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Molecular pharmacology of 4-substituted glutamic acid analogues at ionotropic and metabotropic excitatory amino acid receptors

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Abstract

The pharmacology of (2*S*,4*R*)-4-methylglutamic acid, (2*S*,4*S*)-4-methylglutamic acid and (*S*)- and (*R*)-4-methyleneglutamic acids (obtained in high chemical and enantiomeric purity from racemic 4-methyleneglutamic acid by chiral HPLC using a Crownpak CR(+) column), was examined in binding experiments using rat brain ionotropic glutamate receptors, and in functional assays using cloned metabotropic glutamate (mGlu) receptors. As a notable result of these studies, (2*S*,4*R*)-4-methylglutamic acid and (2*S*,4*S*)-4-methylglutamic acid were shown to be selective for kainic acid receptors and mGlu receptors (subtypes 1α and 2), respectively, whereas (*S*)-4-methyleneglutamic acid showed high but rather non-selective affinity for the (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), kainic acid, NMDA and mGlu receptors (subtypes 1α and 2). Although none of the compounds were specific for any of the receptor subtypes, the results demonstrate that each of these structurally related compounds has a distinct pharmacological profile. © 1997 Elsevier Science B.V.

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(*S*)-Glutamic acid ((*S*)-Glu, **1**), the major excitatory amino acid in the central nervous system, is involved in many important neural processes such as plasticity, learning and memory. Furthermore, (*S*)-Glu is believed to be implicated in serious neurologic disorders such as Alzheimer's disease, epilepsy and schizophrenia (Krogsgaard-Larsen et al., 1996). (*S*)-Glu mediates these effects through two different receptor families. The ionotropic glutamic acid (iGlu) receptors belong to the family of ligand gated ion channel receptors and can be further subdivided into *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and kainic acid receptors. The metabotropic glutamic acid (mGlu) receptors belong to the family of G-protein coupled receptors. Based on sequence homology, second messenger pathways and pharmacology, the eight cloned mGlu receptors have been subdivided into three groups consisting of $mGlu_1$, $mGlu_5$; $mGlu_2$, $mGlu_3$ and $mGlu_4$, $mGlu_6$, $mGlu_7$, $mGlu_8$, respectively (Bräuner-Osborne et al., 1996; Krogsgaard-Larsen et al., 1996).

Recently, (*S*)-Glu analogues with a methyl- or a methylene group in the four position (see structures in Table 1) have attracted attention as potent ligands for kainic acid receptors (Gu et al., 1995) and excitatory amino acid transporters (Vandenberg et al., 1997). In order to further elucidate the pharmacological profile of these closely related (*S*)-Glu analogues we have determined the activities of (2*S*,4*R*)-4-methyl-Glu (**2**), (2*S*,4*S*)-4-methyl-Glu (**3**), (*S*)-4-methylene-Glu (**4**) and (*R*)-4-methylene-Glu (**5**) at $mGlu_{1\alpha}$, $mGlu_2$ and $mGlu_{4a}$, representing the three groups of mGlu receptors, and their affinities for the AMPA, kainic acid and NMDA receptor subtypes.

(2*S*,4*R*)-4-methyl-Glu (**2**) and (2*S*,4*S*)-4-methyl-Glu (**3**) were obtained from Tocris Cookson (Bristol, UK). (*S*)-Glu (**1**) and (*RS*)-4-methylene-Glu were obtained from Sigma (St. Louis, MO, USA). (*RS*)-4-methylene-Glu (154 mg) was resolved into the (*S*) (**4**) and (*R*) (**5**) enantiomers by chiral HPLC in 5 mg injections using a Crownpak CR(+) column (10 × 150 mm, Daicel). The column was con-

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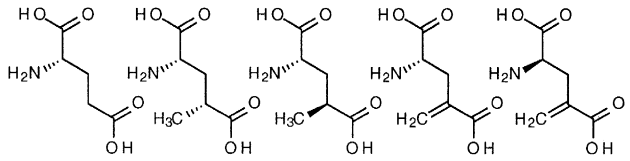
nected to a HPLC system consisting of a Jasco 880PU pump, a Rheodyne 7125 injector, and a Waters M480 detector set at 200 nm connected to a Merck-Hitachi Chromato-Integrator D-2000. The column was eluted at 0–1°C with 1.5 ml/min of aqueous trifluoroacetic acid, pH = 2. Appropriate fractions were pooled and evaporated. In order to removed traces of trifluoroacetic acid, the two enantiomers were, each in four injections, passed through a S-Sepharose ion-exchange column (10 × 100 mm) connected to the above mentioned HPLC system using water at 1.0 ml/min as eluent. After pooling and evaporation of fractions containing (*S*)-(+)-4-methylene-Glu (**4**), the late enantiomer on the Crownpak CR(+) column, the residue was recrystallized (water) to give (*S*)-(+)-4-methylene-Glu (**4**) (68.3 mg, 84%), m.p. 185–186°C (Ezquerra and Pedregal (1994): 192–194°C), $[\alpha]_D^{22} = +13.3°$ ($c = 0.38$, 5 M HCl) (Ezquerra and Pedregal (1994): $+12.8°$ ($c = 0.53$, 5 M HCl)) having ^{1}H NMR and IR spectra similar to those previously reported (Ezquerra and Pedregal, 1994). Treatment of the combined ion-exchanged fractions containing the first eluting enantiomer on the Crownpak CR(+) column as described for (*S*)-4-methylene-Glu (**4**), gave (*R*)-(−)-4-methylene-Glu (62.2 mg, 76%), m.p. 185–186°C, $[\alpha]_D^{22} = -13.5°$ ($c = 0.35$, 5 M HCl), with an IR spectrum virtually identical to that of (*S*)-(+)-4-methylene-Glu (**4**). Anal. $C_6H_9NO_4 \cdot \frac{1}{2}H_2O$: C, H, N. Chiral HPLC analyses of the resolved 4-methylene-Glu isomers, (**4**) and (**5**), using a Crownpak CR(−) column (4.6 × 150 mm, Daicel) eluted at 1°C with 0.4 ml/min of aqueous perchloric acid, pH = 2 and connected to a Waters HPLC system consisting of a M510 pump, a U6K injector, and a M991 Photodiode Array Detector, showed that (*S*)- (**4**) and (*R*)-4-methylene-Glu (**5**) both were obtained with high enantiomeric purities (ee > 99.9% and ee = 99.9%, respectively). Calculation of enantiomeric excess (ee) was done based on peak areas at 200 nm, and the identity of the enantiomeric impurity was, in both cases, verified by spike experiments using small amounts of (*RS*)-4-methylene-Glu.

Activities at the mGlu receptor subtypes were determined as previously described (Bräuner-Osborne et al., 1996). Briefly $mGlu_{1\alpha}$, $mGlu_2$ or $mGlu_{4a}$ receptors were expressed in Chinese hamster ovary (CHO) cells and grown in Dulbecco's modified Eagle medium. Activities at $mGlu_{1\alpha}$ were determined as fold increase in phosphatidylinositol hydrolysis measured by ion-exchange chromatography. At $mGlu_2$ and $mGlu_{4a}$, ligand activities were determined as inhibition of forskolin-induced cyclic AMP levels measured by a scintillation proximity assay (Amersham). Receptor binding assays to rat brain membranes using [^{3}H]AMPA, [^{3}H]kainic acid ([^{3}H]KAIN) or [^{3}H]-4-(3-phosphonoprop-1-yl)piperazine-2-carboxylic acid ([^{3}H]CPP, a NMDA receptor ligand) were also performed as described previously (Bräuner-Osborne et al., 1996).

At the iGlu receptors, (2*S*,4*R*)-4-methyl-Glu (**2**) and (2*S*,4*S*)-4-methyl-Glu (**3**) both showed selectivity for the kainic acid receptor (Table 1). In agreement with a previous study (Gu et al., 1995), we found (2*S*,4*R*)-4-methyl-Glu (**2**) to be approximately 6- and 50-fold more potent than (*S*)-Glu (**1**) and (2*S*,4*S*)-4-methyl-Glu (**3**), respectively, at the kainic acid receptor. In a preliminary electrophysiological study, (*S*)-4-methylene-Glu (**4**) has previously been reported to activate NMDA receptors (Ouerfelli et al., 1993). In agreement with this study, we find (*S*)-4-methylene-Glu (**4**) to bind to NMDA receptors. However, (*S*)-4-methylene-Glu (**4**) is rather non-selective at the iGlu receptors, displaying similar affinities for the AMPA and kainic acid receptors (Table 1). Interestingly, (*R*)-4-methylene-Glu (**5**) selectively binds to the AMPA receptors although with approximately 60-fold lower affinity than the (*S*)-enantiomer, **4**. Due to the very high enantiomeric purity of (*R*)-4-methylene-Glu (**5**) (ee = 99.9%) this activity is most likely a property of the (*R*)-enantiomer, **5**, and not an impurity of the (*S*)-enantiomer, **4**.

The compounds were also tested at representative subtypes of mGlu receptors. Contrary to the results at the iGlu receptors, (2*S*,4*S*)-4-methyl-Glu (**3**) was more potent at the mGlu receptor subtypes than (2*S*,4*R*)-4-methyl-Glu (**2**). Within the mGlu receptors, (2*S*,4*S*)-4-methyl-Glu (**3**) showed selectivity for the $mGlu_{1\alpha}$ and $mGlu_2$ receptors being slightly more potent than (*S*)-Glu (**1**) at both receptors while showing very weak activity at the $mGlu_{4a}$

Table 1
Structure and pharmacological activities of (*S*)-Glu (**1**) and its analogues (**2**–**5**) tested in this study



1 2 3 4 5

	EC_{50} (μM) [a]		
	$mGlu_{1\alpha}$	$mGlu_2$	$mGlu_{4a}$
1	13 ± 1	3.7 ± 0.6	14 ± 1
2	> 1000	95 ± 5	> 1000
3	10 ± 1	2.0 ± 0.3	470 ± 250
4	30 ± 0.4	0.47 ± 0.02	> 1000
5	> 1000	> 1000	> 1000
	IC_{50} (μM) [b]		
	[^{3}H]AMPA	[^{3}H]KAIN	[^{3}H]CPP
1 [c]	0.50	0.27	n.d.
2	> 100	0.047 ± 0.012	34 ± 13
3	> 100	2.4 ± 0.3	81 ± 16
4	0.15 ± 0.03	0.23 ± 0.08	1.2 ± 0.3
5	8.5 ± 2.4	> 100	> 100

[a] Agonist activities at mGlu receptor subtypes expressed in CHO cells.
[b] Binding affinities at ionotropic glutamate receptors using rat brain homogenates.
[c] From Hansen et al. (1989). All values are expressed as mean ± S.E.M. of at least two experiments performed in triplicate.

receptor subtype (Table 1). (*S*)-4-methylene-Glu (**4**) was also selective for the $mGlu_{1\alpha}$ and $mGlu_2$ receptors being inactive at the $mGlu_{4a}$ receptor subtype. The compound displayed highest selectivity for the $mGlu_2$ receptor subtype being 8-fold more potent than (*S*)-Glu (**1**). (*R*)-4-methylene-Glu (**5**) was inactive at all mGlu receptor subtypes, also when tested for antagonist activity against 30 μM (*S*)-Glu (**1**). In a recent study (2*S*,4*R*)-4-methyl-Glu (**2**), (2*S*,4*S*)-4-methyl-Glu (**3**) and (*S*)-4-methylene-Glu (**4**) have been tested exclusively at the $mGlu_{1\alpha}$ receptor subtype and in agreement with our findings (2*S*,4*S*)-4-methyl-Glu (**3**) was found to be equi-active with (*S*)-Glu (**1**), whereas the other two compounds were less active (Todeschi et al., 1997).

As shown in this study, (2*S*,4*R*)-4-methyl-Glu (**2**), (2*S*,4*S*)-4-methyl-Glu (**3**) and (*S*)-4-methylene-Glu (**4**) all display high potency for glutamate receptors. Interestingly, the three compounds show very different pharmacological profiles at the different receptor subtypes in spite of their close structural relationships. However, none of the compounds are specific for one receptor subtype which limits their usefulness as pharmacological tools, but the results indicate that the 4-position of (*S*)-Glu (**1**) is an interesting target for the design of new structural analogues with specificity for either iGlu or mGlu receptor subtypes.

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