Analysis of Agonist and Antagonist Activities of Phenylglycine Derivatives for Different Cloned Metabotropic Glutamate Receptor Subtypes

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The metabotropic glutamate receptors (mGluRs) consist of at least seven different subtypes and are coupled to intracellular signal transduction via G proteins. However, the lack of specific antagonists for the mGluRs limited the precise characterization of the role of the individual mGluRs. In this study, we investigated the agonist and antagonist activities of a series of phenylglycine derivatives for the mGluRs by examining their effects on the signal transduction of representative mGluR1, mGluR2, and mGluR4 subtypes expressed individually in Chinese hamster ovary cells. The phenylglycine derivatives examined included (S)- and (R)-forms of 3-hydroxyphenylglycine (3HPG), 4-carboxy-phenylglycine (4CPG), 4-carboxy-3-hydroxyphenylglycine (4C3HPG), 3-carboxy-4-hydroxyphenylglycine (3C4HPG), and (+)- and (−)-α-methyl-4-carboxyphenylglycine (αM4CPG). Among these 10 compounds, (S)-3HPG acted as an agonist for mGluR1, while (S)-4C3HPG, (S)-3C4HPG, and (S)-4CPG served as effective agonists for mGluR2. The rank order of agonist potencies for mGluR2 was L-glutamate > (S)-4C3HPG > (S)-3C4HPG > (S)-4CPG. No other phenylglycine derivatives showed any definite agonist activity on either mGluR1 or mGluR2. Among the phenylglycine derivatives with no mGluR1 agonist activity, (S)-4C3HPG, (S)-3C4HPG, (S)-4CPG, and (+)-αM4CPG effectively antagonized the action of L-glutamate on mGluR1. The rank order of antagonist potencies was (S)-4C3HPG ≥ (S)-4CPG ≥ (+)-αM4CPG > (S)-3C4HPG. The Schild plot analysis indicated that (RS)-4C3HPG, (S)-4CPG, and (+)-αM4CPG all act as competitive antagonists for mGluR1 with pA2 values of 4.38, 4.46, and 4.38, respectively. Furthermore, (+)-αM4CPG was an effective competitive antagonist for mGluR2 with a pA2 value of 4.29. None of the 10 phenylglycine derivatives showed any agonist or antagonist activity for mGluR4. This investigation demonstrated that (S)-4C3HPG, (S)-4CPG, and (S)-3C4HPG act both as agonists for mGluR2 and as antagonists for mGluR1, and

more importantly, that (+)-αM4CPG serves purely as an antagonist for mGluR1 and mGluR2. The phenylglycine derivatives will be useful for investigating the functions of the mGluR family.

[Key words: phenylglycine derivative, glutamate analog, metabotropic glutamate receptor, agonist potency and selectivity, antagonist potency and selectivity, receptor-expressing cell, signal transduction, Schild plot analysis]

Glutamate receptors play an important role in neuronal plasticity and neurotoxicity in the CNS (Choi and Rothman, 1990; Watkins et al., 1990; Nakanishi, 1992; Bliss and Collingridge, 1993). They are classified into two distinct groups termed ionotropic glutamate receptors and metabotropic glutamate receptors (mGluRs) on the basis of electrophysiological, pharmacological, and molecular studies (Nakanishi, 1992). The ionotropic receptors comprise glutamate-gated, cation-specific channel complexes and are further subdivided into the receptors for NMDA and the non-NMDA receptors for kainate/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) (Nakanishi, 1992; Sommer and Seeburg, 1992). mGluRs are coupled to intracellular second messenger systems via G proteins (Nakanishi, 1992; Schoepp and Conn, 1993). Recent molecular cloning studies have revealed the existence of at least seven different subtypes of mGluRs, termed mGluR1 to mGluR7, which share a common structural architecture with a large extracellular domain preceded by the seven membrane-spanning domains (Houamed et al., 1991; Masu et al., 1991; Abe et al., 1992; Nakanishi, 1992; Tanabe et al., 1992; Nakajima et al., 1993; Okamoto et al., 1994). mGluR1 and mGluR5 are coupled to the stimulation of the phosphatidylinositol (PI) hydrolysis/Ca^{2+} signal transduction, whereas the other five are linked to the inhibition of the cAMP cascade. They also show different agonist selectivities in accordance with their sequence similarities within the mGluR family. mGluR1 and mGluR5 interact selectively with quisqualate, whereas mGluR2 and mGluR3 potently react with trans-1-aminocyclopentane-1,3-dicarboxylate (ACPD) and (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (Abe et al., 1992; Aramori and Nakanishi, 1992; Hayashi et al., 1992; Tanabe et al., 1992, 1993). mGluR4, mGluR6, and mGluR7 effectively respond to L-2-amino-4-phosphonobutyrate (L-AP4), thus corresponding to the putative L-AP4 receptors characterized in many neuronal cells (Thomsen et al., 1992; Nakajima et al., 1993; Tanabe et al., 1993; Okamoto et al., 1994). The seven mGluR subtypes are thus classified into three subgroups

on the basis of their sequence similarities, signal transduction mechanisms, and agonist selectivities.

Numerous lines of recent evidence indicate that mGluRs are involved in the regulation of synaptic transmission in the CNS (Schoepp and Conn, 1993). However, the lack of specific antagonists for the mGluRs has limited the precise characterization of the role of the individual mGluRs in glutamate transmission. Recently, a series of phenylglycine derivatives was chemically synthesized, and the effects of these compounds on glutamate transmission in the brain and spinal cord were studied electrophysiologically and biochemically (Watkins et al., 1987; Bashir et al., 1993; Birse et al., 1993; Eaton et al., 1993a,b; Jane et al., 1993). These studies indicated that some of the compounds are effective as agonists or antagonists for mGluRs. The determination of the accurate potencies and subtype specificity of the effective phenylglycine compounds is thus important for the use of these compounds in studies of the functions of the mGluRs. In this investigation, we adopted clonal cell lines expressing a single mGluR subtype and characterized the agonist and antagonist properties of each phenylglycine compound without any ambiguity due to cross-reactivity with different mGluR subtypes. We tested the effects of five pairs of stereoisomers of phenylglycine derivatives on the signal transduction of representative mGluR1, mGluR2, and mGluR4 expressed individually in Chinese hamster ovary (CHO) cells. We report here the potencies and subtype specificity of the phenylglycine compounds and indicate that $(+)$-α-methyl-4-carboxyphenylglycine (αM4CPG) acts as a selective antagonist for mGluR1 and mGluR2.

Materials and Methods

Materials. The phenylglycine derivatives used in this study were obtained as follows (for the structures of different phenylglycine derivatives, see Table 1): (RS)-4-carboxyphenylglycine (4CPG), (RS)-4-carboxy-3-hydroxyphenylglycine (4C3HPG), and (RS)-αM4CPG were synthesized from the correspondingly substituted benzaldehydes (for 4CPG and 4C3HPG) or acetophenone (for αM4CPG) by the Strecker reaction (a variation of the method of Steiger, 1955), and the racemates were resolved through their L-lysine salts (Evans et al., 1979); synthesis and resolution of (RS)-3-carboxy-4-hydroxyphenylglycine (3C4HPG) were carried out according to the method of Kjær and Larsen (1963); (R)- and (S)-3-hydroxyphenylglycines (3HPG) were prepared from appropriately protected 3-hydroxyphenylacetic acid by enantioselective synthesis, using the method of Evans et al. (1987). Assignment of configuration was by inference from the chiral auxiliaries used in enantioselective synthesis (for 3HPG), sequence of elution in chiral high-performance liquid-chromatography (HPLC) from an asymmetric crown ether column (all except isomers of αM4CPG) (Udvarhelyi and Watkins, 1990), and pH-dependent rotational shifts (Greenstein and Winitz, 1961; Kjær and Larsen, 1963). Since the isomers of αM4CPG were not separated in chiral HPLC, we did not assign absolute stereochemistry in this case. Based on pH-dependent rotational shifts, however, the $(+)$- and $(-)$-isomers (so termed according to the signs of their rotations as determined in acid solution) are likely to be the (S)- and (R)-forms, respectively, as with all the other phenylglycines used in this study. All single enantiomers showed 97% or greater optical purity (most >99%) in analytical chiral HPLC (Udvarhelyi and Watkins, 1990). Observed specific rotations ($[\alpha]_D^{18}$, measured in 6 N HCl) were as follows: (S)-4CPG, $+154°$ (c, 0.25); (R)-4CPG, $-160°$ (c, 0.23), (S)-4C3HPG, $+140°$ (c, 0.38); (R)-4C3HPG, $-141°$ (c, 0.28), (S)-3C4HPG, $+139°$ (c, 0.8); (R)-3C4HPG, $-141°$ (c, 0.9), (S)-3HPG, $+152°$ (c, 0.12); (R)-3HPG, $-163°$ (c, 0.13), $(+)$-αM4CPG, $+93°$ (c, 052); $(-)$-αM4CPG, $-97°$ (c, 0.27). The phenylglycine derivatives were dissolved at 50 mM in an equimolar NaOH solution and used after pH adjustment.

Measurements of PI hydrolysis and cyclic AMP formation. Permanent CHO cell lines expressing individually mGluR1, mGluR2, and mGluR4 were prepared as described previously (Aramori and Nakanishi, 1992; Tanabe et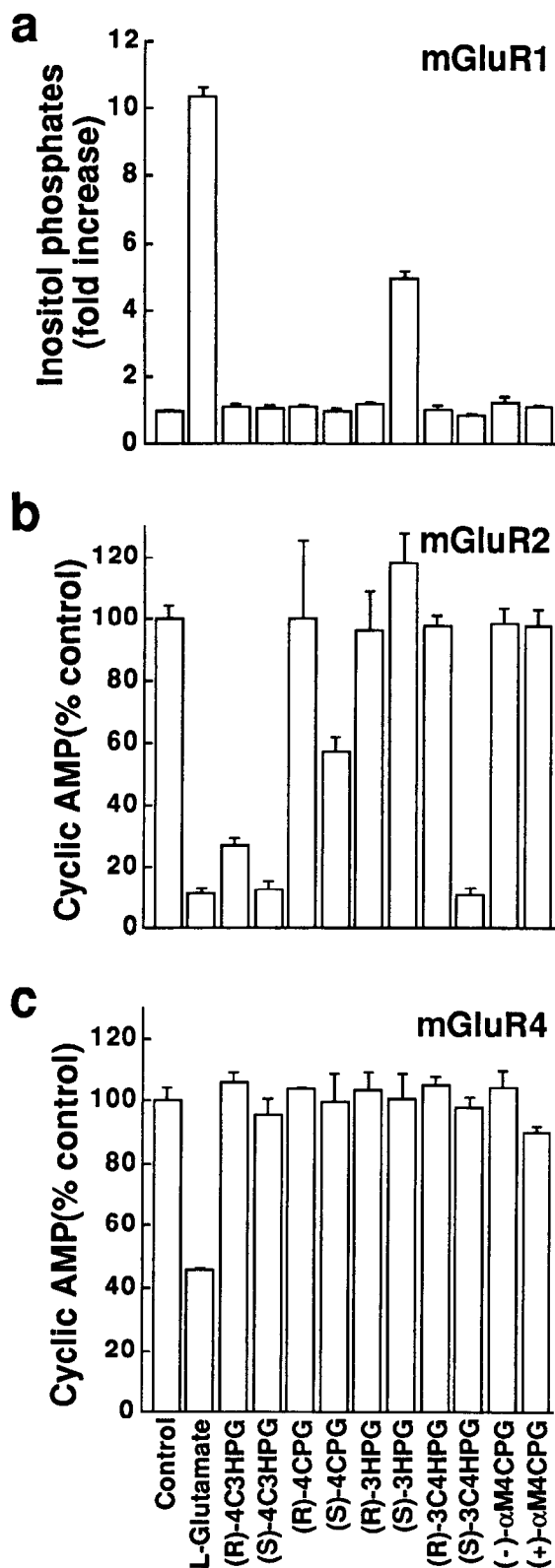 al., 1992, 1993). The agonist and antagonist activities of phenylglycine derivatives for mGluR1 were determined by measuring total inositol phosphate (IP) formation in mGluR1-expressing cells as described previously (Aramori and Nakanishi, 1992; Hayashi et al., 1992); mGluR1-expressing cells were incubated with test reagents for 20 min, and the agonist activity was determined by measuring the formation of a mixture of ³H-labeled inositol mono-, bis-, and trisphosphates; for measurements of antagonist activity, mGluR1-expressing cells were first preincubated with test reagents for 20 min and then incubated with these reagents for 20 min after the addition of a constant or varying concentration of L-glutamate; the inhibitory effects of the test reagents on the L-glutamate–mediated stimulation of total IP formation were then determined. The agonist and antagonist activities of phenylglycine derivatives for mGluR2 and mGluR4 were determined by measuring changes in levels of the forskolin-induced cyclic AMP formation in mGluR2-expressing and mGluR4-expressing cells, respectively, as described previously (Hayashi et al., 1992; Tanabe et al., 1992, 1993); for measurements of the agonist activity, receptor-expressing cells were incubated with test reagents in the presence of 10 μM forskolin for 10 min, and the agonist activity was determined by measuring the inhibition of the forskolin-stimulated cyclic AMP formation; for measurements of the antagonist activity, receptor-expressing cells were first preincubated with test reagents for 20 min and then further incubated with test reagents for 10 min after the addition of 10 μM forskolin together with a constant or varying concentration of L-glutamate. All experiments for measurements of PI hydrolysis and cyclic AMP formation were carried out at least twice in triplicate.

Results

Agonist activities of various phenylglycine derivatives for mGluR1, mGluR2, and mGluR4

The seven mGluR subtypes can be subclassified into three groups on the basis of their sequence similarities, signaling cascades, and agonist selectivities: mGluR1/mGluR5, mGluR2/mGluR3, and mGluR4/mGluR6/mGluR7. We chose mGluR1, mGluR2, and mGluR4 as representatives of each group of the receptors and investigated the effects of 10 phenylglycine derivatives on the signal transduction characteristic of the respective receptor subtypes. We first examined the agonist activities of these phenylglycine derivatives for each of the three mGluR subtypes by the addition of a high concentration (1 mM each) of these compounds to cell lines expressing individual receptor subtypes (Fig. 1). In mGluR1-expressing cells, L-glutamate (1 mM) induced about a 10-fold increase in total IP formation above control levels (Fig. 1a). Among the 10 phenylglycine derivatives, (S)-3HPG showed a weak agonist activity on mGluR1 (Fig. 1a). No other phenylglycine derivative showed any obvious agonist activity on mGluR1 at the concentration of 1 mM. We next analyzed the agonist activities of the 10 phenylglycine derivatives in mGluR2-expressing cells. L-Glutamate inhibited the forskolin-induced cAMP accumulation to about 10–20% of control levels (Fig. 1b). Four of the phenylglycine derivatives, (S)- and (R)-4C3HPG, (S)-4CPG, and (S)-3C4HPG, showed inhibition of the forskolin-stimulated cAMP formation to different extents, but the other six had no such agonist activity in mGluR2-expressing cells (Fig. 1b). When the agonist activities of the phenylglycine derivatives were examined in mGluR4-expressing cells, none of them revealed any appreciable inhibitory effects on the forskolin-stimulated cAMP formation (Fig. 1c). In this experiment, it was confirmed that L-glutamate is effective in reducing the forskolin-stimulated cAMP formation to about 40% of control levels.

The potencies of the four phenylglycine derivatives effective as mGluR2 agonists were analyzed in more detail by determining dose–response curves of these compounds as well as L-glutamate for the inhibition of the forskolin-stimulated cAMP formations in mGluR2-expressing cells (Fig. 2). The rank order of

a mGluR1

b mGluR2

c mGluR4

Figure 1. Agonist activities of 10 phenylglycine derivatives for mGluR1, mGluR2, and mGluR4. In *a*, mGluR1-expressing cells were incubated with L-glutamate (1 mM) or phenylglycine derivatives (1 mM each) for 20 min, and total IP formation was determined. The IP formation is expressed as multiples of IP levels in agonist-untreated cells (*Control*). Basal levels (*Control*) of total inositol phosphates were 912 ± 40 cpm. In *b* and *c*, mGluR2-expressing and mGluR4-expressing cells were incubated with L-glutamate or phenylglycine derivatives (1 mM each) for
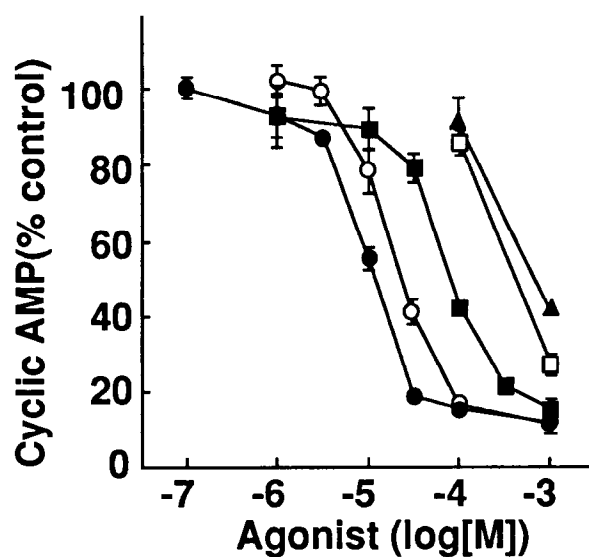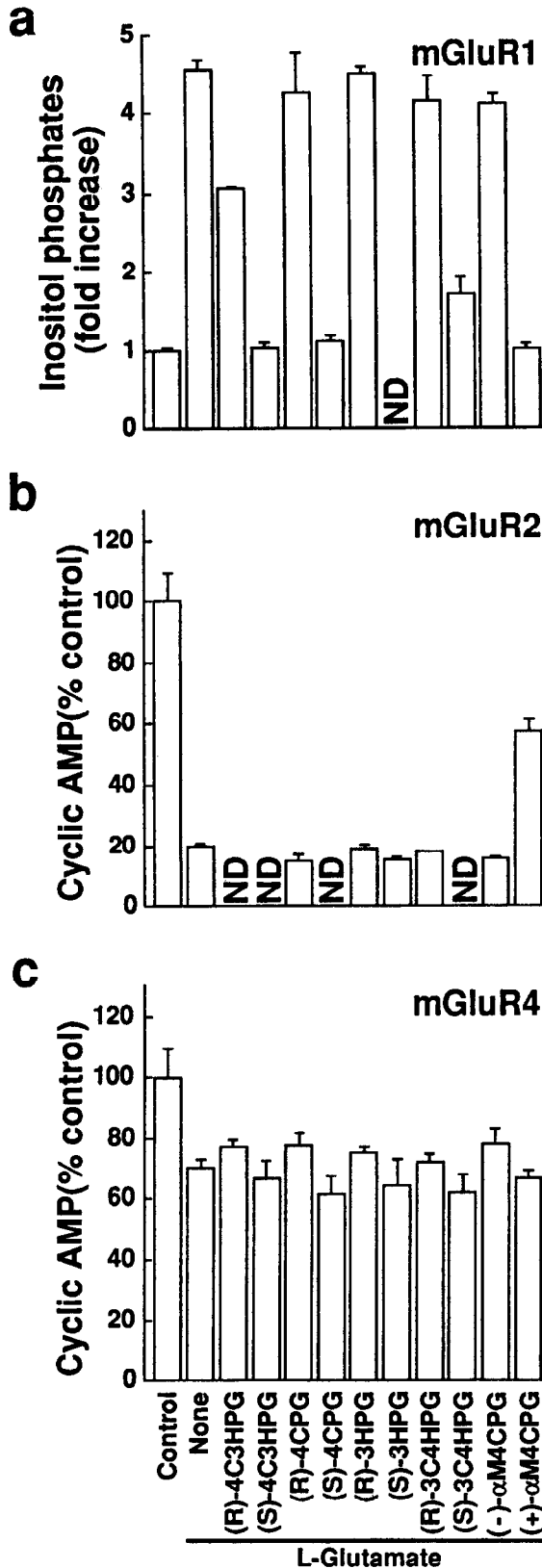

Figure 2. Dose–response curves of phenylglycine derivatives for the inhibition of the forskolin-stimulated cAMP formation in mGluR2-expressing cells. mGluR2-expressing cells were incubated with the indicated concentrations of L-glutamate (●), (*S*)-4C3HPG (○), (*S*)-3C4HPG (■), (*R*)-4C3HPG (□), and (*S*)-4CPG (▲). For other details, see Figure 1.

potencies of these compounds was L-glutamate > (*S*)-4C3HPG > (*S*)-3C4HPG > (*R*)-4C3HPG ≥ (*S*)-4CPG. The half-maximal effective concentrations (EC_{50}) of these agonists were 1×10^{-5}, 2×10^{-5}, 7×10^{-5}, 3×10^{-4}, and 5×10^{-4} M, respectively.

Antagonist activities of various phenylglycine derivatives for mGluR1, mGluR2, and mGluR4

The antagonist effects of nine phenylglycine derivatives on mGluR1 were determined by applying 1×10^{-3} M of different phenylglycine compounds to mGluR1-expressing cells in the presence of 1×10^{-5} M L-glutamate (Fig. 3*a*). This concentration of L-glutamate corresponded to the approximate EC_{50} value of this agonist for mGluR1 and indeed increased IP levels about four- to fivefold. Among the phenylglycine derivatives applied, (*S*)-4C3HPG, (*S*)-4CPG, (*S*)-3C4HPG, and (+)-αM4CPG inhibited L-glutamate–induced IP increase in mGluR1-expressing cells and reduced the IP formation to about control levels (Fig. 3*a*). (*R*)-4C3HPG also partially antagonized the effect of L-glutamate on the mGluR1 activity. Because (*S*)-3HPG showed an agonist activity, this compound was not included in the above analysis. However, (*S*)-3HPG at the concentrations of 1×10^{-4} M and 1×10^{-3} M exhibited weak inhibitory effects (about 15% and 25% inhibition, respectively) on nearly maximally stimulated IP formation evoked by 1×10^{-4} M L-glutamate (data not shown), suggesting that this compound has both agonist and antagonist activities (i.e., partial agonist) on mGluR1. However,

cubated with L-glutamate or phenylglycine derivatives (1 mM each) for

10 min in the presence of 10 μM forskolin, respectively, and intracellular cAMP levels were determined. cAMP levels in cells treated and untreated with 10 μM forskolin were 187.5 ± 7.7 and 5.8 ± 0.5 nmol per well (mGluR2-expressing cells) and 153.1 ± 2.2 and 3.7 ± 0.2 nmol per well (mGluR4-expressing cells), respectively. The cAMP levels in forskolin-stimulated, glutamate-untreated cells (*Control*) are taken as 100%. The data indicated were taken from representative experiments. The values are means ± SD (error bars) of triplicate determinations.
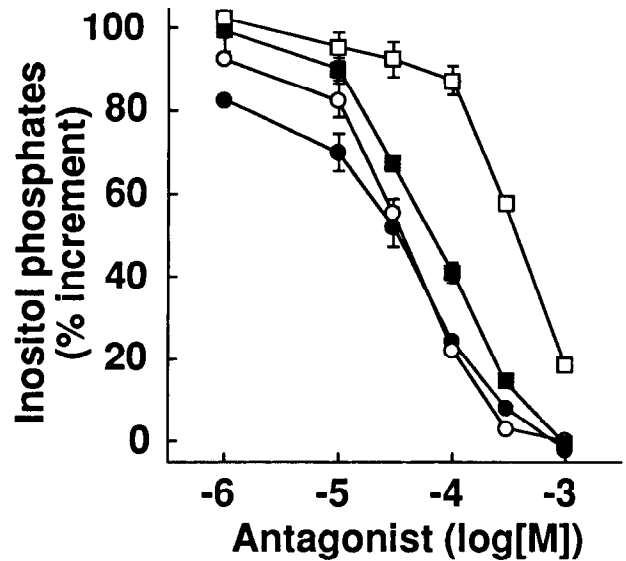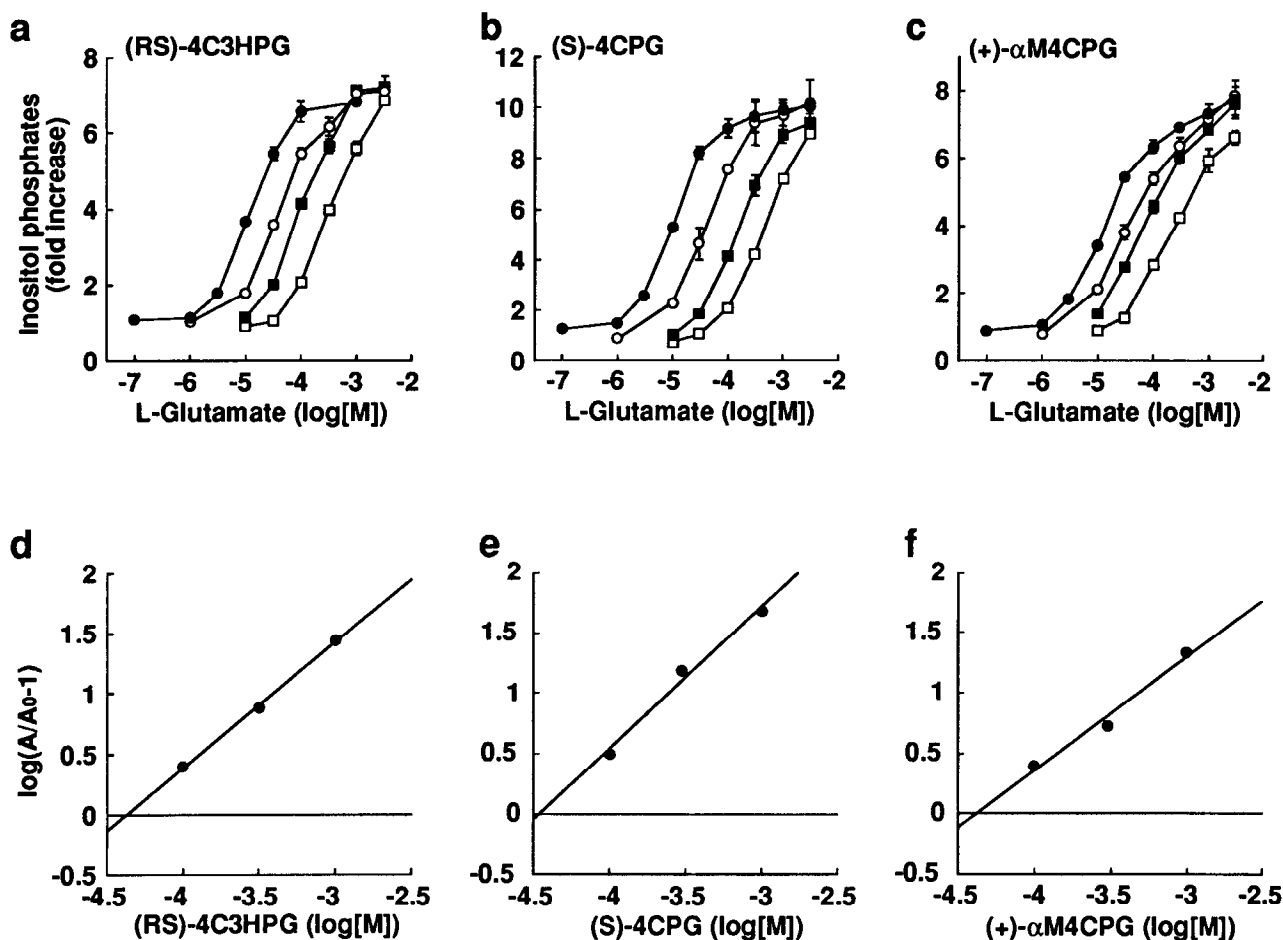
Figure 4. Relative potencies of four phenylglycine derivatives as antagonists for mGluR1. mGluR1-expressing cells were preincubated with the indicated concentrations of (*S*)-4C3HPG (●), (*S*)-4CPG (○), (+)-αM4CPG (■), and (*S*)-3C4HPG (□) for 20 min and further incubated with these compounds for 20 min in the presence of 10 μM L-glutamate. The L-glutamate (10 μM)-stimulated IP formation in the absence of any antagonist was taken as 100%. The basal level of IP formation was 1617 ± 53 cpm and this level increased to 9515 ± 188 cpm with the addition of 10 μM L-glutamate. For other details, see Figure 1.

because both activities were weak, more detailed characterization of (*S*)-3HPG remains to be conducted.

Antagonist activities of six phenylglycine derivatives on mGluR2 were then determined by examining whether the glutamate-mediated inhibition of the forskolin-induced cAMP accumulation was reduced by the addition of 1 × 10⁻³ M of different phenylglycine derivatives in mGluR2-expressing cells (Fig. 3*b*). In this experiment, 3 × 10⁻⁵ M L-glutamate was added, and the analysis of (*R*)- and (*S*)-4C3HPG, (*S*)-4CPG, and (*S*)-3C4HPG was omitted, because they showed effective agonist activities on mGluR2. Among the phenylglycine derivatives examined, (+)-αM4CPG partially antagonized the agonist effect of L-glutamate.

The antagonist effects of the 10 phenylglycine derivatives on mGluR4 were then examined by measuring their antagonist actions on L-glutamate-mediated inhibition of the forskolin-stimulated cAMP formation in mGluR4-expressing cells (Fig. 3*c*). In this experiment, 1 × 10⁻⁵ M glutamate was added to activate mGluR4, and the effects of 1 × 10⁻³ M of different phenylglycine derivatives were analyzed. None of the phenylglycine derivatives showed the antagonist activity on mGluR4. The properties of mGluR4 are thus clearly different from those of mGluR1 and mGluR2, both of which are either activated or

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Figure 3. Antagonist activities of phenylglycine derivatives for mGluR1, mGluR2, and mGluR4. In *a*, mGluR1-expressing cells were preincubated with 1 mM phenylglycine derivatives for 20 min and then incubated with 10 μM L-glutamate in the presence of 1 mM phenylglycine derivatives. Basal levels (*Control*) of total inositol phosphates were 1106 ± 28 cpm. In *b* and *c*, mGluR2-expressing and mGluR4-expressing

Figure 5. Dose–response curves of L-glutamate for determination of antagonist potencies of three phenylglycine derivatives on mGluR1. IP formation in mGluR1-expressing cells was determined by the addition of indicated concentrations of L-glutamate alone (●) or together with 100 μM (O), 300 μM (■), and 1 mM (□) (RS)-4C3HPG (a), (S)-4CPG (b), and $(+)$-αM4CPG (c). Schild plots obtained from the EC_{50} values estimated from a–c are shown in d–f, respectively, where A/A_0 = (EC_{50} for L-glutamate in the presence of phenylglycine derivatives)/(EC_{50} for L-glutamate alone). The pA_2 values and slopes were calculated by linear regression analysis.

antagonized, or both, by several different phenylglycine derivatives.

Dose–response analysis of antagonist effects of phenylglycine derivatives on mGluR1 and mGluR2

The antagonist effects of (S)-4C3HPG, (S)-4CPG, (S)-3C4HPG, and $(+)$-αM4CPG on mGluR1 were examined in more detail by determining relative potencies of these compounds in inhibiting the L-glutamate–induced IP formation. We first determined dose–response curves of the antagonist effects of phenylglycine derivative on the IP formation elicited by a constant amount $(1 \times 10^{-5}$ M) of L-glutamate (Fig. 4). The result indicated that the rank order of potencies is (S)-4C3HPG $\geq$ (S)-4CPG $\geq$ $(+)$-αM4CPG > (S)-3C4HPG. The half-maximal inhibitory concentrations (IC_{50}) of the respective compounds were calculated to be 3×10^{-5} M, 4×10^{-5} M, 7×10^{-5} M, and 4×10^{-4} M.

For more quantitative analysis of the antagonist activities of (RS)-4C3HPG, (S)-4CPG, and $(+)$-αM4CPG for mGluR1, effects of varying concentrations of these compounds in dose–response curves of L-glutamate were determined by changing concentrations of both phenylglycine derivatives and L-glutamate in mGluR1-expressing cells (Fig. 5). When the concentrations of these phenylglycine derivatives were increased, the dose–response curves of L-glutamate shifted to the right in parallel,

indicating that all these compounds act on mGluR1 in a competitive manner. The Schild plot analysis of (RS)-4C3HPG, (S)-4CPG, and $(+)$-αM4CPG gave rise to the pA_2 values of 4.38 (r = 0.999; r, correlation coefficient), 4.46 (r = 0.993), and 4.38 (r = 0.988), respectively. The slopes of the Schild plots of the respective compounds were 1.04, 1.18, and 0.94, confirming that all compounds act as competitive antagonists.

The antagonist effect of $(+)$-αM4CPG on mGluR2 was similarly quantitated by changing concentrations of both $(+)$-αM4CPG and L-glutamate added to mGluR2-expressing cells (Fig. 6). This analysis indicated the dose–response curve of L-glutamate shifts to the right by increasing the concentrations of $(+)$-αM4CPG. The Schild plot analysis of $(+)$-αM4CPG revealed a pA_2 value of 4.29 (r = 0.993) with a slope of 0.891, suggesting a competitive fashion of antagonism of this compound.

In the above experiments, in contrast to the (R)-forms of other phenylglycine derivatives, (R)-4C3HPG was found to behave as an antagonist for mGluR1 and an agonist for mGluR2. However, the (R)-4C3HPG preparation may have contained a trace amount of its enantiomer, and it could not be ascertained whether the observed effects of (R)-4C3HPG reflect its intrinsic activity or arise from the presence of a small amount of (S)-4C3HPG. In subsequent work using chiral HPLC, we have shown
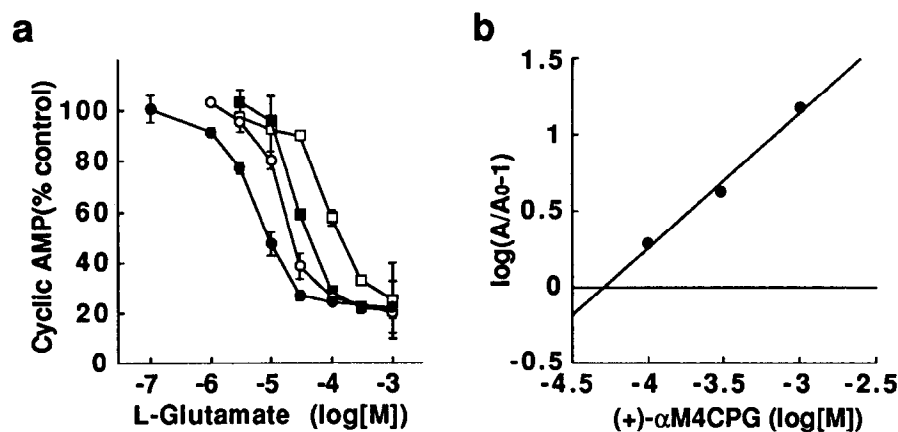
Figure 6. Dose–response curves of L-glutamate for determination of an antagonist potency of (+)-αM4CPG on mGluR2. Inhibition of forskolin-stimulated cAMP accumulation in mGluR2-expressing cells was determined by the addition of the indicated concentrations of L-glutamate alone (●) or together with 100 μM (O), 300 μM (■), and 1 mM (□) (+)-αM4CPG (*a*). Schild plot obtained from the EC_{50} values estimated from *a* is shown in *b*. For other details, see Figures 1 and 5.

that with the frequent freezing and thawing of our sample of (*R*)-4C3HPG over the 1 year period during which this work was conducted, a significant degree of racemization did indeed occur. It is likely, therefore, that the activity reported in this article for (*R*)-4C3HPG can be ascribed predominantly to contamination with the active (*S*)-isomer.

Discussion

In order to investigate the function of specific receptors in the CNS using new pharmacological tools, it is first necessary to establish the specificity of the new agonists and/or antagonists for those receptors. In the present case, the recent development of a series of phenylglycine derivatives for the investigation of mGluR function required initially that the selectivity of the new agents for metabotropic versus ionotropic glutamate receptors be established. A series of electrophysiological experiments on neonatal rat motoneurons *in vitro* and rat thalamic neurons *in*

vivo showed that while the (*R*)-forms of some of these phenylglycines (4CPG, 4C3HPG, 3C4HPG) have significant antagonist activity at NMDA (especially) and AMPA/kainate receptors, the (*S*)-forms of the same and other phenylglycines, including 3HPG and αM4CPG, have little or no such antagonist activity and could even potentiate NMDA- or AMPA-induced responses (Birse et al., 1993; Jane et al., 1993; Jones et al., 1993). The results obtained with the (*S*)-phenylglycine derivatives were consistent with the conclusion that the main effects of these compounds were mediated selectively by mGluRs (Pook et al., 1992; Birse et al., 1993; Eaton et al., 1993b; Glaum et al., 1993; Jane et al., 1993; Jones et al., 1993; Kemp et al., 1994). However, it was important to establish the activity of both forms of all of the substances at particular mGluR subtypes.

In this investigation, we determined the potencies and subtype specificity of the agonist and antagonist activities of phenylglycine derivatives by adopting clonal cell lines expressing a dif-

Table 1. Summary of EC_{50} and IC_{50} values of the agonist and antagonist activities of phenylglycine derivatives on representative mGluR1, mGluR2, and mGluR4 expressed individually in CHO cells

	Chemical structure[a]				mGluR1		mGluR2		mGluR4	
	R_1	R_2	R_3		Agonist	Antagonist	Agonist	Antagonist	Agonist	Antagonist
4C3HPG	–COOH	–OH	–H	(*S*)	–[b]	3×10^{-5} M $pA_2 = 4.38^d$	2×10^{-5} M	ND[c]	–	–
				(*R*)	–	$>10^{-3}$ M	3×10^{-4} M	ND	–	–
4CPG	–COOH	–H	–H	(*S*)	–	4×10^{-5} M $pA_2 = 4.46$	5×10^{-4} M	ND	–	–
				(*R*)	–	–	–	–	–	–
3HPG	–H	–OH	–H	(*S*)	1×10^{-3} M	ND	–	–	–	–
				(*R*)	–	–	–	–	–	–
3C4HPG	–OH	–COOH	–H	(*S*)	–	4×10^{-4} M	7×10^{-5} M	ND	–	–
				(*R*)	–	–	–	–	–	–
αM4CPG	–COOH	–H	–CH₃	(+)	–	7×10^{-5} M $pA_2 = 4.38$	–	$pA_2 = 4.29$	–	–
				(−)	–	–	–	–	–	–

[a] Basic structure is
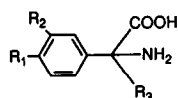
[b] No activity.
[c] Not determined.
[d] This pA_2 value was determined for a mixture of (*R*)- and (*S*)-forms of 4C3HPG.

ferent mGluR subtype. The data obtained in this investigation are summarized in Table 1. The results indicate that the phenylglycine derivatives possess different agonist and antagonist activities, dependent on the species and positions of side chains incorporated into the phenylglycine core structure. The absolute stereochemistry of αM4CPG used remains undetermined, but the (+)- and (−)-isomers of this compound are likely to correspond to the (S)- and (R)-forms, respectively. This study thus indicates that both the agonist and antagonist effects of phenylglycine derivatives on mGluRs are exerted in a stereoselective manner in that the (S)-forms are effective, but the (R)-forms have no or little activity. More importantly, our results disclose that the antagonists of phenylglycine derivatives act in a competitive manner. Although most of these compounds also show agonist effects on a different mGluR subtype, (+)-αM4CPG is a pure antagonist for both mGluR1 and mGluR2. The previous studies indicated that DL-2-amino-3-phosphonopropionate (DL-AP3) is capable of antagonizing some effects of mGluRs in neuronal preparations (Schoepp and Johnson, 1989; Birse et al., 1993). DL-AP3, however, behaves as an uncompetitive antagonist for ACPD-induced PI hydrolysis in rat hippocampal slices (Schoepp et al., 1990) and rat cerebral cortical slices (Birse et al., 1993). Moreover, this compound failed to antagonize the action of the cloned mGluR1, mGluR2, or mGluR4 expressed in CHO cells (Aramori and Nakanishi, 1992; Y. Hayashi and S. Nakanishi, unpublished observations). Thus, the phenylglycine derivatives and DL-AP3 are different in their modes of inhibitory action on mGluRs. Furthermore, (+)-αM4CPG exhibits little effect on either the NMDA receptor or the AMPA/kainate receptor (Jane et al., 1993) and thus represents the first selective antagonist for the mGluR family.

With some exceptions, discussed below, the above characterization of phenylglycine derivatives is consistent with the reports of these compounds studied in neuronal cell and slice preparations. In neonatal rat cerebral cortex slices, (S)-3HPG moderately stimulates PI hydrolysis, whereas (S)-4CPG and (RS)-αM4CPG antagonize ACPD-stimulated PI hydrolysis in a competitive manner (Birse et al., 1993; Eaton et al., 1993b). Furthermore, (S)-4CPG, (S)-4C3HPG, and (RS)-αM4CPG all antagonize ACPD-induced depolarization in neonatal rat motoneurons and ACPD-induced excitation in rat thalamic neurons (Birse et al., 1993; Eaton et al., 1993a,b; Jane et al., 1993). These findings agree well with the actions of these compounds on the PI-coupled mGluR1 subgroup found in the present work. However, some apparent inconsistencies do present themselves. Thus, (S)-4C3HPG was not found to antagonize PI hydrolysis in rat cerebral cortex slices but instead appeared to be a weak agonist (Birse et al., 1993). This response pattern of (S)-4C3HPG is apparently different from that observed for mGluR1 in this investigation, but the reason for this discrepancy remains to be determined. A more important inconsistency concerns the reported action of (+)-αM4CPG as an antagonist of ACPD-induced and L-AP4-induced depressant effects on monosynaptic excitation in neonatal rat motoneurons (Pook et al., 1993; Kemp et al., 1994). Such ACPD and L-AP4 effects (Evans et al., 1982; Cotman et al., 1986; Pook et al., 1992) are considered likely to be mediated by presynaptic mGluRs negatively coupled to the cAMP cascade (Tanabe et al., 1992, 1993; Thomsen et al., 1992; Okamoto et al., 1994). In conformity with this hypothesis, (+)-αM4CPG antagonizes both the ACPD-mediated and L-AP4-mediated inhibition of forskolin-stimulated cAMP formation in guinea pig cerebral cortical slices (Kemp et al., 1994). While the inhibitory effects of (+)-αM4CPG on the ACPD-mediated responses are consistent with the antagonist actions of this compound on the mGluR2 subgroup found in the present work, the action of (+)-αM4CPG on the L-AP4 responses cannot be explained by the properties of the L-AP4–sensitive mGluR4 reported here. The explanation of this inconsistency is currently unclear and may suggest that a different L-AP4–sensitive mGluR subtype is responsible for inducing L-AP4 responses in neonatal motoneurons and the cerebral cortex.

Recently, Bashir et al. (1993) indicated that (RS)-αM4CPG inhibits the ACPD-induced intracellular Ca^{2+} mobilization in CHO cells expressing the cloned mGluR1. They then used (RS)-αM4CPG to examine the involvement of mGluRs in long-term potentiation and indicated that synaptic activation of mGluRs is necessary for the induction of both NMDA receptor-dependent and -independent forms of long-term potentiation in the hippocampus (Bashir et al., 1993). Although the mGluR subtype involved in long-term potentiation remained to be determined, it is already clear that the phenylglycine derivative is very useful as a tool to investigate the role of mGluRs in the integrative brain functions.

The phenylglycine derivatives share the fundamental structure with L-glutamate in that they have an α-amino acid moiety and an ω-anionic group. The characteristic feature of phenylglycine derivatives is the presence of a benzene ring within this structure. This benzene ring directs the ω-anionic group into a particular steric arrangement with respect to the α-carbon moiety (Watkins et al., 1990). The conformational restriction of the glutamate chain has been shown to be very important in providing the specificity of agonists or antagonists not only among the different families of glutamate receptors but also within the members of the mGluR family (Shinozaki et al., 1989; Watkins et al., 1990; Hayashi et al., 1992). Another characteristic feature of phenylglycine derivatives is the possession of π electrons conferred by the benzene ring. These electrons may also contribute to the interaction of the phenylglycine derivatives with particular amino acids in the receptor molecules.

Several properties characteristic of different phenylglycine derivatives may also be discussed on the basis of the comparison of different side chains attached to the phenylglycine core structure. (S)-4C3HPG, (S)-4CPG, and (S)-3C4HPG all show agonist activities on mGluR2 and antagonist activities on mGluR1. The potency difference in both mGluR2 agonist and mGluR1 antagonist activities observed between (S)-4C3HPG and (S)-3C4HPG indicates the importance of the relative positions of the carboxyl group and the hydroxyl group attached to the benzene ring. In contrast, (S)-4C3HPG and (S)-4CPG show an equivalent antagonist potency for mGluR1, but the former is more potent than the latter in the agonist activity for mGluR2. This finding suggests that the presence of a hydroxyl group as a second anionic group may facilitate the interaction of (S)-4C3HPG with the mGluR2 molecule. More interestingly, the agonist activity for mGluR2 is converted into an antagonist activity for the same receptor subtype by the addition of a methyl group on the α-carbon of (S)-4CPG [namely, (+)-αM4CPG]. This methyl group must play a role in preventing activation of the receptor while still allowing binding of the molecule competitively with L-glutamate. Further modification of the phenylglycine would be interesting not only for the understanding of the structure–activity relationship of the mGluR ligands but also for development of new agonists and antagonists for this receptor family.

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