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Phenylglycine derivatives as antagonists of group III metabotropic glutamate receptors expressed on neonatal rat primary afferent terminals

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1 Three novel phenylglycine analogues; (*RS*)-α-methyl-3-chloro-4-phosphonophenylglycine (UBP1110), (*RS*)-α-methyl-3-methoxy-4-phosphonophenylglycine (UBP1111) and (*RS*)-α-methyl-3-methyl-4-phosphonophenylglycine (UBP1112) antagonised the depression of the fast component of the dorsal root-evoked ventral root potential induced by (*S*)-AP4 with apparent K_D values of: 7.4 ± 2.3, 5.4 ± 0.6 and $5.1 \pm 0.3\,\mu$M (all $n = 3$), respectively.

2 A Schild analysis of the antagonism of (*S*)-AP4 induced depression of synaptic transmission by UBP1112 revealed a pA_2 value of 5.3 and a slope of 0.81 ± 0.26 ($n = 9$).

3 None of the phenylglycines tested were potent antagonists of responses mediated by group II mGlu receptors (apparent K_D values $> 480\,\mu$M). UBP1112 when tested at a concentration of 1 mM had little or no activity on (*S*)-3,5-DHPG-, NMDA-, AMPA- or kainate-induced responses on motoneurones.

4 UBP1110, UBP1111 and UBP1112 are at least 100-fold selective for group III over group I and II mGlu receptors expressed in the spinal cord making them the most potent, selective, antagonists yet tested at (*S*)-AP4 sensitive receptors in the spinal cord.
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Keywords: Neonatal rat spinal cord; phenylglycine; mGlu receptors; antagonist; mGlu8; UBP1110; UBP1111; UBP1112

Abbreviations: (1*S*,3*S*)-ACPD, (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid; ACPT-I, (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid; (*S*)-AP4, (*S*)-2-amino-4-phosphonobutanoic acid; (2*R*,4*R*)-APDC, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylic acid; CPPG, (*RS*)-α-cyclopropyl-4-phosphonophenylglycine; (*S*)-3,5-DHPG, (*S*)-3,5-dihydroxyphenylglycine; fDR-VRP, fast component of the dorsal root-evoked ventral root potential; HomoAMPA, (*S*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid; LY341495, (2*S*,1'*S*,2'*S*)-2-(2-carboxycyclopropyl)-2-(9*H*-xanthen-9-yl)glycine; MCPG, (*S*)-α-methyl-4-carboxyphenylglycine; MPPG, (*RS*)-α-methyl-4-phosphonophenylglycine; UBP1110, (*RS*)-α-methyl-3-chloro-4-phosphonophenylglycine; UBP1111, (*RS*)-α-methyl-3-methoxy-4-phosphonophenylglycine; UBP1112, (*RS*)-α-methyl-3-methyl-4-phosphonophenylglycine; UBP1113, (*RS*)-α-cyclopropyl-3-methoxy-4-phosphonophenylglycine; UBP1130, (*RS*)-α-methyl-2-methyl-4-phosphonophenylglycine

Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). The actions of glutamate are mediated by two main groups of receptors, ionotropic glutamate (iGlu) receptors are responsible for mediating fast excitatory transmission, while metabotropic glutamate (mGlu) receptors are thought to play a modulatory role in synaptic transmission (for reviews, see Cartmell & Schoepp, 2000; Schoepp, 2001).

To date, molecular cloning studies have identified eight mGlu receptor subtypes (Conn & Pin, 1997; Schoepp *et al*., 1999) which are subdivided into three groups according to their sequence homology, pharmacology and signal transduction mechanism.

Group I mGlu receptors (mGlu1 and mGlu5) are selectively activated by (*S*)-3,5-dihydroxyphenylglycine ((*S*)-3,5-DHPG) resulting in an increase in phosphoinositide hydrolysis and a rise in intracellular calcium levels. Group II mGlu receptors (mGlu2 and mGlu3) are selectively activated by (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate ((2*R*,4*R*)-APDC) and (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylate ((1*S*,3*S*)-ACPD). Group III mGlu receptors (mGlu4, 6-8) are selectively activated by (*S*)-2-amino-4-phosphonobutanoate ((*S*)-AP4). Group II and III mGlu receptors are negatively coupled to adenylyl cyclase, which causes a decrease in cAMP levels (Conn & Pin, 1997; Schoepp *et al*., 1999). In the neonatal rat spinal cord preparation, it has been shown that activation of either group II or III mGlu receptors reduces the fast component of the dorsal root-evoked ventral root potential (fDR-VRP) likely *via* attenuation of glutamate release (Watkins & Collingridge, 1994). This autoreceptor function of presynaptically located group II or III mGlu receptors has also been reported in other areas of the CNS (Cartmell & Schoepp, 2000; Schoepp, 2001).

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To date, very few potent selective group III mGlu receptor antagonists have been identified (for a review, see Schoepp *et al*., 1999). The therapeutic potential of group III mGlu receptor agonists has received some interest as such compounds have been identified as inhibitors of sound-induced seizures (Chapman *et al*., 1999; Moldrich *et al*., 2001). Additionally, selective activation of group III mGlu receptors has been shown to be neuroprotective (Gasparini *et al*., 1999; Mills *et al*., 2002). Group III mGlu receptors are also believed to play a role in the serotonin model of schizophrenia (Aghajanian & Marek, 2000). The therapeutic potential of group III mGlu receptor antagonists is not yet clear, but despite this they will be useful pharmacological tools for identifying the physiological roles of group III mGlu receptors.

We have previously synthesised a range of phenylglycine analogues among which (*RS*)-α-methyl-4-phosphonophenylglycine (MPPG) and (*RS*)-α-cyclopropyl-4-phosphonophenylglycine (CPPG) were 12- and 30-fold selective, respectively, as antagonists of group III over group II mGlu receptors expressed on primary afferent terminals in the neonatal rat spinal cord (Jane *et al*., 1995; 1996). These phenylglycines were obtained as a result of modification of the first compound reported to antagonise group III mGlu receptor agonist-induced responses, (*S*)-α-methyl-4-carboxyphenylglycine (MCPG, Pook *et al*., 1993; Kemp *et al*., 1994). The main aim of the present study was to examine five new phenylglycine derivatives based on the structures of MCPG, MPPG and CPPG, as antagonists of group III mGlu receptors expressed on primary afferent terminals. To assess selectivity, the most potent of the group III mGlu receptor antagonists were then tested on group II mGlu receptors present on primary afferent terminals and group I mGlu and iGlu receptor subtypes expressed on motoneurones.

As a result of this structure–activity study, a profile of the important structural features required for phenylglycines to interact selectively at native group III mGlu receptors was produced.

The five new phenylglycine derivatives were (*RS*)-α-methyl-3-chloro-4-phosphonophenylglycine (UBP1110), (*RS*)-α-methyl-3-methoxy-4-phosphonophenylglycine (UBP1111), (*RS*)-α-methyl-3-methyl-4-phosphonophenylglycine (UBP1112), (*RS*)-α-methyl-2-methyl-4-phosphonophenylglycine (UBP1130) and (*RS*)-α-cyclopropyl-3-methoxy-4-phosphonophenylglycine (UBP1113) (Figure 1).

Preliminary reports of this work have been published (Miller *et al*., 2000; Conway *et al*., 2001).

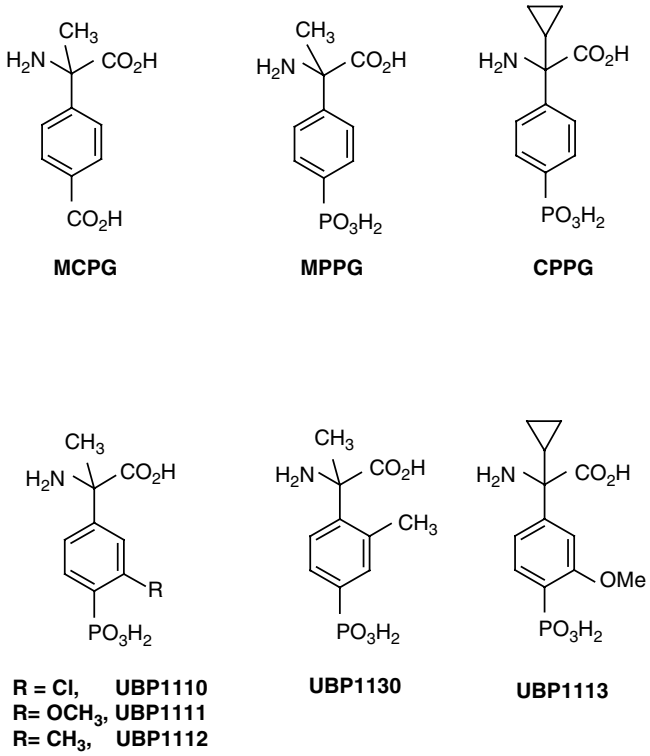

Figure 1 Structures of MCPG, MPPG and CPPG and five new analogues: UBP1110, UBP1111, UBP1112, UBP1130 and UBP1113.

Methods

All experiments were performed on isolated hemisected spinal cords of 1–4-day-old Wistar rats of either sex according to published methods (Evans *et al*., 1982; Howson & Jane, 2003). Briefly, recordings were made from ventral roots after electrical stimulation of the corresponding dorsal root (30 V (16 × threshold), 2 pulses min^{-1}, 0.2 ms pulse width). This supramaximal stimulation procedure ensures the recruitment of large diameter fibres in the lumbar dorsal roots and gives consistent reflex responses from the ventral roots in healthy preparations (Bagust, 1993). The standard superfusion medium contained: (mM) NaCl, 118; $NaHCO_3$, 25; KCl, 3; $CaCl_2$, 2.5; D-glucose, 12; gassed with 5% CO_2/95% O_2. To isolate the fast, non-*N*-methyl-D-aspartate (non-NMDA) component of the fDR-VRP, potentials were recorded in the presence of 2 mM $MgSO_4$ and 50 μM (*R*)-2-amino-5-phosphonopentanoate ((*R*)-AP5). Medium with and without drug additions was superfused over the cord at a rate of 1 ml min^{-1}. After obtaining a control fDR-VRP, the standard solution was changed to an identical one containing the agonist for 5 min. Averages of the peak amplitudes of two consecutive responses in the absence of the agonist and when the minimum response size was achieved after agonist application were used to calculate the percent depression of the fDR-VRP. Data for analysis both in control conditions and in the presence of agonists and antagonists were taken at a time when the fDR-VRP amplitude was at a steady state. Concentrations of agonist were applied that produced depressions of the fDR-VRP ranging from 5 to 80% of the control. The novel phenylglycines were tested for their ability to antagonise either group III ((*S*)-AP4) or group II ((1*S*,3*S*)-ACPD or (2*R*,4*R*)-APDC) mGlu receptor agonist-induced depressions of the fDR-VRP. UBP1112 (30 μM) was also applied for 5 min to the spinal cord in the absence of $MgSO_4$/(*R*)-AP5 and any effect on either the fast or slow component of the fDR-VRP noted.

In cases where antagonist potency on group III mGlu receptors was low, a single concentration of antagonist was applied and observed for its ability to antagonise a single agonist-induced response on the fDR-VRP (experiments were performed on three independent preparations and results were expressed as a percentage antagonism). The agonist-induced depression of the fDR-VRP was again measured after a 30 min washout period to assess recovery from antagonism.

Where little or no antagonist action of a compound was observed when tested at a high concentration (50–100 μM)

against group III mGlu receptor agonist-induced responses, no further characterisation of the antagonist action on group II mGlu receptors was carried out. If, however, after an initial screen, an antagonist appeared to be a potent antagonist at group III mGlu receptors concentration–response curves to (*S*)-AP4 and either (1*S*,3*S*)-ACPD or (2*R*,4*R*)-APDC (to assess selectivity) were constructed in the absence and presence of the novel antagonist and an apparent K_D value obtained. Concentration–response curves to (*S*)-AP4 were constructed in the absence and presence of different concentrations of UBP1112 and Schild's plots constructed.

Further experiments were carried out to determine any antagonist effects of the most potent and selective group III mGlu receptor antagonists, UBP1110 and UBP1112 on group I mGlu receptors or iGlu receptor subtypes expressed on motoneurones. These experiments were conducted in standard medium (excluding Mg^{2+}/(*R*)-AP5) that contained tetrodotoxin (TTX; 10 μM for 2 min, then 0.1 μM continuously). NMDA, kainate, (*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and the selective group I mGlu receptor agonist (*S*)-3,5-DHPG were applied for 1 min at concentrations that produced equi-effective depolarisations. The peak amplitude of agonist-induced depolarisation of motoneurones was measured from d.c. shifts in ventral root polarity after agonist application. Either 1 mM UBP1110 or UBP1112 was then applied (15 min preincubation) and the ability of the antagonist to reduce the peak amplitude of the agonist-induced responses measured. The antagonist was then washed out for 30 min and the agonists reapplied to assess recovery from antagonism.

Materials

UBP1110, UBP1111, UBP1112, UBP1130 and UBP1113 were synthesised in our laboratories using a previously published protocol (Conway *et al*., 2001). NMDA, AMPA, kainate, (*S*)-AP4, (1*S*,3*S*)-ACPD, (2*R*,4*R*)-APDC, (*S*)-3,5-DHPG, (*R*)-AP5 and TTX were obtained from Tocris Cookson Ltd, Avonmouth, Bristol, U.K. All other chemicals were of analytical grade or above.

Novel phenylglycines were made as 100 mM stock solutions dissolved in one equivalent of 100 mM aqueous sodium hydroxide. Stock solutions were then stored at −20°C.

Data analysis

Concentration–response curves were analysed by iterative nonlinear regression (GraphPAD Prism). Apparent K_D values for antagonists were obtained using the Gaddum–Schild equation, K_D = [Antagonist]/DR-1, where for this study the dose ratio (DR) is defined as the EC_{50} in the presence of the antagonist divided by the EC_{50} in the absence of the antagonist. The K_D values obtained are from at least three independent experiments and are given as means ± s.e.m. Where Schild's plots were constructed, pA_2 values were obtained. Paired, two-tailed, Student's *t*-tests were used to assess significance of data.

Results

Four of the antagonists tested UBP1110 (20 μM), UBP1111 (10 μM), UBP1112 (15 μM) and UBP1130 (200 μM) antagonised (*S*)-AP4-induced depressions of the fast component of excitation of neonatal rat motoneurones without affecting the fDR-VRP in the absence of the agonist (for a typical trace and a time course of the antagonist effect of UBP1111, see Figures 2b and 3b). In contrast, UBP1113 showed no antagonism of (*S*)-AP4-induced depressions at a concentration of 50 μM. UBP1112 (30 μM) was also observed to have no effect on either the fast or slow components of the fDR-VRP when applied alone in the absence of $MgSO_4$/(*R*)-AP5 containing medium.

The concentration–response curves obtained in the absence and presence of the antagonists showed that UBP1110, UBP1111 and UBP1112 were almost equipotent antagonists against (*S*)-AP4-induced depressions of the fDR-VRP. These three antagonists produced parallel, rightward shifts of the concentration–response curve to (*S*)-AP4 that, upon analysis, gave apparent K_D values of 7.4 ± 2.3 (n = 6), 5.4 ± 0.6 (n = 3) and 5.1 ± 0.3 (n = 3) μM, respectively (Figure 4a–c, number of independent determinations in parentheses). Schild's analysis of the antagonist effect of UBP1112 on (*S*)-AP4-induced depression of the fDR-VRP revealed a pA_2 value of 5.3 and a slope of 0.81 ± 0.26 (n = 9) (see Figure 4e).

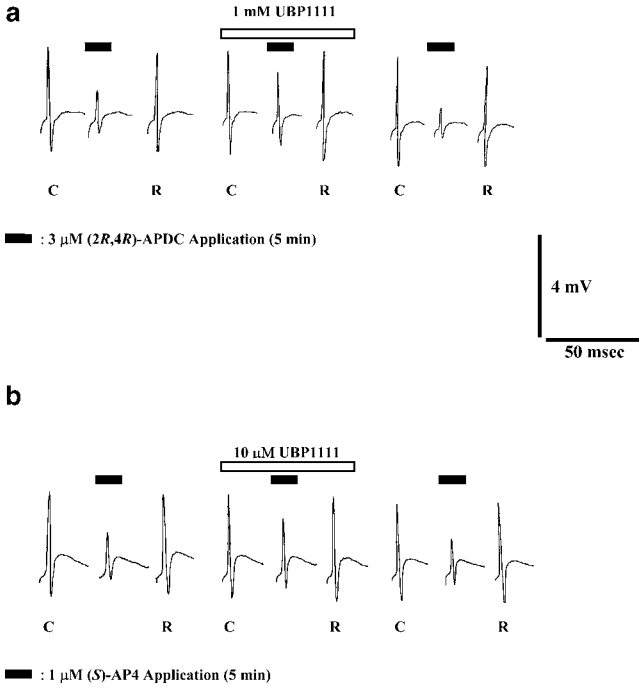

Figure 2 (a) Typical trace showing that (2*R*,4*R*)-APDC (3 μM) depressed the fDR-VRP by 58 and 22% in the absence and presence, respectively, of 1 mM UBP1111. (b) Typical trace showing that (*S*)-AP4 (1 μM) depressed the fDR-VRP by 52 and 32% in the absence and presence, respectively, of 10 μM UBP1111. The experiments were performed in Mg^{2+}/(*R*)-AP5-containing medium. Control depressions of the fDR-VRP were achieved prior to application of antagonist, which was then superfused over the preparation for 15 min before the application of the same concentration of agonist. The reversal of the depressant effects of the agonists can clearly be seen for the antagonist. Recovery of the original response was achieved after a 30 min washout of the antagonist. C, control; R, recovery. Note that the fDR-VRP did not change in amplitude in the presence of UBP1111. Similar results were obtained for all of the novel antagonists.

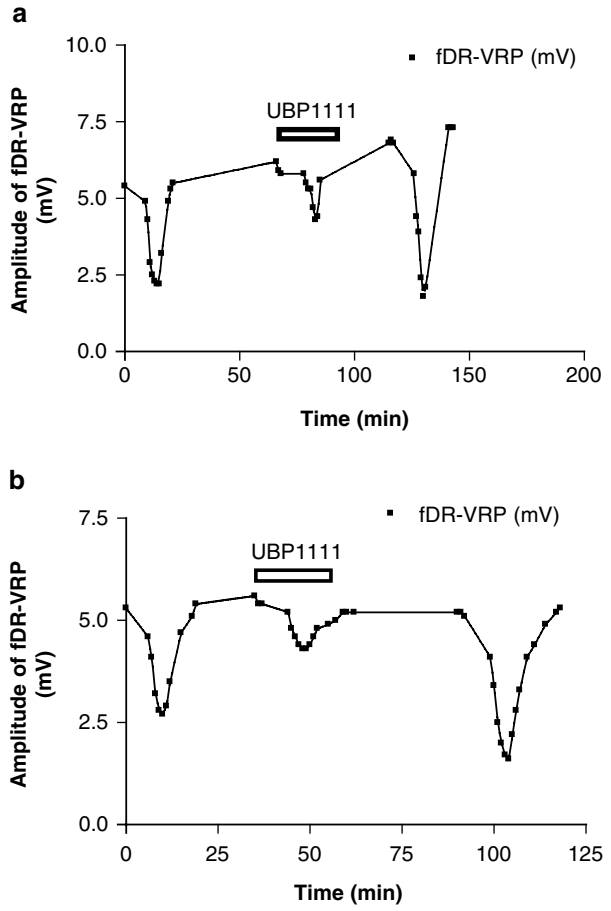

Figure 3 Time course analysis showing the recovery of the fDR-VRP with time. (a) 3 μM (2*R*,4*R*)-APDC produced approximately a 50% reduction in the fDR-VRP. This depression was attenuated in the presence of 1 mM UBP1111. Recovery of the original response occurred within 30 min of washout of UBP1111. (b) 3 μM (*S*)-AP4 produced approximately a 50% reduction in the fDR-VRP. This was attenuated in the presence of 10 μM UBP1111. Recovery of the original response occurred within 30 min of washout of UBP1111. Similar results were obtained for all of the novel antagonists.

In contrast to the compounds described above, UBP1130 did not prove to be a potent group III mGlu receptor antagonist. UBP1130 (200 μM) also produced a parallel rightward shift in the concentration–response curve to (*S*)-AP4 allowing an apparent K_D value of 179 ± 48 μM ($n = 3$, Figure 4d) to be calculated. Due to the low antagonist potency of UBP1130 against group III mGlu receptors, the activity of this compound at group II mGlu receptors was not examined.

The three most potent group III mGlu receptor antagonists, UBP1110, UBP1111 and UBP1112, were also examined for antagonist effects at group II mGlu receptors (for a typical trace and a time course of the antagonist effect of UBP1111 on (2*R*,4*R*)-APDC-induced depression of the fDR-VRP, see Figures 2a and 3a). In the case of UBP1110 (1 mM), concentration–response curves to (1*S*,3*S*)-ACPD were shifted to the right in a parallel fashion allowing an apparent K_D of 737 ± 121 μM ($n = 3$, Figure 5a) to be calculated. Due to the low potency of UBP1111 at group II mGlu receptors, a percent antagonism of (2*R*,4*R*)-APDC-induced depression of the fDR-VRP was calculated rather than a K_D. Using this method, it was shown that at 1 mM UBP1111 produced a 51.3 ± 0.5% antagonism ($n = 3$) of the depression of the fDR-VRP produced by 3 μM (2*R*,4*R*)-APDC. In the presence of 1 mM UBP1112, the concentration–response curve to (2*R*,4*R*)-APDC underwent a parallel, rightward shift allowing an apparent K_D of 488 ± 90 μM ($n = 4$, Figure 5b) to be calculated.

Thus, UBP1110, UBP1111 and UBP1112 are 100-, ~185- and 96-fold selective, respectively, for group III over group II mGlu receptors expressed on primary afferent terminals (see Table 1).

The 3-methoxy analogue of CPPG, UBP1113 (50 μM) did not produce a rightward shift in the (*S*)-AP4 concentration–response curve and was therefore not examined further. A summary of the activity of the novel phenylglycine analogues and the reference compound MPPG on group II and III mGlu receptors is given in Table 1.

It was necessary to establish the selectivity of the most potent and selective group III mGlu receptor antagonists with respect to iGlu and mGlu1 and mGlu5 receptors expressed on spinal motoneurones. Using spinal cords perfused with TTX, depolarisations of motoneurones were produced by application of equieffective concentrations of (*S*)-3,5-DHPG (activates both mGlu1 and mGlu5), NMDA, AMPA and kainate. When the agonists were reapplied in the presence of 1 mM UBP1110, the responses to NMDA, AMPA and kainate were unaffected at 108 ± 6, 100 ± 6 and 103 ± 2% of control values (Figure 6a, $n = 3$). UBP1110 at a concentration of 1 mM did produce a slight reduction in the amplitude of the (*S*)-3,5-DHPG-induced depolarisation of 14 ± 1% of control ($P = 0.003$, paired *t*-test, $n = 3$). Similar results were obtained when 1 mM UBP1112 was used as the antagonist instead of UBP1110. In the presence of 1 mM UBP1112, the responses, expressed as a % of the control, were: AMPA: 73 ± 4%; NMDA: 122 ± 15%; kainate: 112 ± 14% and (*S*)-3,5-DHPG: 86 ± 8% (all $n = 3$, Figure 6b). Upon analysis, only the reduction in the AMPA amplitude was significant ($P = 0.008$, paired *t*-test). Neither UBP1110 nor UBP1112 had depolarising or depressant effects when applied alone.

Discussion

We have previously reported that a series of α-substituted 4-phosphonophenylglycine and α-substituted (*S*)-AP4 analogues are selective antagonists of group III mGlu receptors expressed in the spinal cord (Jane *et al*., 1994; 1995; 1996; Thomas *et al*., 1996). Of the compounds reported so far, the most potent group III mGlu receptor antagonists were MPPG and CPPG; however, these compounds were only 10- and 30-fold selective, respectively, for group III over group II mGlu receptors. As part of a project aimed at the design of more selective group III mGlu receptor antagonists, we have synthesised a range of MPPG and CPPG derivatives with substituents on the phenyl ring (for structures, see Figure 1). The novel antagonists were tested on the neonatal rat spinal cord where a range of mGlu and iGlu receptor subtypes are expressed (Watkins & Evans, 1981; Tölle *et al*., 1993; Schoepp *et al*., 1999; Miller *et al*., 2001; Stegenga & Kalb, 2001). Three of the novel MPPG derivatives tested in this study, UBP1110, UBP1111 and UBP1112, have been found to be slightly more potent than the parent compound and approximately 100-fold selective for group III over group II mGlu receptors expressed in the spinal cord.

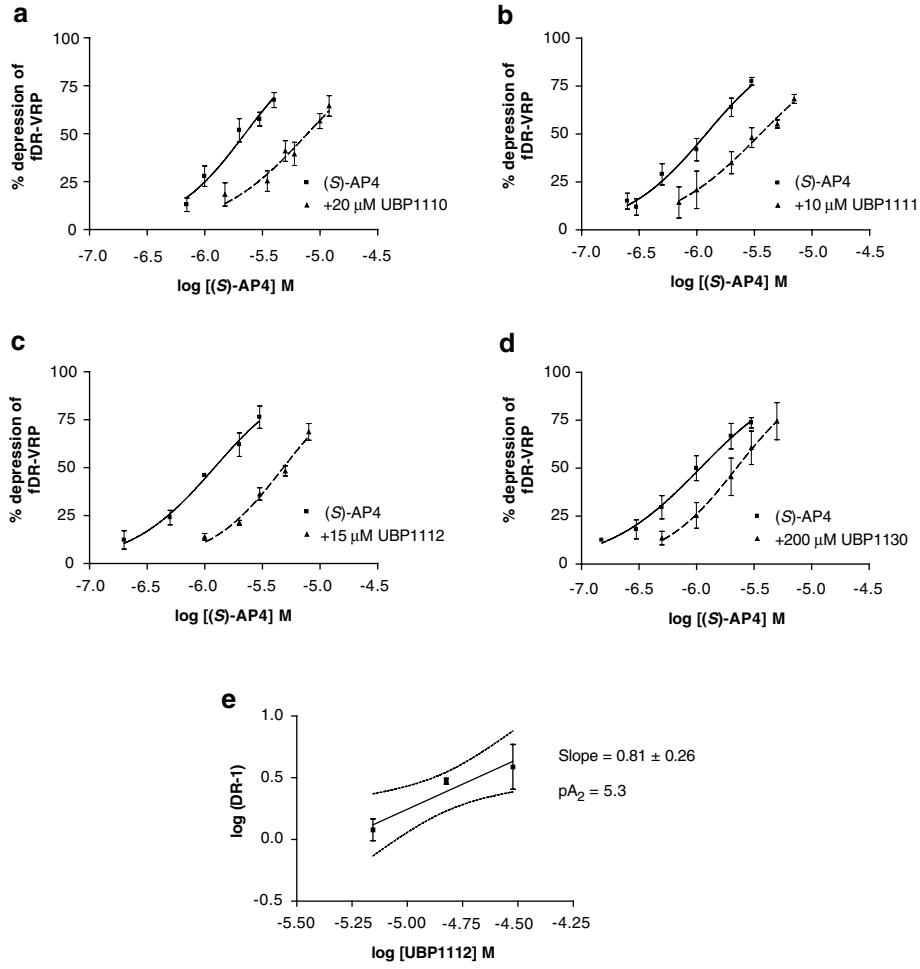

Figure 4 Concentration–respone curves for the depression of the fDR-VRP in neonatal rat motoneurones by (*S*)-AP4 and the antagonism of the (*S*)-AP4 depression by (a) 20 μM UBP1110 ($n=3$), (b) 10 μM UBP1111 ($n=6$), (c) 15 μM UBP1112 ($n=3$) and (d) 200 μM UBP1130 ($n=3$). Each point represents the mean depression $\pm$s.e.m. from at least three independent preparations. (e) Schild's analysis of the antagonism of (*S*)-AP4-induced responses by UBP1112.

In addition, UBP1110 and UBP1112 displayed weak or no significant activity at group I mGlu receptors or iGlu receptors expressed in the spinal cord. In agreement with the antagonist action observed in this study, UBP1111 has also been shown to antagonise mGlu8 receptors in the lateral perforant path synapse of the rat hippocampus (Ayman *et al*., 2001).

Group III mGlu receptors expressed in the neonatal rat spinal cord

Although (*S*)-AP4 is selective for group III vs groups I and II, it is an agonist at all four mGlu receptor subtypes within group III. The rank order of agonist potency determined on cloned mGlu receptors is mGlu6 = mGlu8 > mGlu4 $\gg$ mGlu7 (for a review, see Schoepp *et al*., 1999). One of the group III mGlu receptors thought to be mediating the (*S*)-AP4-induced depression of the fDR-VRP in the spinal cord is mGlu8 (Thomas *et al*., 2001; Howson & Jane, 2003). Evidence for the contribution of mGlu8 receptors to the depression of the fDR-VRP comes from the observation that the high-potency component of the biphasic (*S*)-3,4-dicarboxyphenylglycine ((*S*)-3,4-DCPG) concentration–response curve is mGlu8 receptor mediated (Thomas *et al*., 2001). In addition, immunohistochemical studies have confirmed the expression of mGlu8 receptors in lamina IX (contains synapses of primary afferent terminals with motoneurones) of a lumbar section of the neonatal rat spinal cord (Miller *et al*., 2001). Therefore, the mGlu8 receptor is present in the neonatal rat spinal cord and mediates a depression of the fDR-VRP. Depression of the fDR-VRP in the spinal cord may also be mediated by mGlu7, as immunogold labelling has identified this receptor on primary afferent terminals in the rat spinal cord (Ohishi *et al*., 1995). However, depression of synaptic transmission *via* this receptor is unlikely to occur on application of (*S*)-AP4 or (*S*)-3,4-DCPG due to the low potency of these agonists at the mGlu7 receptor (EC_{50} value 175 ± 39 μM (cAMP assay) and K_i value 211 ± 43 μM (for displacement of [^{3}H]LY341495 ((2*S*,1'*S*,2'*S*)-2-(2-carboxycyclopropyl)-2-(9*H*-xanthen-9-yl)glycine) binding) for (*S*)-AP4 on cloned human mGlu7 receptors) (Wright *et al*., 2000; Thomas *et al*., 2001). Indeed, the fDR-VRP can be completely abolished at low micromolar concentrations of (*S*)-AP4 ($\sim$10 μM), and therefore mGlu7 receptors would not be

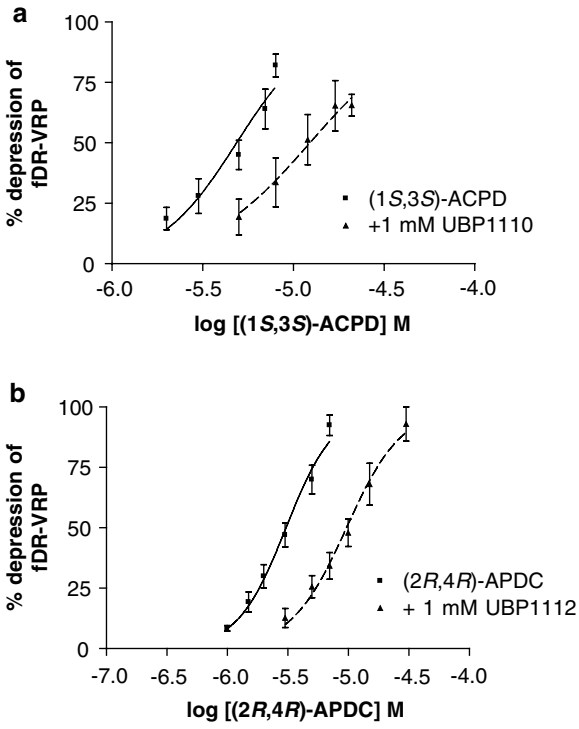

Figure 5 Concentratison–response curves for the depression of the fDR-VRP in neonatal rat motoneurones by group II mGlu receptor agonists. (a) (1*S*,3*S*)-ACPD and the antagonism of the (1*S*,3*S*)-ACPD-induced depression by 1 mM UBP1110. Each point represents the mean depression ± s.e.m. from three independent preparations. (b) (2*R*,4*R*)-APDC and the antagonism of the (2*R*,4*R*)-APDC-induced depression by 1 mM UBP1112. Each point represents the mean depression ± s.e.m. from four independent preparations.

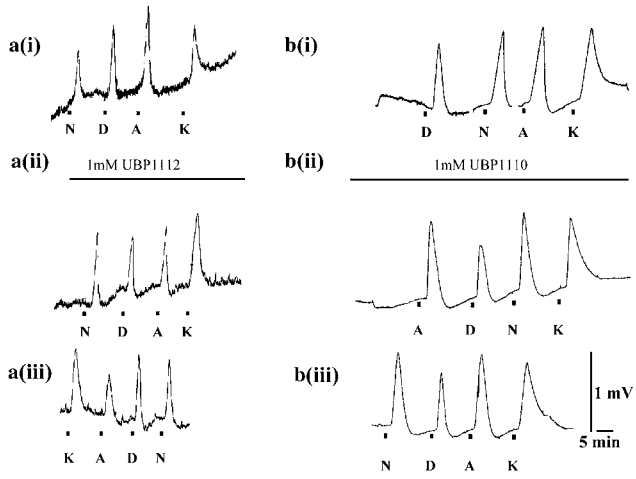

Figure 6 (a) A trace from a typical experiment showing that 1 mM UBP1112 showed little or no significant antagonist effects against NMDA, AMPA, kainate or (*S*)-3,5-DHPG-induced depolarisations of neonatal rat motoneurones: a(i) in the absence of antagonist; a(ii) in the presence of 1 mM UBP1112 (15 min preincubation); a(iii) after a 30 min washout of UBP1112. (b) A trace from a typical experiment showing that 1 mM UBP1110 showed little or no significant antagonist effects against NMDA, AMPA, kainate or (*S*)-3,5-DHPG-induced depolarisations of neonatal rat motoneurones: b(i) in the absence of antagonist; b(ii) in the presence of 1 mM UBP1110 (15 min preincubation); b(iii) after a 30 min washout of UBP1110. It is worth noting that neither UBP1110 nor UBP1112 (1 mM) had depolarising or depressant effects when applied alone.

expected to make a significant contribution to this effect. However, the mGlu7 receptor may be activated by the higher concentrations of (*S*)-AP4 used when constructing concentration–response curves in the presence of the antagonists used in this study. The mGlu4 receptor may also mediate (*S*)-AP4-induced depression of the fDR-VRP, as the mGlu4 receptor has been shown to be expressed in the adult rat spinal cord using immunohistochemical methods (Azkue *et al*., 2001). However, pharmacological evidence suggests that this is unlikely (Schoepp *et al*., 1999). Indeed, the depression of the fDR-VRP by the agonist (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid (ACPT-I), which is equipotent at mGlu8 and mGlu4 receptors (De Colle *et al*., 2000), can be completely blocked by low nanomolar concentrations of LY341495 (LY341495 is >400 selective for mGlu8 over mGlu4, see Howson & Jane, 2002; 2003) strongly suggesting that mGlu8 but not mGlu4 receptors are mediating the depressant effects of ACPT-1. The mGlu6 receptor is unlikely to be present in the spinal cord, as it is thought to be located solely in the retina (Nakajima *et al*., 1993). In addition, there is pharmacological evidence to suggest that mGlu6 receptor activation does not mediate depression of the fDR-VRP, as it has been shown that 100 μM (*S*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid (HomoAMPA), a selective mGlu6 receptor agonist (Ahmadian *et al*., 1997), does not depress the fDR-VRP (Miller and Jane, unpublished observation).

Table 1 Apparent K_D values for antagonism by MPPG, CPPG, UBP1110, UBP1111, UBP1112, UBP1130 and UBP1113 of group II or group III mGlu receptor-mediated depression of the fDR-VRP

Compound	*Group II (1S,3S)-ACPD*	*Group II (2R,4R)-APDC*	*Group III (S)-AP4*	*Selectivity Grp III vs Grp II*
UBP1110	737 ± 121 (3)	—	7.4 ± 2.3 (3)	100
UBP1111	—	51.3 ± 0.5 (3)[a]	5.4 ± 0.6 (6)	~185
UBP1112	—	488 ± 90 (4)	5.1 ± 0.3 (3)	96
UBP1130	—	—	179 ± 48 (3)	
UBP1113	—	—	NE (3)[b]	
MPPG	113 ± 13 (3)[c]	—	9.2 ± 0.3 (9)[c]	12
CPPG	53		1.7	31

Values represent mean apparent K_D's in μM ± s.e.m. (number of determinations given in parentheses) unless otherwise stated. [a]% antagonism of (2*R*,4*R*)-APDC at 3 μM. See text for details. [b]NE = no effect at 50 μM. [c]Reference compound values taken from Jane *et al*. (1995).

The above observations suggest that it is likely that in this study the activity of the new phenylglycines derivatives on group III mGlu receptors has been determined predominantly on a native mGlu8 receptor-mediated effect. However, Schild's analysis of the antagonism of (*S*)-AP4 induced depression of the fDR-VRP by UBP1112 gave a slope of less than 1. It is likely that the reason for this is that at the highest concentration of the antagonist tested (30 μM), high concentrations of (*S*)-AP4 had to be used in order to overcome the antagonist. At these higher concentrations, (*S*)-AP4 may be activating mGlu7 as well as mGlu8 and differential effects of the antagonist UBP1112 on these two receptors may explain the Schild slope of less than unity. We have previously observed a similar effect on Schild's analysis of antagonism of (*S*)-AP4 by the known competitive antagonist LY341495 (Howson & Jane, 2003).

Structural features required for selective antagonism of group III mGlu receptors

From the results we have presented here, we are able to propose a number of structural features that are required for optimal antagonist activity at group III mGlu receptors present on neonatal rat primary afferent terminals:

A terminal phosphono group confers selective activity for native group III over group I mGlu receptors In previous studies, MPPG and CPPG (Jane *et al*., 1995; 1996) were reported to possess little or no antagonist action at group I mGlu receptors. In this study, UBP1110 and UBP1112 have been shown to have little or no antagonist action at group I mGlu receptors expressed on neonatal rat motoneurones.

Substitution at the 3-position of the phenyl ring of MPPG favours antagonist activity at native group III mGlu receptors Group III mGlu receptor antagonist activity is independent of the electronic effects of the substituent, as apparent K_D values for the 3-chloro- (UBP1110) (electron withdrawing) and the 3-methyl- (UBP1112) (electron donating) substituted phenylglycines were similar.

Native group III mGlu receptor-binding site can accommodate substituents at least as large as methoxy at the 3-position of the phenyl ring of MPPG This is illustrated by the potent antagonist activity of UBP1111 on group III mGlu receptors on primary afferent terminals.

Substitution at the 3-position of the phenyl ring of MPPG reduces antagonist activity at group II mGlu receptors We have found that UBP1111 (with a methoxy group at the 3-position) UBP1110 (with a chloro group at the 3-position) and UBP1112 (with a methyl group at the 3-position) are approximately 100-fold selective for group III over group II mGlu receptors. However, MPPG, which has no substitution at the 3-position of the phenyl ring is only approximately 10-fold selective for group III over group II mGlu receptors (Jane *et al*., 1995). This effect is most likely to be due to the steric bulk of the substituent at the 3-position of MPPG being well tolerated by group III but not by group II mGlu receptors.

Substitution at the 2-position of the phenyl ring of MPPG reduces the antagonist potency at native group III mGlu receptors UBP1130 is approximately 20-fold less potent than MPPG. The only structural difference between the two compounds is a methyl substitution at the 2-position of the phenyl ring. A possible reason why substitution at the 2-position is not tolerated is that a change in the preferred conformation of the compound occurs as a result of interaction between the substituents on the alanine side chain and the 2-methyl group on the phenyl ring (see Conway *et al*., 2001).

It is likely that only the *S* enantiomers of the above compounds have activity at mGlu receptors, as the *S* enantiomer of MCPG was a group I, II and III mGlu receptor antagonist, while the *R* isomer was inactive (Kemp *et al*., 1994; Schoepp *et al*., 1999). Thus, once the *S* enantiomers of UBP1110, UBP1111 and UBP1112 have been synthesised and tested they are likely to be twice as potent as the racemates.

A surprising result was the lack of activity of the α-cyclopropyl analogue, UBP1113. One explanation for this is that CPPG and MPPG have different preferred orientations of the glycine unit relative to the phenyl ring. Therefore, the 3-methoxy substituent in UBP1113 may probe excluded space in the receptor-binding site. When energy minimised conformations of UBP1111 and UBP1113 were overlaid, it could clearly be seen that the two phenyl rings of the compounds are perpendicular to each other supporting the above theory (Conway *et al*., 2001).

None of the novel phenylglycines had any effect on the fDR-VRP when applied in the absence of the group II or group III mGlu receptor agonists (for an example, see Figure 2a and b). This is likely due to the supramaximal stimulation protocol being used in these experiments, which obscures any enhancement of the EPSP generated as a result of blocking presynaptic mGluRs and thereby enhancing glutamate release. Indeed, when recordings of whole-cell synaptic responses elicited by electrical stimulation of dorsal roots were made from motoneurones, it was observed that excitatory postsynaptic currents were enhanced in the presence of (*S*)-α-methyl-AP4 (MAP4), a selective group III mGlu receptor antagonist (Jane *et al*., 1994; Cao *et al*., 1997). This observation is consistent with the proposal that activation of group III mGlu receptors on primary afferent terminals depresses synaptic transmission (Cao *et al*., 1997).

Conclusion

We have discussed pharmacological and immunohistochemical evidence suggesting that mGlu8 receptors are likely to be the main group III mGlu receptor subtype responsible for the (*S*)-AP4-induced depression of the fDR-VRP. UBP1110, UBP1111 and UBP1112 have been shown to be approximately 100-fold selective for native group III over group II mGlu receptors with little or no activity on mGlu1, mGlu5 or iGlu receptors expressed on neonatal rat motoneurones. While CPPG is $\sim$3–4-fold more potent than

UBP1110, UBP1111 and UBP1112, it is less selective (see Table 1) making it difficult to use to block group III receptors in tissues selectively, where both group II and III mGlu receptors are expressed. Thus, the novel compounds described in this study are useful replacements for CPPG in studies where selective blockade of group III mGlu receptors is required.

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