Antagonist activity of α-substituted 4-carboxyphenylglycine analogues at group I metabotropic glutamate receptors expressed in CHO cells

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1 We have investigated the antagonist properties of 6 α-substituted phenylglycine analogues based on the structure of 4-carboxyphenylglycine (4-CPG) for group I metabotropic glutamate receptors ($mGlu_{1\alpha}$ and $mGlu_{5a}$) permanently expressed in CHO cells.

2 (S)-4-CPG and (S)-MCPG were the most selective $mGlu_{1\alpha}$ receptor antagonists. Longer chain α-carbon substitutions resulted in a progressive loss of antagonist affinity at $mGlu_{1\alpha}$ receptors but not at $mGlu_{5a}$ receptors. Thus $mGlu_{1\alpha}$ receptor antagonists require small aliphatic groups at the α-position. α-cyclopropyl-4-CPG showed a tendency towards $mGlu_{5a}$ selectivity, suggesting that bulky groups at this position may favour $mGlu_{5a}$ receptor antagonism.

3 We demonstrate that the $mGlu_{5a}$ receptor displays agonist-dependent antagonism. L-glutamate-induced Ca^{2+} release in $mGlu_{5a}$ receptor expressing cells was more susceptible to antagonism by cyclic α-carbon derivatives than (S)-3,5-dihydroxyphenylglycine (DHPG)-induced Ca^{2+} release in the same cell line.

4 The data presented suggests that $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptors have different steric and/or conformational requirements for the binding of antagonists and different amino acids which could interact with agonists.

5 These phenylglycine analogues could provide leads for the development of subtype selective antagonists.

Keywords: Metabotropic glutamate receptor; mGluR; phenylglycine; excitory amino acid; CHO

Abbreviations: (S)-4-CPG, (S)-4-carboxyphenylglycine; (S)-4C3HPG, (S)-4-carboxy-3-hydroxyphenylglycine; (RS)-CHPG, (RS)-2-chloro-5-hydroxyphenylglycine; (RS)-CyCPG, (RS)-α-cyclopropyl-4-carboxyphenylglycine; (S)-3,5-DHPG, (S)-3,5-dihydroxyphenylglycine; DMEM, Dulbecco's Modified Eagle's Medium; (RS)-ECPG, (RS)-α-ethyl-4-carboxyphenylglycine; FBS, foetal bovine serum; (S)-MCPG, (S)-α-methyl-4-carboxyphenylglycine; mGlu receptor, metabotropic glutamate receptor; NMDA, N-methyl-D-aspartate; (RS)-PeCPG, (RS)-α-pentyl-4-carboxyphenylglycine; (RS)-PhCPG, (RS)-α-phenyl-4-carboxyphenylglycine

Introduction

The mGlu family of glutamate receptors are G-protein-linked receptors currently comprising eight members. These are divided into three groups based on structural homology, pharmacology and signal transduction mechanisms when expressed in clonal cell lines (for review, see Pin & Duvoisin, 1995). Group I mGlu receptors ($mGlu_1$ and $mGlu_5$) are linked to phosphoinositide turnover and thus diacylglycerol production and Ca^{2+} mobilization (Tanabe et al., 1992; Abe et al., 1992). These receptors are specifically activated by (S)-3,5-dihydroxyphenylglycine ((S)-3,5-DHPG, (Ito et al., 1992; Schoepp et al., 1994)) and may play a role in long-term potentiation and long-term depression (e.g. Bashir et al., 1993; Bashir & Collingridge, 1994; Cohen & Abraham, 1996; Davis & Laroche, 1996; Fukuda et al., 1997; Manahan-Vaughan, 1997). (S)-3,5-DHPG has also been shown to potentiate NMDA induced depolarizations in the CA1 region of the hippocampus (Harvey & Collingridge, 1993; Fitzjohn et al., 1996) and to induce a novel form of long term depression in the same region (Palmer et al., 1997).

A number of pharmacological tools are available with which to distinguish groups of mGlu receptors and much effort has gone into the synthesis and analysis of phenylglycine derivatives as potential selective agonists and antagonists (Watkins & Collingridge, 1994; Roberts, 1995). (S)-4-carboxyphenylglycine ((S)-4-CPG) and many of it's derivatives display antagonist activity at group I mGlu receptors while (S)-α-methyl-4-carboxyphenylglycine ((S)-MCPG) is a well known antagonist at all groups of metabotropic glutamate receptors (Kemp et al., 1994; Bedingfield et al., 1995; Thomsen et al., 1994; Sekiyama et al., 1996). However, only a few compounds have been synthesized which discriminate between individual subtypes within a group (S)-4-CPG and (S)-MCPG have been shown to be more potent antagonists at $mGlu_1$ than $mGlu_5$ receptors cloned from both rat and human cDNA libraries (Brabet et al., 1995; Kingston et al., 1995) and (+)-2-methyl-4-carboxyphenylglycine was recently shown to be an antagonist at $mGlu_1$ receptors (Clark et al., 1997). Such work has also lead to the discovery of (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG), a partial agonist selective for $mGlu_5$ and an antagonist of $mGlu_1$ receptors (Brabet et al., 1995) as well as (RS)-2-chloro-5-hydroxyphenylglycine ((RS)-CHPG), an $mGlu_5$ selective agonist (Doherty et al., 1997).

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Most of the phenylglycine derivatives described to date which have activity on the group I mGlu receptors have either hydroxyl or carboxyl substitutes on the phenyl ring. Relatively little work, however, has been carried out using α-substituted analogues (Bedingfield *et al*., 1995; Sekiyama *et al*., 1996). These studies have suggested that alkylation of the α-carbon with a methyl or an ethyl group increases the affinity of (S)-4-CPG for mGlu$_1$ receptors. However, with the exception of (S)-MCPG, no studies to date have been reported showing any discrimination between mGlu$_1$ and mGlu$_5$ receptors by such compounds. We have therefore tested a variety of α-substituted derivatives of 4-CPG for their antagonist effects on recombinant mGlu$_{1\alpha}$ and mGlu$_{5a}$ receptors permanently expressed in CHO cells (Figure 1). We report that short-chain alkyl substituents maintain the mGlu$_1$ receptor selectivity of (S)-4-CPG, but that this selectivity is lost when bulkier α-substituents are used. In addition, we report that, in common with the rat mGlu$_{1\alpha}$ receptor (Brabet *et al*., 1995), the mGlu$_{5a}$ receptor displays agonist-dependent antagonism by some α-substituted phenylglycine analogues.

Methods

Synthesis of phenylglycine analogues

(RS)-α-Ethyl-4-carboxyphenylglycine ((RS)-ECPG) was synthesized by the previously reported method (Bedingfield *et al*., 1995). The α-cyclopropyl-, α-pentyl- and α-phenyl-analogues of 4-carboxyphenylglycine were synthesized in a similar manner. The new phenylglycine analogues had ^{1}H and ^{13}C n.m.r. spectra and elemental analytical data consistent with the proposed structure.
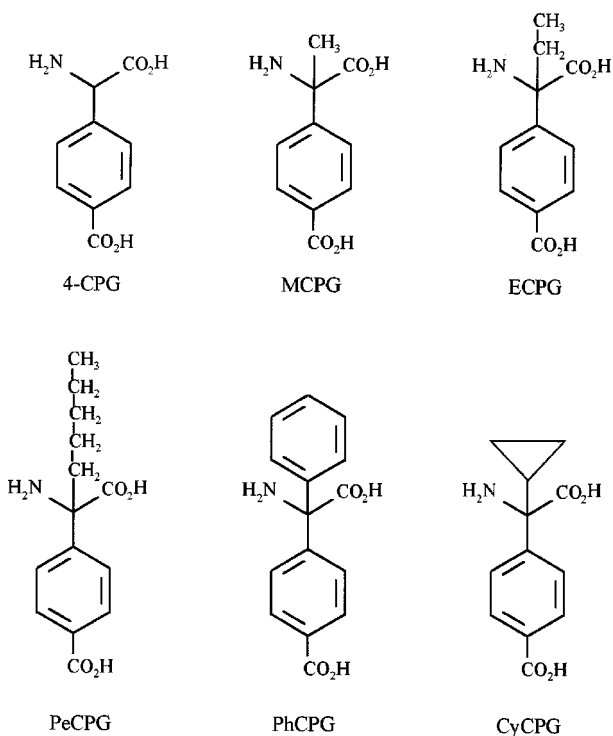

Figure 1 Structures of α-substituted phenylglycine derivatives. Analogues are 4-carboxyphenylglycine (4-CPG); α-methyl-4-CPG (MCPG); α-ethyl-4-CPG (ECPG); α-pentyl-4-CPG (PeCPG); α-phenyl-4-CPG (PhCPG), and α-cyclopropyl-4-CPG (CyCPG).

Cell culture

CHO cells stably expressing mGlu$_{1\alpha}$ and mGlu$_{5a}$ receptors were kindly provided by Professor S. Nakanishi and were cultured in Dulbecco's Modified Eagle's Medium (DMEM, 4.5 g l^{-1} D-glucose) supplemented with 10% dialysed foetal bovine serum (FBS, 10,000 M_r cut-off, Sigma), glutamine (2 mM), 1% L-proline, penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). Cells were maintained at 37°C in a 5% CO$_2$ humidified atmosphere. For imaging experiments, cells were plated onto glass coverslips (22 mm diameter, 1 thickness, 1–2 × 10^5 cells coverslip^{-1}) and used within 48 h.

Ca^{2+} imaging

Cells were washed three times in Krebs buffer (in mM, NaCl 124, KCl 2.9, NaHCO$_3$ 25, NaH$_2$PO$_4$.H$_2$O 1.4, MgSO$_4$.7H$_2$O 1, to which is added 4.5 g l^{-1} D-glucose and CaCl$_2$ (2 mM) and loaded with 5 μM of the membrane permeable Ca^{2+} indicator Fluo3-AM made up in 1 mg ml^{-1} bovine serum albumin/Krebs at 37°C for 20 min. Cells were then washed three times in Krebs buffer and incubated for 30 min in a 5% CO$_2$ atmosphere at 22°C in order to allow for de-esterification of the fluorophore. Cells were then viewed on a Bio-Rad MRC600 confocal microscope equipped with an argon ion laser using standard green filter sets and perfused continuously with Krebs buffer at a rate of approximately 2 ml min^{-1}. Kalman integrations of five individual images were obtained every 10 s. Agonists (2 ml) were bath applied while antagonists were pre-incubated for 1 min prior to co-application with agonist. The fluorescence of between five and ten individual cells in each preparation was measured on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nihimage/) and expressed relative to baseline. The mean peak fluorescence was taken as a measure of mGlu receptor activation.

Data analysis

Dose response profiles were analysed by non-linear least-squares analysis and EC$_{50}$ values derived using SigmaPlot (Jandel Scientific). K$_B$ values were calculated using the equation K$_B$ = L/r − 1 where L is the antagonist concentration and r is the ratio of EC$_{50}$ values in the presence and absence of antagonist. Unless stated otherwise, results are given as means ± s.e.mean (*n*). Statistical analysis was by unpaired *t*-test.

Materials

(S)-4-CPG and (S)-MCPG were obtained from Tocris-Cookson (Bristol, U.K.), DMEM and penicillin/streptomycin solution from GibcoBRL (Paisley, U.K.), and dialysed FBS, L-proline, L-glutamine (cell culture grade) and Fluo3-AM from Sigma Chemical Co. (Poole, U.K.). All other chemicals used were of analytical grade.

Results

Effect of a single concentration of antagonist on L-glutamate-mediated Ca^{2+} release

The ability of 1 mM of each phenylglycine analogue to antagonize L-glutamate (10 μM)-evoked Ca^{2+} release in cells

expressing $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptors was analysed. In each experiment, control responses to L-glutamate at 1, 10 and 100 μM were carried out to ensure that the concentration of agonist used was within the linear phase of the dose response profile. The EC_{50} for L-glutamate-evoked Ca^{2+} release in both these cell lines using this methodology has previously been shown to be 8 μM (Doherty et al., 1997). The results are shown in Figure 2a,b. (S)-4-CPG was the most selective compound tested, depressing responses to 0.9 ± 0.4 (5) and $73\pm10\%$ (4) of control ($P<0.01$) in $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptor-expressing cells followed by (RS)-ECPG, which reduced responses to 2 ± 1 (4) and $34\pm8\%$ (3) of control, respectively ($P<0.01$). (S)-MCPG was an effective antagonist on both cell lines, completely blocking the responses in $mGlu_{1\alpha}$ receptor-expressing cells in all experiments ($n=4$) and reducing responses to $6\pm3\%$ (4) of control in cells expressing the $mGlu_{5a}$ receptor. Conversely, (RS)-CyCPG was a more effective antagonist in cells expressing $mGlu_{5a}$ receptors than in $mGlu_{1\alpha}$ receptor-expressing cells, depressing responses to 7 ± 4 (3) and $20\pm7\%$ (3) of control, respectively, although this did not reach statistical significance.

Effect of a single concentration of antagonist on (S)-3,5-DHPG-mediated Ca^{2+} release in $mGlu_{5a}$ receptor expressing cells

It has previously been reported that the group I mGlu receptors display agonist-dependent antagonism (Brabet et al., 1995). It is therefore of great interest to determine if the antagonist characteristics of these phenylglycine analogues is retained when the commonly used group I mGlu selective agonist (S)-3,5-DHPG is used to elicit the functional response. As with the experiments using L-glutamate as the agonist, a single concentration of antagonist (1 mM) was used to block $mGlu_{5a}$ receptor function mediated by an agonist concentration (50 μM) near it's EC_{50} value (40 ± 8 μM (4)). The results are shown in Figure 2c. The ability of (RS)-PhCPG and (RS)-CyCPG to antagonize $mGlu_{5a}$ receptor-mediated Ca^{2+} release was significantly reduced when using (S)-3,5-DHPG as the agonist compared to that evoked by L-glutamate. Responses were reduced to 63 ± 5 (3) and $46\pm11\%$ (3) of control respective ($P<0.05$). In contrast, (S)-4-CPG appeared to a more effective antagonist when (S)-3,5-DHPG rather than L-glutamate was used as agonist, reducing the maximal Ca^{2+} release to $35\pm18\%$ (3) of control, although this did not reach statistical significance. No difference was detected in the ability of any other analogue to antagonize $mGlu_{5a}$ receptor function evoked by either agonist.

K_B value determination

The experiments carried out above suggest that modification of the α-carbon alters the ability of the phenylglycine derivative to antagonize $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptor function. In order to investigate this in more detail, the aliphatic series of compounds (S)-4-CPG, (S)-MCPG, (RS)-ECPG and (RS)-PeCPG were chosen for further study. Increasing concentrations of L-glutamate were used to evoke Ca^{2+} release in the absence and presence of 1 mM of each compound. Agonist applications were made in a random fashion. EC_{50} values derived from the resulting dose response profiles were then used to calculate a K_B value as described in Methods. The results from a representative experiment are shown in Figure 3. (RS)-PeCPG caused a parallel shift in the dose response profile of L-glutamate in cells expressing the $mGlu_{5a}$ receptor with no loss of maximal activity, a response consistent with

competitive antagonism. Similar parallel shifts were shown by all the compounds which showed antagonism. The EC_{50} values derived from the dose response curves in Figure 3 were 5 ± 2 and 27 ± 11 μM in the absence and presence of (RS)-PeCPG, respectively giving a K_B value of 312 μM.

Similar experiments were carried out for each phenylglycine analogue at least three times with cells expressing either $mGlu_{1\alpha}$ or $mGlu_{5a}$ receptors. The results from these experiments are shown in Figure 4. (The K_B values quoted are those for the single active enantiomer of each compound; ECPG and PeCPG were only available as racemic mixtures in a 50:50
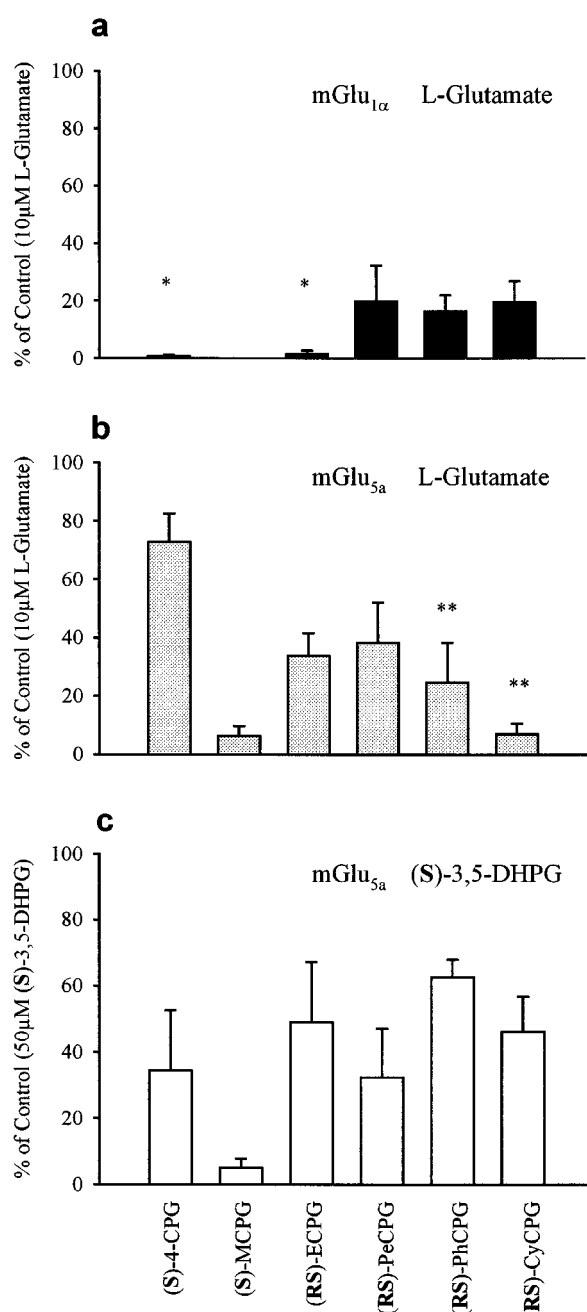

Figure 2 Effects of α-substituted phenylglycine derivatives on agonist-induced Ca^{2+} release in CHO cells expressing either $mGlu_{1\alpha}$ receptors (a) or $mGlu_{5a}$ receptors (b,c). Results are displayed as percentage of control responses (10 μM glutamate (panels a,b) or 50 μM (S)-3,5-DHPG (panel c)) following incubation with 1 mM of each antagonist. *Denotes significant difference between $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptor-expressing cells, $P<0.01$. **Denotes significant difference between effects on L-glutamate and (S)-3,5-DHPG-induced Ca^{2+} release, $P<0.05$.
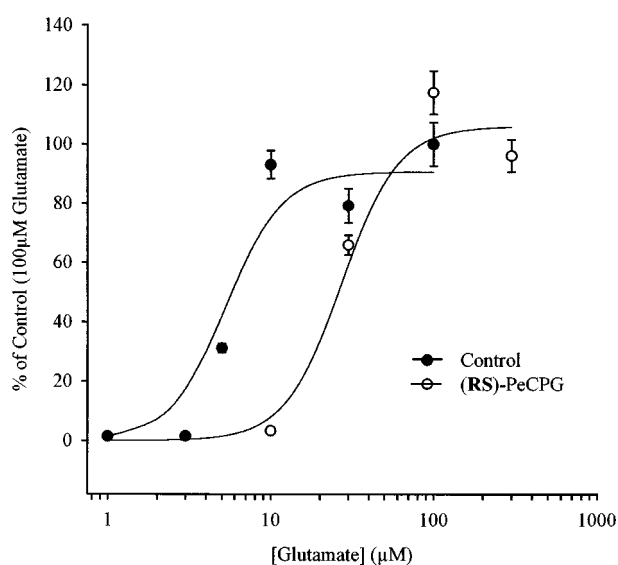
Figure 3 Effect of (RS)-PeCPG on L-glutamate-evoked Ca^{2+} release in CHO cells expressing the mGlu$_{5a}$ receptor. Representative experiment demonstrating the method for determining the K_B value for a single phenylglycine derivative. Increasing concentrations of L-glutamate used to evoke a Ca^{2+} release in the absence and presence of 1 mM (RS)-PeCPG. EC_{50} values derived using non-linear least squares analysis were 5 ± 2 and 21 ± 11 μM, respectively giving a K_B value of 312 μM.
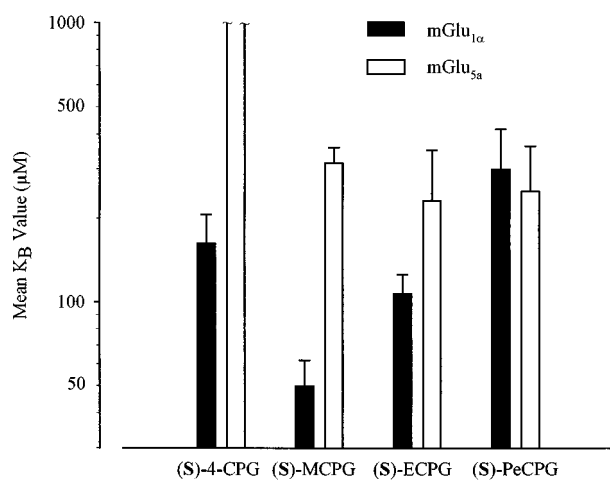

Figure 4 Graphical representation showing K_B values derived for the aliphatic series of α-substituted phenylglycine analogues when used with mGlu$_{1\alpha}$ receptor- and mGlu$_{5a}$ receptor-expressing cells. K_B values were derived from EC_{50} values calculated from dose responses carried out in the absence and presence of 1 mM of each antagonist. Results are means $\pm$ s.e.mean from three independent experiments carried out on each cell line.

ratio). In common with the results for using a single concentration of antagonist, (S)-4-CPG demonstrated selectively for mGlu$_{1\alpha}$-receptor expressing cells ($P < 0.01$), with a K_B value of 163 ± 43 μM. In mGlu$_{5a}$ receptor-expressing cells, no shift in the L-glutamate dose response was detected in two out of three preparations. Methylation of (S)-4-CPG to (S)-MCPG induced antagonist activity at mGlu$_{5a}$ receptor-expressing cells ($K_B = 316 \pm 43$ μM) while maintaining the selectivity for mGlu$_{1\alpha}$ receptors, (K_B value of 50 ± 12 μM, $P < 0.05$). Increasing the chain length of the α-carbon substituent had

no further effect on the ability of the phenylglycine analogues to antagonize mGlu$_{5a}$ receptor function. However, in mGlu$_{1\alpha}$ receptor-expressing cells, increasing the chain length of the substituent group to two and then five carbon atoms results in a progressive lowering of antagonist affinity when used such that (S)-PeCPG has a 6 fold higher K_B value than (S)-MCPG.

Discussion

Selectivity of α-substituted phenylglycine analogues on mGlu$_{1\alpha}$ and mGlu$_{5a}$ receptors

The aim of this study was to investigate the effects of substitution on the α-carbon of 4-CPG on the ability of this compound to discriminate between mGlu$_{1\alpha}$ and mGlu$_{5a}$ receptors. The results show that, in common with previous studies (Kingston *et al.*, 1995; Brabet *et al.*, 1995; Bedingfield *et al.*, 1995), both (S)-4-CPG and (S)-MCPG selectively antagonize mGlu$_{1\alpha}$ receptors over mGlu$_{5a}$ receptors and that (RS)-ECPG is a good antagonist of the mGlu$_{1\alpha}$ receptor. In addition, we have demonstrated that this mGlu$_{1\alpha}$ receptor selectivity is maintained in (RS)-ECPG (Figures 2 and 4).

It has previously been reported that (S)-MCPG is a poor antagonist of L-glutamate-induced rat mGlu$_{5a}$ receptor function (Brabet *et al.*, 1995). In contrast, we have found that although (S)-MCPG displays selectivity for mGlu$_{1\alpha}$ receptors, it is also an effective antagonist of mGlu$_{5a}$ receptors expressed in CHO cells. The reasons for this discrepancy may well be due to the methodologies used in these two studies. Here, we have used cell lines permanently expressing the appropriate receptor, whereas Brabet *et al.* (1995) used receptors transiently expressed in LLC-PK$_1$ cells, thus potentially giving much higher expression levels of receptor. The K_B for (S)-MCPG antagonism of L-glutamate-evoked mGlu$_{1\alpha}$ receptor activation derived in that study was 10 fold higher than we find here. In addition, only 500 μM (S)-MCPG was used to antagonize the functional response. Thus, under those conditions, any effect of (S)-MCPG on the mGlu$_{5a}$ receptor would probably be insignificant although a small shift is discernible in their data. Interestingly, Kingston *et al.* (1995) found that (S)-MCPG acted as an antagonist at the human mGlu$_5$ receptor, with an affinity similar to that derived in this study, when using quisqualate as the agonist.

As well as confirming the mGlu$_{1\alpha}$ receptor selective nature of (S)-4-CPG and (S)-MCPG, this study has extended the analysis to analogues substituted at the α-position with longer chain aliphatic, cyclopropyl or phenyl groups. The data presented here demonstrates that the ability of α-substituted derivatives of 4-CPG to antagonize mGlu receptor activity is dependent on the nature of the substituent group and on the particular receptor subtype involved. Short chain aliphatic substitutions of the α-carbon of 4-CPG produce effective antagonists of mGlu$_{1\alpha}$ receptors, while bulky cyclic substituent groups lead to analogues with greater mGlu$_{5a}$ selectivity (Figure 2). Thus, mGlu$_{1\alpha}$ and mGlu$_{5a}$ receptors have different steric and/or conformational requirements for the binding of these analogues and it should therefore be possible to design selective antagonists for each of these subtypes.

A question remains regarding the effects of these compounds on the other subgroups of mGlu receptors. Many phenylglycine derivates have both agonist and antagonist actions when used with different mGlu receptor subgroups. For example, (S)-4-CPG and (S)-4-carboxy-3-hydroxyphenylglycine ((S)-4C3HPG) are antagonists of group I mGlu receptors but agonists of group II receptors (Hayashi *et al.*,

1994). Previous work has indicated that, in addition to the effects on group I mGlu receptors, (RS)-ECPG is able to antagonize $mGlu_2$ but not $mGlu_6$ receptors when expressed in CHO cells (Sekiyama *et al.*, 1996). However, the ability of (RS)-ECPG to antagonize $mGlu_2$ receptors was reduced compared to $mGlu_{1\alpha}$ receptors, unlike (S)-MCPG which has a similar affinity for both receptors (Hayashi *et al.*, 1994) and is also known to antagonize group III mGlu receptors (Kemp *et al.*, 1994; Bushell *et al.*, 1996). This may make (RS)-ECPG a more useful antagonist for $mGlu_{1\alpha}$ receptors than (S)-MCPG. Little data is available regarding the effects of the other compounds tested here on individual mGlu receptor subtypes, although all the α-substituted compounds used in this study are able to antagonize group II and group III receptors in spinal cord (unpublished observations).

Agonist dependent antagonism

A further important question regarding the ability of compounds to antagonize the group I mGlu receptors is the phenomenon of agonist-dependent antagonism. It has previously been reported that (S)-4-CPG, (S)-MCPG and (S)-4C3HPG were found to display higher affinities for the antagonism of (1S,3R)-ACPD-mediated phosphoinositide turnover in $mGlu_{1\alpha}$-expressing cells than the same functional response mediated by L-glutamate (Brabet *et al.*, 1995). It was therefore of great interest to determine if the same phenomenon was displayed by the $mGlu_{5a}$ receptor. The antagonist effects of the phenylglycine analogues on (S)-3,5-DHPG-mediated Ca^{2+} release were measured (Figure 2c) and compared to the effects on L-glutamate-mediated Ca^{2+} release (Figure 2b). The results indicate that indeed the ability of phenylglycines to antagonize $mGlu_{5a}$ receptor function is also dependent on the agonist used to elicit the functional response. (S)-4-CPG appeared to be a better antagonist of (S)-3,5-DHPG-evoked Ca^{2+} release in this cell line than of L-glutamate-mediated release (65 and 28% inhibition of control responses, respectively). Conversely, (RS)-CyCPG showed a greater ability to antagonize L-glutamate-evoked Ca^{2+} release than that mediated by (S)-3,5-DHPG (93 and 46% inhibition of control responses, respectively) as did (RS)-PhCPG. This effect is unlikely to be simply a matter of a bulky substituent on the α-carbon as (RS)-PeCPG showed no tendency towards agonist dependency. Thus the cyclic α-substituents may block the interaction of L-glutamate with amino-acids which are critical for binding, but which are less important for the binding of (S)-3,5-DHPG. It may well be, therefore, that the ligand binding domain of subgroup I mGlu receptors contains a number of sites within it capable of interacting with different agonists, thus allowing different modes of agonist binding and giving rise to agonist-dependent antagonism.

These data demonstrate the complexity of the pharmacology of the group I mGlu receptors which may provide greater opportunities for the development of subtype selective antagonists. It is also clear that the phenomenon of agonist-dependent antagonism must be taken into consideration in studies using phenylglycine analogues.

Effects of α-alkylation on phenylglycine antagonism of the $mGlu_{1\alpha}$ receptor

In order to investigate the effects of α-alkylation in more detail, experiments were undertaken to measure the affinity of the alkyl series of analogues at both $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptors. The affinities of (S)-4-CPG, (S)-MCPG and (RS)-ECPG for the $mGlu_{1\alpha}$ receptor measured here are in line with previous work using different methodologies (Thomsen *et al.*, 1994; Bedingfield *et al.*, 1995; Kingston *et al.*, 1995). K_B values for the α-alkylphenylglycines showed that alkylation of the α-carbon of 4-CPG with a methyl group increases it's affinity at both $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptors, but substitution with longer alkyl chains results in a chain length-dependent loss of affinity at the $mGlu_{1\alpha}$ receptor (Figure 4). In contrast, at the $mGlu_{5a}$ receptor, no significant effect of either ethyl- or pentyl-alkylation could be detected over that produced by methylation. These results show that the effectiveness of phenylglycine analogues as antagonists of group I mGlu receptors is considerably influenced by α-carbon substitution. In this light, it is interesting that the phenyl- and cyclopropyl-derivatives of 4-CPG also showed a reduced ability to antagonize $mGlu_{1\alpha}$ receptor function (Figure 2a) while showing greater effectiveness against the $mGlu_{5a}$ receptor. Thus the loss of antagonist affinity at $mGlu_{1\alpha}$ receptors may be due to steric hindrance caused by bulky α-substituents or may be due to changes to the preferred conformation of the phenylglycines induced by such α-substituents while such changes may increase $mGlu_{5a}$ selectivity.

The data presented in this paper suggest that the mode of binding of the 4-CPG moiety and thus the ligand binding domains of the $mGlu_{1\alpha}$ and $mGlu_{5a}$ receptors may be very different. The mode of binding and antagonist potency of α-substituted 4-carboxyphenylglycine analogues is dependent on the particular modification made, the agonist chosen to mediate the functional response and the receptor subtype used. In general, short chain aliphatic substitutions retain the $mGlu_{1\alpha}$ receptor selectivity of 4-CPG itself. But this is lost when long chain aliphatic groups or bulky cyclic groups are used. Indeed it may well be that these latter compounds will provide lead compounds from which $mGlu_5$ selective antagonists may be developed.

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