ELSEVIER

European Journal of Pharmacology 309 (1996) 79–85

ejp

Agonists of cyclic AMP-coupled metabotropic glutamate receptors in adult rat cortical slices

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Received 11 April 1996; accepted 16 April 1996

Abstract

A number of potential Group 2 and Group 3 metabotropic glutamate receptor (mGlu receptor) agonists were investigated in adult rat brain cerebrocortical slices. The rank order of their potency in inhibiting forskolin-stimulated adenylyl cyclase was found to be: (*S*)-2-amino-2-methyl-4-phosphonobutyric acid (MAP4) > (2*S*,1′*S*,2′*S*)-2-(2-carboxycyclopropyl)glycine (L-CCG-I) > (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*S*-ACPD) > (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*)-ACPD) > (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) > (*S*)-2-methylglutamate ((*S*)-MG) > L-glutamate > (2*S*,1′*S*,2′*S*)-2-(2-carboxycyclopropyl)alanine (MCCG) > L-2-amino-4-phosphonobutyric acid (L-AP4) > L-serine-*O*-phosphate (SOP). The finding that (*S*)-2-amino-2-methyl-4-phosphonobutyric acid was the most potent agonist at these metabotropic glutamate receptors is in contrast to its observed potent mGlu receptor antagonist action in the neonatal rat spinal cord.

Keywords: Metabotropic glutamate receptor; Cerebral cortex slice; cAMP; (Agonist)

1. Introduction

Metabotropic glutamate (mGlu) receptors represent a unique family of G-protein-coupled receptors. They have seven transmembrane-spanning domains, large extracellular N-terminal domains and exhibit little or no sequence homology with other G-protein-coupled receptors (for review, see Pin and Duvoisin (1995)). Eight mGlu receptors have so far been cloned (Houamed et al., 1991; Masu et al., 1991; Abe et al., 1992; Nakajima et al., 1993; Tanabe et al., 1992; Okamoto et al., 1994; Saugstad et al., 1994; Duvoisin et al., 1995) and these have been provisionally classified into three groups according to their sequence homology (members of each group have 60–75% sequence homology), second messenger coupling and agonist preference.

Group 1 consists of mGlu receptors 1 and 5 which are positively linked to phosphoinositide hydrolysis. They are predominantly located postsynaptically (Shigemoto et al., 1993), although the $mGlu_5$ receptor is also present presynaptically (Romano et al., 1995) and is thought to mediate a feedback facilitatory effect on glutamatergic transmission (Herrero et al., 1992, 1994).

Group 2 ($mGlu_1$ and $mGlu_2$ receptors) and group 3 (mGlu receptors 4, 6, 7, 8) mGlu receptors are negatively coupled to adenylyl cyclase and are thought to be mainly presynaptic and, serving as autoreceptors to modulate the release of glutamate. Their activation results in inhibition of cyclic AMP accumulation following forskolin stimulation of adenylyl cyclase. Group 2 mGlu receptors, stably expressed in Chinese Hamster Ovary (CHO) cells, have an agonist rank order of potency of: (2*S*,1′*S*,2′*S*)-2-(2-carboxycyclopropyl)glycine (L-CCG-I) > L-glutamate ≥ (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*)-ACPD) > ibotenate > quisqualate (Tanabe et al., 1992) and lack sensitivity to L-2-amino-4-phosphonobutyrate (L-AP4).

In contrast, group 3 mGlu receptors are potently activated by L-AP4. The $mGlu_4$ receptor in rat is found predominantly in the cerebellum and the agonist activity profile at this receptor is: L-AP4 > L-glutamate ≥ L-CCG-I ≥ L-serine-*O*-phosphate (SOP) (Tanabe et al., 1993). Human $mGlu_{4a}$ receptor transfected into baby hamster kidney (BHK) cells has the following profile (Eriksen and Thomsen, 1995): L-AP4 = serine-*O*-phosphate (SOP) > L-

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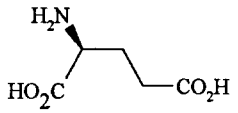
PII S0014-2999(96)00314-7

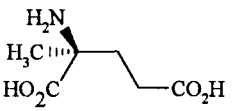
glutamate = L-CCG-I > 1-amino-3-(phosphonomethylene)-cyclobutanecarboxylate >> (1*S*,3*R*)-ACPD = quisqualate > ibotenate. $MGlu_6$ receptors are restricted to the ON-bipolar cells of the retina (Nakajima et al., 1993). Similarly $mGlu_8$ receptor immunoreactivity is found in the retina and in the olfactory bulb. It is also present, albeit sparsely, in the cerebral cortex (Duvoisin et al., 1995). The $mGlu_8$ receptor has the agonist profile: L-glutamate > L-AP4 >> (1*S*,3*R*)-ACPD. $MGlu_7$ receptor mRNA is more widely distributed and the receptor has an agonist profile of: L-AP4 = SOP > L-glutamate when expressed in CHO cells (Okamoto et al., 1994; Saugstad et al., 1994).

In this paper we report the agonist actions of L-glutamate, (*S*)-2-methylglutamate ((*S*)-MG), L-AP4, (*S*)-2-

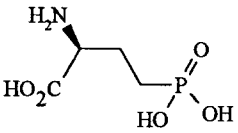
Table 1
Structures of agonists



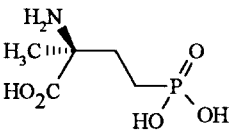
(L)-Glutamic acid (L-glu)



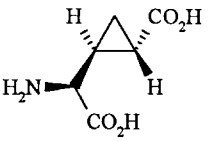
(S)-2-Methylglutamic acid ((S)-MG)



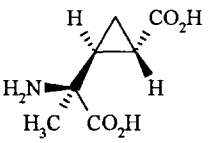
(L)-2-Amino-4-phosphonobutyric acid (L-AP4)



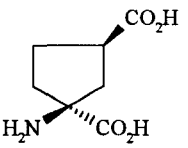
(S)-2-Amino-2-methyl-4-phosphonobutyric acid (MAP4)



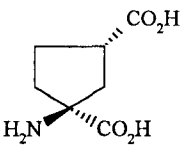
(2S,1'S,2'S)-2-(2-Carboxycyclopropyl)glycine (L-CCG-I)



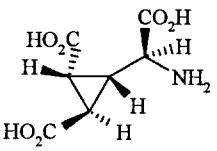
(2S,1'S,2'S)-2-(2-Carboxycyclopropyl)alanine (MCCG)



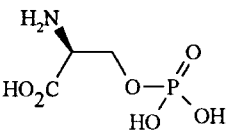
(1S,3R)-1-Aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD)



(1S,3S)-1-Aminocyclopentane-1,3-dicarboxylic acid ((1S,3S)-ACPD)



(2S,1'R,2'R,3'R)-2-(2,3-Dicarboxy-cyclopropyl)glycine (DCGIV)



L-Serine-O-phosphate (SOP)

amino-2-methyl-4-phosphonobutyric acid (MAP4), L-CCG-I, (2*S*,1′*S*,2′*S*)-2-(2-carboxycyclopropyl)alanine (MCCG), 2-(2,3-dicarboxycyclopropyl)-glycine (DCG-IV), (1*S*,3*S*)-ACPD, (1*S*,3*R*)-ACPD and SOP (see Table 1 for structures) on mGlu receptors negatively coupled to adenylyl cyclase in adult rat cerebral cortical slices; a tissue which, according to reported mGlu receptor mRNA distribution and immunohistochemical data, primarily contains $mGlu_3$ and $mGlu_7$ receptors. Some caution is however necessary as quantitatively minor populations of mGlu receptor subtypes 2, 4 and 8 are almost certainly present as may be other, as yet unreported, mGlu receptor subtypes.

2. Materials and methods

2.1. Materials

DCG-IV was a gift from Y. Ohfune. SOP, forskolin and adenosine deaminase were obtained from the Sigma Chemical Company, Poole, Dorset, UK. All other compounds, were obtained from Tocris Cookson, Langford, Bristol, UK. Other reagents were of the highest grade available.

2.2. Methods

Experiments were conducted as previously described (Bedingfield et al., 1995). Briefly; adult rat cortical slices (300 × 300 μm prisms) were prepared and left to equilibrate for 60 min in several changes of oxygenated Krebs medium at 37°C. Aliquots of gravity-packed slices (25 or 50 μl according to the cyclic AMP assay system used) were preincubated with agonist, adenosine deaminase (0.4 units) and 100 μM of the phosphodiesterase inhibitor Ro 20-1724 for 20 min at 37°C. Forskolin (30 μM) was added, to directly stimulate cyclic AMP production by adenylyl cyclase, in a final volume of 250 μl. After 10 min the reaction was terminated and the amount of cyclic AMP estimated.

Cyclic AMP accumulation was estimated by either ion-exchange chromatography as described by Kemp et al. (1994) or by a binding protein displacement assay as described by Bedingfield et al. (1996). The assay methods gave identical results in control experiments (data not presented).

Data are expressed as a percentage of the maximum cyclic AMP concentration produced following stimulation

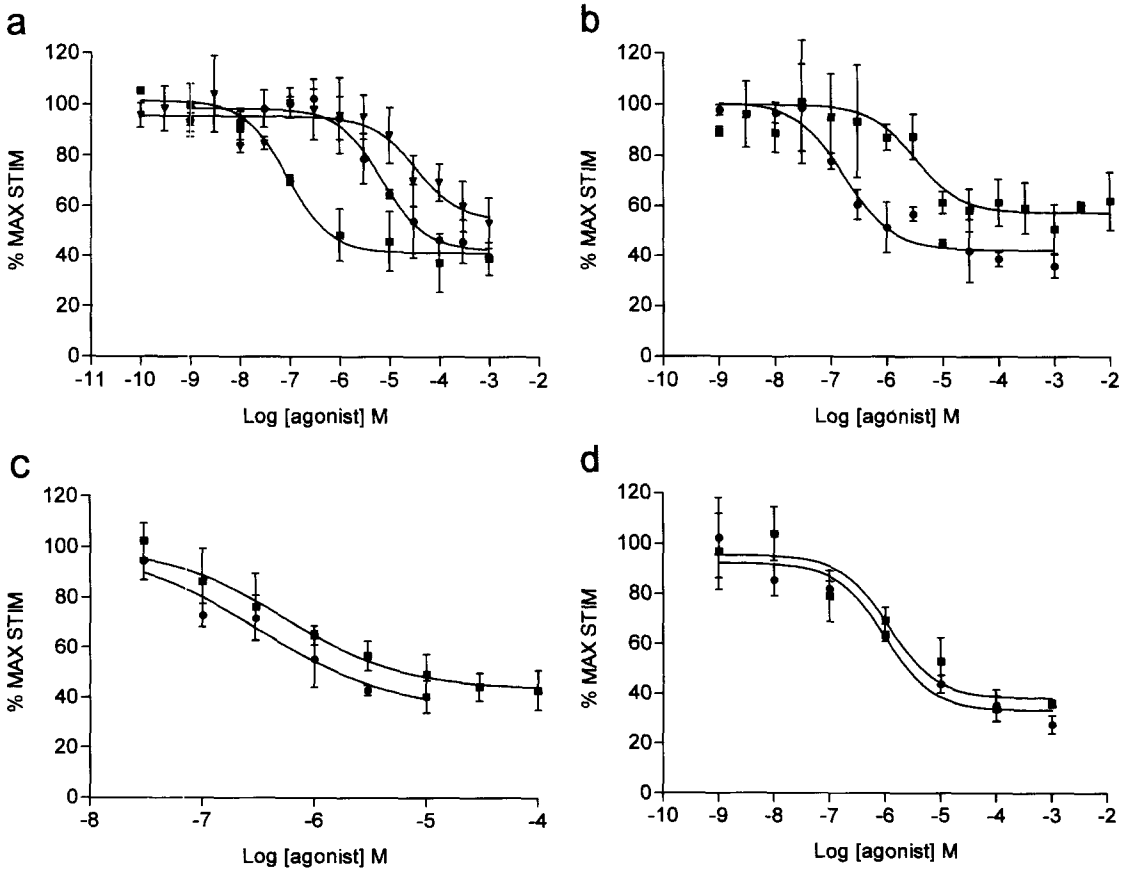


Fig. 1. Inhibition of forskolin (30 μM)-stimulated formation of cyclic AMP in adult rat cortical slices by agonists of metabotropic glutamate receptors negatively coupled to adenylyl cyclase. None of the agonists completely reversed the stimulation of cyclic AMP production. All results are the means ± S.E.M. of at least three experiments performed in triplicate. Concentration-dependent agonism was exhited by: a: MAP4 (■), L-AP4 (●), SOP (▼); b: L-CCG-I (●) and MCCG (■); c: (1*S*,3*R*)-ACPD (■), (1*S*,3*S*)-ACPD (●); d: (*S*)-MG (●), L-glu (■).

by 30 μM forskolin. All experiments were performed a minimum of three times in triplicate.

3. Results

MAP4 was the most potent agonist tested with an EC_{50} of 0.11 ± 0.1 μM. Thus, introduction of an α-methyl group into L-AP4 resulted in an 80-fold increase in agonist potency over the non-methylated parent compound (EC_{50} for L-AP4 = 8.82 ± 1.07 μM). MAP4 is some 350 times more potent than SOP ($EC_{50} = 38.0 \pm 26.6$ μM). L-AP4 and SOP are group 3 mGlu receptor-specific agonists (Fig. 1).

With the group 2 selective agonists; L-CCG-I showed high potency ($EC_{50} = 0.24 \pm 0.13$ μM). The α-methyl analogue, MCCG, also retained agonist activity, although its potency was 13-fold lower ($EC_{50} = 3.16 \pm 0.74$ μM) than for L-CCG-I (Fig. 2). The (1*S*,3*S*)- and (1*S*,3*R*)-isomers of ACPD had similar potencies, with EC_{50} values of 0.30 ± 0.30 μM and 0.48 ± 0.11 μM respectively (Fig. 2). L-Glutamate and its α-methyl form had similar EC_{50} values of 1.12 ± 1.20 μM and 0.74 ± 0.72 μM (Fig. 2).

DCG-IV, in addition to its activity at group 2 mGlu receptors, is a potent NMDA agonist at higher concentrations (Ishida et al., 1993). In cerebrocortical slices, at concentrations above 30 μM, there was a reversal of the inhibition of cyclic AMP formation by DCG-IV. When the potent and selective NMDA antagonist (*R*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (*R*-CPP) (10 μM) was also included, the NMDA stimulation at higher concentrations of DCG-IV was abolished and the EC_{50} was calculated to be 0.56 ± 0.09 μM. This indicates that activation of NMDA receptors can cause a rise in the accumulated cyclic AMP, most likely consequent to its elevating intracellular calcium concentrations (Fig. 2 and Table 2). None of the agonists tested were able to completely inhibit the forskolin-stimulated accumulation of cyclic AMP. All observed agonist effects were likely to be directly receptor-mediated since we have found no evidence for an action on the glutamate high-affinity transporter (data not shown).

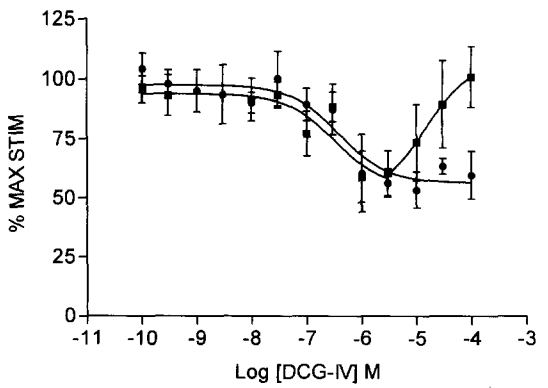


Fig. 2. Inhibition of forskolin (30 μM)-stimulated formation of cyclic AMP in adult rat cortical slices by the mGlu receptor agonist DCG-IV: (■) alone, (●) in the presence of the potent selective NMDA antagonist 10 μM (*R*)-CPP. (*R*)-CPP prevents DCG-IV from stimulating cyclic AMP production via NMDA activation at concentrations above 30 μM. DCG-IV was not able, even in the presence of *R*-CPP, to completely inhibit the forskolin-stimulated cyclic AMP production. Results represent the means ± S.E.M. of four experiments performed in quadruplicate.

Table 2
Summary of agonist potencies

Agonist		EC_{50} μM
(*S*)-2-Amino-2-methyl-4-phosphonobutyric acid	MAP4	0.11 ± 0.10
(2*S*,1′*S*,2′*S*)-2-(2-Carboxycyclopropyl)glycine	L-CCG-I	0.24 ± 0.13
(1*S*,3*S*)-1-Aminocyclopentane-1,3-dicarboxylic acid	(1*S*,3*S*)-ACPD	0.30 ± 0.30
(1*S*,3*R*)-1-Aminocyclopentane-1,3-dicarboxylic acid	(1*S*,3*R*)-ACPD	0.48 ± 0.11
(2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-Dicarboxycyclopropyl)glycine	DCG-IV	0.56 ± 0.09
(*S*)-2-Methylglutamate	(*S*)-MG	0.74 ± 0.72
L-Glutamate	L-Glutamate	1.12 ± 1.20
(2*S*,1′*S*,2′*S*)-2-(2-Carboxycyclopropyl)alanine	MCCG	3.16 ± 0.74
L-2-Amino-4-phosphonobutyric acid	L-AP4	8.82 ± 1.07
L-Serine-*O*-phosphate	SOP	38.0 ± 26.6

Agonist EC_{50} values were calculated from graphs of agonist concentration vs. percentage depression of maximum forskolin stimulated cyclic AMP accumulation. The EC_{50} value for DCG-IV was derived in the presence of 10 μM R-CPP. Values (± S.E.M.) are from at least three experiments performed in triplicate.

4. Discussion

Rat cerebrocortical slices contain members of each of the three groups of mGlu receptors. Although mRNA distribution and receptor immunohistochemical studies indicate that the prodominant types are $mGlu_3$, $mGlu_5$ and $mGlu_7$ (Abe et al., 1992; Jane et al., 1995; Shigemoto et al., 1993; Ohishi et al., 1993; Tanabe et al., 1993; Okamoto et al., 1994; Saugstad et al., 1994), the presence, in unknown proportion, of the other types is almost certain. Some are probably present in very low density and others may comprise important minor populations. This receptor heterogeneity together with an absence of information regarding the efficiency of coupling to effector systems, and particularly the lack of subtype specific agonists or antagonists, makes it impossible to ascribe specific effects absolutely to individual subtypes.

In previous studies we have described the activity of a number of phenylglycine compounds acting at mGlu receptors negatively coupled to adenylyl cyclase. Several of these were found to be both potent and specific antagonists (Bedingfield et al., 1995, 1996; Kemp et al., 1994; Jane et

al., 1995; Watkins and Collingridge, 1994). However, even when compounds are used which show relative mGlu receptor group selectivity, comparison between data derived from transfected cell lines and those obtained from native receptors, do not always correlate well. The discrepancies may be ascribed to a number of factors including: cloned cell lines being deficient in particular mechanisms necessary for efficient effector coupling; possible alternate coupling and, also, the absence in transfected receptors of specific post-translational modifications which may occur in native receptors. An example of this is the $mGlu_6$ receptor, which is restricted to the outer zone of the inner nuclear layer of the retina where ON-bipolar cells are located (Nakanishi, 1995). Activation of these receptors is thought to stimulate a cyclic GMP phosphodiesterase, resulting in membrane hyperpolarisation due to cyclic GMP-regulated calcium channels. In contrast, $mGlu_6$ receptors in transfected CHO cells couple negatively to adenylyl cyclase (Nakajima et al., 1993; Akazawa et al., 1994). All group 3 mGlu receptors appear to be weakly coupled to adenylyl cyclase when expressed in CHO/BHK cells, although different groups report various inhibition maxima, which may reflect variability in levels of receptor expression (Duvoisin et al., 1995; Thomsen et al., 1992; Tanabe et al., 1993; Okamoto et al., 1994, Saugstad et al., 1994). The complex web of intraneuronal inhibitory and stimulatory systems, positive and negative feedback and other regulatory mechanisms, which are present in whole tissue, are of course lacking in cell lines.

While mGlu receptors that are negatively coupled to adenylyl cyclase have been reported to be localised solely presynaptically, and to have a depressant action on glutamate transmission, $mGlu_5$ receptors are found both pre- and post-synaptically (Jane et al., 1995; Shigemoto et al., 1993). Presynaptic effects on mGlu receptors, leading to enhanced glutamate release from synaptosomes, have also been reported (Herrero et al., 1992, 1994); such dual actions add a further level of complexity to studies carried out in whole nervous tissue and may preclude simple comparisons to be made with data derived from clonal cell lines.

Notwithstanding these constraints, of particular interest in this present study are the α-methyl substituted forms of known group-specific agonists. (*S*)-MG did not demonstrate any change of potency from its parent non-specific agonist, glutamate. MAP4 did however show a marked enhancement in potency relative to that of L-AP4; conversely, MCCG displayed a considerable reduction in potency.

Recently East et al. (1995) reported that MAP4 effectively inhibited 4-aminopyridine-stimulated glutamate release from striatal synaptosomes and was devoid of any antagonist action against either L-AP4 or (1*S*,3*S*)-ACPD-mediated inhibition of release. Cassidy and O'Connor (1995) demonstrated that MAP4 was unable to block L-AP4-induced reduction of paired pulse depression (PPD) evoked in the medial perforant pathway of the rat dentate gyrus. Indeed MAP4 applied alone enhanced PPD. This implies that MAP4 was acting as an agonist at a receptor not susceptible to L-AP4. Knöpfel et al. (1995b) found that MAP4 was a weak antagonist of L-AP4 at both human cloned $mGlu_{4a}$ and $mGlu_2$ receptors. They also reported that, at high concentrations, MAP4 exhibited weak agonist activity at human $mGlu_{4a}$ receptors. These results are particularly striking since MAP4 has been demonstrated to be a highly selective antagonist of L-AP4-mediated depression of monosynaptic excitation in neonatal rat spinal cord (Jane et al., 1994) and of L-AP4 and L-CCG-I disinhibition of GABAergic inhibitory responses in rat ventrobasal thalamic neurones (Salt and Eaton, 1995). Vignes et al. (1995) have recently demonstrated that MAP4 was able to antagonise the group 3 agonist L-AP4 but not the group 2 agonists (1*S*,3*R*)- and (1*S*,3*S*)-ACPD in Schaffer collateral-commisural pathway of rat hippocampus. Manzoni et al. (1995) demonstrated similar findings in hippocampal mossy fibres, using L-CCG-1 as the group 2 agonist. Bushell et al. (1995) found that MAP4 had no effect alone in the dentate gyrus, following stimulation of the lateral perforant pathway. MAP4 did however antagonise L-AP4-induced depression of the synaptic response.

These apparently opposite effects may be explained by different mGluR subtypes being present. Alternatively, while the electrophysiological studies are observing functional transmission, in the rat cortical slices accumulation of cyclic AMP is being measured. Evidence has been provided which suggests that group 2 and 3 mGlu receptors have direct (presumably through G-protein coupling) effects on N, P, Q and L-type calcium channels (Chavis et al., 1994, 1995; Glaum and Miller, 1995; Lu and Knöpfel, 1994; Trombley and Westbrook, 1992) and potassium channels (Brown, 1988; Schoepp and Conn, 1993; Knöpfel et al., 1995a). It may be that measurement of cyclic AMP accumulation does not accurately reflect the functional effects of mGlu receptors on neuronal transmission.

Assuming that neonatal rat spinal cord and rat ventrobasal thalamic neurones contain different mGlu receptor subtypes from those present in rat cortical slices or striatum, and that the effects seen in cortex are real and functionally relevant, MAP4 must be able to discriminate between L-AP4-sensitive receptors. Also a receptor must be present which is capable of being activated by MAP4, is negatively coupled to adenylyl cyclase and may act as a presynaptic autoreceptor.

MCCG has been reported to antagonise the presynaptic depressant effects of (1*S*,3*S*)- ACPD, (1*S*,3*R*)-ACPD and L-CCG-I in neonatal rat spinal cord (Jane et al., 1994). It exhibited no antagonist activity vs. L-AP4-induced depression and did not depress monosynaptic excitation. Salt and Eaton (1995) found that MCCG blocked the disinhibitory action of L-CCG-I on GABAergic inhibitory responses in rat ventrobasal thalamic neurones, in a similar way to which MAP4 blocked the agonist effect of L-AP4. Knöpfel

et al. (1995b) have recently reported that MCCG antagonizes (1*S*,3*R*)-ACPD-mediated depression of forskolin-stimulated cyclic AMP formation in CHO cells expressing human $mGlu_2$ receptors. They also reported that MCCG had no effect on L-AP4 responses in cells expressing human $mGlu_4$ receptors and that MCCG, alone, was without effect. Vignes et al. (1995) and Manzoni et al. (1995) showed that in rat hippocampal Schaffer collateral-commisural pathway and mossy fibres, respectively, MCCG behaves as a group 2 antagonist, reversing the effects of (1*S*,3*R*)- and (1*S*,3*S*)-ACPD and L-CCG-1, whilst being inactive against L-AP4.

In contrast to these findings, in the present study MCCG behaved as an agonist at rat cortical mGlu receptors negatively coupled to cyclic AMP formation. It was, however, the weakest agonist acting at these, presumably, group 2 receptors, being 13-fold less potent than its parent compound L-CCG-I. The reason for this discrepancy might be explained by (a) lack of $mGlu_2$ receptors in the cortex and (b) the possible presence of another mGlu receptor which could be activated by MCCG.

(1*S*,3*S*)- and (1*S*,3*R*)-ACPD, L-CCG-I and DCG-IV all had broadly similar EC_{50} values, which is likely to reflect their relative selectivity for group 2 mGlu receptors that are present in the cortex. The agonist action of DCG-IV at NMDA receptors was clearly demonstrated, by the observation that, at higher concentrations (> 30 μM), the inhibition of cyclic AMP production was lost, presumably reflecting interaction with NMDA receptors, since some forms of adenylyl cyclase are calcium sensitive. This action was completely blocked by the potent selective NMDA antagonist (*R*)-CPP. The fact that NMDA activation can stimulate cyclic AMP production is noteworthy. No other agonists tested gave a similar increase in cyclic AMP at higher concentrations.

L-AP4 and SOP, presumably acting at group 3 mGlu receptors, were much less potent than the agonists acting at group 2 mGlu receptors. L-AP4 was some four times more potent than SOP. This lack of potency in comparison to group 2 mGluR agonists, may imply that either group 3 mGluRs have a lower coupling efficiency to adenylyl cyclase or that L-AP4 and SOP are not very good ligands. However, in electrophysiological studies on neonatal rat spinal motoneurones, L-AP4 is more potent than ACPD (Koerner and Cotman, 1981; Evans et al., 1982; Davies and Watkins, 1982; Monaghan et al., 1989; Baskys and Malenka, 1991; Pook et al., 1992). Thus, it may be that the coupling to adenylyl cyclase is less efficient; but again differences in the receptor subtypes present in the two preparations must be considered. L-glutamate and (*S*)-MG had similar EC_{50} values and neither caused enhancement of cyclic AMP at higher concentrations. Why L-glutamate failed to enhance cyclic AMP levels via its activation of NMDA receptors is not known. The role of glutamate uptake mechanisms and their interaction with mGlu receptor-mediated effects is currently unclear.

The agonist actions of the compounds in reducing the accumulation of cyclic AMP in rat cortical slices are in general agreement with their agonist actions reported in other experimental systems, the notable exceptions being the α-methyl forms of L-AP4 and L-CCG-I, which have been previously reported as antagonists, although corroborating evidence for MAP4 being an agonist in some preparations has been reported (Chavis et al., 1995; Cassidy and O'Connor, 1995: Knöpfel et al., 1995b).

Acknowledgements

This work was supported by the Medical Research Council, Tocris Cookson and the US Public Health Service. The authors would also wish to thank Professor Jeff Watkins for his valuable input to this work.

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