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Novel potent selective phenylglycine antagonists of metabotropic glutamate receptors

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Abstract

The metabotropic glutamate (mGlu) receptor antagonist properties of novel phenylglycine analogues were investigated in adult rat cortical slices (mGlu receptors negatively coupled to adenylyl cyclase), neonatal rat cortical slices and in cultured rat cerebellar granule cells (mGlu receptors coupled to phosphoinositide hydrolysis). (*RS*)-α-methyl-4-phosphonophenylglycine (MPPG), (*RS*)-α-methyl-4-sulphonophenylglycine (MSPG), (*RS*)-α-methyl-4-tetrazolylphenylglycine (MTPG), (*RS*)-α-methyl-3-carboxymethyl-4-hydroxyphenylglycine (M3CM4HPG) and (*RS*)-α-methyl-4-hydroxy-3-phosphonomethylphenylglycine (M4H3PMPG) were demonstrated to have potent and selective effects against 10 μM L-2-amino-4-phosphonobutyrate (L-AP4)- and 0.3 μM (2*S*,1′*S*,2′*S*)-2-(2-carboxycyclopropyl)glycine (L-CCG-1)-mediated inhibition of forskolin-stimulated cAMP accumulation in the adult rat cortex. In contrast, these compounds demonstrated either weak or no antagonism at mGlu receptors coupled to phosphoinositide hydrolysis in either neonatal rat cortex or in cultured cerebellar granule cells. These compounds thus appear to be useful discriminatory pharmacological tools for mGlu receptors and form the basis for the further development of novel antagonists.

Keywords: Metabotropic glutamate receptor; cAMP; Phosphoinositide hydrolysis; Cortex; Cerebellar granule cell; Phenylglycine

1. Introduction

Eight subtypes of metabotropic glutamate ($mGlu_{1-8}$) receptor have been cloned and demonstrated to exert their effects through G-proteins with the subsequent modulation of second messenger systems. Having no sequence homology with previously characterised G-protein-coupled receptors, except for a bovine calcium-sensing protein (Brown et al., 1993), they represent a new family of receptors (Houamed et al., 1991; Masu et al., 1991; Abe et al., 1992; Nakajima et al., 1993; Tanabe et al., 1992). MGlu receptors can be classified into 3 groups according to their second messenger association, agonist selectivity and sequence homology (Pin and Duvoisin, 1995).

Group 1 receptors ($mGlu_{1,5}$) are coupled to phosphoinositide hydrolysis with an agonist potency profile of: quisqualate > L-glutamate (L-Glu) ≥ ibotenate > (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*)-ACPD). Both the 2nd ($mGlu_{2,3}$) and 3rd ($mGlu_{4,6-8}$) receptor groups are negatively coupled to adenylyl cyclase and are thought to act as presynaptic autoreceptors regulating glutamate transmission (Forsythe and Clements, 1990; Baskys and Malenka, 1991). Group 2 receptors have an agonist rank order potency of: (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) ≥ (2*S*,1′*S*,2′*S*)-2-(2-carboxycyclopropyl)glycine (L-CCG-1) > L-Glu ≥ *trans*-ACPD > ibotenate > quisqualate and are insensitive to L-2-amino-4-phosphonobutyrate (L-AP4) (Tanabe et al., 1992, 1993; Ohishi et al., 1993; Ishida et al., 1993). Group 3 receptors show differing agonist profiles: $mGlu_4$ receptor (L-AP4 > L-serine-*O*-phosphate > L-Glu > L-CCG-1), $mGlu_6$ receptor (L-AP4 > L-serine-*O*-phosphate > L-Glu), $mGlu_7$ receptor (L-AP4 = L-serine-*O*-phosphate > L-Glu) and $mGlu_8$ receptor (L-Glu > L-AP4 > > (1*S*,*R*)-ACPD) (Tanabe et al., 1993; Nakajima et al., 1993; Okamoto et al., 1994; Saugstad et al., 1994; Duvoisin et al., 1995).

Variations in the distribution of mRNA, as demonstrated by in situ hybridisation studies, and the different pharmacological profiles for the different subtypes of mGlu

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receptors suggest that they may possess distinct functions within the central nervous system. Whilst (1*S*,3*R*)-ACPD, L-CCG-1 and L-AP4 have been demonstrated to be selective agonists for the mGlu receptor family, they are not subtype specific. Similarly, no mGlu receptor antagonist has been reported which fulfils the criteria of specificity and potency. The lack of mGlu receptor ligands has considerably hindered the elucidation of mGlu receptor subtype function and has initiated vigorous research in several laboratories.

Over the past few years, several phenylglycine derivatives have been shown to be active on mGlu receptors in cloned cell lines, neonatal rat spinal cord, neonatal and adult rat cortex (Watkins and Collingridge, 1994; Roberts, 1995). Recently, we have reported the activity of a series of phenylglycine-derived compounds active at both phosphoinositide hydrolysis and adenylyl cyclase-coupled mGlu receptors (Bedingfield et al., 1995). Based on the structure-activity relationships demonstrated by these compounds, a series of novel compounds has been developed. In this paper, we report the actions of these compounds on both phosphoinositide hydrolysis and cAMP metabolism. The effects of some of these compounds on monosynaptic excitation in neonatal rat motoneurones has already been reported (Jane et al., 1995).

2. Materials and methods

2.1. Materials

Phenylglycine derivatives were synthesised in our own laboratories (see section 2.6. for summary of syntheses and abbreviations). [8-^{3}H]Adenosine-3′,5′-cyclic phosphate (60 Ci/mmol) was purchased from Amersham International. D-*myo*-[^{3}H]inositol (20 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO. USA). Adenosine deaminase and forskolin were from Sigma (Poole, UK). Ro 20-1724, (1*S*,3*R*)-ACPD, L-AP4 and L-CCG-1 were gifts from Tocris-Cookson (Bristol, UK). Fetal calf serum, basal Eagle's medium, penicillin (10 000 U/ml), streptomycin (10 000 μg/ml) solution and versene were purchased from Gibco. 12-well culture clusters were obtained from Costar. Other chemicals were of the highest grade available

2.2. cAMP measurement in adult rat cerebrocortical slices

The effect of the compounds on mGlu receptors negatively coupled to adenylyl cyclase was investigated using a modification of previously reported methods (Brown et al., 1972; Voss and Wallner, 1992). Briefly, cerebral cortices from adult male Wistar rats (250–300 g) were removed and cross-chopped (300 μm × 300 μm) on a McIlwain chopper. Slices were allowed to recover in oxygenated Krebs bicarbonate medium (pH 7.4) for 1 h at 37°C, with four changes of medium.

Aliquots (25 μl) of gravity-sedimented slices were added to medium containing test compounds, 0.4 U of adenosine deaminase (included to minimise the activation of adenylyl cyclase via positively coupled adenosine A_2 receptors) and 0.1 mM of the specific phosphodiesterase IV inhibitor Ro 20-1724, in microfuge tubes. After incubating for 20 min at 37°C to equilibrate, 25 μl forskolin was added to give a concentration of 30 μM in a final volume of 250 μl. After 10 min, the microfuge tubes were transferred to ice, the reaction was stopped and the accumulated cAMP extracted with 100 μl 1 : 1 mixture of methanol-chloroform. After centrifugation, 100 μl of the aqueous supernatant was added to 100 μl of cAMP binding protein prepared from bovine adrenal medulla and 100 μl of [^{3}H]cAMP (20 nCi) in a total volume of 500 μl made up with 50 mM Tris-acetate buffer, pH 7.4, containing 4 mM EDTA. After incubating at 4°C for 90 min, 200 μl of 0.5% charcoal/0.2% bovine serum albumin suspension in buffer, was added. Following separation by centrifugation, a 500-μl aliquot was taken for liquid scintillation spectrometry using a Wallac 1409 spectrometer.

Agonists were used to inhibit the forskolin-stimulated rise in cAMP. Potential antagonists were then used to challenge the agonist effects of 10 μM L-AP4 and 0.3 μM L-CCG-1 (approximate EC_{50} concentrations in this system). Determinations were carried out a minimum of 4 times in quadruplicate. cAMP concentrations were determined by reference to a standard cold cAMP curve and results are expressed as a percentage of the maximum stimulation of cAMP concentration by forskolin.

2.3. Measurement of phosphoinositide hydrolysis in neonatal rat cortical slices

The method used was that previously described by Bedingfield et al. (1995). Briefly, cerebral cortices from 6–8-day neonatal Wistar rats were rapidly dissected out into ice-cold Krebs bicarbonate medium. These were then cross-chopped (300 μm × 300 μm) on a McIlwain tissue chopper, allowed to equilibrate in 250 ml Krebs at room temperature for 45 min with two changes of buffer. Slices were incubated for a further 2 h at room temperature in 15 ml Krebs medium containing 75 μCi D-*myo*-[^{3}H]inositol. Slices were then washed 5 times with 20 ml Krebs medium, allowed to settle under gravity, and the Krebs medium aspirated off following each wash. 50-μl aliquots of gravity-packed slices were transferred to tubes containing Krebs medium, antagonist and 10 mM LiCl (final volume 300 μl). After pre-incubation for 20 min at 37°C, 25 μl agonist was added, after which, the tubes were incubated for 45 min at 37°C. The reaction was terminated by the addition of; 1 ml CHCl/CHOH (1 : 2 v/v), 300 μl deionised water and 300 μl $CHCl_3$. After centrifugation, [^{3}H]inositol monophosphate ([^{3}H]IP_1) was separated from the aqueous phase by ion exchange chromatography using the formate form of Dowex-1 resin (mesh 100–200) and counted by

liquid scintillation counting. Compounds were used to antagonise (1*S*,3*R*)-ACPD-stimulated phosphoinositide hydrolysis at concentrations of 1 mM and 3 mM in order to determine K_B values. Data are presented as a percentage of basal phosphoinositide hydrolysis stimulation.

2.4. Rat cerebellar granule cell culture

Cultured cerebellar granule cells were prepared from 8-day-old rat cerebella as described previously (Toms et al., 1995). Dissociated cells were plated onto poly-L-lysine-coated 12-well culture dishes (1.1×10^6 cells/well), in basal Eagle's medium supplemented with fetal calf serum (10% v/v), KCl (25 mM), L-glutamine (4 mM), penicillin (50 U/ml) and streptomycin (50 μg/ml)). Cells were incubated at 37°C in a humidified atmosphere of 6% CO_2 – 94% air. Cytosine β-D-arabino-furanoside (10 μM) was added 20 h after plating to prevent glial cell proliferation.

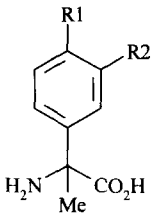
2.5. Measurement of phosphoinositide hydrolysis in cultured granule cells

Phosphoinositide hydrolysis in granule cells was determined by monitoring the accumulation of $[^3H]IP_1$ in the presence of LiCl (10 mM) in a modified protocol as described by Aronica et al. (1993). Cultures (4–6 days old) were pre-labelled with 2 μCi/ml D-*myo*-[^{3}H]inositol for 24 h. The culture medium was then removed and the cells washed extensively with buffer (composition, mM: NaCl 154, KCl 5.6, $MgSO_4$ 1, $NaHCO_3$ 3.6, glucose 5.6, $CaCl_2$ 1.3, HEPES 5, LiCl 10, pH 7.4). To each well, 500 μl of buffer, supplemented with 50 μM D-2-amino-5-phosphonopentanoic acid (D-AP5), 30 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 1 μM tetrodotoxin, was added. If required, the antagonist was then added, immediately followed by the agonist, and incubated at 37°C for 30 min. The assay was terminated by buffer aspiration and the addition of ice-cold 500 μl $HClO_4$ (7.5% v/v) and left on ice for 15 min. The cell extract was then neutralized with Na_2CO_3 and $[^3H]IP_1$ isolated via ion exchange chromatography and quantified by liquid scintillation spectrometry.

2.6. Synthesis and purification of phenylglycine compounds

All α-methyl phenylglycine analogues used in this study, α-methyl-4-hydroxyphenylglycine (M4HPG), α-methyl-4-phosphonophenylglycine (MPPG), α-methyl-4-sulphonophenylglycine (MSPG), α-methyl-4-tetrazolylphenylglycine (MTPG), α-methyl-4-hydroxy-3-phosphonomethylphenylglycine (MCM4HPG) and α-methyl-3-phosphonomethyl-4-hydroxyphenylglycine (M4H3PMPG) (Table 1), were synthesized by a Bucherer-Bergs reaction on the appropriately substituted acetophenone using the method of Allan et al. (1990). The intermediate hydantoins so formed were hydrolysed in 6 N HCl and the amino acid purified by ion-exchange chromatography and crystallisation from a suitable solvent. All compounds had satisfactory physico-chemical data.

Table 1
Structures of phenylglycine-derived compounds



Compound		R1	R2
α-Methyl-4-hydroxyphenylglycine	M4HPG	OH	H
α-Methyl-4-phosphonophenylglycine	MPPG	PO_3H_2	H
α-Methyl-4-sulphonophenylglycine	MSPG	SO_3H	H
α-Methyl-4-tetrazolylphenylglycine	MTPG	Tetrazolyl	H
α-Methyl-3-carboxymethyl-4-hydroxyphenylglycine	M3CM4HPG	OH	CH_2COOH
α-Methyl-4-hydroxy-3-phosphonomethylphenylglycine	M4H3PMPG	OH	$CH_2PO_3H_2$

3. Results

3.1. Antagonism of mGlu receptors negatively coupled to adenylyl cyclase in adult rat cortical slices

The effects of the compounds on L-AP4- and L-CCG-1-mediated inhibition of forskolin-stimulated cAMP are shown in Fig. 1Fig. 2, respectively (IC_{50} values summarised in Table 2). The rank order of antagonist potency against both agonists is: MPPG > MSPG > M3CM4HPG > M4H3PMPG > MTPG (M4HPG inactive). However, when the potencies against each agonist are compared, MPPG is more than twice as potent against L-CCG-1 (69.5 ± 0.5 nM) as compared to L-AP4 (156 ± 29 nM). MSPG is approximately equipotent against both agonists (170 ± 76 nM and 163 ± 41 nM), respectively. M3CM4HPG is slightly more potent against L-CCG-1 (311 ± 62 nM) than L-AP4 (463 ± 71 nM). M4H3PMPG is approximately 10-fold more potent against L-AP4 (2.23 ± 1.24 μM) than L-CCG-1 (25.6 ± 6.4 μM). MTPG is also 10 times more potent against L-AP4 (59.4 ± 7. μM) than L-CCG-1 (607 ± 135 μM).

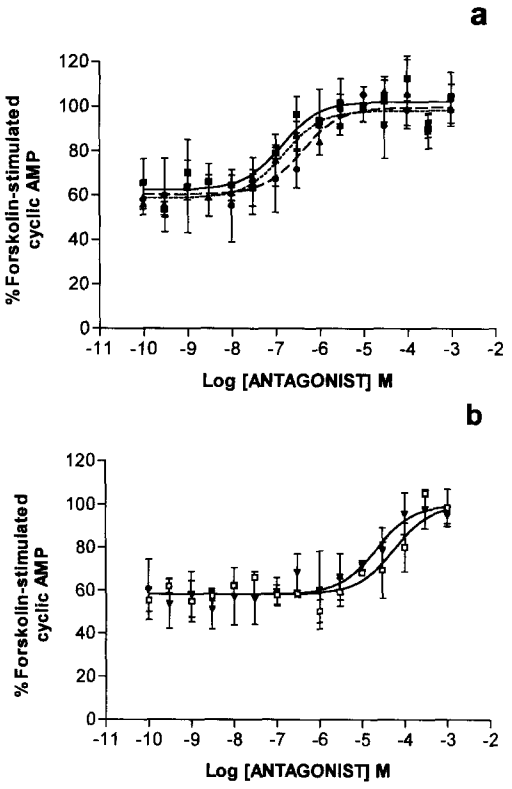


Fig. 1. Dose-response curves for the antagonism by phenylglycine compounds of (Fig. 1) L-AP4 (10 μM)- and (Fig. 2) L-CCG-1 (0.3 μM)-induced inhibition of forskolin-stimulated cAMP production via mGlu receptors present in adult rat cortical slices: (a) MPPG, ——■; MSPG, ...▲; M3CM4HPG, ------●; (b) M4H3PMPG, ▼; MTPG, □. All data are means ± S.E.M. of 4 experiments performed in quadruplicate.

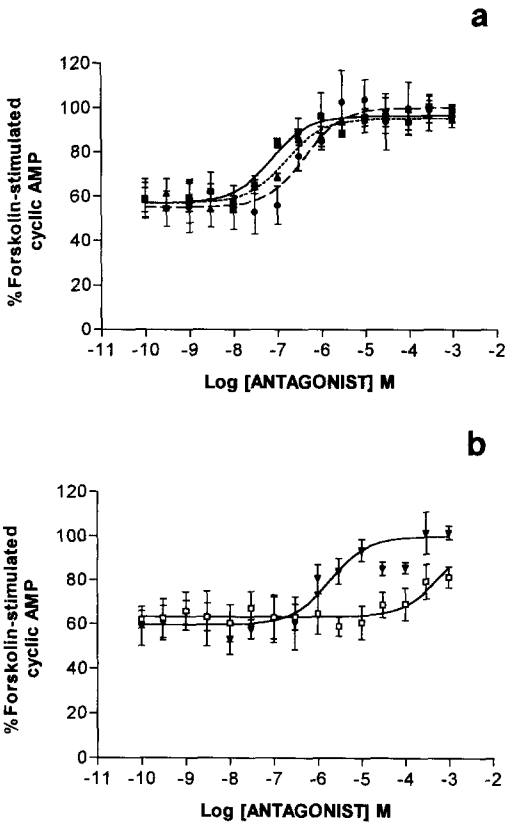


Fig. 2. For legend, see Fig. 1.

3.2. Antagonism of mGlu receptors coupled to phosphoinositide hydrolysis in neonatal rat cortical slices

MPPG, MTPG, MSPG and M3CM4HPG at 1 mM and 3 mM were used to challenge the effect of (1*S*,3*R*)-ACPD (Fig. 3a–d). All were antagonists of moderate potency causing a parallel displacement of the concentration-response curve allowing K_B values to be calculated. The derived rank order of potency ($K_B \pm$ S.E.M.): MPPG (0.91 ± 0.11 mM) > MTPG (1.00 ± 0.16 mM) > MSPG (1.17 ± 0.34 mM) > M3CM4HPG (1.83 ± 0.25 mM). We were unable to test M4H3PMPG due to limited availability. These results are summarised in Table 3.

3.3. Action of compounds at mGlu receptor(s) coupled to phosphoinositide hydrolysis in cultured granule cells

MPPG, MSPG, MTPG and M3CM4HPG were tested at 100 μM against the agonist quisqualate at its EC_{50} concentration of 2 μM. Results are expressed as percentage of quisqualate-stimulated phosphoinositide hydrolysis. All had negligible effects, the results are summarised in Fig. 4. Other phenylglycine compounds were also tested at 100 μM for antagonist properties. Only 4CPG (35.3 ± 3.7% of

Table 2
Summary of antagonist effects of novel phenylglycine compounds on mGlu receptors negatively coupled to adenylyl cyclase in adult rat cortical slices

Compound	10 μM L-AP4	0.3 μM L-CCG-1
MPPG	156 ± 29 nM	69.5 ± 0.5 nM
MSPG	170 ± 76 nM	163 ± 41 nM
M3CM4HPG	463 ± 71 nM	311 ± 62 nM
M4H3PMPG	2.23 ± 1.24 μM	25.6 ± 6.4 μM
MTPG	59.4 ± 7.3 μM	607 ± 135 μM
M4HPG	No effect	No effect

Data are IC_{50} (± S.E.M.) concentrations derived from at least $n = 4$ experiments performed in quadruplicate. Agonist concentrations are approximate EC_{50} concentrations in this system.

Table 3
Summary of antagonist effects of novel phenylglycine compounds on 1*S*,3*R*-ACPD acting at mGlu receptors positively coupled to phosphoinositide hydrolysis in neonatal rat cortical slices

Compound	K_B
MPPG	0.91 ± 0.11 mM
MTPG	1.00 ± 0.16 mM
MSPG	1.17 ± 0.34 mM
M3CM4HPG	1.83 ± 0.25 mM

Data are K_B (± S.E.M.) concentrations derived from at least $n = 3$ experiments.

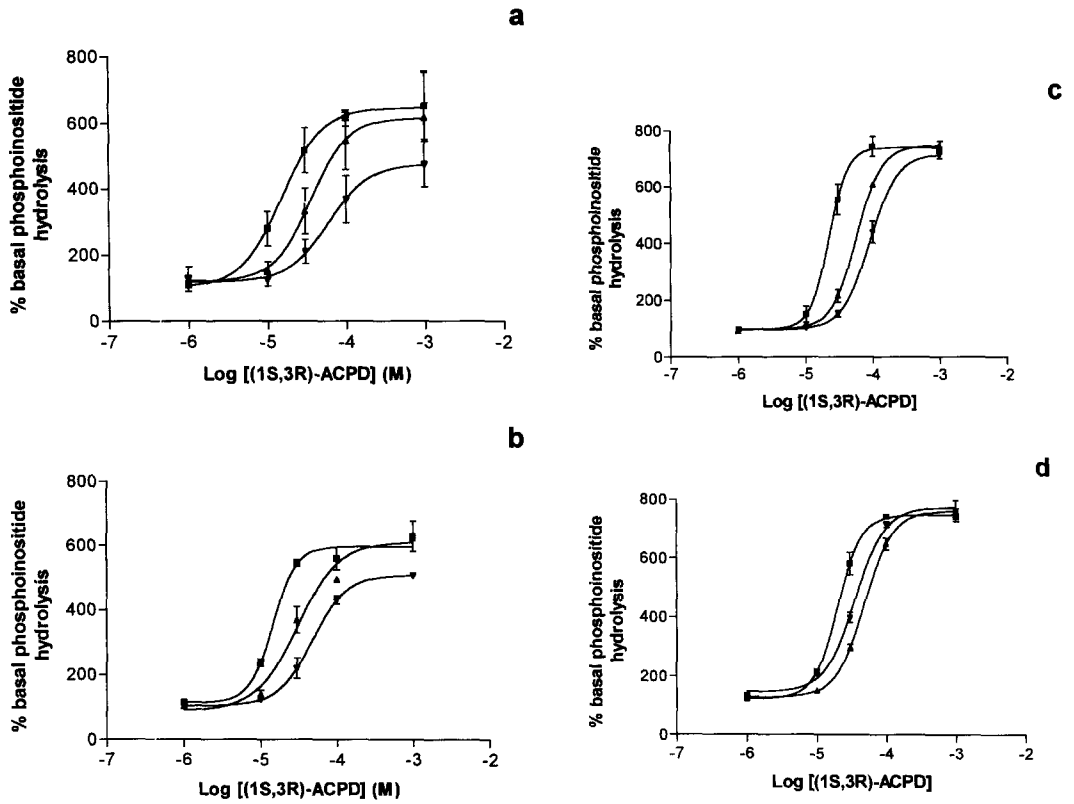


Fig. 3. Antagonism of mGlu receptor-mediated increase in phosphoinositide production in neonatal rat cortical slices following stimulation by (1*S*,3*R*)-ACPD. (a) (1*S*,3*R*)-ACPD, ■; +1 mM MPPG, ▲; +3 mM MPPG, ▼. (b) (1*S*,3*R*)-ACPD, ■; +1 mM MSPG, ▲; +3 mM MSPG, ▼. (c) (1*S*,3*R*)-ACPD, ■; +1 mM MTPG, ▲; +3mM MTPG, ▼. (d) (1*S*,3*R*)-ACPD, ■; +1 mM M3CM4HPG, ▼; +3 mM M3CM4HPG, ▲. All data are means ± S.E.M. of 3 experiments performed in triplicate.

Table 4
Rank orders of potency of phenylglycine analogues as antagonists of mGlu receptor group specific agonists in different tissue preparations

Coupling	Adenylyl cyclase				Phosholipase C	
Tissue	Adult rat cortex		Neonatal spinal cord		Neonatal cortex	Granule cells
Rank	L-CCG-1	L-AP4	(1*S*,3*S*)-ACPD	L-AP4	(1*S*,3*R*)-ACPD	L-Quisqualate
1	MPPG	MPPG	MTPG [b]	MPPG [b]	(+)-M4CPG [a]	(*S*)-4CPG
2	MSPG	MSPG	MPPG [b]	MSPG [b]	4C2IPG	4C2IPG
3	M3CM4HPG	M3CM4HPG	MSPG [b]	MTPG [b]	(*S*)-4CPG [a]	(+)-M4CPG
4	M3CMPG [a]	M3CMPG [a]	(+)-M4CPG [a]	(+)-M4CPG [a]	MPPG	
5	M3C4HPG [a]	M4H3PMPG			M4C3HPG [a]	
6	(+)-M4CPG [a]	M3C4HPG [a]			MTPG	
7	E4CPG [a]	(+)-M4CPG [a]			M4CMPG [a]	
8	M3CPG [a]	E4CPG [a]			MSPG	
9	M4H3PMPG	M3CPG [a]			M3CM4HPG	
10	M4CMPG [a]	MTPG			M4C3C1PG [a]	
11	(−)M4CPG [a]	M4C3ClPG [a]			M3CMPG [a]	
12	MTPG	(−)M4CPG [a]			M3CPG [a]	
13	M4C3ClPG [a]	M4CMPG [a]			M3C4HPG [a]	

Abbreviations: (*RS*)-α-methyl-4-phosphonophenylglycine (MPPG; (*RS*)-α-methyl-4-sulphonophenylglycine (MSPG); (*RS*)-α-methyl-3-carboxymethyl-4-hydroxyphenylglycine (M3CM4HPG); (*RS*)-α-methyl-3-carboxymethylphenylglycine (M3CMPG); (*RS*)-α-methyl-3-carboxy-4-hydroxyphenylglycine (M3C4HPG); (+)-α-methyl-4-carboxyphenylglycine (M4CPG); (*RS*)-α-ethyl-4-carboxyphenylglycine (E4CPG); (*RS*)-α-methyl-3-carboxyphenylglycine (M3CPG); (*RS*)-α-methyl-4-hydroxy-3-phosphonomethylphenylglycine (M4H3PMPG); (*RS*)-α-methyl-4-carboxymethylphenylglycine ((+)-M4CMPG); (*RS*)-α-methyl-4-tetrazolylphenylglycine (MTPG); (*RS*)-α-methyl-4-carboxy-3-chlorophenylglycine (M4C3ClPG); (*S*)-4-carboxyphenylglycine ((*S*)-4CPG); (*RS*)-4-carboxy-2-iodophenylglycine (4C2IPG).
Previously published data from (a) Bedingfield et al. (1995); (b) Jane et al. (1995).

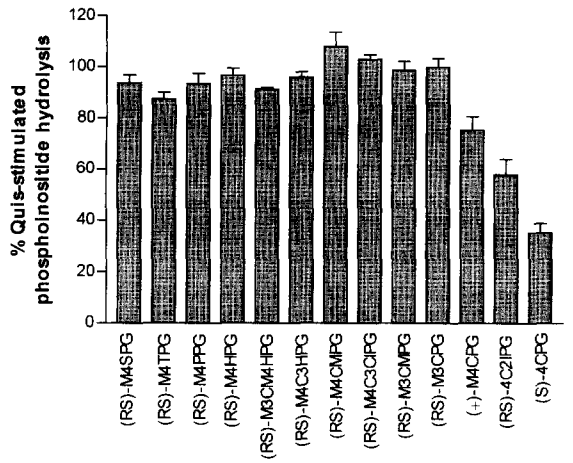


Fig. 4. Effects of several phenylglycine derivatives (100 μM) on 2 μM quisqualate-stimulated [^{3}H]inositol monophosphate production in cultured rat cerebellar granule cells (4–6 days in vitro). All data are means ± S.E.M. of 3 experiments performed in triplicate. See legend to Table 4 for full names of abbreviations.

control stimulation), (*RS*)-4-carboxy-2-iodophenylglycine (4C2IPG) (58.0 ± 6.0%) and M4CPG (75.3 ± 5.4%) showed antagonist activity.

4. Discussion

Previously, we have demonstrated that phenylglycine analogues can act as agonists and/or antagonists in various preparations. They have been found to have varying specificity and efficacy, according to their stereochemistry, ω-acidic group, positions of substituents and inter-acidic chain length. By examining the structural features required for antagonism, novel compounds based on the phenylglycine structure have been developed with the object of enhancing specificity and potency towards the differing mGlu receptor groups and individual subtypes. It was with this objective in mind that MPPG, MSPG, MTPG, M3CM4HPG and M4H3PMPG were synthesized. The data presented in this paper (summarised in Table 4) permit some conclusions to be drawn as to the structural requirements of compounds acting at differing mGlu receptor subtypes. These new data must be considered in conjunction with previously published data on other phenylglycine compounds (Bedingfield et al., 1995; Jane et al., 1995; Hayashi et al., 1994; Watkins and Collingridge, 1994).

In the present study, mGlu receptor-stimulated phosphoinositide hydrolysis in neonatal rat cortical slices, most likely reflects the activity of $mGlu_5$ since $mGlu_1$ receptor mRNA distribution is sparse in the neonatal rat cortex (Nomura et al., 1993; Catania et al., 1994). The mGlu receptor(s) coupled to phosphoinositide hydrolysis in cultured rat cerebellar granule cells is unclear at present. In our hands, 1 mM 4C3HPG, a reported $mGlu_{5a}$ receptor partial agonist and $mGlu_{1a}$ receptor antagonist (Brabet et al., 1995), fails to stimulate phosphoinositide hydrolysis (Toms et al., 1995). Additionally, cultured granule cells have been reported to contain high levels of $mGlu_1$ receptor mRNA and negligible amounts of $mGlu_5$ receptor mRNA (Santi et al., 1994; Prézeau et al., 1994). In contrast, recent immunocytochemical data have indicated prevalent $mGlu_5$ receptor expression in cultured granule cells at 4 days in vitro (Copani et al., 1995). mGlu receptors negatively coupled to adenylyl cyclase in adult rat cerebral cortical slices, are likely to be $mGlu_3$ and $mGlu_7$ receptors based on reported mRNA localization and immunohistochemical data. However, caution needs to be observed as other, as yet undescribed, mGlu receptor subtypes may also be present.

There appear to be considerable differences in the structural requirements for group 1 and group 2/3 mGlu receptor antagonism. Most striking is the preference of group 1 receptor(s) present in neonatal rat cortex and cultured cerebellar granule cells for a planar carboxyl group in the 4 position of the phenyl ring. Although the tetrazole group of MTPG is planar, this compound has a longer inter-acidic chain length (a more distal N-atom in the tetrazole ring being possibly responsible for receptor interaction). Any variation from this geometry, i.e. for the tetrahedral geometry of phosphono or sulphono groups, markedly decreases potency. In contrast, an opposite effect is seen for adenylyl cyclase-coupled mGlu receptors where ω-acidic groups with tetrahedral geometry and lower pK_a values appear to be more potent. An α-alkyl group is essential for antagonism of group 2/3 mGlu receptors, but is not vital for group 1 mGlu receptor antagonism (although its presence does enhance antagonist potency (M4CPG > 4CPG) in neonatal rat cortical slices). In contrast, an α-methyl group reduces antagonist potency at group 1 mGlu receptor(s) in cultured cerebellar granule cells (4CPG > M4CPG with other α-methyl-substituted phenylglycines being inactive). It is noteworthy that group 2 mGlu receptors have been reported to augment group 1 mGlu receptor-stimulated phosphoinositide hydrolysis in hippocampal slices, without any significant effect in either adult rat cortical slices or cultured cerebellar granule cells (Genazzani et al., 1994). However, we cannot exclude the possibility of a group 2 mGlu receptor facilitatory action on phosphoinositide hydrolysis in the neonatal rat cortex in the present study. It is therefore conceivable that the increased potency of M4CPG (compared to 4CPG) in the neonatal cortex could be due to an additional removal of a group 2 mGlu receptor facilitatory action.

One characteristic common to all mGlu receptors is the stereospecific nature of the antagonism; the (+)-form (believed to be the (*S*)-enantiomer) of M4CPG is the more active enantiomer (Hayashi et al., 1994; Jane et al., 1993). Inter-acidic chain length and conformational effects are also important since M4CMPG is less potent than M4CPG at group 2/3 mGlu receptors in the cortex and conversely, M3CMPG is more potent than M4CPG (both have similar

chain lengths) at either group 2 or 3 mGlu receptors in the cortex.

The neonatal rat spinal cord preparation has been used to study mGlu receptors of group 2/3 by observing the reversal of the monosynaptic depression produced by (1*S*,3*S*)-ACPD (group 2) and L-AP4 (group 3) (Pook et al., 1992; Kemp et al., 1994). Previously, the antagonist actions of MPPG, MSPG, MTPG and M4CPG have been described in this preparation. Against L-AP4 antagonist orders of potency were: MPPG > MSPG > MTPG > M4CPG, and against (1*S*,3*S*)-ACPD: MTPG > MPPG > MSPG > M4CPG (Kemp et al., 1994; Jane et al., 1995). The salient features of these spinal cord data are: (1) the potent action of MTPG against (1*S*,3*S*)-ACPD (in contrast to its weak action against L-CCG-1 in the cortex) and (2) the selectivity of MPPG against L-AP4 responses (in contrast to the slight selectivity of MPPG for group 2 mGlu receptors in the cortex). Possible explanations are: (a) the existence of different mGlu receptor subtypes; or (b) differences in the receptor signalling mechanism in these preparations. Further work is required fully to explain this observation.

In summary, from these data we can conclude that; MPPG, MSPG and M3CM4HPG are potent and selective antagonists which may be used to discriminate between group 2/3 and group 1 mGlu receptors in the rat CNS. In rat cortex and spinal cord, MPPG is the most potent antagonist of L-AP4-sensitive mGlu receptors and is also the most potent antagonist of the rat cortical L-CCG-1-sensitive mGlu receptor yet reported. These compounds represent an important advance on what has become the standard mGlu receptor antagonist, M4CPG.

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