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Structure-activity relationships for a series of phenylglycine derivatives acting at metabotropic glutamate receptors (mGluRs)

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1 The actions of a series of twelve phenylglycine derivatives at metabotropic glutamate receptors (mGluRs) linked to both phosphoinositide hydrolysis (PI) and cyclic AMP were investigated.

2 PI hydrolysis was determined by the accumulation of [^{3}H]-inositol-monophosphate ([^{3}H]-IP$_1$) in neonatal rat cortical slices prelabelled with [^{3}H]-*myo*-inositol. The non-selective mGluR agonist (1S,3**R**)-1 - aminocyclopentane - 1,3 - dicarboxylic acid ((1S,3**R**)-ACPD) produced a concentration-dependent increase in [^{3}H]-IP$_1$ (EC_{50} ≈ 20 μM). This agonist was subsequently used to investigate potential antagonist activity of the phenylglycine derivatives. Of the compounds tested (+)-α-methyl-4-carboxyphenylglycine (M4CPG) and (**RS**)-α-ethyl-4-carboxyphenylglycine (E4CPG) were the most active with K_B values of 0.184 ± 0.04 mM and 0.367 ± 0.2 mM respectively.

3 Activity at adenylyl cylase-coupled mGluRs was investigated by determining the accumulation of [^{3}H]-cyclic AMP in adult rat cortical slices. [^{3}H]-cyclic AMP accumulation, elicited by 30 μM forskolin, was inhibited by (2S,3S,4S)-α-(carboxycyclopropyl)glycine (L-CCG-1) and L-2-amino-4-phosphonobutanoate (L-AP4) with respective EC_{50} values of 0.3 μM and 10 μM. Neither agonist was able to inhibit completely forskolin stimulated cyclic AMP accumulation; this is evidence that not all adenylyl cyclase is susceptible to modulation by mGluRs. Phenylglycine derivatives were examined for their ability to antagonize the inhibition of [^{3}H]-cyclic AMP accumulation by L-CCG-1 or L-AP4 at their EC_{50} concentrations.

4 A rank order of potency of the phenylglycine derivatives as antagonists of L-AP4 and L-CCG-1 was obtained. The most effective compound, (**RS**)-α-methyl-3-carboxymethylphenylglycine (M3CMPG) had IC_{50} values in the order of 1 μM against L-AP4 and 0.4 μM against L-CCG-1.

5 The results from this study indicate that phenylglycine-derived compounds can discriminate between groups of metabotropic glutamate receptors and may also display some selective activity between subtypes within groups. Future work based on these findings may lead to the development of more selective and potent compounds as important pharmacological tools.

Keywords: Metabotropic glutamate receptors; cyclic AMP; phosphoinositide turnover; L-AP4; L-CCG-1, (1S,3**R**)-ACPD; phenylglycine

Introduction

L-Glutamate is the dominant excitatory neurotransmitter within the vertebrate central nervous system (Monaghan *et al.*, 1989) and its receptors can be classified into two main groups, namely: ionotropic glutamate receptors (N-methyl-D-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate and kainate) and metabotropic glutamate receptors (mGluRs).

The mGluRs exert their effects through coupling to G-proteins and the subsequent activation of second messenger systems. Structurally they all possess seven membrane spanning domains (analogous to other G-protein linked receptors) separated by short intracellular and extracellular loops. However, apart from some similarity to a bovine Ca^{2+}-sensing receptor (Brown *et al.*, 1993), mGluRs show no sequence homology to the already classified G-protein coupled receptors and therefore 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).

To date eight subtypes of mGluRs (mGluR1-8) have been cloned and stably expressed in cell lines. These can be classified into three groups according to their second messenger association, agonist selectivity and sequence homology (for review see Pin & Duvoisin, 1995).

The first group (Group 1) comprising mGluRs 1 and 5 have the same signal transduction mechanism and are predominantly located postsynaptically (Shigemoto *et al.*, 1993; Catania *et al.*, 1994). Both couple to phosphoinositide (PI) hydrolysis, whereby activation of the G-protein leads to the breakdown of phosphatidylinositol-bis-phosphate (PIP_2) and the subsequent formation of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Interaction of IP_3 with its specific receptors on the membrane of the endoplasmic reticulum results in the release of calcium. DAG causes, amongst other effects, the activation of protein kinase C (PKC) leading to protein phosphorylation of an array of substrate proteins.

mGluRs 1 and 5 are found to have 60.8% sequence homology (Okamoto *et al.*, 1994) and have similar potencies for agonists, with the rank order being: quisqualate > L-glutamate ≥ ibotenate > (2S,3S,4S)-α-(carboxycyclopropyl)-glycine-(L-CCG-1) > (1S,3**R**)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3**R**)-ACPD) when expressed in Chinese hamster ovary (CHO) cells (Abe *et al.*, 1992).

The second and third groups of mGluRs, comprising mGluRs 2 and 3, and mGluRs 4, 6, 7 and 8 respectively, are all negatively coupled to adenylyl cyclase and their activation leads to a reduction in the formation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and a consequent inhibition of processes mediated by cyclic AMP. Both of these groups of mGluRs are thought to act presynaptically as autoreceptors regulating glutamate transmission (Forsythe & Clements, 1990; Baskys & Malenka, 1991).

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Agonists acting at group II mGluRs (2 and 3), when expressed in (CHO) cells, have a rank order of potency of: L-CCG-1 > L-glutamate ≥ *trans*-ACPD > ibotenate > quisqualate (Tanabe *et al.*, 1992). These receptors are insensitive to L-2-amino-4-phosphonobutanoate (L-AP4). mGluR3 mRNA has a different pattern of expression from mGluR2, mGluR3 being predominant in the cortex with mGluR2 having a significant level of expression in cortical granule cells and pyramidal cells (Tanabe *et al.*, 1992; 1993; Ohishi *et al.*, 1993).

The third group consists of mGluR 4, 6, 7 and 8; these receptors are also negatively linked to adenylyl cyclase but show different pharmacological profiles and mRNA expression patterns from members of the second group. The rank order of potency of agonists at mGluR4 in CHO cells is: L-AP4 > Glu ≥ L-CCG-1 ≥ L-serine-O-phosphate (SOP) (Tanabe *et al.*, 1993). mGluR6 has a slightly different agonist potency profile namely, L-AP4 > SOP > Glu and it is exclusively restricted to the retina (Nakajima *et al.*, 1993). The most recently described subtype, mGluR7 (Okamoto *et al.*, 1994; Saugstad *et al.*, 1994), has an agonist profile similar to mGluR6 when expressed in CHO cells (Okamoto *et al.*, 1994). When expressed in baby hamster kidney (BHK) cells, L-AP4 was again more potent than glutamate at reducing cyclic AMP levels, but lower overall potencies were observed (Saugstad *et al.*, 1994). The authors attributed this to either low receptor expression or less efficient coupling to adenylyl cyclase which may indicate that coupling of mGluR7 to adenylyl cyclase is not its primary transduction mechanism.

In order to elucidate the functions of different mGluR subtypes in the CNS, it is necessary to have specific and selective pharmacological tools. (1S,3R)-ACPD, L-CCG-1 and L-AP4 are selective agonists for the mGluR family. Recently phenylglycine-derived agonists and antagonists of mGluRs in cloned cell lines, rat neonatal spinal cord, phosphoinositide (PI) linked mGluRs in rat neonatal cortex and adenylyl cyclase linked mGluR in adult rat cortex have been developed (Watkins *et al.*, 1987; Birse *et al.*, 1992; Hayashi *et al.*, 1994). (±)-α-Methyl-4-carboxyphenylglycine (M4CPG) is a stereoselective antagonist of moderate potency (Eaton *et al.*, 1993; Jane *et al.*, 1993; Kemp *et al.*, 1994), but limited selectivity. It affects both PI and adenylyl cyclase-coupled mGluRs although, in common with other phenylglycines it is devoid of activity at mGluR4 (Hayashi *et al.*, 1994).

Kingston *et al.* (1995) have recently shown that the antagonists (S)-4-carboxy-3-hydroxyphenylglycine (4C3HPG), (S)-4-carboxyphenylglycine (4CPG) and M4CPG exhibit differential potencies at the PI-linked mGluRs 1α and 5a, with each demonstrating higher potencies at the mGluR1α subtype. These compounds are, however, also active at the cyclic AMP linked mGluRs (Pook *et al.*, 1993).

Compounds which exhibit high potency and selectivity for a particular group of mGluRs are lacking. Here we describe the actions of twelve phenylglycine derivatives on (i) PI hydrolysis in rat neonatal cortical slices. This most probably reflects their activity at mGluR5 since mGluR1 is poorly expressed in the cortex; (ii) on mGluRs negatively coupled to adenylyl cyclase in adult rat cerebral cortical slices which, from reported mGluR mRNA expression and immunohistochemical data, should contain subtypes mGluR3 and 7. Caution, however, needs to be observed as other as yet undescribed mGluR subtypes may be present.

Methods

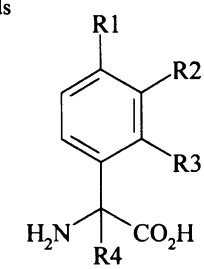
Measurement of PI hydrolysis

The method used was that previously described by Birse *et al.* (1993). Briefly, cerebral cortices from 6–8 day neonatal Wistar rats were rapidly dissected out, cross-chopped (300 μm × 300 μm) and allowed to equilibrate at room temperature for 45 min in 250 ml oxygenated Krebs bicarbonate medium (pH 7.4) with two changes of buffer. Slices were then incubated for 2 h at room temperature in 15 ml buffer containing 75 μCi D-*myo*-[^{3}H]-inositol (20 Ci mmol^{-1}). Slices were allowed to settle under gravity and washed in 5 × 20 ml medium. Aliquots (50 μl) of 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, in the presence of the antagonist, 25 μl agonist was added before a final incubation for 45 min at 37°C.

The reaction was terminated by the addition of: 1 ml $CHCl_3/CH_3OH$ (1:2 v/v), 300 μl deionised water and 300 μl $CHCl_3$. After centrifugation, [^{3}H]-IP_1 was separated from the aqueous phase by ion exchange chromatography by use of the formate form of Dowex-1-chloride (mesh 100–200) and counted in a Wallac 1409 liquid scintillation spectrometer.

Compounds were initially tested for agonist activity at a concentration of 1 mM and for their ability to antagonize 20 μM (1S,3R)-ACPD-stimulated PI hydrolysis (approximate EC_{50} value in this assay) at concentrations of 50 μM and 3 mM.

Table 1 Structures of phenylglycine derived compounds



Phenylglycine

Compound		*R1*	*R2*	*R3*	*R4*
3-Carboxyphenylglycine	3CPG	H	COOH	H	H
α-Methyl-3-carboxyphenylglycine	M3CPG	H	COOH	H	CH_3
α-Methyl-3-carboxy-4-hydroxyphenylglycine	M3C4HPG	OH	COOH	H	CH_3
α-Methyl-3-carboxymethylphenylglycine	M3CMPG	H	CH_2COOH	H	CH_3
4-Carboxyphenylglycine	4CPG	COOH	H	H	H
2-Iodo-4-carboxyphenylglycine	2I4CPG	COOH	H	I	H
α-Methyl-4-carboxyphenylglycine	M4CPG	COOH	H	H	CH_3
α-Methyl-3-hydroxy-4-carboxyphenylglycine	M3H4CPG	COOH	OH	H	CH_3
α-Methyl-3-chloro-4-carboxyphenylglycine	M3Cl4CPG	COOH	Cl	H	CH_3
α-Methly-4-carboxymethylphenylglycine	M4CMPG	CH_2COOH	H	H	CH_3
α-Ethyl-4-carboxyphenylglycine	E4CPG	COOH	H	H	CH_2CH_3

Those compounds which were found to be active were investigated further in order to determine K_B values. Data are presented as a percentage of basal PI stimulation.

Determination of phenylglycine activity at mGluRs negatively coupled to adenylyl cyclase

The effect of compounds on adenylyl cyclase-linked mGluRs was investigated by use of a modification of the method previously described (Kemp *et al.*, 1994). Briefly, cerebral cortices from adult male Wistar rats (250–300 g) were removed, cross chopped (300 × 300 μm) and incubated in oxygenated Krebs bicarbonate medium (pH 7.4) for 1 h at 37°C, with four changes of medium. Slices were then resuspended in 20 ml medium per cortex containing 40 μCi [8-^{3}H]-adenine (25 Ci mmol^{-1}) and incubated for 40 min. Slices were sedimented under gravity and washed with 3 × 50 ml of medium. Aliquots (50 μl) of slices were added to medium containing test compounds and 0.8 units of adenosine deaminase (to minimize endogenous adenosine activating cyclic AMP production via adenosine A_2 receptors) in microfuge tubes. After incubating for 20 min at 37°C, 25 μl forskolin was added to give a concentration of 30 μM in a final volume of 250 μl. After a further 10 min incubation, the microfuge tubes were transferred to ice and 200 μl 1 M HCl added followed by 750 μl H_2O. The slices were homogenized, pelleted in a microcentrifuge and 1 ml supernatant applied to a minicolumn containing 1 ml Dowex 50x-400. Each column was calibrated by application of [^{3}H]-cyclic AMP. The cyclic AMP-containing fraction was eluted with 4 ml H_2O and adsorbed onto 0.5 ml alumina. The [^{3}H]-cyclic AMP synthesized from the labelled adenine was then eluted with 4 ml 0.1 M imidazole and determined by liquid scintillation spectrometry.

Agonists (i.e. inhibiting adenylyl cyclase via mGluR activation) were tested against the forskolin-stimulated rise in cyclic AMP. Potential antagonists were examined in the presence of either 10 μM L-AP4 or 0.3 μM L-CCG-1 (approximate EC_{50} values in this system). Determinations were carried out a minimum of three times performed in triplicate. Data were analysed by use of GraphPAD-PRISM and Lotus 1-2-3.

Synthesis and purification of phenylglycine compounds

The phenylglycine derivatives used in this study, the structures of which are given in Table 1, were obtained as follows: (**RS**)-2-iodo-4-carboxyphenylglycine ((**RS**)-2I4CPG), (**S**)-4-carboxyphenylglycine ((**S**)-4CPG), (**RS**)-3-carboxyphenylglycine ((**RS**)-3CPG) and (+)- and (−)-α-methyl-4-carboxyphenylglycine (M4CPG) were prepared according to the methods described by Hayashi *et al.* (1994). The (**RS**)-α-methyl and α-ethyl phenylglycine derivatives were prepared by the Bucherer-Bergs synthesis (Allan *et al.*, 1990) from the corresponding substituted acetophenone or propiophenone (for (**RS**)-E4CPG) derivatives followed by acid hydrolysis of the intermediate hydantoins. All phenylglycine derivatives were purified by ion-exchange chromatography and crystallisation from an appropriate solvent (usually water). All compounds had ^{1}H and ^{13}C n.m.r. spectra and elemental analytical data consistent with the proposed structure.

Chemicals Phenylglycine compounds were synthesized in our own laboratories (see methods). D-*myo*-[^{3}H]-inositol (20 Ci mmol^{-1}) was purchased from ARC (St. Louis). [8-^{3}H]-adenine (25 Ci mmol^{-1}) and [8-^{3}H]-adenosine 3′, 5′ -cyclic phosphate (60 Ci mmol^{-1}) were purchased from Amersham International plc. Adenosine deaminase, Dowex 50W, Dowex-1-chloride alumina and forskolin were from Sigma (Poole). (1S,3R)-ACPD, L-AP4 and L-CCG-1 were gifts from Tocris-Cookson (Bristol). 3-Isobutyl-1-methyl-xanthine (IBMX) was from Aldrich Chemical Co. Other chemicals were of the highest grade available.

Results

Table 1 details the structures of the twelve phenylglycine derivatives analysed in this study.

Activity at PI-coupled mGluRs

Compounds were tested for agonist activity at a concentration of 1 mM. All compounds had little agonist effects on basal PI levels. (+)-MCPG and (S)-4CPG appear to reduce basal PI levels modestly at 1 mM, this observation was not evident in the presence of 1 μM (1S,3R)-ACPD (Figure 1).

Possible antagonist activity of the twelve phenylglycine derivatives was assessed by their ability to inhibit the [^{3}H]-IP_1 accumulation generated by the non-selective mGluR agonist (1S,3R)-ACPD. It can be seen that the compounds show varying degrees of antagonist activity with (**RS**)3CPG and (−)-M4CPG being largely without effect, while the non-selective mGluR antagonist (+)-M4CPG was the most effective of those tested (K_B = 0.184 ± 0.04 mM) (Table 2). Figure 2(a–e)

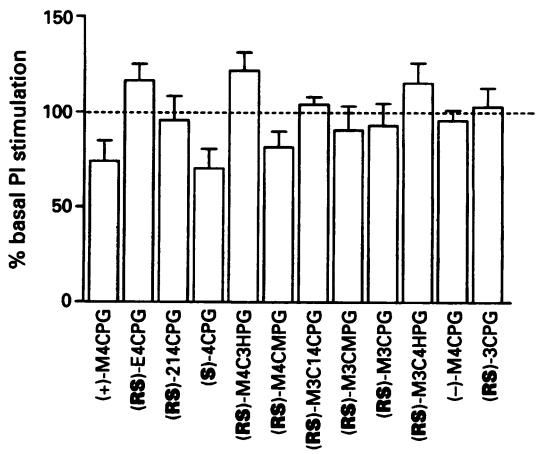


Figure 1 Effect of phenylglycine derivatives on basal phosphoinositide (PI) stimulation when tested for agonist activity at a concentration of 1 mM. None of the compounds displayed significant agonist activity. Represented are means (±s.e.mean) values of three experiments performed in triplicate.

Table 2 Antagonist properties of phenylglycine compounds against PI linked neonatal rat cortical mGluRs

Compound	K_B (mM)
(+)-M4CPG	0.184 ± 0.04
(RS)-E4CPG	0.367 ± 0.2
(RS)-2I4CPG	0.624 ± 0.05
(S)-4CPG	0.660 ± 0.08
(RS)-M4C3HPG	0.955 ± 0.06
(RS)-M4CMPG	1.083 ± 0.09
(RS)-M3Cl4CPG	3.028 ± 0.5
(RS)-M3CMPG	3.154 ± 0.3
(RS)-M3CPG	5.571 ± 1.08
(RS)-M3C4HPG	5.960 ± 0.4
(−)-M4CPG	40% antagonism of 20 μM (1S,3R)-ACPD at 3 mM
(RS)-3CPG	20% antagonism of 20 μM (1S,3R)-ACPD at 3 mM

K_B values were determined from the extent of the dextral shift in the concentration-response curve to (1S,3R)-ACPD. Presented, in descending rank order of potency, are the mean K_B values ± s.e.means for three experiments performed in triplicate for two concentrations (1 and 3 mM) of antagonist. K_B values for (−)-M4CPG and (**RS**)-3CPG were not determined due to insufficient activity.

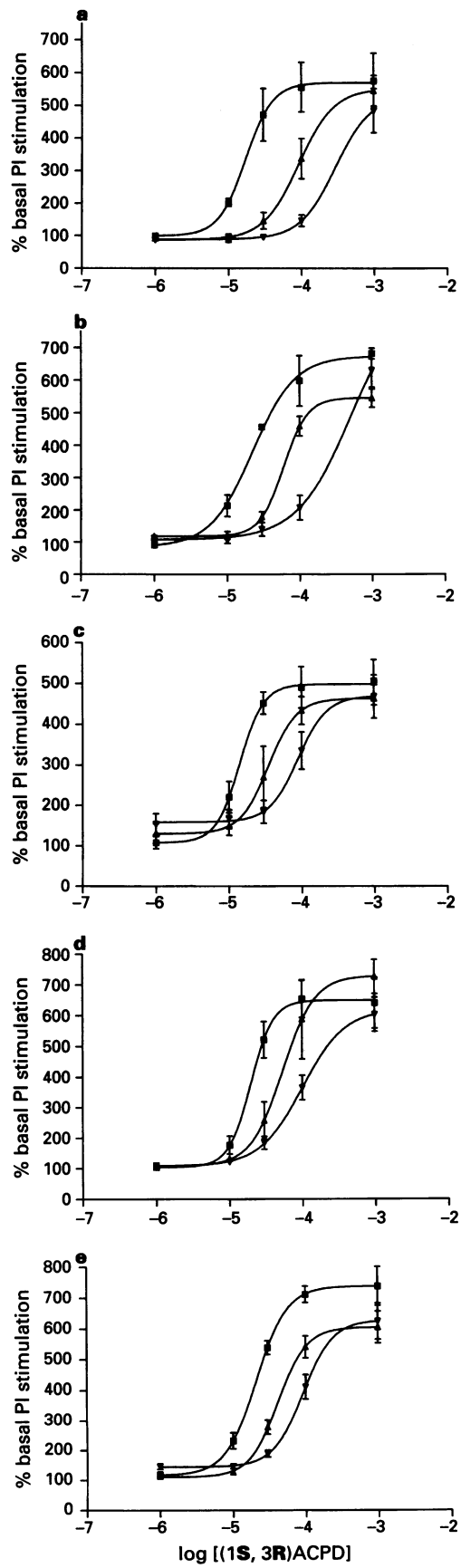


Figure 2 Concentration-response curves to (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD) in the absence (■) and presence of 1 mM (▲) and 3 mM (▼) antagonist. Antagonists caused a dose dependent dextral shift in the concentration-response to (1S,3R)-ACPD. (a) (+)-M4CPG: $K_B = 0.184 \pm 0.04$ mM. (b) (RS)-E4CPG: $K_B = 0.367 \pm 0.2$ mM. (c) (RS)-2I4CPG: $K_B = 0.624 \pm 0.05$ mM. (d) (RS)-4CPG: $K_B = 0.660 \pm 0.08$ mM. (e) (RS)-M4C3HPG: $K_B = 0.955 \pm 0.06$ mM. Compounds with K_B values greater than 1 mM are not presented graphically. Data are represented as means (±s.e.mean) of three experiments performed in triplicate.

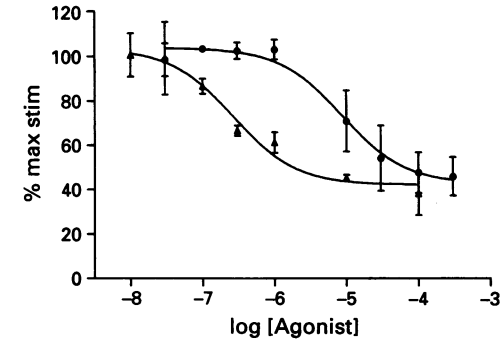


Figure 3 Agonist effect of L-2-amino-4-phosphonobutanoate (L-AP4) (●) and (2S,3S,4S)-α-(carboxycyclopropyl) glycine (L-CCG-1) (▲) on 30 μM forskolin stimulated adenylyl cyclase negatively coupled to mGluR in rat cortical slices. EC_{50} values L-AP4 = 8.90 ± 1.07 μM, L-CCG-1 = 0.24 ± 0.13 μM. Complete inhibition of the stimulated response is not possible. Results are means (±s.e.mean) of 3–4 preparations in triplicate or quadruplicate.

shows the activity of the five most potent compounds against (1S,3R)-ACPD. All compounds showing antagonist activity resulted in a dose-dependent dextral shift of the (1S,3R)-ACPD concentration-response curve, with little or no reduction in the maximum consistent with competitive antagonism.

Activity at adenylyl cyclase-coupled mGluRs

L-CCG-1 and L-AP4 were chosen as agonists since they exhibit good selectivity for group II and group III mGluRs respectively. L-AP4 inhibited forskolin-stimulated cyclic AMP production in cortical slices with an EC_{50} of 8.9 ± 1.1 μM (Figure 3). The concentration-effect curves for both L-AP4 and LCCG-1 displayed incomplete inhibition of the stimulated response, even at 1 mM; this phenomenon has been observed with all agonists tested. A likely explanation is that all (or most) adenylyl cyclase is stimulated by forskolin, while not all adenylyl cyclase is localized in cells possessing negatively-coupled mGluRs, thus leaving a considerable fraction of adenylyl cyclase activity which cannot be depressed by mGluR agonists. The range of concentrations between maximal and minimal effect is quite narrow (less than two log units) implying that L-AP4 is primarily active at only one receptor. L-CCG-1 is more potent than L-AP4 with an EC_{50} of 0.24 ± 0.13 μM (Figure 3), but the concentration-effect curve is not as steep as that for L-AP4 suggesting that L-CCG-1 may have agonist properties at more than one receptor but with differing potencies. The effect of L-CCG-1 on mGluR7 has not been obtained.

Forskolin-stimulated cortical slices in the presence of the agonists 10 μM L-AP4 and 0.3 μM L-CCG-1 were challenged by the antagonists. The resulting [^{3}H]-cyclic AMP accumulation was expressed as a percentage of maximum stimulation. A similar antagonist rank order against each agonist was obtained for the six most potent compounds, namely: (RS)-M3CMPG > (RS)-M3C4HPG > (+)M4CPG > (RS)-E4CPG > (RS)-3CPG = (RS)-M3CPG. The potency of the first four compounds was greater against L-CCG-1 than L-AP4. This was particularly notable for (RS)-E4CPG which exhibits an eleven fold difference. (RS)-M3C14CPG has an IC_{50} of 76.63 ± 14.74 μM against L-AP4, but proved inactive against L-CCG-1. (RS)-M4C3HPG has an IC_{50} of 83.25 ± 40.34 μM against L-CCG-1 and was inactive against L-AP4. (RS)-M4CMPG has low potency for both agonists but was more

potent against L-AP4. The graphs for those compounds demonstrating antagonism against L-AP4 and LCCG-1 are represented in Figures 4 and 5 respectively. The data are summarized in Table 3. The compounds that were effectively inactive ($IC_{50} > 1$ mM) are not represented graphically. None of the compounds tested had a significant effect on basal cyclic AMP levels. (S)-4CPG and (RS)-2I4CPG displayed weak agonist properties (<30%) at 1 mM.

Discussion

The principle objective of this study was to determine the rank order of potency of a series of novel phenylglycine compounds acting at native PI-linked mGluRs (principally mGluR5) and at least two mGluRs negatively-coupled to adenylyl cyclase in rat cerebral cortex. From this ranking some conclusions concerning structure activity relationships can be drawn which provide indicators for the development of more specific and potent compounds.

Structure-activity at PI linked mGluRs

(i) Antagonist activity resides mainly in the (S)-enantiomer of 4CPG and the (+)-isomer of M4CPG (stereoselectivity will probably be found with other α-alkyl-phenylglycines in this study when the separate isomers have been isolated and tested). As demonstrated previously (Hayashi *et al.*, 1994) it is likely that the (+)-isomer of M4CPG has the (S)-configuration, but this awaits confirmation by X-ray crystallography. The antagonism observed at higher doses of (−)-M4CPG could be explained by relatively (<5%) minor contamination of the (−)-isomer by the active (+)-isomer of M4CPG. (ii) Addition of an α-alkyl group to both 4CPG and 3CPG en-

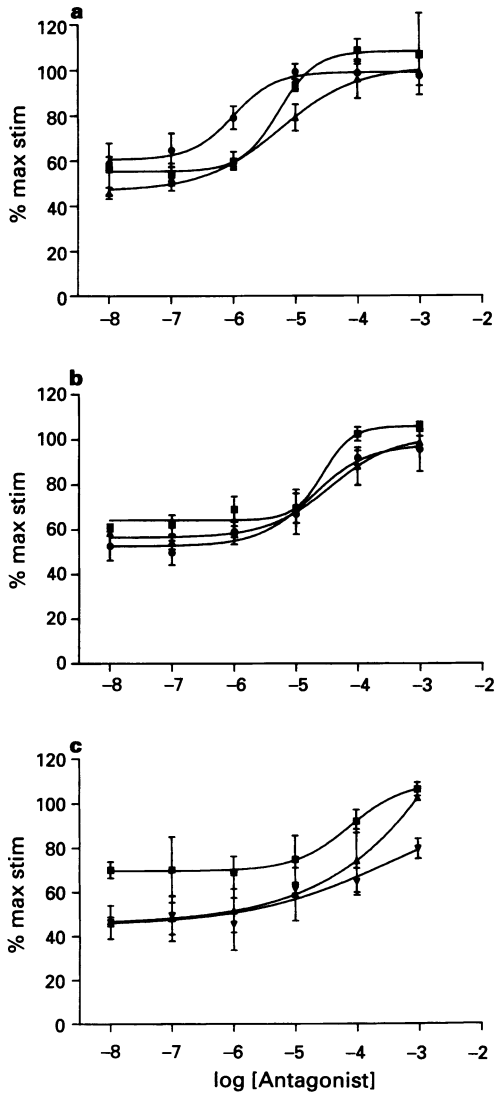


Figure 4 Antagonism by phenylglycine derivatives of 10 μM L-2-amino-4-phosphonobutanoate (L-AP4) induced inhibition of cyclic AMP production from adenylyl cyclase negatively coupled to mGluR. Effect is expressed as % of maximum stimulated cyclic AMP concentration achieved by 30 μM forskolin acting on adenylyl cyclase. Compounds are in rank order of potency. (a) (●)(RS)-M3CMPG; (■)(RS)-M3C4HPG; (▲)(+)-M4CPG. (b) (●) (RS)-E4CPG; (■)(RS)-3CPG; (▲)(RS)-M3CPG. (c) (●)(RS)-M3C14CPG; (■)(−)-M4CPG; (▲)(RS)-M4CMPG. Compounds which were inactive are not presented graphically. Results are means (±s.e.mean) of 3–4 preparations in triplicate or quadruplicate.

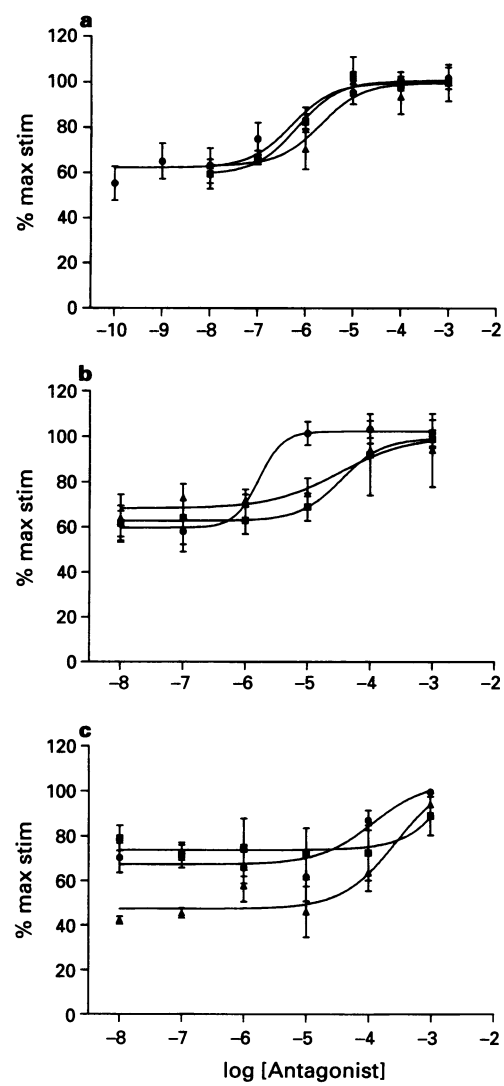


Figure 5 Antagonism by phenylglycine derivatives of 0.3 μM (2S,3S,4S)-α-(carboxycyclopropyl) glycine (L-CCG-1)-induced inhibition of cyclic AMP production from adenylyl cyclase negatively coupled to mGluR. Effect is expressed as % of maximum stimulated cyclic AMP concentration achieved by 30 μM forskolin acting on adenylyl cyclase. Compounds are in rank order of potency. (a) (●)(RS)-M3CMPG; (■)(RS)-M3C4HPG; (▲)(+)-M4CPG. (b) (●) (RS)-E4CPG; (■)(RS)-M3CPG; (▲)(RS)-3CPG. (c) (●)(RS)-M4C3HPG; (■)(RS)-M4CMPG; (▲)(−)-M4CPG. Compounds which were inactive are not presented graphically. Results are means (±s.e.mean) of 3–4 preparations in triplicate or quadruplicate.

Table 3 Antagonist properties of phenylglycine compounds acting at mGluRs negatively coupled to adenylyl cyclase

Compound	IC_{50} (μM) v. 10 μM L-AP4	v. 0.3 μM L-CCG-1	
(RS)-M3CMPG	1.07 ± 0.22	0.40 ± 0.31	(1)
(RS)-M3C4HPG	5.35 ± 0.81	0.77 ± 0.33	(2)
(+)M4CPG	5.63 ± 1.64	1.35 ± 0.29	(3)
(RS)-E4CPG	18.42 ± 9.53	1.63 ± 0.54	(4)
(RS)-3CPG	27.47 ± 15.03	34.62 ± 4.57	(6)
(RS)-M3CPG	35.10 ± 12.34	32.36 ± 26.25	(5)
(RS)-M3Cl4CPG	76.63 ± 14.74	> 1000	
(−)-M4CPG	92.87 ± 42.06	235.12 ± 34.34	(8)
(RS)-M4CMPG	376.93 ± 37.56	658.6 ± 391.9	(9)
(RS)-M4C3HPG	> 1000	83.25 ± 40.34	(7)
(RS)-4CPG	> 1000	> 1000	
(RS)-2I4CPG	> 1000	> 1000	

Antagonist properties of the above compounds in forskolin-stimulated adult rat cortical slices, mean IC_{50} values ± s.e. means, are presented in rank order as opposed to 10 μM L-AP4. Antagonist rank order versus 0.3 μM L-CCG-1 in parentheses. Values were determined from at least three experiments in triplicate.

hances potency. (iii) Replacement of the α-methyl group of M4CPG with an α-ethyl group does not result in a loss of potency suggesting that there is a pocket in this region of the receptor which is capable of accommodating groups of at least the size of the ethyl moiety. (iv) The optimum interacidic chain length for antagonism seems to be that found in (+)-M4CPG. Both M4CMPG and M3CPG (especially) are less potent. Steric factors are also important since M3CMPG, which has the same interacidic chain length as (+)-M4CPG, is of lower activity. M3CMPG may not be able to assume the correct confirmation for optimal binding to the receptor. (v) Substitution of an iodo group at the 2-position of (RS)-4CPG enhances potency (if it is assumed that the (S)-form of 2I4CPG is the active form and the (R)-form is relatively inactive, as appears to be the case with the two isomers of 4CPG (see Table 2 and Hayashi *et al.*, 1994)). It is possible that the iodo group is interacting with a lipophilic pocket in the receptor thereby enhancing binding. (vi) Substitution of a chloro group or a hydroxy group in the 3-position of MCPG results in loss of potency; a 3-hydroxy substituent being better tolerated than 3-chloro.

Structure-activity at cyclic AMP linked mGluRs

(i) The preferred chain length for antagonism of both L-AP4 (group III) and L-CCG-1 (group II) activated mGluRs in rat cortical slices seems to be that found in M3CMPG or M4CPG since both (RS)-M4CMPG and (RS)-M3CPG are much less active. (ii) An α-alkyl group is necessary for antagonism by 4-carboxy substituted phenylglycines of both L-AP4 and L-CCG-1 activated mGluRs since (S)-4CPG and (RS)-2I4CPG are inactive or show agonist activity on their own, whereas 3-carboxy substituted phenylglycines may not require an α-alkyl group as (RS)-3CPG is as active as (RS)-M3CPG. (iii) In the compounds studied, a degree of conformational flexibility in the interacidic group chain length is advantageous in order for the ω-acidic group to interact optimally with the complimentary receptor interaction point (since (RS)-M3CMPG is more potent than (+)-M4CPG at both L-AP4 and L-CCG-1 activated mGluRs). (iv) Replacement of the α-methyl group of M4CPG with an α-ethyl group enhances selectivity for L-CCG-1 activated mGluRs suggesting that a larger hydrophobic interaction pocket (situated at a site proximal to the α-position of the phenylglycine) exists in these receptors compared to L-AP4 activated mGluRs. (v) Antagonist activity of M4CPG resides mainly in the (+) isomer (it is likely that similar stereoselectivity will be seen also in the other α-alkyl phenylglycines in this study when the separate isomers have been prepared and tested). As shown previously (Hayashi *et al.*, 1994) it is likely that the (+)-isomer of M4CPG has the (S) configuration. (vi) A chloro substituent in the 3-position of M4CPG increases selectivity at L-AP4-activated mGluRs (but at the same time reduces potency). However a hydroxy substituent at the 3-position of M4CPG increases selectivity at L-CCG-1 activated mGluRs (again with a reduction in potency). This is evidence that L-AP4 and L-CCG-1 activate different mGluRs in the rat cortex, and suggests further lead structures (along with the α-alkyl differential discussed in (iv) above) for optimising specificity of antagonist action. (vii) The activity of (RS)-M3C4HPG is anomalous in that although it has a 3-carboxy substituent it is more potent than (+)-M4CPG (and much more potent than (RS)-M3CPG). This may be due to the 4-hydroxyl substituent interacting at an additional site in the receptor thereby enhancing binding. It would appear that the effect is not due to the increased acidity of the ortho-hydroxy carboxylic acid group, since (RS)-M4C3HPG is less active than (+)-M4CPG.

Overall, these results indicate that different properties are required for antagonist activity at the different mGluR groups, for example, for 4-carboxy substituted phenylglycines to have antagonist activity at mGluRs coupled to adenylyl cyclase an α-alkyl group is necessary, whereas for mGluRs coupled to PI hydrolysis an α-alkyl group enhances potency but is not essential for antagonist activity. Also (RS)-M3CMPG was the most potent compound tested at adenylyl cyclase-coupled mGluRs but was almost devoid of activity at PI-linked mGluRs.

In addition to the primarily expressed mGluRs 3, 5, and 7 in rat cerebral cortex, mGluR2 is certainly present and possibly also mGluR1 and 4, albeit at low density. The presence of other mGluRs (as yet not demonstrated or otherwise) cannot be ruled out, nor can the presence of splice variants, which may have differing potencies to ligands and varying coupling efficiencies. Whilst rat cortical mGluRs coupled to PI hydrolysis are mainly of the mGluR5 subtype (Catania *et al.*, 1994), it remains to be determined whether the phenylglycine antagonists in this study can discriminate between the mGluR1 and mGluR5 receptor subtypes. Similarly further development of phenylglycine antagonists are required before the selectivity necessary to discriminate between cyclic AMP coupled subtypes is achieved.

This study demonstrates the progress being made into the development of subgroup selective compounds. The observations presented will be useful in designing new antagonists capable of discriminating between mGluRs groups and subtypes.

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