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European Journal of Pharmacology 338 (1997) 111–116

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Dicarboxyphenylglycines antagonize AMPA- but not kainate-induced depolarizations in neonatal rat motoneurones

Nicola K. Thomas, Peter Clayton, David E. Jane *

Department of Pharmacology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK

Received 10 April 1997; revised 5 August 1997; accepted 9 September 1997

Abstract

Ionotropic glutamate receptors have been categorized into three main groups according to the selective agonists that activate them, the *N*-methyl-D-aspartate (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA) and (2*S*,3*S*,4*S*)-3-carboxymethyl-4-isopropenylpyrrolidine-2-carboxylic acid (kainate) receptors. Both AMPA and kainate induce depolarizations in neonatal rat spinal motoneurones. However, selective antagonists capable of discriminating between the effects of these two antagonists are not widely available. As part of a search for such compounds we report the actions of (*RS*)-3,4-dicarboxyphenylglycine (DCPG) and (*RS*)-3,5-dicarboxyphenylglycine on agonist-induced motoneuronal depolarizations in the neonatal rat spinal cord preparation. In addition, the actions of (*R*)- and (*S*)-3,4-DCPG are also described. (*RS*)-3,4-DCPG and (*RS*)-3,5-DCPG antagonized AMPA-induced depolarizations (apparent $K_d = 137$ μM ($n = 3$) and 167 μM ($n = 5$), respectively). However, (*RS*)-3,5-DCPG (1 mM) potentiated responses due to kainate ($n = 5$) while (*RS*)-3,4-DCPG (1 mM) displayed weak antagonism of these responses (apparent $K_d > 12$ mM, $n = 3$). (*RS*)-3,4- and (*RS*)-3,5-DCPG at 500 μM both displayed antagonism at the NMDA receptor (apparent $K_d = 472$ μM and 346 μM, respectively) and a postsynaptic subgroup I metabotropic glutamate receptor activated by (1*S*,3*R*)-ACPD. The AMPA receptor antagonist activity of (*RS*)-3,4-DCPG was shown to reside in the (*R*)-enantiomer (apparent $K_d = 77$ μM, $n = 3$). The same isomer was responsible for the NMDA receptor antagonism while showing little or no antagonism of kainate-induced depolarizations (apparent $K_d > 3$ mM, $n = 3$), and a weak antagonistic effect at (1*S*,3*R*)-ACPD receptors. (*S*)-3,4-DCPG (500 μM) was unable to antagonize kainate-induced depolarizations, showed weak or no antagonism of NMDA- and AMPA-induced depolarizations, but antagonized (1*S*,3*R*)-ACPD-induced depolarizations. Thus (*RS*)-3,4-, (*RS*)-3,5- and (*R*)-3,4-DCPG demonstrate useful discrimination of responses due to AMPA and kainate, strongly suggesting that pharmacologically distinct AMPA and kainate receptors exist in motoneurones in the neonatal rat spinal cord.

Keywords: Glutamate receptor; Novel antagonist; Neonatal rat spinal cord

1. Introduction

Excitatory amino acid receptors have been categorized as ionotropic glutamate (iGlu) receptors which mediate fast synaptic transmission through ligand-gated ion-channels and metabotropic glutamate (mGlu) receptors which are G-protein coupled to second messenger systems and have been implicated in the modulation of synaptic transmission in the central nervous system. To date, eight mGlu receptors have been characterized (Pin and Duvoisin, 1995), these falling into two main groups: those coupled to phosphoinositide hydrolysis ($mGlu_1$ and $mGlu_5$, comprising subgroup I) and those negatively coupled to cAMP formation ($mGlu_{2+3}$ and $mGlu_{4+6-8}$, comprising subgroups II and III, respectively). Three main groups of iGlu receptors have been categorized according to the selective agonists which activate them, the *N*-methyl-D-aspartate (NMDA), (*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA) and (2*S*,3*S*,4*S*)-3-carboxymethyl-4-isopropenylpyrrolidine-2-carboxylic acid (kainate) receptors (reviewed by Watkins et al., 1991). It is known that selective agonists of all three groups of iGlu receptors as well as agonists of subgroup I mGlu receptors induce depolarizations in neonatal rat spinal motoneurones (Watkins and Collingridge, 1994). Antagonists capable of discriminating between NMDA and non-NMDA receptors are widely available (Jane et al., 1995); however, antagonists capable of differentiating AMPA and kainate receptors are less common. Pook et al. (1993) have reported that

* Corresponding author. Tel.: (44-117) 928-9000, ext. 4948; Fax: (44-117) 927-9839.

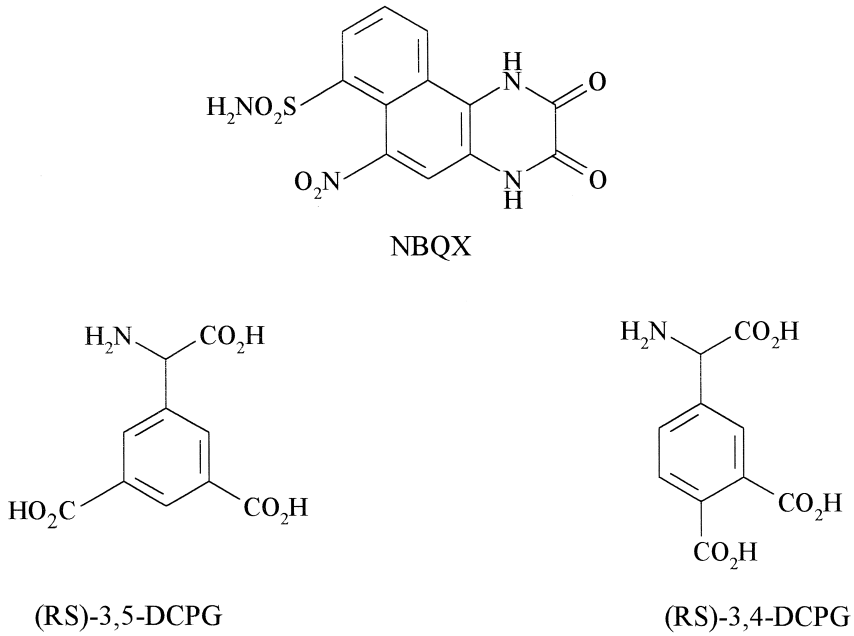

Fig. 1. Structures of the AMPA receptor antagonist NBQX and the novel compounds (*RS*)-3,5-DCPG and (*RS*)-3,4-DCPG.

6-nitro-7-sulphamobenzo(f)quinoxaline-2,3-dione (NBQX) is 12-fold more selective as an antagonist of AMPA vs. kainate-induced spinal motoneuronal depolarization. Additional, and preferably more selective, antagonists would be desirable, particularly in view of the diversity of sub-types of AMPA and kainate receptors now known to exist (Nakanishi and Masu, 1994).

As part of a search for such compounds we have synthesised (*RS*)-3,5-dicarboxyphenylglycine (3,5-DCPG, Fig. 1) and (*RS*)-3,4-dicarboxyphenylglycine (3,4-DCPG, Fig. 1) and separated the individual enantiomers of 3,4-DCPG by chiral HPLC in order to clarify the modes of action of this compound. We report here the actions of these compounds on NMDA, AMPA, kainate and (1*S*,3*R*)-ACPD-induced depolarizations and show that (*RS*)-3,4-, (*RS*)-3,5-DCPG and (*R*)-3,4-DCPG selectively antagonize depolarizations due to AMPA relative to those due to kainate.

2. Materials and methods

(*RS*)-3,4- and (*RS*)-3,5-DCPG were synthesised in our own laboratory from the corresponding substituted aldehydes using the Strecker reaction (Steiger, 1955), followed by hydrolysis of the intermediate aminonitriles in 6 M HCl. Purification was achieved by ion-exchange resin chromatography. Both compounds had physico–chemical properties consistent with the proposed structures. Racemic 3,4-DCPG was separated into the *R* and *S* forms by chiral HPLC using a Crownpak column as previously described for phenylglycine analogues (Udvarhelyi and Watkins, 1990). The isomer which eluted first was tentatively assigned the *R* absolute stereochemistry based on the elution order of enantiomers of known phenylglycines (Udvarhelyi and Watkins, 1990). The (*R*)-isomer had a rotation of $\alpha_D = -55°$ ($c = 0.58$, H_2O) and the (*S*) enantiomer an $\alpha_D = +60.1°$ ($c = 0.70$, H_2O).

The novel compounds were tested for their ability to antagonize a variety of glutamate receptor-mediated responses. Experiments were performed on hemisected spinal cords from 1–5 day old neonatal rat pups (Evans et al., 1982). The standard superfusion medium contained (mM): NaCl 118, $NaHCO_3$ 25, KCl 3, $CaCl_2$ 2.5, D-glucose 12, gassed with 95%O_2/5%CO_2, with all solutions being superfused over the preparation at a rate of 1 ml/min. In order to investigate the effects of the novel compounds on postsynaptically-mediated depolarizations, tetrodotoxin (10 μM for 2 min then 0.1 μM continuously) was included in the standard Ringer solution enabling us to study agonist-induced depolarizations generated directly in the motoneurones.

The selectivity of the novel compounds was first established by investigating the effects of (*RS*)-3,4 and (*RS*)-3,5-DCPG on both metabotropic and ionotropic receptors. Thus, the ability of 500 μM (*RS*)-3,4- and (*RS*)-3,5-DCPG to antagonize depolarizations generated by the mGlu receptor agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) and the ionotropic glutamate receptor agonists AMPA, NMDA and kainate was studied. Each of the agonists was superfused over the preparation for 1 min with at least 20 min intervals between subsequent additions of (1*S*,3*R*)-ACPD to prevent desensitization of the mGlu receptor (Birse et al., 1993).

To determine the potency of (*RS*)-3,4-DCPG and (*RS*)-3,5-DCPG at the various receptor subtypes,

concentration–response curves for (1*S*,3*R*)-ACPD, AMPA, kainate and NMDA were performed both in the absence and presence of (*RS*)-3,4- and (*RS*)-3,5-DCPG (500 μM or 1 mM) allowing calculation of apparent K_d values.

The abilities of 500 μM (*R*)-3,4-DCPG and (*S*)-3,4-DCPG to antagonize (1*S*,3*R*)-ACPD-, AMPA-, kainate- and NMDA-induced depolarizations were also investigated in order to identify the isomer responsible for the observed antagonist effects. Further concentration–response curves were then constructed in the absence and presence of 100 μM and 1 mM (*R*)-3,4-DCPG for AMPA and kainate respectively, and apparent K_d values calculated.

With the exception of (*RS*)-3,4-DCPG and (*RS*)-3,5-DCPG which were synthesised in our own laboratory, all other compounds were obtained from Tocris Cookson (Bristol) or Sigma (Poole).

3. Results

Table 1 summarises the effects of (*RS*)-3,4- and (*RS*)-3,5-DCPG and the (*R*)- and (*S*)-forms of 3,4-DCPG on depolarizations in motoneurones induced by AMPA, kainate, NMDA and (1*S*,3*R*)-ACPD. (*RS*)-3,4-DCPG, (*RS*)-3,5-DCPG (500 μM, Fig. 2) and (*R*)-3,4-DCPG (500 μM, Fig. 3) produce a hyperpolarization when applied to a tetrodotoxin treated spinal cord and this effect is reversed upon wash out of the drugs; this is a common effect with excitatory amino acid antagonists (Evans et al., 1982). Both (*RS*)-3,5-DCPG (500 μM, Fig. 2) and (*RS*)-3,4-DCPG (500 μM) antagonized depolarizations due to AMPA ($K_d = 167 \pm 13$ μM ($n = 5$) and 137 ± 20 μM ($n = 3$) respectively), but not those due to kainate. In addition, both (*RS*)-3,5-DCPG (Fig. 4a) and (*R*)-3,4-DCPG (100 μM, Fig. 5) produced rightward parallel shifts of the concentration–response curve for AMPA consistent with a competitive mode of antagonism. In contrast, (*RS*)-3,5-DCPG (1 mM) enhanced depolarizations due to kainate, producing a leftward parallel shift of the concentration–response curve (Fig. 4b), while (*RS*)-3,4-DCPG, at high concentrations, antagonized responses due to kainate ($K_d \geq 12$ mM), but clearly at a much higher concentration than that needed to antagonize responses due to AMPA. (*RS*)-3,5-DCPG weakly antagonized responses due to NMDA ($K_d = 472 \pm 50$ μM ($n = 3$) and (1*S*,3*R*)-ACPD (K_d not determined, Fig. 2). Similarly, (*RS*)-3,4-DCPG weakly antagonized responses due to NMDA and (1*S*,3*R*)-ACPD, with K_d values of 346 ± 45 μM and 397 ± 44 μM ($n = 3$ for both compounds), respectively. The AMPA and NMDA receptor antagonist activity of 3,4-DCPG was found to reside in the *R* enantiomer (Fig. 3). (*R*)-3,4-DCPG antagonized responses due to AMPA with a $K_d = 77 \pm 6$ μM ($n = 3$) (Fig. 5), while at a concentration of 500 μM complete antagonism of the NMDA-induced depolarization was also observed (Fig. 3; Table 1). Weak antagonism

Table 1
Summary of effects of (*RS*)-3,4- and (*RS*)-3,5-DCPG and (*R*)- and (*S*)-3,4-DCPG on agonist-induced depolarizations in the neonatal rat spinal cord preparation

Compound	% of control response			
	AMPA	kainate	NMDA	(1*S*,3*R*)-ACPD
(*RS*)-3,5-DCPG	28 ± 3 (5)	118 ± 9 (5) [b]	26 ± 4 (3)	56 ± 8 (3)
(*RS*)-3,4-DCPG	21 ± 5 (3)	92 ± 1 (3) [b]	21 ± 6 (3)	39 ± 6 (3)
(*R*)-3,4-DCPG	39 ± 7 (3) [a]	83 ± 11 (3) [b]	0 (3)	87 ± 5 (3)
(*S*)-3,4-DCPG	83 ± 12 (3)	107 ± 7 (3)	83 ± 11 (3)	52 ± 13 (3)

Values are expressed as mean % of control response, in presence of 500 μM (*RS*)-3,5-, (*RS*)-3,4-, (*R*)-3,4- or (*S*)-3,4-DCPG ± S.E.M. (number of experiments) unless otherwise indicated.
[a] 100 μM (*R*)-3,4-DCPG used.
[b] 1 mM (*RS*)-3,5-, (*RS*)-3,4- and (*R*)-3,4-DCPG used.

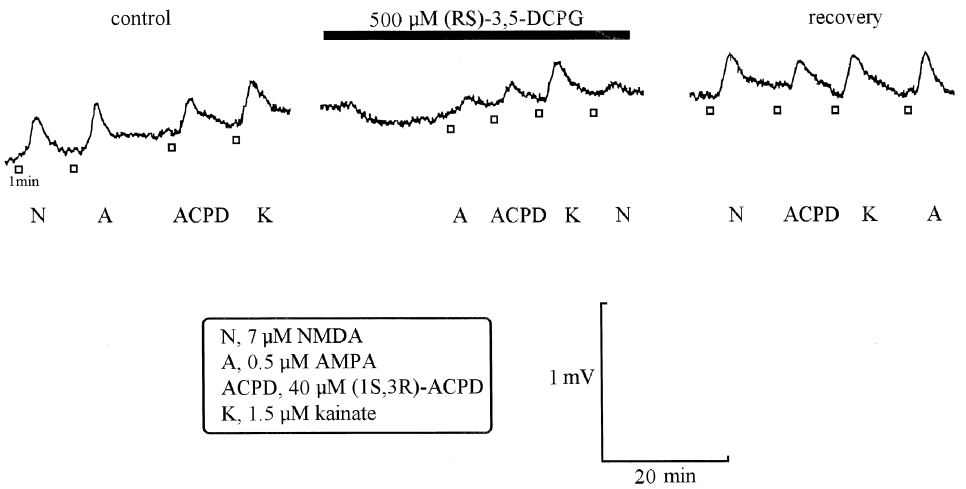

Fig. 2. A trace from a typical experiment showing the antagonist effects of 500 μM (*RS*)-3,5-DCPG on depolarizations generated directly in motoneurones by NMDA, AMPA, kainate and (1*S*,3*R*)-ACPD.

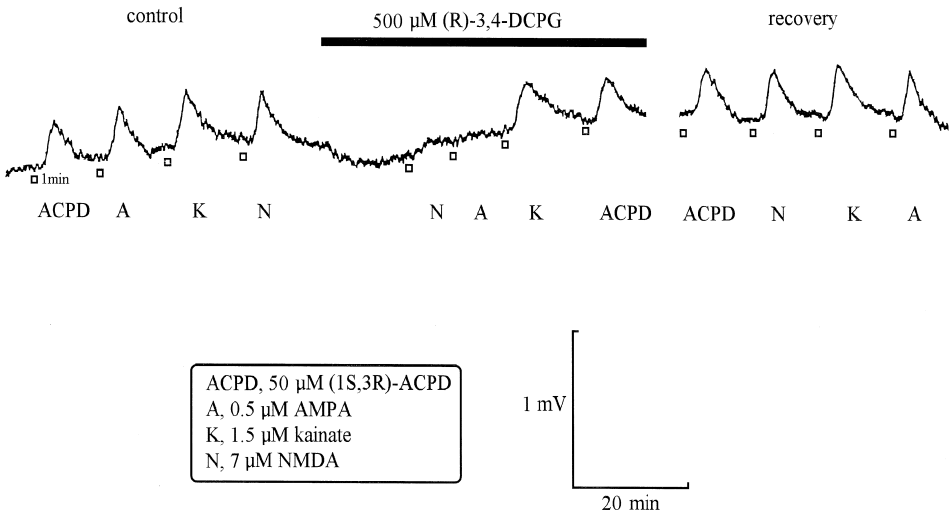

Fig. 3. A trace from a typical experiment showing the antagonist effects of 500 μM (*R*)-3,4-DCPG on depolarizations generated directly in motoneurones by NMDA, AMPA, kainate and (1*S*,3*R*)-ACPD.

of depolarizations induced by (1*S*,3*R*)-ACPD was also observed in the presence of 500 μM (*R*)-3,4-DCPG (Fig. 3; Table 1). (*S*)-3,4-DCPG (500 μM) weakly antagonized NMDA- and AMPA-induced depolarizations whereas those due to kainate were unaffected (Table 1). In additions 500 μM (*S*)-3,4-DCPG antagonized depolarizations induced

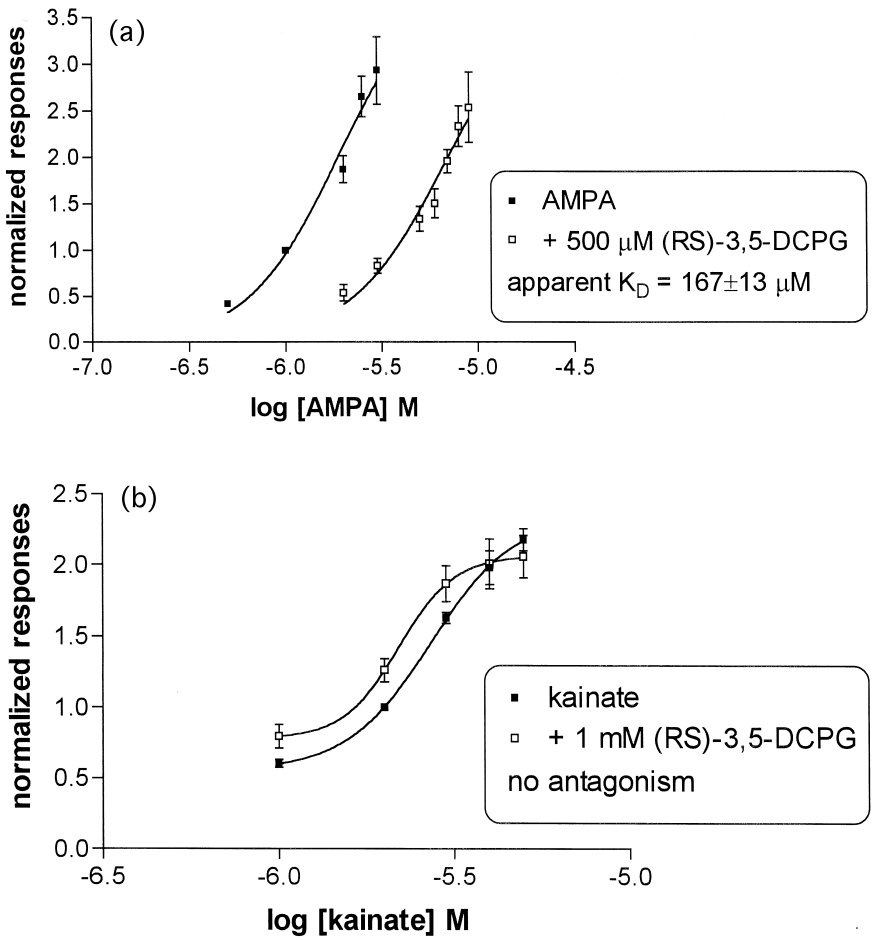

Fig. 4. Concentration–response curves for motoneuronal depolarizations produced by (a) AMPA and (b) kainate in the absence (■) and presence (□) of (*RS*)-3,5-DCPG. 500 μM (*RS*)-3,5-DCPG shifts the concentration–response curve to AMPA in a rightward manner, whereas the concentration–response curve to kainate is shifted to the left in the presence of 1 mM (*RS*)-3,5-DCPG. Each point represents the mean normalised response ± S.E.M. from 5 preparations.

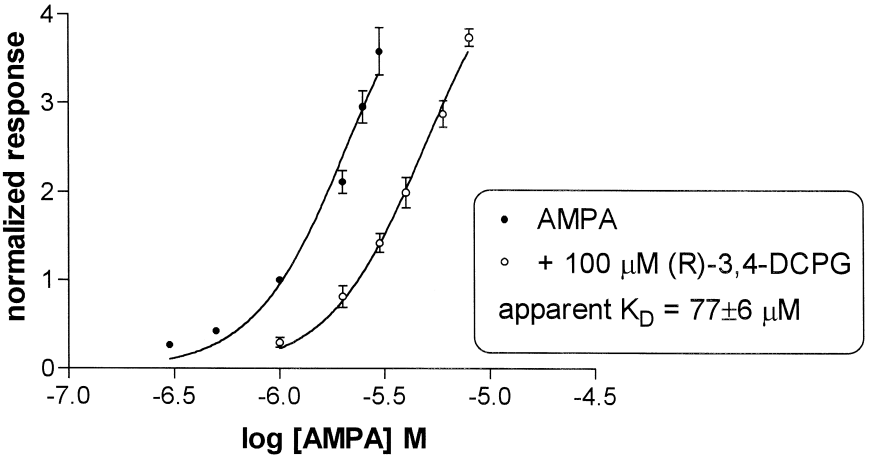

Fig. 5. Concentration–response curve for AMPA in the absence (●) and presence (○) of 100 μM (*R*)-3,4-DCPG. In the presence of (*R*)-3,4-DCPG, the AMPA concentration–response curve is shifted to the right in a parallel manner. Each point represents mean normalised response ± S.E.M. from 3 preparations.

by (1*S*,3*R*)-ACPD by 48% when compared to those depolarizations determined in the absence of antagonist (Table 1). Due to limitation of compound availability complete quantitative characterization of the actions of (*R*)- and (*S*)-3,4-DCPG on ionotropic and metabotropic glutamate receptors could not be undertaken. For similar reasons the apparent K_d for antagonism of (1*S*,3*R*)-ACPD-induced depolarizations by (*RS*)-3,5-DCPG could not be calculated.

4. Discussion

Both (*RS*)-3,4- and 3,5-DCPG were selective antagonists of AMPA-relative to kainate-induced motoneuronal depolarizations. Neither of the phenylglycines significantly antagonized responses due to kainate. However, (*RS*)-3,5-DCPG slightly enhanced kainate-induced responses. The mechanism of this enhancement is currently unknown, but may involve interaction with a modulatory site on the receptor. It would appear therefore that at the concentrations used in this study (up to 10 μM) kainate is selectively activating kainate receptors. Higher concentrations of kainate may be necessary to activate AMPA receptors present on the spinal motoneurones.

The AMPA receptor antagonist activity (as well as the NMDA receptor antagonism) of (*RS*)-3,4-DCPG was found to reside in the *R* enantiomer, while the *S* enantiomer weakly antagonized responses due to (1*S*,3*R*)-ACPD and had little effect on AMPA, kainate and NMDA receptors. Due to a limitation of compound availability complete quantitative characterization of the actions of (*R*)- and (*S*)-3,4-DCPG on ionotropic and metabotropic glutamate receptors could not be undertaken at this stage. The individual enantiomers of (*RS*)-3,5-DCPG are required to further clarify the actions of this compound and an attempted resolution of the racemate is currently underway.

The present results give a strong indication that both (*RS*)-3,5-DCPG and (*R*)-3,4-DCPG exhibit useful selectivity between AMPA and kainate receptors and indicate the likelihood that pharmacologically distinct AMPA and kainate receptors exist in motoneurones in the neonatal rat spinal cord. However, the possibility exists that some of the depolarization may be due to effects on presynaptic ionotropic receptors. It has recently been reported that presynaptic NMDA receptors are present in the spinal dorsal horn (Liu et al., 1994) and the possibility that AMPA and kainate receptors are present on presynaptic terminals cannot be ruled out. The hyperpolarization resulting from application of either 3,4- or 3,5-DCPG could result from the blockade of depolarizations caused by leakage of glutamate from surrounding terminals or glial cells. Alternatively, this hyperpolarization could be the result of antagonism of a residual tonic excitatory input incompletely blocked by tetrodotoxin (Evans et al., 1982).

The mixed actions of these compounds on other iGlu and mGlu receptors may limit the usefulness of these compounds as selective AMPA receptor antagonists, unless used in conjunction with specific antagonists of the other receptors. New analogues of these compounds are currently being synthesised in an attempt to improve both the selectivity and the potency of the present compounds.

Acknowledgements

We would like to thank Professor J.C. Watkins for helpful discussion during preparation of this manuscript. This work was funded by the Medical Research Council, UK.

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