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Pharmacological characterization of the rat metabotropic glutamate receptor type 8a revealed strong similarities and slight differences with the type 4a receptor

Cyril De Colle [a], Anne-Sophie Bessis [b], Joël Bockaert [a], Francine Acher [b], Jean-Philippe Pin [a,*]

[a] *Centre INSERM-CNRS de Pharmacologie-Endocrinologie, UPR 9023-CNRS, rue de la Cardonille, 34094 Montpellier Cedex 5, France*
[b] *Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601-CNRS, Université René Descartes-Paris V, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France*

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Abstract

In the brain, group-III metabotropic glutamate (mGlu) receptors $mGlu_4$, $mGlu_7$ and $mGlu_8$ receptors play a critical role in controlling the release process at many glutamatergic synapses. The pharmacological profile of $mGlu_4$ receptor has been studied extensively, allowing us to propose a pharmacophore model for this receptor subtype. Surprisingly, the activity of only a few compounds have been reported on $mGlu_7$ and $mGlu_8$ receptors. In order to identify new possibilities for the design of selective compounds able to discriminate between the members of the group-III mGlu receptors, we have undertaken a complete pharmacological characterization of $mGlu_8$ receptor and compared it with that of $mGlu_4$ receptor, using the same expression system, and the same read out. The activities of 32 different molecules revealed that these two mGlu receptors subtypes share a similar pharmacological profile. Only small differences were noticed in addition to that previously reported with *S*-carboxyglutamate (*S*-Gla) being a partial agonist at $mGlu_4$ receptor and a full antagonist at $mGlu_8$ receptor. These include: a slightly higher relative potency of the agonists 1*S*,3*R* and 1*S*,3*S*-aminocyclopentane-1,3-dicarboxylic acid (ACPD), *S*-4-carboxyphenylglycine (*S*-4CPG) and *S*-4-carboxy-3-hydroxyphenylglycine (*S*-4C3HPG), and a slightly higher potency of the antagonists 2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) and *RS*-α-methyl-4-phosphonophenylglycine (MPPG) on $mGlu_8$ receptor. When superimposed on the $mGlu_4$ receptor pharmacophore model, these molecules revealed three regions that may be different between the ligand binding sites of $mGlu_8$ and $mGlu_4$ receptors.

Keywords: mGlu8 receptor; mGlu4 receptor; Pharmacophore model; (Rat)

1. Introduction

Glutamate, the neurotransmitter of most fast excitatory synapses, activates two major families of receptors, the ionotropic and the metabotropic receptors (mGlu receptors). These later receptors, which are coupled to G-proteins, are often not directly involved in the fast synaptic transmission, but modulate the efficacy of glutamatergic synapses (Nakanishi, 1995; Conn and Pin, 1997). Therefore, these receptors constitute good targets for drugs modulating glutamate function in the brain. Eight mGlu receptors have been characterized and classified into three groups based on sequence homology, pharmacology and transduction mechanism (Nakanishi, 1992; Conn and Pin, 1997). Group-I includes $mGlu_1$ and $mGlu_5$ receptors and their splice variants (1a, 1b, 1c, 1d, 5a and 5b) that all activate phospholipase C. Both group-II ($mGlu_2$ and $mGlu_3$ receptors) and group-III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ receptors and their splice variants 4a, 4b, 7a, 7b, 8a and 8b) inhibit adenylyl cyclase when expressed in heterologous expression systems.

Our knowledge of the pharmacology of each mGlu receptors subtypes has expanded rapidly within the last few years (Roberts, 1995; Conn and Pin, 1997; Pin et al., 1999). Of interest, many potent and selective agonists and

* Corresponding author. CCIPE-CNRS-UPR9023, Laboratoire des Mécanismes Moléculaires des Communications Cellulaires, 141 rue de la Cardonille, F-34094 Montpellier Cedex 5, France. Tel.: +33-467-14-2933; fax: +33-467-54-24-32.

E-mail address: pin@ccipe.montp.inserm.fr (J.-P. Pin).

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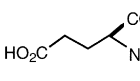
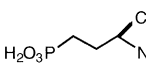
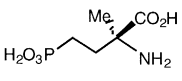
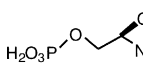
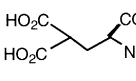
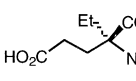
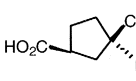
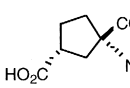
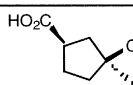
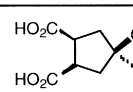
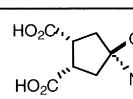
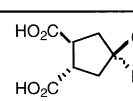
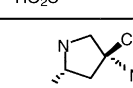
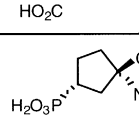
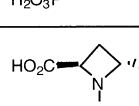
S-Glutamate		+++	+++	+++
S-AP4*		0	0	++++
S-MAP4		0	-	--
S-SOP		0	--	++++
S-Gla		0	--	± ±
S-E-Glu		?	--	-
1*S*,3*S*-ACPD		+	+++	+
1*S*,3*R*-ACPD		+++	+++	+
1*R*,3*R*-ACPD		0	0	0
ACPT-I		0	0	+++
ACPT-II		--	--	--
3*S*, 4*S*-ACPT		-	-	+++
2*R*,4*R*-APDC		0	++++	0
2*S*,4*S*-CPeAP4 *		?	?	++
2*R*,4*R*-ADA		++	0	0

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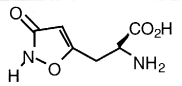
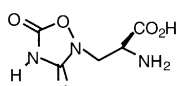
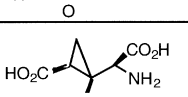
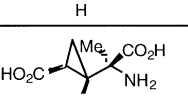
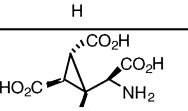
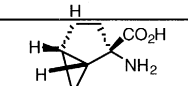
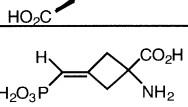
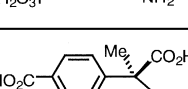
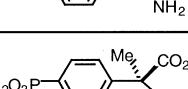
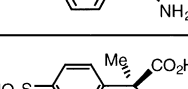
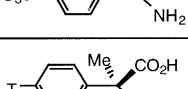
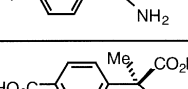
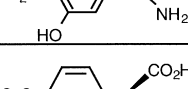
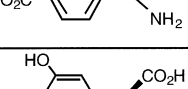
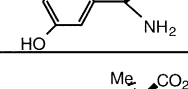
S-Ibotenate		+++	++	+
S-Quisqualate		++++++	0	+
2*S*-CCG-I		+++	++++	+++
2*S*-MCCG-I		0	---	0
2*S*-DCG-IV		-	++++	---
LY354740		-	+++++	--
CBAP5		?	?	+++
S-MCPG *		--	--	0
S-MPPG *		0	--	--
S-MSPG *		0	--	0
S-MTPG *		-	-	0
S-4C3HPG		--	+++	0
S-4CPG		--	+	0
S-3,5-DHPG *		++	0	0
S-MSOPPE *		?	?	?

Fig. 1. Structure and activity at $mGlu_1$ (1), $mGlu_2$ (2) and $mGlu_4$ (4) receptors of the compounds used in this study. The acronymes are defined in the text. * indicates that, although one stereoisomer is shown, the activity of the racemic mixture has been examined. The absence of activity at 1 mM is indicated by 0, the agonist activity is indicated by +, the antagonist activity by − and the partial agonist activity by ±. The number of symbols is directly function of the reported potency of the compound (one symbol per order of magnitude, starting by at 1 mM). In the structure of MTPG, T means tetrazolyl. The potencies were taken from (Conn and Pin, 1997; Pin et al., 1999).

antagonists have been characterized for group-I and group-II receptors (Jane et al., 1995; Annoura et al., 1996; Pellicciari et al., 1996; Thomsen et al., 1996; Schoepp et al., 1997; Kingston et al., 1998; Ornstein et al., 1998a,b; Gasparini et al., 1999b; Litschig et al., 1999; Varney et al., 1999) (see Fig. 1). However, though the pharmacological profile of $mGlu_4$ receptor has been extensively studied (Johansen et al., 1995; Conn and Pin, 1997), the activities of very few compounds have been examined on the other group-III mGlu receptors. These receptors play, however, very important roles in the central nervous system, and many possible applications are expected from selective group-III mGlu receptors ligands. Among these receptors, $mGlu_6$ receptor is responsible for the on-bipolar response in the retina (Nakanishi, 1995). All other group-III receptors are mostly localized on glutamatergic nerve terminals acting as autoreceptors. Accordingly, activation of group-III mGlu receptors has been shown to be neuroprotective in many experimental systems (Bruno et al., 1995, 1996; Gasparini et al., 1999a; Lafon-Cazal et al., 1999). The $mGlu_4$ receptors are involved in motor performance and spatial learning as shown by studies performed with $mGlu_4$ receptor knock-out mice (Pekhletski et al., 1996; Gerlai et al., 1998). These $mGlu_4$ receptors have also been proposed to play a role in the detection of glutamate taste (umami), as revealed by their expression in vallate and foliate taste buds (Chaudhari et al., 1996). The $mGlu_7$ receptors are involved in taste aversion and in epilepsy as revealed by the phenotype of the $mGlu_7$ receptor knock-out mice (Masugi et al., 1999). They are also likely to play a role in the nociceptive transmission due to their localization in the terminals of c-fibers (Ohishi et al., 1995). Less is known about the putative roles of $mGlu_8$ receptor, which has been described in olfactory bulb, pontine gray, lateral reticular nucleus of the thalamus, piriform cortex and retina (Duvoisin et al., 1995; Saugstad et al., 1997; Koulen et al., 1999).

It has been proposed that the pharmacological profile of $mGlu_8$ receptor may be intermediate between that of the group-II $mGlu_2$ receptor and that of the group-III $mGlu_4$ receptor (Saugstad et al., 1997; Wu et al., 1998). Such an observation could be of great help to identify possible residues in the glutamate binding pocket of mGlu receptors responsible for their specific pharmacological profiles. Such information may then be useful for the design of potent and selective ligands for each mGlu receptors subtypes.

The aim of this study was to carefully examine the pharmacological profile of the rat $mGlu_8$ receptor and to compare it with that of the other group-III receptor $mGlu_4$ receptor, and of the group-II receptor $mGlu_2$ receptor. To that aim, the activity of several group-II and group-III ligands were examined on these three receptor subtypes using the same assay. Among the 32 compounds tested (either agonists, antagonists or inactive), most display a similar potency on $mGlu_8$ receptor and $mGlu_4$ receptor. Small differences reveal, however, possibilities to develop new selective compounds.

2. Materials and methods

2.1. Materials

S-glutamate, *S*-4-carboxyglutamate (*S*-Gla), *RS*-2-amino-4-phosphonobutanoate (AP4) and most other chemicals were obtained from Sigma (Lisle d'abeau, France) unless otherwise specified. (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl) glycine (2*S*-CCG-I), (2*S*,2′*R*,3′*R*)-2-(2,3-dicarboxy-cyclopropyl)glycine (DCG-IV), *RS*-3,5-dihydroxyphenylglycine (3,5-DHPG), 1-amino-3-(phosphonomethylene) cyclobutane carboxylic acid (CBAP5), 2*SR*,4*SR*-cyclopentyl-AP4 (2*SR*,4*SR*-CPeAP4), *S*-4-carboxyphenylglycine (*S*-4CPG), *S*-4-carboxy-3-hydroxyphenylglycine (*S*-4C3HPG), *S*-ibotenate, *S*-Quisqualate, *S*-serine-*O*-phosphate (*S*-SOP), (*S*)-2-ethylglutamate (*S*-E-Glu), *S*-2-methyl-AP4 (*S*-MAP4), 2*S*-2-methyl-CCG-I (MCCG-I), *RS*-2-methyl-4-phosphonophenylglycine (MPPG), *RS*-2-methyl-4-sulfonophenylglycine (MSPG), *RS*-2-methyl-4-tetrazolylphenylglycine (MTPG) and *RS*-2-methylserine-*O*-phosphate monophenylphosphorylester (MSOPPE) were from Tocris Cookson (Essex, England). The *RS*-2-methyl-4-carboxyphenylglycine (MCPG), aminobicyclo [2.2.1.] heptane dicarboxylate (ABHD) and 1-amino-1,3,4-cyclopentane tricarboxylate (ACPT) isomers were synthesized as previously described (Coudert et al., 1996; Acher et al., 1997; Tellier et al., 1998). The nomenclature of the different stereoisomers of ACPT was according to Acher et al. (1997): 1*S*,3*R*,4*S*-ACPT (ACPT-I), 1*R*,3*R*,4*S*-ACPT (ACPT-II), 3*SR*,4*SR*-ACPT (ACPT-III). The isomers of 1-amino-1,3-cyclopentane dicarboxylate (ACPD) were a kind gift from Dr. Kenneth Curry (Vancouver, Canada). 2*R*,4*R*-azetidine-2,4-dicarboxylate (2*R*,4*R*-ADA or CGP61920) were kindly provided by Novartis Pharma (Basel, Switzerland). 2-Aminobicyclo [3.1.0.] hexane-2,6-dicarboxylate (LY354740, Lot. 346SB5) and 2*R*,4*R*-4-aminopyrrolidin-2,4-dicarboxylate (2*R*,4*R*-APDC, LY314593, Lot. 115C) were gifts from the Eli Lilly (Indianapolis, USA). The construction of the plasmids expressing $mGlu_{1a}$, $mGlu_2$ and $mGlu_4$ receptors has been described previously (Joly et al., 1995; Gomeza et al., 1996). The *Xho*I and *Xba*I fragment of the $pmGR_8$ ($mGlu_8$ receptor cDNA in the pBluescript vector) containing the entire coding sequence of the rat $mGlu_8$ receptor clone (gift of Dr. J. Saugstad) was subcloned in the eucaryotic expression vector carrying a CMV promoter pRK7.

2.2. Culture and transfection of human embryonic kidney 293 (HEK 293) cells

HEK 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, France) supplemented with 10% fetal calf serum and transfected by

electroporation as previously described (Gomeza et al., 1996). Electroporation was carried out in a total volume of 300 μl with 10 μg carrier DNA, plasmid DNA containing $mGlu_2$ receptor (2 μg), $mGlu_4$ receptor (5 μg), or $mGlu_8$ receptor (5 μg) and 10 million cells. To allow $mGlu_2$, $mGlu_{4a}$ and $mGlu_{8a}$ receptors to activate phospholipase C, these receptors were co-expressed with the chimeric G-protein Gq_{i9} as previously described (Gomeza et al., 1996).

2.3. Determination of inositol phosphates accumulation

Determination of inositol phosphate accumulation in transfected HEK 293 cells was performed after labelling the cells overnight with [^{3}H] myo-inositol (23.4 Ci/mol, NEN, France). The stimulation was conducted for 30 min in a medium containing 10 mM lithium chloride (LiCl) and the indicated concentration of agonist. The basal inositol phosphate formation was determined after 30-min incubation in the presence of 10 mM LiCl and the glutamate pyruvate transaminase (glutamate-degrading enzyme, 1 U/ml) and 2 mM pyruvate to avoid the possible action of glutamate released from cells. Results are expressed as the amount of inositol phosphate produced over the radioactivity contained in the membranes.

2.4. Data analysis

The dose–response curves were fitted using the equation $y = [(y_{max} - y_{min})/1 + (x/EC_{50})^n] + y_{min}$ and the Kaleidagraph program (Synergy Software, USA). The possible correlation between the potencies of the compounds tested on different receptors was assayed using the program JMP (SAS Institute, Cary, USA).

2.5. Molecular modeling

Molecular modeling experiments were performed as previously described (Bessis et al., 1999).

Table 1
Activities and potencies of several S-glutamate analogs on the rat $mGlu_8$ receptor co-expressed in HEK293 cells with the chimeric Gq_{i9} protein, and comparison with the data obtained with $mGlu_4$ receptor
The maximal effect obtained with 1 mM of the drug (percent of the *S*-glutamate effect, or percent inhibition) are indicated. The EC_{50} and IC_{50} values and the Hill coefficients were determined as described in the text and are means ± S.E.M. of at least three independent experiments performed in triplicates. Each dose–response curves consisted of at least seven different concentrations of the drug (see Figs. 2 and 3 for typical dose–response curves).

Agonists	mGlu8 receptor			mGlu4 receptor		
	Percent of Glu effect	EC_{50}	nH	Percent of Glu effect	EC_{50}	nH
S-glutamate	100	9.5 ± 2.5[a]	1.27 ± 0.14	100	16.4 ± 2.0[b]	1.96 ± 0.17
1*S*,3*S*-ACPD	85.3 ± 11.3	243 ± 39	1.93 ± 0.63	48.1 ± 3.8	1000	–
1*S*,3*R*-ACPD	97.7 ± 3.5	163 ± 12	2.20 ± 0.20	25.8 ± 4.6	> 1000[b]	–
1*R*,3*S*-ACPD	49.2 ± 11.6	1000	–	23.8 ± 6.1	> 1000[b]	–
ACPT-I	66.9 ± 23.1	8.2 ± 4.9	1.53 ± 0.28	85.6 ± 12.9	7.2 ± 2.3[c]	1.28 ± 0.14
ACPT-III	75.1 ± 2.0	11.6 ± 3.2	1.13 ± 0.10	78.5 ± 14.5	47.5 ± 8.4[c]	1.03 ± 0.21
(3*S*,4*S*) ACPT	69,6	7.0	1.4	89.3 ± 10.2	8.8 ± 3.2[c]	1.72 ± 0.13
AP4	106.9 ± 21.0	0.56 ± 0.07[d]	1.38 ± 0.10	98.5 ± 8.7	0.46 ± 0.26[b]	1.10 ± 0.04
2*R*,4*R*-APDC	16.3 ± 0.9	–	–	*inactive*	–	–
CBAP5	96.3 ± 2.2	14.8 ± 4.6	1.35 ± 0.04	85.9 ± 9.2	33 ± 15	0.98 ± 0.19
2*S*-CCG-I	110.1 ± 9.01	2.77 ± 1.08[a]	1.32 ± 0.40	109 ± 9	8.9 ± 3.3[b]	1.42 ± 0.06
2*SR*,4*SR*-CPeAP4	75.6 ± 1.2	62.7 ± 2.72	1.65 ± 0.02	77.5 ± 2.5	136 ± 66	1.33 ± 0.49
S-4C3HPG	47.0 ± 2.9	126 ± 19	1.62 ± 0.47	*Inactive*	–	–
S-4CPG	39.2 ± 1.85	35.3 ± 5.5	1.73 ± 0.27	*Inactive*	–	–
S-ibotenate	67.4 ± 2.6	311 ± 49	1.10 ± 0.17	62.1 ± 5.6	406 ± 105	1.14 ± 0.15
S-SOP	77.1 ± 12.3	1.55 ± 0.28[d]	1.21 ± 0.21	102 ± 11	2.3 ± 1.5[b]	1.42 ± 0.32
Antagonists	**Percent inhibition**	**IC_{50}**	**nH**	**Percent inhibition**	**IC_{50}**	**nH**
ACPT-II	95.0 ± 7.1	123 ± 18	0.93	95.9 ± 3.4	125[c]	1.03
2*S*-DCG-IV	112.0 ± 8.7	32.4 ± 17.5[a]	1.21 ± 0.12	95.1 ± 6.7	22.5 ± 6.8[a]	2.3 ± 0.6
S-E-Glu	68.0 ± 3.0	689 ± 89	1.17 ± 0.05	37.5 ± 7.5	> 1000	–
S-Gla	81.3 ± 3.8	298 ± 81[b]	0.92 ± 0.04	Partial agonist	135 ± 26[a]	1.66 ± 0.30
LY354740	82.5 ± 3.4	293 ± 128	0.88 ± 0.14	62.9 ≠ 10.2	1009 ± 164	–
S-MAP4	97.7 ± 2.0	105 ± 10	0.85 ± 0.13	98.1 ± 2.5	88.1 ± 10.7[b]	1.74 ± 0.35
2*S*-MCCG-I	44.0 ± 2.5	> 1000	–	16.5 ± 2.4	> 1000	–
MCPG	8.0 ± 5.0	–	–	*Inactive*	–[a]	–
MPPG	106 ± 2	20.0 ± 1.7	1.14 ± 0.10	99.9 ± 1.7	54.6 ± 14.1[b]	1.22 ± 0.25
MSPG	75.5 ± 2.9	476 ± 97	1.27 ± 0.19	30.0 ± 3.6	> 1000[a]	–
MTPG	67.3 ± 2.5	558 ± 78	1.00 ± 0.16	37.4 ± 12.0	> 1000[a]	–

[a] Values were taken from Brabet et al. (1998).
[b] Values were taken from Gomeza et al. (1996).
[c] Values were taken from Acher et al. (1997).
[d] Values were taken from Corti et al. (1998).

3. Results

The action of several compounds known for their agonist or antagonist activity at mGlu receptors subtypes (Fig. 1) was examined on HEK 293 cells transiently expressing the rat $mGlu_8$ receptor isoform. We previously reported that the activation of group-II or group-III mGlu receptors was difficult to assess by measuring the inhibition of adenylyl cyclase activity in a transient transfection assay. We, therefore, examined the activation of $mGlu_8$ receptor via the stimulation of phospholipase C activity mediated by the chimeric G-protein Gq_{i9} (Conklin and Bourne, 1993; Conklin et al., 1993; Gomeza et al., 1996; Blahos et al., 1998; Parmentier et al., 1998; Milligan and Rees, 1999). This G-protein α subunit corresponds to Gαq in which the last nine carboxyl-terminal residues have been replaced by those of $G\alpha i_2$. We previously reported that the pharmacological profiles of $mGlu_2$ and $mGlu_4$ receptors determined using this functional assay were identical to those reported using the more physiological measurement of the inhibition of adenylyl cyclase (Gomeza et al., 1996).

The rank order of potency of agonists at the $mGlu_8$ receptor was: AP4 > *S*-SOP > 2*S*-CCG-I > 3*S*,4*S*-ACPT > *S*-glutamate > ACPT-I = CBAP5 > 2*SR*,4*SR*-CPeAP4 > 1*S*,3*R*-ACPD > 1*S*,3*S*-ACPD > *S*-ibotenate > 1*R*,3*S*-ACPD (Table 1, Fig. 2). Most of these ligands maximally stimulated inositol phosphate formation to levels similar to those obtained with *S*-glutamate or the other potent group-III agonists AP4 or *S*-SOP (Table 1, Fig. 2). *S*-Quisqualate, *S*-4CPG and *S*-4C3HPG were also found to stimulate inositol phosphate formation in cells expressing $mGlu_8$ receptor and $G\alpha q_{i9}$. The maximal effects obtained with these compounds were, however, lower than that obtained with S-glutamate (Table 1, Fig. 3a). Surprisingly, such an effect of *S*-Quisqualate on $mGlu_8$ receptor was not observed in other expression systems (Saugstad et al., 1997; Wu et al., 1998). We, therefore, examined whether or not the effect of *S*-Quisqualate and other partial agonists could be the consequence of the release of glutamate from the HEK293 cells, rather than a direct action on $mGlu_8$ receptor. In the presence of glutamate–pyruvate transaminase (glutamate-degrading enzyme) and 2 mM pyruvate, *S*-Quisqualate no longer stimulated phospholipase C in cells expressing $mGlu_8$ receptor and Gq_{i9}, but the response generated by *S*-4CPG and *S*-4C3HPG remained the same (Fig. 3b). This indicates that the agonist-like effect of *S*-Quisqualate is indirect, resulting from an increase in the extracellular glutamate concentration in the extracellular medium. However, *S*-Quisqualate had no effect within this range of concentration on $mGlu_4$ receptor-expressing cells (estimated EC_{50} around 1 mM). This could be explained

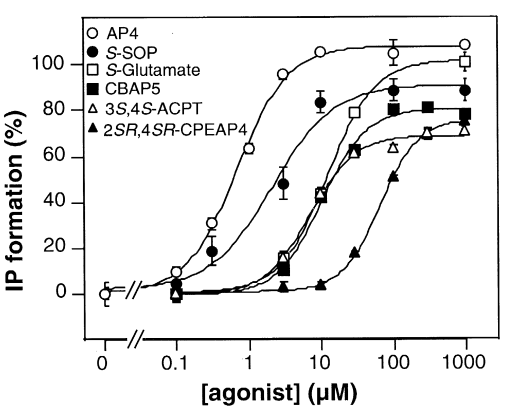


Fig. 2. Typical agonists dose–response curves obtained by measuring inositol phosphate formation in HEK293 cells transiently expressing $mGlu_8$ receptor and Gq_{i9}. The effect of various concentrations of AP4 (open circles), *S*-SOP (closed circles), *S*-glutamate (open squares), CBAP5 (closed squares), 3*S*,4*S*-ACPT (open triangles) and 2*SR*,4*SR*-CPeAP4 (closed triangles) are shown. Data are expressed as percentage of the inositol phosphate formation induced by 1 mM *S*-glutamate in the same cells and are means ± S.E.M. of triplicate determinations from a typical experiment.

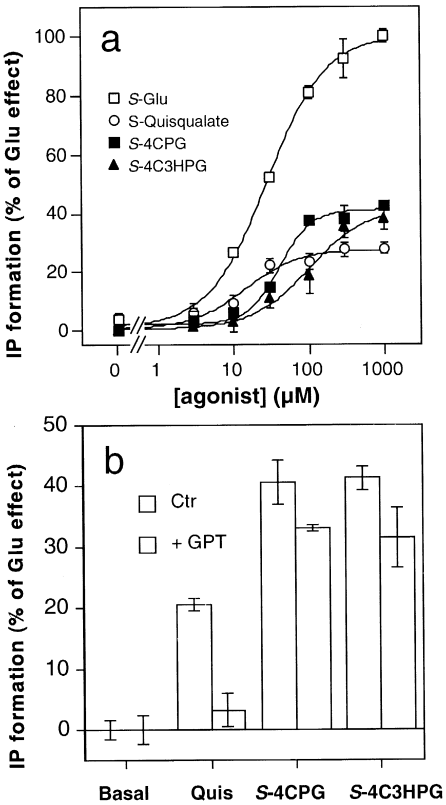


Fig. 3. The apparent partial agonist activity of *S*-Quisqualate on $mGlu_8$ receptor, but not that of *S*-4CPG and *S*-4C3HPG, results from the endogenous glutamate released from the cells rather than from a direct action of *S*-Quisqualate on $mGlu_8$ receptor. (a) Typical dose–response curves obtained with various concentration of *S*-glutamate (open squares), *S*-Quisqualate (open circles), *S*-4CPG (closed squares) and *S*-4C3HPG (closed triangles). (b) The inositol phosphate formation induced by *S*-Quisqualate (300 μM), *S*-4CPG (1 mM) ad *S*-4C3HPG (1 mM) were measured in the absence (clear bars) or presence (dark bars) of the glutamate-pyruvate transaminase (glutamate degrading enzyme) and 2 mM pyruvate. Data are expressed as percentage of the inositol phosphate formation induced by 1 mM *S*-glutamate in the same cells and are means ± S.E.M. of triplicate determinations from a typical experiment.

by the twofold higher potency of glutamate on $mGlu_8$ receptor (Table 1) such that a small increase in the extracellular glutamate concentration can activate $mGlu_8$ receptor without significantly activating $mGlu_4$ receptor.

The rank order of potency of antagonists at $mGlu_8$ receptor was: DCG-IV = MPPG > *S*-MAP4 > ACPT-II > *S*-Gla = LY354740 > MSPG = MTPG = *S*-E-Glu (Table 1 and Fig. 4). In our expression system, MCPG inhibited the glutamate effect on $mGlu_8$ receptor only at concentrations higher than 1mM (estimated IC_{50} of 3 mM), and cannot, therefore, be considered as an active $mGlu_8$ receptor antagonist in our assay.

Other compounds, 1*R*,3*R*-ACPD, 2*R*,4*R*-ADA, 3,5-DHPG and MSOPPE, were found to have neither agonist nor antagonist properties at either $mGlu_{4a}$ or $mGlu_{8a}$ receptors.

The potencies of the compounds tested on $mGlu_8$ receptor in this study were compared to those found with the same functional assay with $mGlu_{4a}$ (Table 1), $mGlu_2$ and $mGlu_{1a}$ receptors (Gomeza et al., 1996; Brabet et al., 1998; Parmentier et al., 1998; Parmentier et al., 2000). A highly significant correlation ($r = 0.852$; $p < 0.0001$) was found when the agonists' potencies at $mGlu_4$ receptor were compared to those at $mGlu_8$ receptor (Fig. 5a). The same was true when the potencies of the antagonists are compared ($r = 0.861$; $p < 0.003$). However, no correlation could be found when the potencies of these compounds at $mGlu_8$ receptor are compared to those determined at $mGlu_2$ receptor (Fig. 5b) ($r = -0.365$; $p < 0.24$ for the agonist potencies; and $r = 0.231$; $p < 0.58$ for the antagonist potencies) or $mGlu_{1a}$ receptor ($r = 0.07$; $p < 0.75$).

Some agonists and antagonists have a better potency on $mGlu_8$ receptor than $mGlu_4$ receptor. These include 1*S*,3*S*-ACPD, 1*S*,3*R*-ACPD, *S*-4CPG and *S*-4C3HPG for the agonists, and LY354740 for the antagonists.

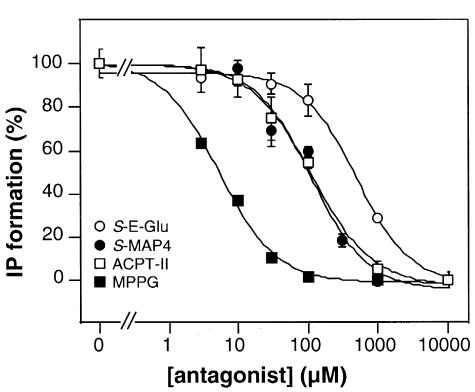


Fig. 4. Typical antagonists dose–response curves obtained by inositol phosphate formation in HEK293 cells transiently expressing $mGlu_8$ receptor and Gq_{i9}, and stimulated with 20 μM *S*-glutamate. The effect of various concentrations of MPPG (closed squares), *S*-MAP4 (closed circles), ACPT-II (open squares) and *S*-E-Glu (open circles) are shown. Data are expressed as percentage of the inositol phosphate formation induced by 20 μM *S*-glutamate in the same cells and are means ± S.E.M. of triplicate determinations from a typical experiment.

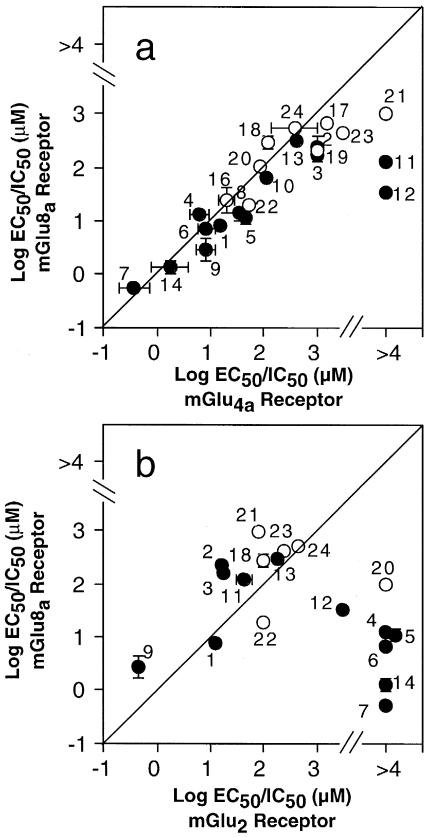


Fig. 5. Correlation between the potencies of several agonists (closed circles) and antagonists (open circles) at $mGlu_8$ receptor with those determined at $mGlu_4$ receptor (a) or $mGlu_2$ receptor (b). The EC_{50} and IC_{50} values were determined as described in the text using the same assay. The points represent the values determined for: (1) *S*-glutamate; (2) 1*S*,3*S*-ACPD; (3) 1*S*,3*R*-ACPD; (4) ACPT-I; (5) ACPT-III; (6) 3*S*,4*S*-ACPT; (7) AP4; (8) CBAP5; (9) 2*S*-CCG-I; (10) 2*SR*,4*SR*-CPeAP4; (11) *S*-4C3HPG; (12) *S*-4CPG; (13) *S*-Ibotenate; (14) *S*-SOP; (16) DCG-IV; (17) *S*-E-Glu; (18) *S*-Gla; (19) LY354740; (20) *S*-MAP4; (21) MCCG-I; (22) MPPG; (23) MSPG; (24) MTPG. The lines in (a) and (b) correspond to the equation $y = x$. The correlation line in (a) is not shown for clarity, its equation is $y = 0.63x + 0.35$, $r = 0.85$ if a value of 4 is taken for the log(EC_{50}) for *S*-4CPG (12) and *S*-4C3HPG (11) on $mGlu_4$ receptor; and $y = 0.80x + 0.12$, $r = 0.95$ if *S*-4CPG (12) and *S*-4C3HPG (11) are excluded.

The great similarity between $mGlu_{8a}$ and $mGlu_{4a}$ receptors pharmacology allows the construction of a similar pharmacophore model. The $mGlu_8$ receptor model (Fig. 6) is, thus, analogous to that of $mGlu_4$ receptor, which has been recently described (Bessis et al., 1999). *S*-glutamate adopts an extended amino acid conformation (see definition in Bessis et al., 1999) and would bind to the receptor S1, S2a, S2b, S3a and S3b sites by means of its amino and carboxylic functions. Phosphonic or phosphoric analogues, such as *S*-AP4, reveal an additional S3c binding site, while ACPT-I and 3*S*,4*S*-ACPT disclose an S4 site. Coulombic interactions or hydrogen bonds anchor the ligands to the S1 to S4 sites. The alkyl rings of 2*SR*,4*SR*-CPeAP4, ACPD, LY354740 are located in the R5 region which is perpendicular to the R6 region where the cyclopropyl ring

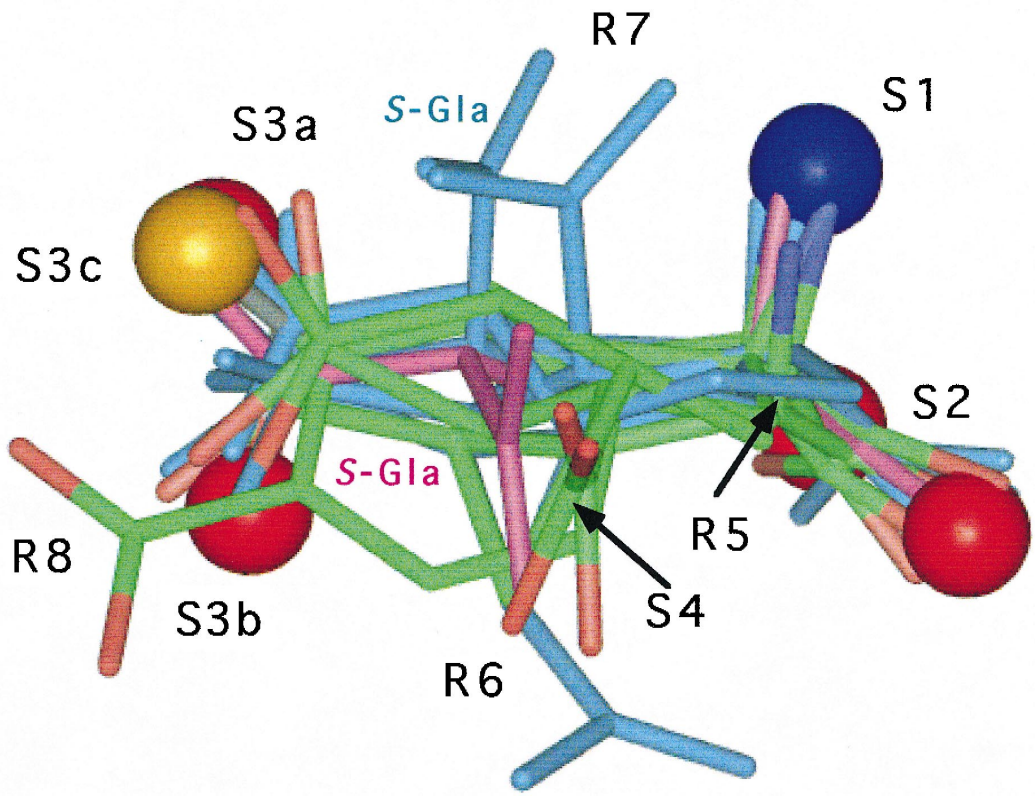


Fig. 6. Superposition of the possible bioactive conformation of several group-III mGlu receptors agonists (carbons in green, oxygens in red and nitrogens in dark blue) and antagonists (in blue) according to the agonist pharmacophore model of $mGlu_4$ receptor. The S1 (blue sphere) S2 (red spheres) and S3 (red and orange spheres) sites are indicated. The agonists shown are: S-4CPG (more potent on mGlu8 receptor than mGlu4 receptor) with its distal carboxylic group in the R8 region, 2*S*-CCG-I, *S*-AP4; ACPT-I and 3*S*,4*S*-ACTP with their additional carboxylic group in the S4 region. The antagonists shown are: ACPT-II with its additional carboxylic group in the region R7, DCG-IV with its additional carboxylic group in the R6 region, LY354740 with the methylene groups of its cyclopentane ring located in the R5 region. The possible agonist (magenta) and antagonist (blue) conformations of *S*-Gla are shown.

of 2*S*-CCG-I and DCG-IV are found (Fig. 5). We have previously proposed that the ACPT-II third carboxylic group would lie in an R7 region as shown in Fig. 5 opposite to region R6. A particular comment should be made on *S*-Gla. *S*-Gla is a S-glutamate analogue bearing an additional acidic function at carbon 4. It can adopt two extended conformations that both allow S1, S2a, S2b, S3a and S3b binding. In the first one, the third carboxylate points to the S4 site, while in the second conformation, it points to the R7 region.

4. Discussion

Our study represents the first complete pharmacological characterization of the rat $mGlu_{8a}$ receptor. It reveals no major difference between the activities of most compounds tested on $mGlu_{8a}$ and $mGlu_{4a}$ receptors. It also reveals no major similarity between the pharmacological profiles of $mGlu_{8a}$ and $mGlu_2$ receptors.

The potencies of some of the compounds reported here have already been determined on the rat $mGlu_8$ receptor expressed in *Xenopus* oocytes (Saugstad et al., 1997), and on the human $mGlu_8$ receptor stably expressed in the AV12 cells (Wu et al., 1998). In these two studies, the potencies of the agonists are often 2–10 times higher than those measured in the present study. This may simply be explained by a more efficient coupling of the $mGlu_{8a}$ receptor to its physiological transduction cascades (activation of GIRK or inhibition of adenylyl cyclase) than to the phospholipase C pathway via the chimeric G-protein Gq_{i9}. However, in all three studies, the rank order of potency of S-glutamate, *S*-AP4, 2*S*-CCG-I and 1*S*,3*R*-ACPD is the same. Some differences were, however, noticed. A major difference is that Wu et al. (1998) reported an agonist activity of LY354740 at the human $mGlu_{8a}$ with an EC_{50} of 36 μM, whereas we found this compound to be a full antagonist with an IC_{50} of about 300 μM in our assay. This difference might be explained by the different amino acids between the human and the rat sequences. Another possible explanation is that LY354740 is a very partial agonist at $mGlu_8$ receptor, and that only the very efficient coupling obtained in the AV12 cells overexpressing this receptor allowed the detection of its agonist activity. Another difference is that 1*S*,3*S*-ACPD is more potent than 1*S*,3*R*-ACPD on the human receptor expressed in AV12

cells (Wu et al., 1998), whereas we found it slightly less potent than 1*S*,3*R*-ACPD on the rat receptor in our expression system. Whether this is a species difference, or the consequence of the different assays used remains to be elucidated.

The activity and potency of most compounds on $mGlu_8$ receptor were found to be very similar to those obtained on $mGlu_4$ receptor. Among these are the recently developed $mGlu_4$ agonists ACPT-I, 3*S*,4*S*-ACPT (Acher et al., 1997), CBAP5, and 2*SR*,4*SR*-CPeAP4 (Johansen et al., 1995), and the $mGlu_4$ antagonists *S*-MAP4, MPPG and ACPT-II. Interestingly, ACPT-II was previously found to antagonize with a similar affinity $mGlu_1$, $mGlu_2$ and $mGlu_{4a}$ receptors (Acher et al., 1997). Our data extend the antagonistic action of this molecule to $mGlu_{8a}$ receptors, further suggesting it is a general mGlu receptors antagonist.

Taken all together, our data revealed an excellent correlation between the potencies of agonists and antagonists on these two receptor types. Accordingly, the proposed pharmacophore model for $mGlu_4$ receptor is also valid for $mGlu_8$ receptor, with the same regions S1-4 and R5-7 as defined previously (Bessis et al., 1999). Indeed all agonists bearing a distal phosphonic or phosphoric group display the same potency on $mGlu_4$ receptor and $mGlu_8$ receptor. Since they show no activity at group-I and group-II receptors, they define the same selective S3c binding site (Fig. 5). Analogously, the S4 hydrophilic site defined in the $mGlu_4$ receptor model is conserved, since ACPT-I and 3*S*,4*S*-ACPT display similar potencies at both receptors. Moreover ligands with substituents in the R7 (ACPT-II) regions display similar activities, also indicating this region is similar on these two group-III mGlu receptors. The high sequence similarity between $mGlu_4$ and $mGlu_8$ receptors amino terminal domain is in favor of similar binding sites and similar pharmacophore models.

However, the slight difference in potency and activity of some ligands revealed three possible differences in the binding site of these receptors. The first one is revealed by the slightly higher potency on $mGlu_8$ receptor than on $mGlu_4$ receptor of the agonists 1*S*,3*R*-ACPD, 1*S*,3*S*-ACPD and 2*S*-CCG-I, and the antagonist LY354740. This may result from a different interaction of their alkyl rings (R5 and R6 regions, Fig. 5) with the protein. This part of the $mGlu_8$ receptor binding site might be more hydrophobic or sterically less hindered.

The second difference is revealed by our previous observation that *S*-Gla acts as a partial agonist at $mGlu_4$ receptor, but as a full antagonist at $mGlu_8$ receptor (Brabet et al., 1998). This difference cannot be explained by a difference in expression between these two receptors, since western blots performed with an antibody directed against the same epitope added on these two receptors revealed that $mGlu_8$ receptor is indeed expressed at a slightly higher density than $mGlu_4$ receptor (Blahos et al., unpublished observation). We found that at least two possible conformations of *S*-Gla could fit the $mGlu_{4a/8a}$ receptor pharmacophore model. In the first one, the additional acidic group points to the S4 site which is defined by the carboxylic substituent of the agonists ACPT-I and 3*S*,4*S*-ACPT (Bessis et al., 1999). Such a conformation of *S*-Gla would, therefore, act as an agonist. In the second conformation of *S*-Gla, the additional carboxylic group fits into the R7 region, where the additional carboxylic group of ACPT-II is found. Since ACPT-II is an antagonist, whereas ACPD is an agonist, it is likely that the conformation of *S*-Gla that occupies this R7 region will act as an antagonist. Accordingly, *S*-Gla appears to be a mixture of two possible active molecules, one being an agonist and the other an antagonist. Such a mixture of conformations with opposite activities can behave either as a full agonist, partial agonist or full antagonist depending on (1) the ratio of the affinities of the two conformers and (2) the relative amount of these two conformers (Ebert et al., 1994, 1996). Accordingly, one would propose that S4 or R7 regions are slightly different between $mGlu_4$ receptor and $mGlu_8$ receptor, such that the binding of the antagonist conformer is favored in $mGlu_8$ receptor.

The third difference is shown by the phenylglycine derivatives *S*-4CPG and *S*-4C3HPG, which behave as potent $mGlu_8$ receptor partial agonists and which are both inactive at 1 mM on $mGlu_4$ receptor. In both molecules, the carboxylic group on the fourth position on the phenyl group occupies a new specific $mGlu_8$ receptor region (called R8) in the pharmacophore model (Fig. 6). This may easily explain their distinct activities at these two group-III mGlu receptors if this region is different between these two receptors. In agreement with this hypothesis, the replacement of the carboxylic group in *S*-4CPG by a phosphono group leads to a phosphonophenylglycine, a group-III mGlu receptors agonist, which is more potent on $hmGlu_8$ receptor than on $hmGlu_4$ receptor (Gasparini et al., 1999a). Moreover, we found that the alpha-methyl derivative of the phosphonophenylglycine, MPPG, is slightly more potent on $mGlu_8$ receptor than on $mGlu_4$ receptor.

The present structure-activity analysis is a working hypothesis that should help to further define the structural requirements for $mGlu_4/mGlu_8$ receptors selectivity. It will be refined with new experiments, such as the construction of a 3D-model of the amino-terminal domain of the receptors and the docking of the ligands.

It has previously been suggested that the pharmacological properties of $mGlu_8$ receptor resemble those of the group-II $mGlu_2$ receptor (Saugstad et al., 1997). This proposal was based on the relatively good potency of the group-I/II antagonist MCPG to inhibit the glutamate-induced activation of the rat $mGlu_{8a}$ receptor expressed in *Xenopus* oocytes (IC_{50} about 300 μM), whereas this drug was known to be inactive on $mGlu_8$ receptor (Saugstad et al., 1997). The relatively high agonist potency of 2*S*-CCG-I, which was believed at that time to be much less potent on the group-III $mGlu_4$ receptor, also supported the au-

thors' hypothesis. However, in our system, MCPG displayed a very low potency on the rat $mGlu_{8a}$ receptor, and similar data were obtained on the human receptor (Wu et al., 1998). Although we and others confirmed that 2*S*-CCG-I is a potent agonist on $mGlu_8$ receptor (Brabet et al., 1998; Wu et al., 1998), this compound has also been shown to be a potent agonist of the group-III $mGlu_4$ receptor (Gomeza et al., 1996; Brabet et al., 1998; Wu et al., 1998). However, we found that 1*S*,3*R*-ACPD, 1*S*,3*S*-ACPD and *S*-4C3HPG, which have a high affinity for group-II mGlu receptors, display a higher potency on $mGlu_8$ receptor than on $mGlu_4$ receptor, suggesting that the regions R5 and R_8 in the $mGlu_8$ receptor are somewhat similar to those of $mGlu_2$ receptor.

In conclusion, our study reveals a strong similarity between the pharmacological profiles of $mGlu_8$ and $mGlu_4$ receptors. Accordingly, it is not possible to discriminate between $mGlu_4$- and $mGlu_8$-mediated responses in the brain using the actual commercially available tools. However, our study revealed some small differences that can be explained by our $mGlu_{4a/8a}$ pharmacophore models and may be used to generate more selective ligands.

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