SPECIAL REPORT

Antagonism of the mGlu5 agonist 2-chloro-5-hydroxyphenylglycine by the novel selective mGlu5 antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) in the thalamus

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Our previous work has shown that Group I mGlu receptors participate in thalamic sensory processing *in vivo*. However, unequivocal demonstration of mGlu5 participation has not been possible due to the lack of specific ligands. We have therefore made a preliminary study of the *in vivo* actions of the agonist (R,S)-2-Chloro-5-hydroxyphenylglycine [CHPG] and the novel mGlu5 antagonist 6-methyl-2-(phenylethynyl)-pyridine [MPEP] in order to characterize their suitability for functional studies. Iontophoretically administered MPEP selectively antagonized excitatory responses of single rat thalamic neurones to CHPG compared to the broad-spectrum mGlu agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate. In contrast, the established mGlu1 and mGlu5 antagonist (S)-4-carboxyphenylglycine reduced responses to both agonists. These findings are the first demonstration of an *in vivo* action of CHPG and its antagonism by a selective mGlu5 antagonist. Furthermore MPEP appears to be a good tool for functional studies of mGlu5.

Keywords: Metabotropic glutamate receptor; mGlu5; 6-methyl-2-(phenylethynyl)-pyridine; MPEP; (R,S)-2-Chloro-5-hydroxyphenylglycine; CHPG; thalamus; sensory system; iontophoresis

Abbreviations: 4CPG, 4-carboxyphenylglycine; ACPD, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate; CHPG, (R,S)-2-Chloro-5-hydroxyphenylglycine; MPEP, 6-methyl-2-(phenylethynyl)-pyridine; NMDA, N-methyl-D-aspartate

Introduction The metabotropic glutamate (mGlu) receptors is a family of receptors whose activation by L-glutamate and other excitatory amino acids is coupled to a cascade of intracellular second-messenger events. There are at least eight of these receptors, and they can be placed into three groups: Group I, mGlu1, mGlu5; Group II, mGlu2, mGlu3; Group III, mGlu4, mGlu6, mGlu7, mGlu8, on the basis of sequence homology, agonist/antagonist pharmacology and coupling to intracellular transduction mechanisms (Nakanishi, 1992; Watkins & Collingridge, 1994; Conn & Pin, 1997). Group I receptors are known to couple to postsynaptic inositol phosphate hydrolysis, and there is considerable evidence to suggest that these receptors participate in synaptic processes in several brain regions (for review see Conn & Pinn, 1997). In the rat thalamus *in vivo*, work from this laboratory has shown that excitatory responses to mGlu agonists can be reduced by a variety of antagonists active at Group I receptors and that these antagonists can reduce responses of thalamic neurones to noxious thermal stimulation (Eaton *et al*., 1993; Salt & Eaton, 1994). More recently, we have shown that an antagonist with selectivity towards mGlu1 can have similar actions (Salt & Turner, 1998). This leaves the question open as to whether there is a distinct effect of mGlu5 receptors on neuronal activity in the thalamus. Recently, the novel compound 6-methyl-2-(phenylethynyl)-pyridine (MPEP) has been described as a selective non-competitive mGlu5 antagonist which shows activity in a number of *in vitro* and *in vivo* systems (Gasparini *et al*., 1999). This offers the possibility of unequivocally identifying responses which may be mediated by mGlu5, and we have therefore made a preliminary evaluation of this compound on the responses of thalamic neurones to the selective mGlu5 agonist (R,S)-2-Chloro-5-hydroxyphenylgly-

cine [CHPG] (Doherty *et al*., 1997) and the broad-spectrum mGlu agonist ACPD. We have compared the effects of MPEP with the activity of the established Group I antagonist (S)-4-carboxyphenylglycine [(S)-4CPG].

Methods Experiments were carried out in adult Wistar rats anaesthetized with urethane (1.2 g kg^{-1} i.p.) in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines, as detailed previously (Salt, 1987). Extracellular single-neurone recordings were made with multi-barrel iontophoretic electrodes, the outer barrels of which contained one of the following aqueous solutions: ACPD [(1S,3R)-1-aminocyclopentane-1,3-dicarboxylate] 50 mM, pH 8; CHPG [(R,S)-2-Chloro-5-hydroxyphenylgly-cine] 100 mM, pH 8; NMDA [N-methyl-D-aspartate] 50 mM, pH 8; (S)-4CPG [(S)-4-carboxyphenylglycine] 50 mM, pH 8; MPEP [6-methyl-2-(phenylethynyl)-pyridine] 2 mM or 10 mM in 150 mM NaCl, pH 5. In addition, one barrel contained 1 M NaCl for automatic current balancing. All drugs were ejected iontophoretically as anions (with the exception of MPEP), and prevented from diffusing out of the pipette by a retaining current (10–20 nA) of opposite polarity to the ejection current. MPEP was provided by Novartis (Basel, Switzerland), other drugs were purchased from Tocris (Bristol, U.K.). Neurones in the ventrobasal thalamus and immediately overlying thalamus were identified on the basis of their stereotaxic position and responses to somatosensory stimuli, as described previously (Salt, 1987; Salt & Eaton, 1994).

Results Neurones were excited by regular cyclical applications of the agonists CHPG (150–350 nA), ACPD (50–100 nA) and NMDA (20–80 nA) (Figure 1), and the resulting excitatory responses were quantified as the number of action

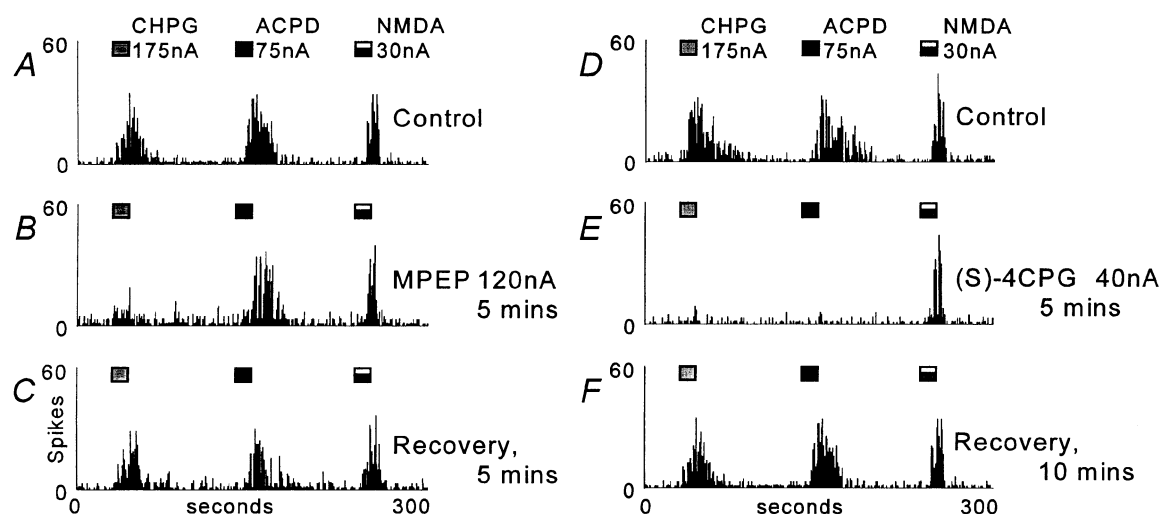* Author for correspondence; E-mail: t.salt@ucl.ac.uk

Figure 1 Peristimulus time histograms (PSTHs) showing responses of a thalamic neurone to iontophoretically applied CHPG, ACPD and NMDA. Each PSTH shows action potentials ('spikes') counted into successive 1000 ms epochs, agonists were applied as indicated by the marker bars above the records. (*A*) Control responses. (*B*) Responses during the co-application of MPEP (note the selective reduction of the CHPG response). (*C*) Recovery following termination of the MPEP ejection. (*D*) Control responses. (*E*) Reduction of both CHPG and ACPD responses during co-application of (S)-4CPG. (*F*) Recovery following termination of (S)-4CPG ejection.

potentials evoked. Agonist ejection parameters were adjusted so as to produce sub-maximal responses. Application of the antagonist MPEP onto 13 thalamic neurones throughout two or more agonist ejection cycles was found to reduce responses to CHPG with less effect on responses to ACPD or NMDA (Figure 1, Table 1). In contrast, application of (S)-4CPG onto six neurones (four of which were also studied with MPEP) produced a reduction of responses to both ACPD and CHPG, again with little effect on NMDA responses (Figure 1, Table 1).

Discussion The results of this study are consistent with previous data which show that MPEP is a selective mGlu5 antagonist (Gasparini *et al.*, 1999) and that CHPG is a selective mGlu5 agonist (Doherty *et al.*, 1997). The present data extend previous *in vitro* findings (Doherty *et al.*, 1997) in demonstrating unequivocally for the first time that CHPG is able to activate mGlu5 receptors *in vivo*. It is noteworthy that MPEP was found to reduce responses to CHPG selectively compared to ACPD. This would suggest that ACPD does not exert its excitatory effects in the thalamus *via* mGlu5, even though this agonist has a broad spectrum of activity. *In situ* hybridization studies and immunohistochemistry indicate that although mGlu5 is present in the thalamus (Abe *et al.*, 1992; Romano *et al.*, 1996) there are considerably higher levels of mGlu1 in this brain region (Masu *et al.*, 1991; Shigemoto *et al.*, 1992; Martin *et al.*, 1992). This would suggest that the excitatory action of ACPD in the thalamus is mediated *via* mGlu1, and this is supported by the finding that thalamic ACPD responses can be antagonized by the mGlu1-selective antagonist (+)-2-methyl-4-carboxyphenylglycine (Salt & Turner, 1998). This of course does not preclude the possibility that there is activation of mGlu5 as well as mGlu1 by ACPD but that the effects *via* mGlu5 are masked by the activation of mGlu1 receptors, which

Table 1 Effects of MPEP and (S)-4CPG on responses to CHPG, ACPD and NMDA

Antagonist	CHPG % control	ACPD % control	NMDA % control	Current nA
MPEP n = 13	33 ± 3.2**	83 ± 9.5*	107 ± 10.6	70 ± 9.0
(S)-4CPG n = 6	26 ± 5.2*	22 ± 5.4*	102 ± 13.3	68 ± 17.4

Values are means ± s.e.mean of agonist responses in the presence of antagonist expressed as percentages of control values, and the mean ± s.e.mean iontophoretic current of antagonist. Values are significantly different from controls (Wilcoxon Signed Rank Test) as indicated: *$P < 0.05$; **$P < 0.01$.

are numerically predominant (Masu *et al.*, 1991; Shigemoto *et al.*, 1992). This could explain the observed lack of effect of MPEP against ACPD. This matter could be resolved if more potent specific agonists for mGlu1 and mGlu5 become available. Interestingly, in the hippocampus, MPEP is able to antagonize excitatory responses to a broad-spectrum Group I agonist, (S)-3,5-dihydroxyphenylglycine, (Gasparini *et al.*, 1999): this is likely to reflect the predominance of mGlu5 compared to mGlu1 in the hippocampus.

In conclusion, our results demonstrate that it is possible to selectively activate and antagonize mGlu5 receptors in the thalamus. Furthermore, the novel antagonist MPEP appears to be a powerful tool for the identification of mGlu5 synaptic contributions in forthcoming functional studies.

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