostere group as in quisqualate **2** or ibotenate **3**. Thus some molecules of the models show a different carboxy-

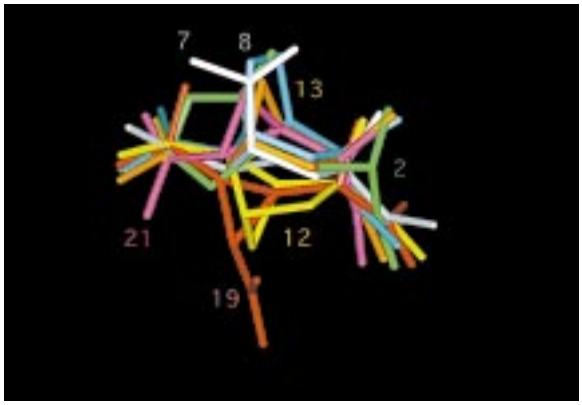


Figure 4. mGluR1 pharmacophore model C. Molecules of interest to describe the pharmacophore bulk environment (**2**, **12**, **13**, **21** agonists; **7**, **8**, **19** antagonists) have been used for superpositions.

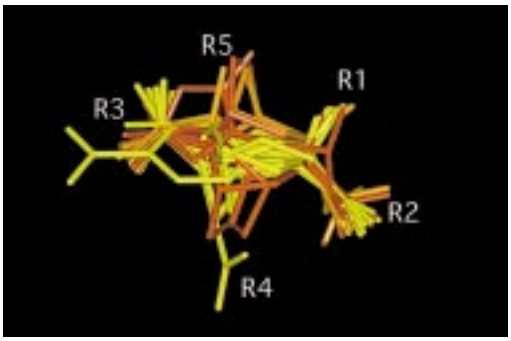


Figure 5. mGluR2 pharmacophore model generated by the Apex-3D program using compounds of the training set. Ligands of the validation set have been added by superposition of the functional groups of adequate conformers. Active molecules (EC_{50} ratio < 8) are drawn in yellow, moderately active ones in orange (8 < EC_{50} ratio < 25), and inactive ones (EC_{50} ratio > 25) in red. Thus compounds **1**, **(−)-4**, **(+)-5**, **6**, **12**, **17**, **18**, **19**, **(+)-24**, and **(−)-24**, **34** are shown in yellow, **(+)-4**, **(+)-25**, and **28** in orange, and **2** (mGluR1 model A conformer), **8**, **10**, **11**, **13**, **27**, and **29** in red. Binding sites R1, R2, R3, allowed R4, and forbidden R5 regions are indicated.

late orientation from the others. However rotating these groups to optimize an interaction with the receptor should be of low energy cost.

mGluR1. By using the criteria previously described, it was not possible to select one of the two pharmacophore models (A or B) extracted by Apex-3D for mGluR1 and represented in Figure 2, since some molecules fit model A but not model B, whereas some others fit model B only. Model A can be described by the $d_1 = 4.5-5.4$ Å and $d_2 = 4.3-5.1$ Å distances (as defined in the Introduction) and by S1, S2, S3a, and S3b binding sites (Figure 2A). S1 is a hydrogen-bond donor site; S2, S3a, and S3b are hydrogen-bond acceptor sites. The glutamate conformer extracted by Apex-3D and fitting this model can be described as a g^-a extended conformation (Figure 1). For model B, the glutamate molecule is in a ag^+ conformation, corresponding to a folded form (Figure 1). This model is characterized by shorter d_1 and d_2 distances (4.7−4.9 and 3.3−3.4 Å, respectively) and by S1, S2, S3b, and S3c binding sites (Figure 2B). S1 to S3b are defined as for model A; S3c is a hydrogen-bond acceptor site. Yet in both models, S2 and S3 sites can also be defined as ionic interaction sites, since carboxylic groups bear a negative charge at neutral pH. The two

models were probed by superimposing the molecules of the validation sets (Table 1). However, these two models did not account for the higher activity of the quisqualate molecule, able to fit both models. After examination of the quisqualate conformations corresponding to A and B models, we noticed that the conformer extracted from model A could be also easily superimposed onto model B with its additional carbonyl group. This led us to propose a new pharmacophore model (model C) for mGluR1, combining A and B features and describing an additional anchor site for mGluR1 agonists (Figure 3). This model C is described by S1, S2, S3a, S3b, and S3c binding sites and accounts for the higher activity of the quisqualate compound (Figure 3). On this unique model, extended rigid agonists that accommodate only model A (**10**, **12**) and folded rigid agonists that accommodate only model B (**14**, **15**[47]) can be fitted together with extended and folded conformations of flexible agonists (**1**, **(+)-4**). Glutamic acid in an extended form is preferred as rigid extended ligands (**12** and carboxycyclopropylglycine **18**) display higher activities than folded ones (**14** and **15**, Table 1). Quisqualic acid (**2**) is the only molecule that fits simultaneously model A and model B and thus all binding sites of model C (Figure 3). Moreover, inactive molecules with d_1 and d_2 distances compatible with the pharmacophore model have been added to the superposition, with the aim to define zones of excluded volumes (Figures 2 and 4). Hydrophilic (S3c) and hydrophobic (S4 and foreground region of Figure 2) regions are also defined (Figures 2 and 4). The selective agonist (*S*)-3,5-dihydroxyphenylglycine (**33**) was not superposed to any model by the Apex program. Yet it could be manually fitted to sites S1 to S3a of the model (Figure 2). The antagonists **8**[48] and aminocyclopentanetricarboxylic acid **7**[49] that fit sites S1, S2, S3a, and S3b of the model were superposed to model C (Figure 4). They show that an additional volume in the S3c region might be responsible for the antagonist property. Other antagonists such as **16**[50] or carboxyphenylglycines[51,52] display longer distances d_1 and d_2 and were not superposed to the model.

mGluR2. For mGluR2, we ended with one pharmacophore model that can be described by the following distances between the functional groups: $d_1 = 4.6-5.4$ Å and $d_2 = 4.2-4.9$ Å (Figure 5). This model is fitted by a glutamate molecule in an *aa* extended conformation. The various glutamate analogues bearing a methyl group in positions 2, 3, and 4 and adopting this *aa* conformation were used to define the bulk tolerance regions around the glutamate protons positions (α, 3 or 4, *proS* or *proR*). The mGluR2 binding site can be described by three major anchor points: R1 is a hydrogen-bond donor site; R2 and R3 are hydrogen-bond acceptor sites. R2 and R3 can also be defined as ionic interaction sites as explained for S2 and S3 sites of mGluR1 model. Two sterically allowed regions are delineated by **18**, dicarboxycyclopropylglycine **19**,[53] and **12** in region R4 and by **(+)-24**, **(+)-5**, **(−)-4**, and LY354740 (**17**)[34] in the foreground region of Figure 5. This foreground region can be defined by the glutamate α- and 4-*proS*-proton region. The phenyl ring of (*S*)-4-carboxy-3-hydroxyphenylglycine (**34**) or (*S*)-4-carboxyphenylglycine (not shown) lies in this foreground region. However distances between the distal acidic group and proximal amino and acidic groups are larger than those of the model. Region R4 is limited by a forbidden region around the glutamate 3-*proS* position and defined by **10**, **11**, and **27**. A moderately tolerated to forbidden zone located around the glutamate 4-*proR* position (region R5) is disclosed by **(+)-25**, **13**, and **8**. When quisqualic acid (**2**) is superposed to the mGluR2 model in the conformation of the mGluR1 model A, binding sites R1 to R3 are accommodated; however the second carbonyl group is located in this restricted R5 region (Figure 5). Agonists of D configuration such as **(+)-4** or **(−)-24** show a good fit of their functional groups to the model, while their carbon chains lie in the background of Figure 5. The methyl group of **28** is located as well in this weakly tolerated background region.

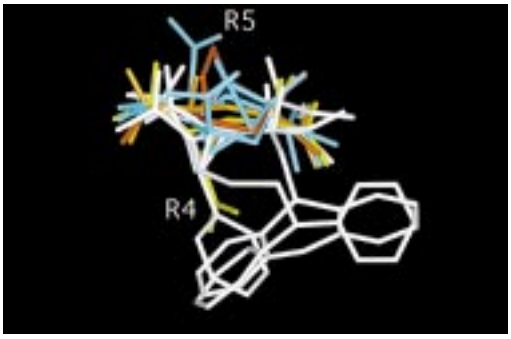


Figure 6. Superposition of mGluR2 antagonists (**20**, **23**, **31a** in white; **7**, **9**, **30** in blue) onto the pharmacophore model. Agonists **19** (yellow), **(+)-25** (orange), and **8** (red) are colored according to their activity as in Figure 5.

Finally amino and carboxylic groups of competitive antagonists such as α-methyl-2-(carboxycyclopropyl)-glycine (**22**), LY341495 (**23**),[54] LY307452 (**31b**),[55] 2-(2′carboxy-3′-phenylcyclopropyl)glycine (**20**), **7**, and γ-carboxyglutamic acid (**30**)[53] can be perfectly superposed to those of the extended *aa* conformation of glutamate. Indeed, among the conformations adopted by **23** and **31b**, one can be found that fits the binding sites of the model (R1 to R3) together with the aromatic group superposed to the phenyl group of **20** in region R4, as depicted in Figure 6. Among the **7** conformers, the one that best fulfills the mGluR2 model requirements displays the additional carboxylic group in the R5 region. Interestingly, a **27** conformer that locates its additional acidic group in the same R5 region can be found (Figure 6). These superpositions would thus reveal that specific groups positioned in the R4 (aromatic) or R5 (acidic) regions would enable binding but prevent complete activation of the receptor.

Discussion

mGluR1. For mGluR1, several interpretations for the coexistence of the two models A and B can be proposed. It can be speculated that glutamate binds to two different sites with a higher affinity for one of the two. Alternatively, these two sites may be combined in one that can accommodate either an extended or a folded conformation of Glu. Such a possibility may originate from the flexibility and mobility of the side chain of a basic residue of the receptor (such as an arginine) that interacts with the distal acidic group of glutamate. The extended form would then allow a better interaction.

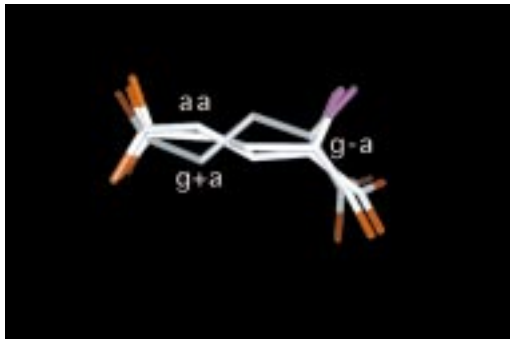


Figure 7. Superposition of the amino and carboxylic groups of the three extended glutamate conformations (*aa*, g^-a, g^+a). Oxygen atoms are colored in red, nitrogens in purple, and carbons in white.

However neither of these two situations would account for the increased affinity of quisqualic acid, the most potent mGluR1 agonist. Thus it can be proposed that folded conformations would interact at an additional site so that quisqualate would be linked to the recognition site by features (ionic interaction or hydrogen bond) of both models A and B. In such a model, functional groups of both open (^{1}E) and closed (E_1) conformations of **(−)-4**[29] can be superimposed onto quisqualic acid, taking advantage of the three distal binding sites of quisqualate. Sites S3a and S3b are accommodated by all good mGluR1 agonists (Table 1), sites S3b and S3c by folded ones. Finally a unique model C (Figure 3) combining features of model A (extended glutamate) and model B (folded glutamate) is proposed. It is characterized by distances d_1 and d_2 from model A and an additional binding site (S3c) from model B. These distances are in agreement with the good affinity of conformationally restricted extended ligands such as carboxycyclopropylglycines, aminobicyclohexanedicarboxylic acids, and aminobicycloheptanedicarboxylic acids mimicking *aa* or g^+a conformations. Yet a g^-a glutamate conformation is displayed by the Apex-3D model. Examination of the mGluR1 model shows that all three extended forms can fit their functional groups to this model (d_1, $d_2 > 4.5$ Å) (Figure 7). Thus no accurate choice can be established for glutamic acid and its linear analogues. Because of this ambiguity, precise allowed and forbidden volumes cannot be described using activities of methylglutamates as is the case for the mGluR2 model. However rigid analogues with minimal bulk that mimic extended forms of glutamic acid provide mapping probes for the binding site. Indeed functional groups of **18**, **12**[31] (conformation *aa*) and **21**,[56] **13** (conformation g^+a) can be superposed to model C (Figure 4), so that the lower affinity of the latter ones (Table 1) would be due to the location of a hydrophobic group at the hydrophilic site S3c defined in model C, rather than to the difference in glutamate conformations. On the contrary the analogous group in **18** or **12** is well-accepted by the receptor residues at the S4 site (Figure 4). Yet when this group becomes larger than one carbon atom as in **10**, **19**, or **20**, activity is drastically decreased showing that the S4 region (Figure 2) is sterically restricted. In agreement with Kozikowski[31] and Costantino,[57] the superposition of **17** to the model evidences an excluded volume located in the foreground region of Figure 2 and defined by the cyclic carbon chain of **17**. The methyl group of the most stable conformer (g^-a) of **29** lies in this forbidden region (Figure 2), possibly accounting for the lack of activity of this compound.

Yet model C does not account for the good affinity of **33** as superposition shows a good fit for only sites 1−3a as for **32** (Figure 2). Because the hydroxyl group in position 5 (in the background of Figure 2) does not fit with any hydrophilic sites of our model and because **33** is more active than **32**, a new stabilizing interaction through hydrogen bonds between the 5-hydroxyl and the receptor can be proposed. However to date no other mGluR1 ligand supports this hypothesis.

Finally the functional groups of **8** and **7** mGluR1 competitive antagonists can fit to the model (Figure 4) showing that region S3c is sterically sensitive for the complete activation of the receptor. On the other hand, the larger pharmacophoric distances (d_1, d_2) of **16** and 4-carboxyphenyl glycines such as **34** (not shown in Figure 2) are probably responsible for their antagonist nature, as described for NMDA competitive antagonists.[58] In the case of 4-carboxyphenylglycines, the bulk of the phenyl ring is obviously not the cause of this property, since **32** and **33** are endowed with similar phenyl bulk but shorter distances and display agonist activities.

Costantino and Pelliciari[35] have previously proposed a folded conformation for glutamate at the agonist binding site of mGluR1. These results were deduced from a 3D-model of the amino terminal domain of the mGluR1 receptor. They are not in contradiction with our hypothesis since the additional binding site S3c that we propose can accommodate folded ligands. However our pharmacophore model suggests that an extended form would bind to the receptor with higher affinity. New homology modeling studies of mGluR1 using our pharmacophore data will enable us to refine the 3D-model of the agonist binding site.

mGluR2. The present pharmacophore model of mGluR2 corresponds unambiguously to an extended *aa* conformation in accordance with previous results.[25,31−34,57] This statement is reinforced by the excellent superposition to the model of amino and carboxylic groups of agonists such as aminopyrrolidinedicarboxylic acid **6**,[59] **17**,[34] and **12**[31] which were not used in the training set. The mGluR2 model is characterized by the three anchor sites R1, R2, and R3, by the tolerated R4 and Figure 5 foreground regions and by the restricted R5 and Figure 5 background regions. The extended form is further confirmed by the agonist property of 4-carboxyphenylglycines such as **34** (Figure 5). Although the distal carboxylate appears to be away from the glutamate one, they could both interact with the same basic residue that possesses some flexibility or two basic sites such as an arginine that would accommodate larger d_1, d_2 distances. This is currently under investigation. The size of region R4 can be estimated by considering that **18** and **19** exhibit the same very good affinity and that **20**, where the additional carboxylate of **19** is replaced by a phenyl group, becomes a good antagonist as its epimer on the 3′ carbon (PCCG-12)[33] is inactive. This R4 region is also restricted on its right side of Figure 5 around the glutamate 3-*proS* position. The R5 region appears as a forbidden region. Thus the large decrease of affinity between **12** and **13** which both fit the R1, R2, and R3

binding sites could be explained by the location of the bridge carbon in either the R4 region (**12**) or R5 region (**13**). Yet as noted for the mGluR1 model, when this bridge becomes larger than one carbon atom as for **10**, it is no more accepted in region R4. It can be noted that when quisqualic acid (**2**) is superposed to the mGluR2 model in the conformation of the mGluR1 model, a carbonyl group of the quisqualic cycle lies in the R5 forbidden region and could be responsible for the lack of affinity of quisqualic acid. The background region of Figure 5 appears to be of modest tolerance as exemplified by D isomers and **28**. Opposite, a large tolerated hydrophobic zone is revealed in the foreground region of Figure 5 with **(+)-24**, **(+)-5**, **(−)-4**, **17**, and **34**. Indeed several α-substituted glutamic acid analogues exhibit good [**(+)-5**, **(−)-4**] to excellent (**17**) affinities for mGluR2. Yet the 2-methylglutamate **29** is inactive. When the functional groups of these compounds are superimposed, a critical restriction for the localization of the α-substituent is revealed. The 2-methylglutamate **29** in the *aa* conformation can be perfectly superposed to **17**; nevertheless in the g^-a conformation, which is predominant at pH 7,[44] the methyl group lies in a probably excluded region (data not shown). The lack of activity for the 2-methylglutamate **29** could be explained by the high energy barrier between the g^-a and *aa* conformations.[44] As a matter of fact, energy barriers range from 3 to 12 kcal·mol^{-1} for 3- and 4-methylglutamates and from 20 to 45 kcal·mol^{-1} for the 2-methyl analogue.[44] Yet we have no explanation for the antagonist property of **22** for which the α-methyl substituent is superposed to the α carbon of the agonist **17**. However when this α substituent is an ethyl chain bearing an aromatic group such as in **23**, antagonist affinities are increased up to 160-fold.[54] This affinity gain may be due to a hydrophobic interaction between aromatic groups of the ligand and the protein. A similar stabilization could account for the high affinities of **31a**, **31b**, N-substituted aminopyrrolidinedicarboxylic acid,[55,60,61] and **20**[33] as depicted in Figure 6. It is assumed that competitive antagonists bind to the same site as agonists (R1 to R3), and a slight difference can turn an agonist into an antagonist as shown by **8** and **9**, for example.[30] Moreover the antagonist property seems to be linked to the interaction of some specific substituents such as aromatic groups in the R4 region or acidic groups in the R5 region that would prevent complete activation of the receptor. The suggested features that would be responsible for antagonism are based on flexible molecules and need to be confirmed with additional molecules.

mGluR1/mGluR2 Comparison. Glutamic acid adopts an extended conformation at the two binding sites of mGluR1 and mGluR2. As a consequence, selectivity between the receptors is mostly due to sterical factors or results from additional selective interactions with particular ligands.

As shown in Figure 7 functional groups of all extended glutamate conformations can be superposed so that S1, S2, and S3 (a and b) sites of the mGluR1 model coincide with R1, R2, and R3 sites of the mGluR2 model (Figure 8). The R4 and foreground regions of the mGluR2 model display a large cavity which accommodates **19** and **17** agonists or the **20** antagonist. The bulk tolerance of mGluR1 is much more restricted, and these compounds

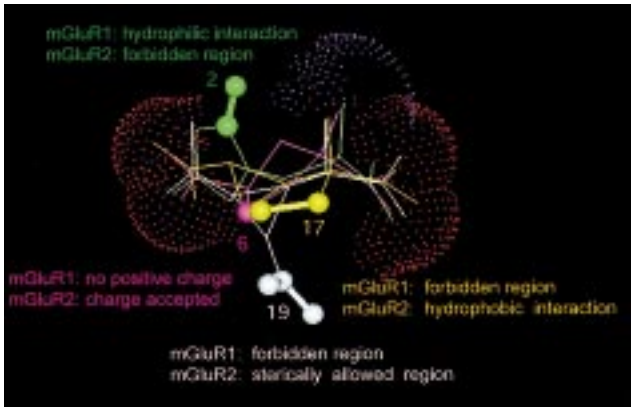


Figure 8. Comparison of mGluR1 and mGluR2 models by using selective compounds (**2**, **6**, **17**, **19**). The dot spheres (Connolly solvent-accessible surfaces) evidence the chemical features (α- and γ-COOH in red, NH_2 in blue) shared by the two receptors. The putative selective regions are shown with ball-and-stick representation.

are inactive (**20**) or weak antagonists (**17** and **19**). On the contrary, atoms of the quisqualic cycle would not be tolerated in the R5 region of the mGluR2 model and would be well-accepted at the S3c mGluR1 site. The selective mGluR2 agonist **6** adopts analogous conformations[59] and is an isostere of **(−)-4** which is an agonist of both mGluR1 and mGluR2; its selectivity could then be due to a negative electrostatic interaction at mGluR1 as was previously suggested.[59] A specific hydrophobic interaction of aryl groups of **23**, **31a**, and **20** antagonists at the mGluR2 binding site and a specific hydrophilic interaction of **33** at the mGluR1 binding site attest to other sites of selectivity which need to be confirmed. In contrast **7**, a common mGluR1/mGluR2 antagonist, may define an analogous interaction between the additional acidic group and the protein that would be responsible for the inactivation. Some phenylglycines (4-carboxyphenylglycine, 3-carboxy-4-hydroxyphenyl, and **34**) are agonists at mGluR2 and antagonists at mGluR1. This could be explained by larger tolerance of pharmacophoric distances in the mGluR2 model compared to the mGluR1 one.

Conclusion

In the present work, we have shown that glutamate activates both mGluR1 and mGluR2 receptors in an analogous extended conformation. However the pharmacophore models evidence selective features for each binding site. These data will be used for the docking of agonists in the LIVBP-like model of the mGluR binding sites[24] and should help in the identification of essential residues. They will also allow database searches to identify new potential ligands that would possess the structural requirements without containing the glutamate structure. In fine, the enclosed results should bring a major contribution to the rational design of new potent molecules with therapeutic effects.

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