Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1569–1572

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

New Probes of the Agonist Binding Site of Metabotropic Glutamate Receptors

Anne-Sophie Bessis,[a] Jean Bolte,[b] Jean-Philippe Pin[c] and Francine Acher[a,*]

[a]*Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR8601-CNRS, Université René Descartes, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France*

[b]*Laboratoire de Synthèse, Electrosynthèse et Etude de Systèmes d'Intérêt Biologique, UMR6504-CNRS, Université Blaise Pascal, 63177 Aubière Cedex, France*

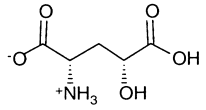
[c]*Centre INSERM-CNRS de Pharmacologie-Endocrinologie, UPR9023-CNRS, 141 rue de la Cardonille, 34094 Montpellier Cedex 5, France*

Received 18 December 2000; accepted 7 March 2001

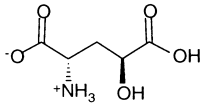
Abstract—The (2*S*,4*R*)- and (2*S*,4*S*)-4-hydroxyglutamates activate cloned $mGlu_{1a}$, $mGlu_2$, and $mGlu_{8a}$ receptors with different potencies. Best results were obtained with the (2*S*,4*S*) isomer being almost as potent as glutamate on $mGlu_{1a}R$ and $mGlu_{8a}R$. Data are interpreted on the basis of the binding site model and X-ray structure. © 2001 Elsevier Science Ltd. All rights reserved.

Glutamic acid (Glu) plays a major role in the mammalian central nervous system, as the neurotransmitter of most excitatory synapses. It is involved in many brain functions such as motor control and vision, as well as in learning, memory, and brain development. However glutamate is also the main endogenous neurotoxin and plays an important part in several neuropathologies and brain disorders.[1] Accordingly glutamate receptors are considered as potential therapeutic targets. Two main types of receptors have been characterized: the ionotropic (iGluR) and metabotropic receptors (mGluRs).[1,2] So far, the development of compounds acting on iGluRs has been hampered by the essential role of these receptors. Nevertheless, since metabotropic receptors have modulatory functions, they are perceived as promising new therapeutic targets.[3–6] Indeed the first mGluR agonist with nanomolar affinity, which was discovered recently by the Eli Lilly group, is now in clinical trial for anxiety.[6–8]

The eight cloned subtypes of mGluRs that have been identified so far are classified into three groups according to sequence similarity, transduction mechanism, and pharmacological profile.[3] Group-I receptors ($mGlu_{1,5}R$) stimulate PLC hydrolysis, while group-II ($mGlu_{2,3}R$) and -III ($mGlu_{4,6,7,8}R$) receptors inhibit adenylyl cyclase in heterologous expression systems. As previously detailed, potential therapeutic applications are mostly expected from group-I antagonists and group-II, -III agonists.[4] Most ligands are moderately potent with micromolar affinities in the same range as glutamate itself. Moreover, although group-selective agonists are known, potent subtype-selective ones are still lacking.[9] In the present letter, we wish to report on the effect of (2*S*,4*R*)-4-hydroxyglutamate **1** and (2*S*,4*S*)-isomer **2** assayed at cloned $mGlu_{1a}R$, $mGlu_2R$, and $mGlu_{8a}R$, representing the three groups mentioned above. These analogues serve as topological hydrophilic probes for the glutamate binding site around the distal acidic function. A similar investigation has been previously carried out on cloned excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2).[10] The new data will allow a refinement of the agonist pharmacophore models[11–13] and of receptor binding site models.[14] They might also be interpreted with the recently published crystal structure of the $mGlu_1R$ lingand-binding region (LBR) when the coordinates are available.[15] The final objective is to better define the elements that govern selectivity, these in turn might be useful for the discovery of new selective ligands.



(2*S*,4*R*)-4-OHGlu **1**



(2*S*,4*S*)-4-OHGlu **2**

*Corresponding author. Tel.: +33-142-86-3321; fax: +33-142-868387; e-mail: acher@biomedicale.univ-paris5.fr

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00158-5

Several syntheses of diastereomeric 4-hydroxyglutamic acids, which are naturally occurring, have been described.[16–22] In the present study, a diastereoisomeric mixture of 4-hydroxy-L-glutamic acids was obtained by enzymatic transamination of racemic 4-hydroxy-2-oxoglutaric acid catalysed by glutamic oxaloacetic transaminase as previously reported.[19] Separation of the isomers **1** and **2** was achieved by anion exchange chromatography,[16–19] diastereomeric ($\geq$99.6%) and enantiomeric (ee$\geq$99.3%) purity were checked by GC on derivatized samples.[23]

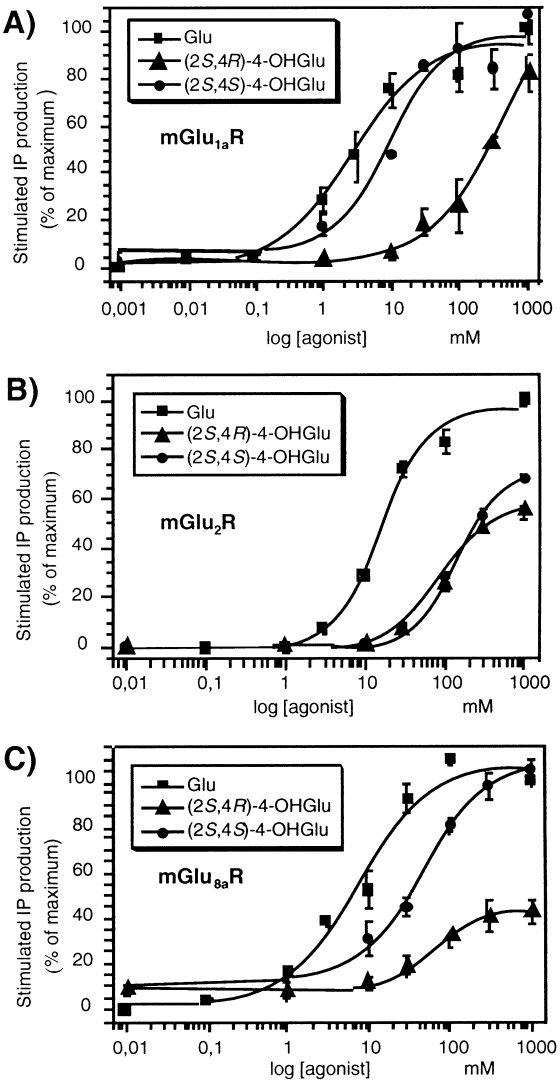


Figure 1. Agonist effect of glutamate, 4-hydroxyglutamate **1** and **2** on cells expressing $mGlu_{1a}$ (A), $mGlu_2$ (B) and $mGlu_{8a}$ (C) receptors. The total IP production was measured and expressed as the percentage of the maximal IP production induced by glutamate.[24,25] Curves were fitted as described in Table 1.

The effect of (2*S*,4*R*)-4-hydroxyglutamate **1** and (2*S*,4*S*)-**2** were examined on representative members of the three mGluR groups, rat $mGlu_{1a}R$, $mGlu_2R$ and $mGlu_{8a}R$, respectively, transiently expressed in HEK 293 cells as previously described.[24,25] Both compounds activated all three receptors in a dose-dependent manner but with different potencies (Fig. 1). On $mGlu_{1a}R$, **2** showed an EC_{50} value close to that of Glu and appears to be a full agonist, but **1** was much less potent with an EC_{50} around 400 μM (Fig. 1A, Table 1). Although the maximal effect of this drug could not be determined precisely due to its low affinity, curve fitting suggests **1** is a full agonist at this mGlu receptor type. On $mGlu_2R$, the two isomers behave similarly as partial agonist (60% of the maximal response measured with 1 mM Glu) with analogous potencies (Fig. 1B, Table 1). On $mGlu_{8a}R$, the maximal response obtained with **1** was about 60% of that obtained with Glu, indicating this compound is a partial agonist. In contrast, **2** behaved as a full agonist in our assay. Both compounds display a similar EC_{50} value on this receptor subtype (Fig. 1C, Table 1).

We have shown with pharmacophore models, that glutamic acid adopts an extended conformation on $mGlu_{1a}$, $mGlu_2$, $mGlu_{4a}$, and $mGlu_{8a}$ receptors and have deduced that ligand selectivity with respect to receptor groups and subtypes would result from a different environment at the protein binding site.[4,11–13] Indeed, our model of the $mGlu_{4a}R$ binding site as well as the crystal structure of the $mGlu_1R$ binding domain[15] together with a sequence alignment of all mGluR subtypes,[14] allow to identify several residues that are likely involved in ligand recognition selectivity (Fig. 2). Further information on the impact of these residues can be obtained from the functional analysis of the effect of glutamate analogues bearing hydrophobic[11,12] or hydrophilic[25,26] substituents. Models[14,27] and X-ray structure[15] reveal that the ligand binding domain folds in two lobes which delineate an interdomain cleft where glutamate binds. The closed bilobate structure trapping the agonist (Fig. 2) would be a critical step in the activation mechanism. Accordingly, residues from both lobes surrounding the ligand (glutamate analogue) were proposed to either bind to the functional groups of the glutamate moiety embedded in the ligand or to interact with its selective substituents. This is supported by mutagenesis experiments[27–29] and the recent structure of

Table 1. Activities and potencies of glutamate, 4-hydroxyglutamates **1** and **2** on rat $mGlu_{1a}$, $mGlu_2$, $mGlu_{8a}$ receptors expressed in HEK293 cells as described in refs 24 and 25. R_{max} indicates the maximal effect obtained with 1 mM of the drug expressed as percent of the maximal effect of glutamate. EC_{50} values (μM) and Hill coefficients (nH) were determined by fitting the dose–response curves to the equation $y=[(y_{max}-y_{min})/1+(x/EC_{50})^{nH}]+y_{min}$. Data are means±SEM of at least 3 independent experiments performed in triplicate

	Glutamate	(2*S*,4*R*)-4-OHGlu **1**			(2*S*,4*S*)-4-OHGlu **2**		
Receptor	EC_{50}	EC_{50}	nH	R_{max}	EC_{50}	nH	R_{max}
$mGlu_{1a}R$	3±1	373±118	1.06±0.13	70±6	11±2	0.88±0.19	95±6
$mGlu_2R$	16±2	161±21	1.00±0.02	52±8	137±10	1.48±0.23	60±9
$mGlu_{8a}R$	8±2	69±21	1.11±0.11	54±6	53±14	0.90±0.08	94±5

a glutamate bound to the binding domain of $mGlu_1R$.[15] It is now of interest to compare the effect of different glutamate analogues with the proposed binding sites of the three mGluRs representing each group.

$mGlu_{1a}R$. As (2*S*,3*S*,4*S*)-3,4-dihydroxyglutamic acid (DHGA),[26] (2*S*,4*S*)-4-hydroxyglutamic acid (**2** X = OH, Y = H, Fig. 2A, Table 1) is an $mGlu_{1a}R$ agonist with potency comparable to glutamate (Glu X = Y = H Fig. 2A, Table 1). Yet it is about 10-fold less potent than the (2*S*,4*S*)-4-methylglutamic acid (4-MeGlu X = CH_3, Y = H, Fig. 2A, EC_{50} = 0.9 μM).[11] In both cases the (2*S*,4*R*) isomer is a much weaker agonist (Table 1 and ref 11). These data tend to show that the glutamate 4-pro*R* substitution is sterically restricted and that a methyl or a hydroxyl substituent are accepted in the 4-pro*S* position. Since (2*S*,4*S*)-4-MeGlu is more active than its hydroxy analogue, we suggest that the methyl substituent interacts favorably with the aromatic moiety of Tyr74 while the hydroxyl of **2** is not optimally oriented to allow any stabilizing interaction (Fig. 2A). A larger group such as a carboxylate in γ-carboxyglutamate (Gla X = CO_2^-, Y = H, Fig. 2A) prevents from any binding[25] and apparently no attractive coulombic interaction takes place between this group and Arg323. We previously noted that the $mGlu_1R$ binding site is quite restricted;[11] we now suggest that it is due to the bulk of Tyr74 in the vicinity of the distal acidic function of glutamate (Fig. 2A).

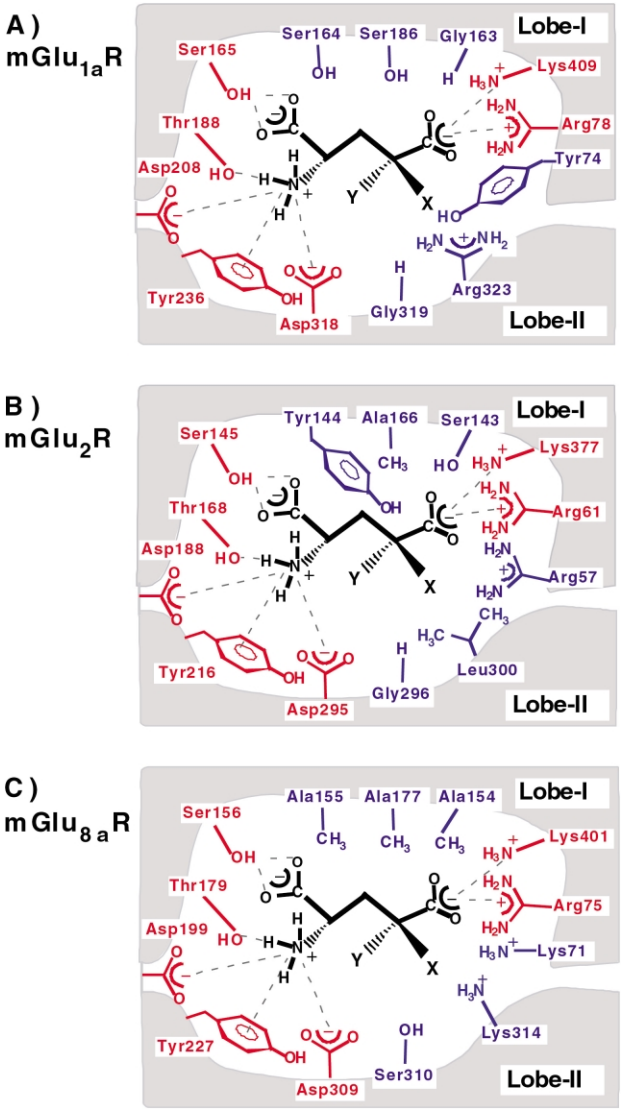


Figure 2. Schematic binding site of $mGlu_{1a}R$, $mGlu_2R$ and $mGlu_{8a}R$ derived from our previous model of $mGlu_{4a}R$ LBR[14] and the structure of the $mGlu_1R$ LBR.[15] Asp (208 for A, 188 for B, 199 for C) located in the hinge, is shown although it is quite distant (5.3 Å,[14] 5.0 Å[15]) from the amino group of glutamate. Interaction with Lys (409 for A, 377 for B, 401 for C) has been added according to the $mGlu_1R$ crystal structure.[15] Glutamate (X = Y = H) and its analogues (X, Y = H, OH, CH_3 or CO_2^-) have been positioned according to the $mGlu_{4a}R$ model[14] and to the $mGlu_1R$ crystal structure.[15] Conserved residues in red bind the amino and acidic functions of glutamate, group specific or subtype specific residues are shown in blue.

$mGlu_2R$. Both **1** and **2** are partial agonists with moderate potency at $mGlu_2R$, their affinity is about 10-fold lower than that of glutamate in the same assay (Table 1). We have shown that (2*S*,3*S*,4*S*)-3,4-DHGA does not bind to $mGlu_2R$, in contrast with (2*S*,4*S*)-4-MeGlu which is a more potent agonist than glutamate itself (EC_{50} = 3.5 μM).[11] Indeed the pharmacophore model showed the glutamate 4-pro*S* region (X region in Fig. 2B) to be hydrophobic.[11] We, thus, suggest that Tyr144 strongly contributes to hydrophobic interactions in this region all the more since Leu300 side chain appears to be too short to interact with the ligands (Fig. 2B).

$mGlu_{8a}R$. While **1** is a partial agonist at $mGlu_8R$ with an EC_{50} value of 69 μM, **2** is a full agonist with similar affinity (EC_{50} 53 μM). According to the pharmacophore model,[13] the hydroxyl function of **2** would lie in a hydrophilic region that could be defined by Lys71, Lys314 and Ser310 (Fig. 2C). Although this substituent is well accepted it does not allow additional binding of **2** to the receptor that would increase the activity of the agonist. On the other hand, such a favorable effect has been detected when the agonist bears an additional acidic function which has been shown to make polar interactions with the basic cluster of lysines.[14] However, if this acidic group adopts a different orientation, it can cause a repulsive interaction that prevents activation so that the ligand behaves as an antagonist, as it is the case with γ-carboxyglutamic acid.[13,25] Although a hydrophobic cluster of alanines is present on the first lobe of $mGlu_{8a}R$ (Fig. 2C), it seems that the basic cluster surrounding the distal carboxylate of glutamate is more critical for group selectivity. This result is in contrast with the preferred hydrophobic environment of $mGlu_2R$ binding site.

In conclusion, we have shown that (2*S*,4*S*)-4-hydroxyglutamate **2** is almost as potent as glutamate at $mGlu_{1a}R$ and $mGlu_{8a}R$. Moreover **2** and its (2*S*,4*R*)-isomer **1** can be used as hydrophilic probes of the mGluR binding sites. The pharmacological data are tentatively interpreted on the basis of a previous $mGlu_{4a}R$ model, the crystal structure of $mGlu_1R$ (coordinates not yet available) and the sequence alignment.

Acknowledgements

This work was supported by grants from the CNRS (PCV00-134 and 'Action Molécules et Cibles Thérapeutiques') and RETINA France. The authors wish to thank Parke-Davis Pharmaceutical Research (Ann Arbor, MI) for allowing a fulbright scholarship to A.-S. Bessis. We are also grateful to Dr J. Rossi (UMR6504) for his technical assistance in the preparation of hydroxyglutamates, to Drs. R. Azerad (UMR8601), M.-L. Parmentier (UPR9023) and L. Prézeau (UPR9023) for their advise and constructive discussions.

References

1. Nakanishi, S. *Science* **1992**, *258*, 597.
2. Hollmann, M.; Heinemann, S. *Annu. Rev. Neurosci.* **1994**, *17*, 31.
3. Conn, P. J.; Pin, J.-P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
4. Pin, J.-P.; De Colle, C.; Bessis, A.-S.; Acher, F. *Eur. J. Pharmacol.* **1999**, *375*, 277.
5. Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, B.; Madsen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 2609.
6. Monn, J. A.; Schoepp, D. D. *Annu. Rep. Med. Chem.* **2000**, *35*, 1.
7. Monn, J. A.; Valli, M. J.; Massey, S. M.; Wright, R. A.; Salhoff, C. R.; Johnson, B. G.; Howe, T.; Alt, C. A.; Rhodes, G. A.; Robey, R. L.; Griffey, K. R.; Tizzano, J. P.; Kallman, M. J.; Helton, D. R.; Schoepp, D. D. *J. Med. Chem.* **1997**, *40*, 528.
8. Helton, D. R.; Tizzano, J. P.; Monn, J. A.; Schoepp, D. D.; Kallman, M. J. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 651.
9. Schoepp, D. D.; Jane, D. E.; Monn, J. A. *Neuropharmacology* **1999**, *38*, 1431.
10. Vandenberg, R. J.; Mitrovic, A. D.; Chebib, M.; Balcar, V. J.; Johnston, G. A. R. *Mol. Pharmacol.* **1997**, *51*, 809.
11. Jullian, N.; Brabet, I.; Pin, J.-P.; Acher, F. *J. Med. Chem.* **1999**, *42*, 1546.
12. Bessis, A.-S.; Jullian, N.; Coudert, E.; Pin, J.-P.; Acher, F. *Neuropharmacology* **1999**, *38*, 1543.
13. De Colle, C.; Bessis, A.-S.; Bockaert, J.; Acher, F.; Pin, J.-P. *Eur. J. Pharmacol.* **2000**, *394*, 17.
14. Bessis, A.-S.; Bertrand, H.-O.; Galvez, T.; De Colle, C.; Pin, J.-P.; Acher, F. *Protein Sci.* **2000**, *9*, 2200.
15. Kunishima, N.; Shimada, Y.; Tsuji, Y.; Sato, T.; Yamamoto, M.; Kumasaka, T.; Nakanishi, S.; Jingami, H.; Morikawa, K. *Nature* **2000**, *407*, 971.
16. Benoiton, L.; Winitz, M.; Birnbaum, S. M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1957**, *79*, 6192.
17. Kristensen, E. P.; Larsen, L. M.; Olsen, O.; Sørensen, H. *Acta Chem. Scand.* **1980**, *B34*, 497.
18. Ducrocq, C.; Decottignies-Le Maréchal, P.; Azerad, R. *J. Labelled Comp. Radiopharm.* **1984**, *22*, 61.
19. Passerat, N.; Bolte, J. *Tetrahedron Lett.* **1987**, *28*, 1277.
20. Hanessian, S.; Vanasse, B. *Can. J. Chem.* **1993**, *71*, 1401.
21. Gefflaut, T.; Bauer, U.; Airola, K.; Koskinen, A. M. P. *Tetrahedron: Asymmetry* **1996**, *7*, 3099.
22. Heinz, L. J.; Lunn, W. H. W.; Murff, R. E.; Paschal, J. W.; Spangle, L. A. *J. Org. Chem.* **1996**, *61*, 4838.
23. Maurs, M.; Ducrocq, C.; Righini-Tapie, A.; Azerad, R. *J. Chromatogr.* **1985**, *25*, 444.
24. Gomeza, J.; Mary, S.; Brabet, I.; Parmentier, M.-L.; Restituito, S.; Bockaert, J.; Pin, J.-P. *Mol. Pharmacol.* **1996**, *50*, 923.
25. Brabet, I.; Parmentier, M.-L.; De Colle, C.; Bockaert, J.; Acher, F.; Pin, J.-P. *Neuropharmacology* **1998**, *37*, 1043.
26. Dauban, P.; de Saint-Fuscien, C.; Acher, F.; Prézeau, L.; Brabet, I.; Pin, J.-P.; Dodd, R. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 129.
27. O'Hara, P. J.; Sheppard, P. O.; Thogersen, H.; Venezia, D.; Haldeman, B. A.; Mc Grane, V.; Houamed, K. H.; Thomsen, C.; Gilbert, T. L.; Mulvihill, E. R. *Neuron* **1993**, *11*, 41.
28. Hampson, D. R.; Huang, X.-P.; Pekhletski, R.; Peltekova, V.; Hornby, G.; Thomsen, C.; Thøgersen, H. *J. Biol. Chem.* **1999**, *274*, 33488.
29. Jensen, A. A.; Sheppard, P. O.; O'Hara, P. J.; Krogsgaard-Larsen, P.; Bräuner-Osborne, H. *Eur. J. Pharmacol.* **2000**, *397*, 247.