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Endogenous sulphur-containing amino acids: potent agonists at presynaptic metabotropic glutamate autoreceptors in the rat central nervous system

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1 We have recently demonstrated that presynaptically located metabotropic glutamate (mGlu) autoreceptors regulate synaptic glutamate release both *in vitro* and *in vivo*. We now report a positive modulatory action of the sulphur-containing amino acids (SCAAs), L-cysteic acid (CA) and L-cysteine sulphinic acid (CSA), at presynaptic group I mGlu receptors, specifically of the mGlu5 subtype, acting to enhance synaptic glutamate release from the rat forebrain *in vitro*.

2 Neuronal glutamate release was monitored using electrically-evoked efflux of preloaded [^{3}H]-D-aspartate from rat forebrain hemisections.

3 Both CA (3–100 μM) and CSA (1–100 μM), in addition to the selective group I mGlu receptor agonist, (S)-3,5-dihydroxyphenylglycine ((S)-DHPG), concentration-dependently enhanced electrically-stimulated efflux of [^{3}H]-D-aspartate from the rat forebrain slices. Basal efflux of label remained unchanged.

4 The inhibitory activity of the broad spectrum mGlu receptor antagonist, (±)-α-methyl-4-carboxyphenylglycine ((±)-MCPG; 200 μM), coupled with the inactivity of the selective mGlu1 receptor antagonists, (R,S)-1-aminoindan-1,5-dicarboxylic acid ((R,S)-AIDA; 100–500 μM) and the more potent (+)-2-methyl-4-carboxyphenylglycine (LY367385; 10 μM) against these responses, indicates an action of the SCAAs at the mGlu5 receptor subtype. This proposal is supported by the potent inhibition of these responses by the selective, non-competitive mGlu5 receptor antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP; 10 μM). The observed enhancement of the responses to high concentrations of CA by the selective mGlu5 receptor desensitization inhibitor, cyclothiazide (CYZ; 10 μM), is also consistent with this concept.

5 Administration of the agonists in the presence of bovine serum albumin (BSA; 5–15 mg ml^{-1}) markedly attenuated the positive modulatory responses observed, strongly supporting a role for arachidonic acid in the expression of these mGlu5 receptor-mediated responses.

6 The regulatory actions of SCAAs on synaptic glutamate release demonstrated in the present study may provide a physiological function for these putative neurotransmitter amino acids in the mammalian brain. These central actions of the SCAAs may have wide-ranging implications for a range of neurological and neuropsychiatric disease states and their treatment.
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Keywords: Sulphur-containing amino acids; L-cysteic acid; L-cysteine sulphinic acid; mGlu receptors; [^{3}H]-D-aspartate; glutamate release; presynaptic; autoreceptors

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; BSA, bovine serum albumin; CA, L-cysteic acid; CSA, L-cysteine sulphinic acid; CYZ, cyclothiazide; [^{3}H]-D-asp, D-[2,3-^{3}H] aspartate; KA, kainic acid; LY367385, (±)-2-methyl-4-carboxyphenylglycine; (±)-MCPG, (±)-α-methyl-4-carboxyphenylglycine; MPEP, 2-methyl-6-(phenylethynyl)pyridine; NMDA, N-methyl-D-aspartate; phaclofen, (R,S)-3-amino-2-(4-chlorophenyl)-propylphosphonic acid; (R,S)-AIDA, (R,S)-1-aminoindan-1,5-dicarboxylic acid; SCAA, sulphur-containing amino acid; (S)-DHPG, (S)-3,5-dihydroxyphenylglycine; TTX, tetrodotoxin

Introduction

The dicarboxylic amino acid glutamate, is the principal excitatory neurotransmitter in the mammalian central nervous system and is known to play a key role in learning and memory processes in addition to certain pathological disorders including epilepsy and ischaemic brain damage (Croucher & Bradford, 1989; Lipton & Rosenberg, 1994; Palmer, 1999). Its actions are mediated by both ionotropic and metabotropic glutamate receptors. Ionotropic glutamate receptors are homomeric or heteromeric cation-specific ligand-gated ion channels which are divided into three groups according to their agonist selectivity, namely N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate (KA) receptors. The metabotropic glutamate (mGlu) receptors are G-protein-coupled receptors, of which there are currently eight

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identified subtypes, which can also be divided into three groups based on their sequence homologies, pharmacology and signal transduction mechanisms (Pin & Duvoisin, 1995). Group I mGlu receptors (mGlu1 and 5, each with splice variants) are positively coupled to phosphoinositide hydrolysis and the mobilisation of intracellular Ca^{2+}, whilst group II (mGlu2 and 3) and group III (mGlu4, 6, 7 and 8) receptors are both negatively coupled to adenylate cyclase. We, and others, have recently demonstrated that *presynaptically* located group I mGlu autoreceptors mediate a positive modulatory control on synaptic glutamate release both *in vitro* (rat cerebrocortical synaptosomes: Herrero *et al*., 1992; rat brain slices: Croucher *et al*., 1997; Thomas *et al*., 2000) and *in vivo* (intrastriatal microdialysis: Patel & Croucher, 1998).

Evidence is also accumulating to suggest that some sulphur-containing amino acids (SCAAs) may act as central excitatory neurotransmitters (for reviews, see Griffiths, 1990; Thompson & Kilpatrick, 1996). SCAAs are analogues of glutamate or aspartate containing sulphonic or sulphinic functional groups and include cysteic acid, cysteine sulphinic acid, homocysteic acid and homocysteine sulphinic acid. Many of the criteria for identification of a neurotransmitter substance are fulfilled by these amino acids, and in particular L-cysteic acid (CA) and L-cysteine sulphinic acid (CSA) (Recasens *et al*., 1982; Griffiths, 1990; Griffiths & Dunlop, 1991; Thompson & Kilpatrick, 1996). Thus, CA and CSA are heterogeneously distributed in the mammalian brain and spinal cord and, whilst the brain concentrations of the SCAAs are at least two orders of magnitude less than those of glutamate and aspartate, this likely reflects the greater involvement of the latter amino acids in intermediary metabolism (Baba *et al*., 1982; Kilpatrick & Mozley, 1986; Waller *et al*., 1991; Porter & Roberts, 1993). Synthetic and degradative enzymes for the metabolism of these SCAAs (from the essential amino acid, methionine) are also heterogeneously distributed in the brain and are localized in nerve terminals (Misra & Olney, 1975; Griffiths, 1990). Calcium-dependent, depolarisation-induced release of endogenous or radiolabelled CA and CSA has been demonstrated in a range of rat brain preparations *in vitro* (Iwata *et al*., 1982a; Recasens *et al*., 1984a; Do *et al*., 1986; Griffiths, 1990; Klancnik *et al*., 1992) and SCAAs have been shown to produce excitatory actions comparable to glutamate and aspartate on both mammalian and amphibian central neurones (Curtis & Watkins, 1960; 1963; Curtis *et al*., 1961; Mewett *et al*., 1983). SCAAs, including CA and CSA, also inhibit sodium-independent binding of [^{3}H]-L-glutamate to rat brain membranes (Iwata *et al*., 1982b; Recasens *et al*., 1982; 1983; Mewett *et al*., 1983) indicating that they have binding sites in common with glutamate. Distinct, saturable and stereospecific, sodium-independent membrane binding sites selective for CSA have also been described (Recasens *et al*., 1983; 1984b). However, despite the burgeoning evidence in support of a neurotransmitter role for some SCAAs in the mammalian brain, no specific functional roles for these amino acids have been identified to date.

Recently, SCAAs have been shown to stimulate phosphoinositide hydrolysis in neonatal rat cerebrocortical slices, primary cultures of rat cerebellar granule cells and mGlu receptor-transfected cell lines, with similar or greater potency to glutamate itself (Porter & Roberts, 1993; Gorman & Griffiths, 1994; Kingston *et al*., 1998). In the present study, we now investigate the possibility that the SCAAs, and in particular CA and CSA, may act to modulate central neuronal glutamate release by interaction with presynaptic group I mGlu receptors. A role for the retrograde intercellular messenger, arachidonic acid, in the mediation of these actions is proposed. Preliminary accounts of some of this work have been presented to the British Pharmacological Society and the Federation of European Neuroscience Societies (Croucher *et al*., 2000a, b).

Methods

In the present study glutamate release was monitored using [^{3}H]-D-aspartate ([^{3}H]-D-asp) as a non-metabolisable marker for glutamate. Depolarisation-induced outflow of [^{3}H]-D-asp from rat brain slices is a widely used technique for the study of presynaptic receptor mechanisms controlling glutamate release (e.g. Lombardi *et al*., 1994; 1996; Palmer & Reiter, 1994; Patel & Croucher, 1997; Thomas *et al*., 2000) and full details of the current protocol are published elsewhere (Patel & Croucher, 1997). A brief summary is given below.

Experimental protocol

Male Wistar rats (230–250 g) were sacrificed by decapitation, the brains were removed rapidly and a modified brain slicer was used to cut three serial forebrain coronal slices of 500 μm thickness. Each slice contained areas of the amygdaloid complex, hippocampus and cerebral cortex, all regions known to contain high densities of glutamatergic synapses. The slices were divided down the mid-sagittal plane providing six hemisections per animal. Each hemisection was preincubated individually for 45 min in a Krebs-bicarbonate buffer, pH 7.4 at 37°C, constantly gassed with 95%O_2/5%CO_2. The composition of the buffer solution was as follows (mM): NaCl 120, KCl 4.75, KH_2PO_4 1.2, $CaCl_2$ 2.6, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 10. The slices were then 'loaded' with [^{3}H]-D-asp by incubating for a further 45 min in buffer containing 40 nM [^{3}H]-D-asp (specific activity 25 Ci mmol^{-1}). The individual hemisections were transferred to gold microelectrode superfusion chambers, placed in a waterbath at 37°C and superfused with oxygenated Krebs buffer at a rate of 0.4 ml min^{-1} for 45 min to wash off excess radiolabel. Superfusate samples were subsequently collected every 2 min using a multiple fraction collector (Retriever IV, ISCO, U.S.A.). Six basal superfusate samples were collected prior to each of two periods of electrical stimulation (S_1 and S_2) given 32 min apart. The stimuli comprised 5 min trains of biphasic rectangular pulses (36 mA, 2 msec) at a frequency of 20 Hz, delivered to the tissue using an HSE Type 215/T constant current stimulator. Superfusate fractions were collected for a total of 74 min and at the end of the experiment the slice sections were removed from the electrode chambers and solubilized for liquid scintillation counting. Scintillation fluid (Optiphase Hi-Safe III, Fischer Ltd, U.K.) was added to the superfusate samples and solubilized tissue samples and the radioactive content was quantified by liquid scintillation spectrometry using an LKB Wallac RackBeta II scintillation counter (65% counting efficiency). Mean basal efflux levels (B_1 and B_2) were calculated as the mean level of radioactivity

within the four samples immediately preceding the respective periods of electrical stimulation. Responses to electrical stimulation (S_1 and S_2) were calculated as the total levels of stimulated efflux minus mean basal levels. Drugs were applied in the superfusate 10 min prior to S_2 and the ratios of basal and electrically-stimulated efflux of [^{3}H]-D-asp before and after drug application (B_2/B_1 and S_2/S_1, respectively) were calculated to assess the influence of drugs or other treatments on basal and stimulated release. Previous studies have confirmed that the calculation of ratios in this manner provides a reliable measure of release against which the effects of drug treatments can be monitored (Butcher *et al*., 1987; Martin *et al*., 1991; Barnes *et al*., 1994; Patel & Croucher, 1997).

Data analysis

Results are presented as mean ± s.e.mean of n independent observations. Statistical significance of differences in responses was determined using Student's 2-tailed t-test for independent groups. Values were considered to be significantly different from controls if $P<0.05$.

Materials

The following drugs and reagents were used, with their sources in parenthesis: (S)-3,5-dihydroxyphenylglycine ((S)-DHPG), (±)-α-methyl-4-carboxyphenylglycine ((±)-MCPG), (R,S)-1-aminoindan-1,5-dicarboxylic acid ((R,S)-AIDA), cyclothiazide (CYZ) and (R,S)-3-amino-2-(4-chlorophenyl)-propylphosphonic acid (phaclofen) (Tocris Cookson, U.K.). L-Cysteic acid (CA), L-cysteine sulphinic acid (CSA), γ-aminobutyric acid (GABA), anhydrous calcium chloride ($CaCl_2$), tetrodotoxin (TTX), bovine serum albumin (BSA) and EGTA were purchased from Sigma Chemicals (Dorset, U.K.). (+)-2-Methyl-4-carboxyphenylglycine (LY367385) was synthesized in the laboratories of Eli Lilly and Company Ltd (Windlesham, U.K.) by Mr John Harris. 2-Methyl-6-(phenylethynyl)pyridine (MPEP) was a kind gift from Dr Fabrizio Gasparini of Novartis Pharma AG, Switzerland. D-[2,3-^{3}H] Aspartate ([^{3}H]-D-asp), specific activity 25 Ci mmol^{-1}, was supplied by Amersham International, U.K. All other reagents were supplied by Merck Ltd (Dorset, U.K.).

Results

The basal release of preloaded [^{3}H]-D-asp from the rat forebrain slices in the absence of experimental manipulations remained essentially constant during the course of the experiments, as shown by the closeness to unity of the calculated mean ratio B_2/B_1 (mean ± s.e.mean = 1.26 ± 0.15; $n=6$; $P>0.05$) (Table 1). Following each period of electrical stimulation, a significant increase in efflux of radiolabel was observed in comparison to basal, pre-stimulation levels. The calculated mean ratio of these responses (S_2/S_1) in the untreated slices was 1.11 ± 0.13; $n=6$ (Table 1). The lack of deviation of this figure from unity confirms that the response to the first electrical stimulation did not significantly influence the response to that of the second, using the current protocol. Ratios of basal (B_2/B_1) and stimulated (S_2/S_1) release obtained in untreated brain tissue were subsequently compared with similar ratios obtained from slices exposed to agonists and/or antagonists or other experimental manipulations, as described below.

Control experiments: Ca^{2+}-dependency, tetrodotoxin-insensitivity and inhibitory action of GABA on [^{3}H]-D-aspartate efflux

Consistent with previous studies using this preparation (Patel & Croucher, 1997), electrically-evoked responses in the rat forebrain slices were strongly Ca^{2+}-dependent. Thus, in Ca^{2+}-free buffer and in the presence of 1 mM EGTA, electrically-evoked efflux of [^{3}H]-D-asp was reduced to 31.8% of the mean control response (Figure 1). Also consistent with earlier findings (Patel & Croucher, 1997), the basal release of label showed no clear Ca^{2+}-dependency (Figure 1). In addition, the presence of the voltage-sensitive Na^+-channel blocker, tetrodotoxin (TTX; 100 nM), in the superfusate did not influence either electrically-stimulated or basal efflux of label from the slices (Figure 1).

In agreement with previous studies using rat brain slices or synaptosome preparations (Pende *et al*., 1993; Waldmeier *et al*., 1994; Patel & Croucher, 1997), the presence of GABA (50 μM) in the superfusate significantly inhibited the electrically-evoked release of [^{3}H]-D-asp (Figure 1). This effect was abolished by co-administration of the agonist with the selective $GABA_B$ receptor antagonist, phaclofen (300 μM) (Figure 1), consistent with the mediation of this response by presynaptic $GABA_B$ inhibitory heteroreceptors (Waldmeier *et al*., 1994). Phaclofen alone did not significantly influence either basal or electrically-stimulated efflux (Figure 1).

Enhancement of [^{3}H]-D-aspartate efflux by the selective group I mGlu receptor agonist, (S)-3,5-dihydroxyphenylglycine

To demonstrate facilitation of electrically-evoked release by presynaptic group I mGlu receptor activation in the present study, a concentration-response curve to the selective group I mGlu receptor agonist, (S)-DHPG (Schoepp *et al*., 1994) was constructed. (S)-DHPG, over the concentration range 0.1–3.0 μM, concentration-dependently enhanced the electrically-evoked release of [^{3}H]-D-asp without influencing basal efflux (Figure 2). At a higher concentration (10 μM), (S)-DHPG failed to significantly alter the electrically-evoked efflux of label, although co-application of the group I mGlu receptor desensitisation inhibitor, CYZ (10 μM), with the agonist, restored a near-maximal response (Figure 2).

Effects of the sulphur-containing amino acids, L-cysteine sulphinic acid and L-cysteic acid, on [^{3}H]-D-aspartate efflux

As with the selective group I mGlu receptor agonist, (S)-DHPG (Figure 2), L-cysteine sulphinic acid (CSA), 1–100 μM, concentration-dependently enhanced electrically-stimulated efflux of [^{3}H]-D-asp from the rat forebrain slices, without influencing basal efflux (Figure 3). The maximum response observed, at 100 μM CSA, was a 28.05-fold enhancement of electrically-evoked release ($P<0.005$).

Table 1 The effects of (±)-MCPG and (R,S)-AIDA on basal and electrically-stimulated release of [^{3}H]-D-aspartate from rat forebrain slices in the presence or absence of CSA, 10 μM or CSA, 100 μM

Treatment		*Efflux ratio ± s.e.mean*		
Agonist	*Antagonist*	B_2/B_1	S_2/S_1	(n)
Electrical stimulation alone (control)	–	1.26±0.15	1.11±0.13	6
CSA, 10 μM	–	1.27±0.11	12.84±3.31[b]	5
CSA, 10 μM	(±)-MCPG, 200 μM	1.43±0.20	3.64±0.78[b,c]	4
CSA, 10 μM	(R,S)-AIDA, 100 μM	1.24±0.08	10.55±2.41[b]	6
CSA, 100 μM	–	1.12±0.08	28.05±4.81[b]	4
CSA, 100 μM	(±)-MCPG, 200 μM	1.53±0.10	6.30±3.04[d]	4
–	(±)-MCPG, 200 μM	1.07±0.12	1.25±0.53	6
–	(R,S)-AIDA, 100 μM	1.26±0.14	0.96±0.16	4

[a]$P<0.05$ compared to control S_2/S_1; [b]$P<0.005$ compared to control S_2/S_1; [c]$P<0.05$ compared to S_2/S_1 obtained with 10 μM CSA alone; [d]$P<0.01$ compared to S_2/S_1 obtained with 100 μM CSA alone (Student's *t*-test for independent groups). (*n*) = number of independent observations.

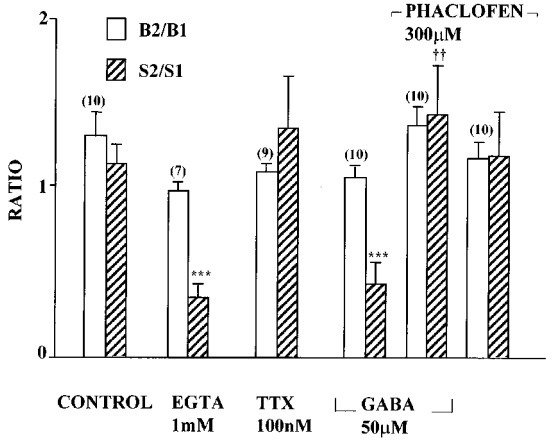

Figure 1 Calcium-dependency and tetrodotoxin-insensitivity of, and the action of GABA on, electrically-stimulated [^{3}H]-D-aspartate release from rat forebrain slices. Each value is the mean of *n* (in parenthesis) independent observations (±s.e.mean). ***$P<0.005$ versus electrical stimulation alone; ††$P<0.01$ versus agonist alone (Student's *t*-test for independent groups).

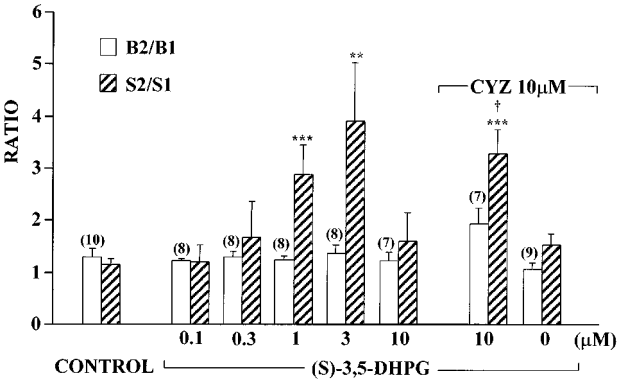

Figure 2 Concentration-dependent enhancement of electrically-stimulated [^{3}H]-D-aspartate release from rat forebrain slices by the selective group I mGlu receptor agonist, (S)-3,5-DHPG. Each column represents the mean of *n* (in parenthesis) independent observations (±s.e.mean). **$P<0.01$, ***$P<0.005$ versus electrical stimulation alone; †$P<0.05$ versus (S)-DHPG, 10 μM (Student's *t*-test for independent groups).

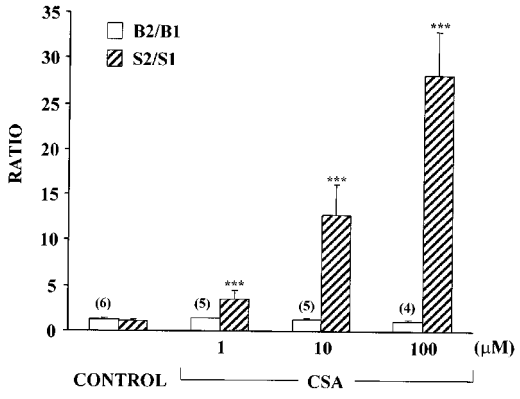

Figure 3 The effects of cysteine sulphinic acid (CSA) on basal and electrically-stimulated release of [^{3}H]-D-aspartate from rat forebrain slices. Each column represents the mean of *n* (in parenthesis) independent observations (±s.e.mean). ***$P<0.005$ versus electrical stimulation alone (Student's *t*-test for independent groups).

L-cysteic acid (CA), 3–100 μM, also markedly potentiated the electrically-evoked efflux of label, with a maximum 6.1-fold enhancement seen following CA, 3 μM (Figure 4). Again, basal efflux remained unchanged (Figure 4). As with (S)-DHPG-evoked responses, the smaller mean response seen following CA, 10 μM (compared with CA, 3 μM) was highly sensitive to the benzothiadiazide group I mGlu receptor desensitisation inhibitor, CYZ (S_2/S_1 ratios: CA, 10 μM alone = 3.86±0.88, $n=7$; CA, 10 μM plus CYZ, 10 μM = 8.13±1.45, $n=7$; $P<0.05$). Interestingly, the lowest concentration of CA tested (1 μM), caused a consistent and significant *reduction* in electrically-stimulated efflux of [^{3}H]-D-asp from rat forebrain slices (to 39.1% of control values; $P<0.005$) (Figure 4).

Effects of broad-spectrum and subtype-selective mGlu receptor antagonists on L-cysteine sulphinic acid-induced responses

The broad spectrum mGlu receptor antagonist, (±)-α-methyl-4-carboxyphenylglycine ((±)-MCPG; 200 μM), decreased the electrically-evoked responses to CSA, 10 μM and 100 μM, by 78.4% ($P<0.05$) and 80.8% ($P<0.01$), respectively (Table 1). However, the selective mGlu1 receptor

antagonist, (R,S)-1-aminoindan-1,5-dicarboxylic acid ((R,S)-AIDA; 100 μM), was inactive in this respect (Table 1). Consistent with this finding, the more potent and recently developed mGlu1 receptor antagonist, (+)-2-methyl-4-carboxyphenylglycine (LY367385; 10 μM), also failed to influence the response to CSA, 10 μM (Figure 5).

Effects of broad-spectrum and subtype-selective mGlu receptor antagonists on L-cysteic acid-induced responses

(±)-MCPG, 200 μM, similarly inhibited the action of CA, 3 μM on electrically-evoked [^{3}H]-D-asp efflux (79.0% reduction; $P<0.05$) (Table 2). Moreover, (R,S)-AIDA, 100–500 μM and LY367385, 10 μM again failed to influence the agonist-evoked responses (Table 2 and Figure 5). Interestingly, the *inhibitory* effect of CA, 1 μM on [^{3}H]-D-asp efflux from the forebrain slices was also fully reversed by (±)-MCPG, 200 μM (S_2/S_1 ratio = 1.47 ± 0.56 in the presence of CA and (±)-MCPG; $n=6$; $P>0.05$ compared to control, drug-free response).

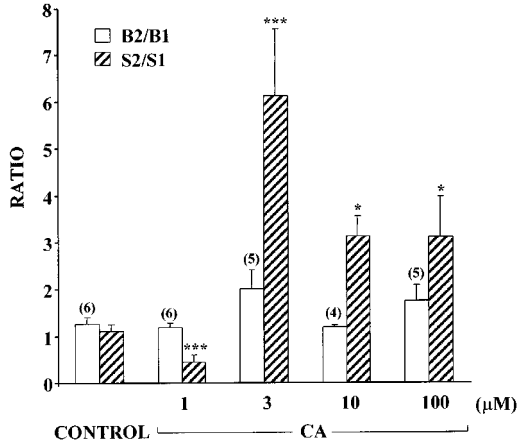

Figure 4 The effects of cysteic acid (CA) on basal and electrically-stimulated release of [^{3}H]-D-aspartate from rat forebrain slices. Each column represents the mean of n (in parenthesis) independent observations (±s.e.mean). *$P<0.05$, ***$P<0.005$ versus electrical stimulation alone (Student's t-test for independent groups).

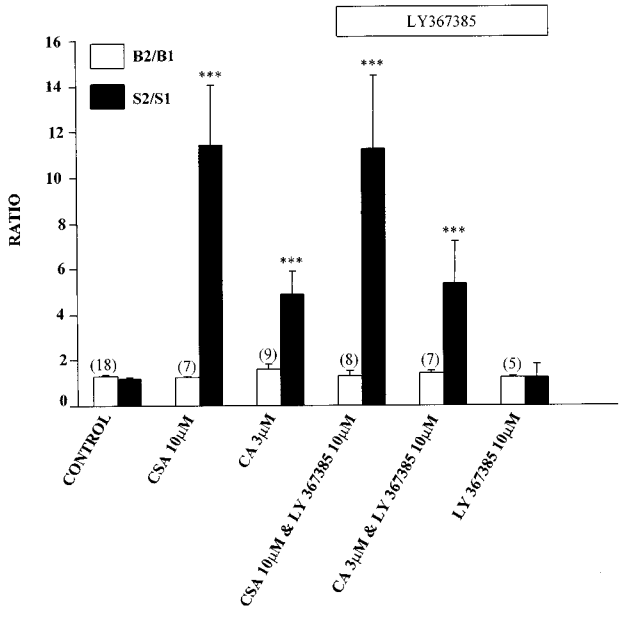

Figure 5 Lack of effect of the selective mGlu1 receptor antagonist, LY367385, on SCAA-induced responses in rat forebrain slices. Each column represents the mean of n (in parenthesis) independent observations (±s.e.mean). ***$P<0.005$ versus electrical stimulation alone (Student's t-test for independent groups).

Influence of the novel, selective mGlu5 receptor antagonist, 2-methyl-6-(phenylethynyl)pyridine, on L-cysteine sulphinic acid- and L-cysteic acid-induced responses

Finally, and importantly, the newly synthesized selective, non-competitive mGlu5 receptor antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP; 10 μM), fully inhibited the positive modulatory responses to both CSA, 10 μM ($P<0.005$) and CA, 3 μM ($P<0.005$) (Figure 6).

Neither MPEP nor any of the other broad-spectrum or subtype-selective mGlu receptor antagonists utilised in the present study influenced basal or stimulated efflux of label when given alone (Tables 1 and 2; Figures 5 and 6).

Effects of bovine serum albumin on sulphur-containing amino acid-induced enhancement of electrically-stimulated [^{3}H]-D-aspartate efflux

In order to examine whether the potentiating actions of the SCAAs on [^{3}H]-D-asp release require the presence of endogenous arachidonic acid, as suggested previously for group I mGlu receptor-mediated responses in other preparations (Herrero *et al*., 1992; Lombardi *et al*., 1996), responses to the SCAAs were compared in the presence and absence of bovine serum albumin (BSA), an inactivator of locally produced fatty acids.

BSA, 5 mg ml^{-1} markedly and significantly ($P<0.05$) attenuated the responses to CSA, 10 μM (Table 3). A slightly higher concentration of BSA (15 mg ml^{-1}) reduced the responses to CA, 3 μM to a similar level ($P<0.05$) (Table 3). When given alone at the same concentrations (5–15 mg ml^{-1}), BSA did not influence basal or electrically-evoked release of label in the rat forebrain slice preparation (Table 3). These results are consistent with a key role for arachidonic acid in the mediation of positive modulatory SCAA-evoked responses.

Discussion

We have recently shown that neuronal excitatory amino acid release in the rat forebrain is facilitated by *presynaptic* group I mGlu receptors both *in vitro* (Croucher *et al*., 1997; Thomas *et al*., 2000) and *in vivo* (Patel & Croucher, 1998). The results of the present study now demonstrate a positive modulatory action of the endogenous SCAAs, CA and CSA, on neuronal excitatory amino acid release via an action at these group I mGlu autoreceptors.

In control experiments, the electrically-evoked release of [^{3}H]-D-asp from rat forebrain slices was shown to be strongly Ca^{2+}-dependent, insensitive to tetrodotoxin and inhibited by GABA *via* an action at $GABA_B$ receptors. These observa-

Table 2 The effects of (±)-MCPG and (R,S)-AIDA on basal and electrically-stimulated release of [^{3}H]-D-aspartate from rat forebrain slices in the presence or absence of CA, 3 μM

Treatment		*Efflux ratio ± s.e.mean*		
Agonist	*Antagonist*	B_2/B_1	S_2/S_1	(n)
Electrical stimulation alone (control)	–	1.26±0.15	1.11±0.13	6
CA, 3 μM	–	2.01±0.40	6.12±1.44[b]	5
CA, 3 μM	(±)-MCPG, 200 μM	1.45±0.17	1.89±0.38[a,c]	9
CA, 3 μM	(R,S)-AIDA, 100 μM	1.41±0.21	4.71±1.34[a]	9
CA, 3 μM	(R,S)-AIDA, 500 μM	1.28±0.18	4.65±0.90[a]	7
–	(±)-MCPG, 200 μM	1.07±0.12	1.25±0.53	6
–	(R,S)-AIDA, 500 μM	1.16±0.07	1.65±0.49	7

[a]$P<0.05$ compared to control S_2/S_1; [b]$P<0.005$ compared to control S_2/S_1; [c]$P<0.05$ compared to S_2/S_1 obtained with 3 μM CA alone (Student's *t*-test for independent groups). (*n*) = number of independent observations.

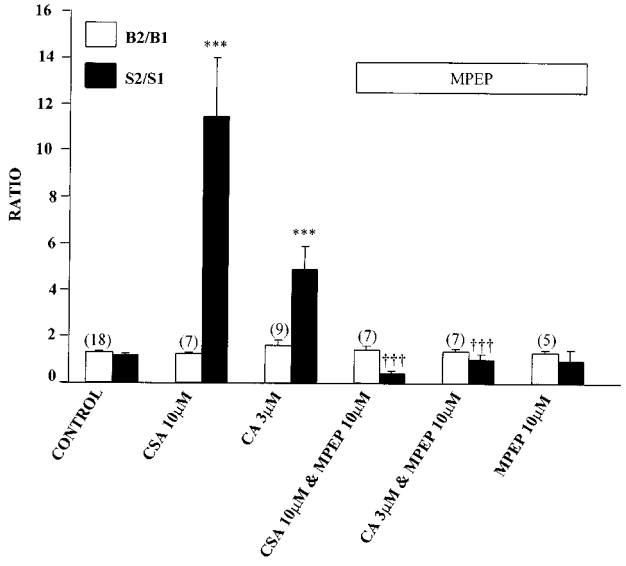

Figure 6 Abolition of SCAA-induced responses in rat forebrain slices by the novel, selective, non-competitive mGlu5 receptor antagonist, MPEP. Each column represents the mean of *n* (in parenthesis) independent observations (±s.e.mean). ***$P<0.005$ versus electrical stimulation alone; †††$P<0.005$ versus agonist alone (Student's *t*-test for independent groups).

tions indicate a neuronal origin for the electrically-evoked release of [^{3}H]-D-asp from presynaptic excitatory amino acidergic nerve terminals. Consistent with this concept was the observed concentration-dependent enhancement of electrically-evoked release of label by the selective group I mGlu receptor agonist, (S)-DHPG. The loss of effectiveness of this agonist at the highest concentration tested (10 μM) was likely due to desensitization of the presynaptic group I mGlu receptors, as originally demonstrated by Herrero *et al.* (1994), and the marked potentiation of the response to (S)-DHPG, 10 μM by co-application of the group I mGlu receptor desensitization inhibitor, CYZ (Sharp *et al.*, 1994), strongly supports this proposal. Cyclothiazide is also known to enhance AMPA subtype ionotropic glutamate receptor-mediated responses (see Patel & Croucher, 1997). However, this action would not be expected to influence the directly-evoked mGlu receptor-mediated responses in the present study.

As with the group I mGlu receptor agonist, (S)-DHPG, both of the SCAAs tested in the present study enhanced electrically-evoked efflux of [^{3}H]-D-asp from rat forebrain slices in a concentration-dependent manner, without influencing basal levels of efflux (Figures 3 and 4). However, the response profiles of the two agonists showed some clear differences. Cysteic acid markedly potentiated efflux over the concentration range 3–100 μM, with a maximum 6.1 fold enhancement apparent following CA, 3 μM. Consistent with the actions of (S)-DHPG at group I mGlu receptors, the concentration-response curve to CA in this preparation was bell-shaped with the highest concentrations of CA (10–100 μM) producing only small, but still just-significant responses. A similar response profile has been reported for CA in stimulating phosphatidylinositol hydrolysis in rat pup cerebrocortical slices (Porter & Roberts, 1993). The significant enhancement of the responses to CA, 10 μM in the presence of the desensitization inhibitor CYZ (10 μM) strongly supports a role for group I mGlu receptors in the mediation of these agonist-evoked responses. Moreover, the recent demonstration of a selective action of CYZ at mGlu5 as opposed to mGlu1 receptors (Sharp *et al.*, 1994) is indicative of a selective agonist action of CA at presynaptic positive modulatory mGlu5 receptors (see also below).

Interestingly, at the lowest concentration tested (1 μM), CA caused a small but consistent and significant reduction in electrically-evoked efflux of label from the forebrain slices. This inhibitory response was fully blocked by the group I/II mGlu receptor antagonist, (±)-MCPG (Hayashi *et al.*, 1994). These findings are consistent with low concentrations of CA inhibiting synaptic glutamate release by an interaction with presynaptic group II subtype mGlu autoreceptors. Indeed, we have previously demonstrated powerful inhibitory actions of selective group II mGlu receptor agonists on synaptic glutamate release from cerebrocortical synaptosome preparations, actions that were paralleled by antiepileptogenic and anticonvulsant effects of these mGlu receptor ligands in an animal model of complex partial epilepsy (Attwell *et al.*, 1995; 1998).

Cysteine sulphinic acid also markedly enhanced electrically-stimulated release of [^{3}H]-D-asp in a concentration-dependent manner and over a similar concentration range to that of CA (1–100 μM). However, even at the highest concentration of CSA tested (100 μM), there was no evidence of a diminution in response, as seen following high concentrations of CA. This lack of apparent desensitization of the CSA-evoked response may be the result of an additional interaction of CSA with the excitatory amino acid re-uptake carriers (see below) acting to mask any fall in

Table 3 Influence of BSA on basal and electrically-stimulated release of [^{3}H]-D-aspartate from rat forebrain slices in the presence or absence of CSA, 10 μM or CA, 3 μM

Treatment		*Efflux ratio ± s.e.mean*		
Agonist	*Antagonist*	B_2/B_1	S_2/S_1	(n)
Electrical stimulation alone (control)	–	1.26 ± 0.15	1.11 ± 0.13	6
CSA, 10 μM	–	1.23 ± 0.08	11.41 ± 2.60[a]	7
CSA, 10 μM	BSA, 5 mg ml^{-1}	1.54 ± 0.19	2.17 ± 0.63[b]	4
CA, 3 μM	–	1.60 ± 0.28	4.88 ± 0.98[a]	9
CA, 3 μM	BSA, 5 mg ml^{-1}	1.84 ± 0.26	4.89 ± 1.16[ns]	4
CA, 3 μM	BSA, 15 mg ml^{-1}	1.45 ± 0.13	2.26 ± 0.22[b]	4
–	BSA, 5 mg ml^{-1}	1.42 ± 0.13	0.97 ± 0.40	6
–	BSA, 15 mg ml^{-1}	1.36 ± 0.11	1.42 ± 0.41	6

[a] $P<0.005$ compared to control S_2/S_1; [b] $P<0.05$ compared to agonist alone; ns = non-significant (Student's *t*-test for independent groups). (*n*) = number of independent observations.

receptor-mediated response. Alternatively, it may be the result of differences in receptor binding modalities of CSA compared with CA, and with other group I mGlu receptor agonists, as previously suggested by Kingston *et al.* (1998) following studies of the actions of SCAAs at group I mGlu receptors expressed in clonal RGT cell lines.

Cysteine sulphinic acid and CA showed similar potencies in enhancing electrically-stimulated release of [^{3}H]-D-asp from rat forebrain slices. This is in accordance with previous reports of equivalent potencies of CSA and CA in stimulating phosphoinositide hydrolysis in both neonatal rat cerebrocortical slices (Porter & Roberts, 1993) and in clonal cell lines expressing human mGlu5 receptors (Kingston *et al.*, 1998). However, CSA was clearly more efficacious than CA in enhancing electrically-evoked efflux of label, with an apparent maximum response of 28.05 fold enhancement seen following CSA, 100 μM. This may well be attributable to an interaction of CSA at this high concentration with the glutamate transport processes. Indeed, SCAAs are known to be competitive substrates of the glutamate transporters, albeit with lower affinities than glutamate itself (see Thompson & Kilpatrick, 1996; Kingston *et al.*, 1998). However, lower concentrations of the SCAAs, as used to further characterise the amino acid-evoked responses in the present study, do not appear to significantly influence amino acid transport in the rat forebrain slice preparation (see below).

To further characterise the mechanisms of action of the SCAAs in the rat forebrain *in vitro*, a range of broad spectrum or more receptor subtype-selective mGlu antagonists were tested for their effects on CA- and CSA-evoked responses. Responses to both CA, 3 μM and CSA, 10 μM were markedly attenuated by the non-selective group I/II mGlu receptor antagonist, (±)-MCPG (Hayashi *et al.*, 1994), consistent with an action of these SCAAs at presynaptic group I mGlu receptors. This finding is in agreement with the previously reported ability of other phenylglycine derivatives to attenuate SCAA-evoked responses in the neonatal rat spinal cord (Thompson *et al.*, 1995), which further supports an action of SCAAs at mGlu receptors in the mammalian CNS. Moreover, the findings of the present study that the newly synthesized, non-competitive, and highly selective mGlu5 receptor antagonist, MPEP (Gasparini *et al.*, 1999), completely abolished the positive modulatory responses to both CA, 3 μM and CSA, 10 μM, is convincing evidence that these responses are mediated specifically by the mGlu5 subtype of mGlu receptors. Our observation of an enhancement of CA-induced responses by the selective mGlu5 receptor desensitization inhibitor, CYZ (see Results) is also in line with this conclusion. The full inhibition of the SCAA-evoked responses by MPEP further suggests that, at the concentrations tested here, the SCAAs have no significant inhibitory activity on glutamate transport processes. Of relevance in this regard, Kingston *et al.* (1998) have recently shown that SCAAs induce group I mGlu receptor-mediated responses in clonal RGT cell lines in the absence of any influence on the glutamate uptake system (GLAST) expressed in these cells.

Although SCAAs have also previously been shown to bind to mGlu1 receptors, at least in clonal cell lines transfected with human mGlu1α (Kingston *et al.*, 1998), CA and CSA showed 10–20 fold lower affinity in this respect than the widely used broad spectrum mGlu receptor agonist, (1S,3R)-ACPD (Kingston *et al.*, 1998). The lack of any significant inhibitory activity of the selective mGlu1 receptor antagonists (R,S)-AIDA (Moroni *et al.*, 1997) and LY367385 (Clark *et al.*, 1997) against SCAA-mediated responses in the present study (Tables 1 and 2; Figure 5), coupled with the complete abolition of these responses by the mGlu5 receptor antagonist MPEP, suggests that any interaction of the SCAAs with mGlu1 receptors in the rat forebrain does not contribute significantly to the positive modulatory responses observed. Also of note, the lack of effect of (R,S)-AIDA (Tables 1 and 2), LY367385 (Figure 5), MPEP (Figure 6) or (±)-MCPG (Tables 1 and 2) on basal levels of [^{3}H]-D-asp efflux when given alone, implies a lack of *tonal* stimulation of phosphoinositide hydrolysis by SCAAs (or other endogenous agonists e.g. glutamate) acting at mGlu receptors in the rat forebrain *in vitro*.

With regard to the intracellular mechanism(s) of the SCAA-mediated responses reported here, Herrero and co-workers have previously demonstrated that group I mGlu receptor-mediated responses in rat cerebrocortical synaptosomes show an absolute requirement for arachidonic acid (Herrero *et al.*, 1992). It was subsequently hypothesized by these authors that arachidonic acid sensitizes presynaptically located PKC to its physiological activator DAG, the production of which is stimulated by mGlu receptor activation (Coffey *et al.*, 1994; Vázquez *et al.*, 1994). We have since reported a similar arachidonic acid-dependency of (S)-DHPG-evoked positive modulatory responses in the rat forebrain slice preparation (Thomas *et al.*, 2000). The marked attenuation of both CA- and CSA-evoked responses by BSA,

an inactivator of locally reproduced fatty acids (Lombardi *et al*., 1996), now shown in the present study (Table 3), both suggests a key role for arachidonic acid in the expression of positive modulatory SCAA-evoked responses in the rat forebrain *in vitro* and further supports our proposal of group I mGlu receptor-mediation of these responses. Interestingly, it has also been suggested that mGlu1 receptors may positively couple to PLA_2 and arachidonic acid formation, at least in transfected CHO cells (Aramori & Nakanishi, 1992). However, the lack of inhibitory activity of the selective mGlu1 receptor antagonists (R,S)-AIDA and LY367385 against SCAA-mediated responses in rat forebrain slices, clearly indicates that activation of any such native receptors in the rat forebrain does not contribute significantly to the responses observed here.

Sulphur-containing amino acids are known to show a broad spectrum of activity at postsynaptic ionotropic glutamate receptors, with some selectivity for the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtypes (Mewett *et al*., 1983; Murphy & Williams, 1987; Pullan *et al*., 1987). Previous reports have also demonstrated SCAA-induced release of [^{3}H]-D-asp and endogenous glutamate from rat brain synaptosome fractions and from hippocampal slices (Dunlop *et al*., 1989; Minc-Golomb *et al*., 1989). However, the lack of effect of selective ionotropic glutamate receptor antagonists (up to 1 mM concentrations) on these responses (Dunlop *et al*., 1989) demonstrated the non-involvement of presynaptic ionotropic glutamate receptors in mediating this release. In accordance with these reports, our own results showing full inhibition of CA- and CSA-evoked responses by MPEP are consistent with the positive modulatory responses observed being mediated solely *via* presynaptic mGlu5 receptors in the rat forebrain slice preparation. The lack of activity of SCAAs at presynaptic ionotropic glutamate receptors may be a reflection of the distinct pharmacological profiles of these receptor populations, compared to their postsynaptic counterparts, as previously demonstrated for presynaptic AMPA receptors in the rat forebrain *in vitro* (Thomas *et al*., 1997; Croucher *et al*., 1998). Moreover, SCAAs have also been shown to stimulate the release of a range of other neurotransmitters, including acetylcholine, GABA, noradrenaline and dopamine from rat CNS tissue preparations (see Thompson & Kilpatrick, 1996). It now seems likely that many of these responses may be mediated, at least in part, by activation of presynaptically located mGlu5 receptors. Sulphur-containing amino acids, and in particular CSA, have also been proposed as potential endogenous agonists of a novel PLD-linked subtype of mGlu receptor (Boss *et al*., 1994; Pellegrini-Giampietro *et al*., 1996). However, the precise functions and pharmacology of these receptors are currently undefined and their potential role in regulating neuronal glutamate release remains to be determined.

In recent years there has been a renewed interest in the SCAAs as endogenous neuroexcitatory agonists of physiological and pathological importance. Indeed, their actions in the CNS may have wide-ranging implications for a range of neurological and neuropsychiatric disease states and their treatment. Abnormalities in SCAA metabolism have been implicated in the aetiology of several such disorders, including Parkinson's and Alzheimer's diseases (Heafield *et al*., 1990), intractable depression (Francis *et al*., 1989) and epilepsy (Folbergrová *et al*., 2000). The results of the present investigation extend our knowledge of the mechanisms of action of these proposed endogenous neurotransmitter substances and indicate that many of their known physiological and/or pathological actions, including their excitatory (see Introduction), cytotoxic (Olney *et al*., 1971; Kim *et al*., 1987) and epileptogenic (Turski, 1989) effects in the mammalian CNS may be mediated, at least in part, by stimulation of mGlu receptors of the mGlu5 subtype. Blockade of the actions of endogenous SCAAs by mGlu receptor antagonists may contribute significantly to the known pharmacological, behavioural and potentially therapeutically beneficial effects of these agents.

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References

ARAMORI, I. & NAKANISHI, S. (1992). Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. *Neuron,* **8,** 757–765.

ATTWELL, P.E.J., KAURA, S., SIGALA, G., BRADFORD, H.F., CROUCHER, M.J., JANE, D.E. & WATKINS, J.C. (1995). Blockade of both epileptogenesis and glutamate release by (1S,3S)-ACPD, a presynaptic glutamate receptor agonist. *Brain Res.,* **698,** 155–162.

ATTWELL, P.E.J., KENT, N.S., JANE, D.E., CROUCHER, M.J. & BRADFORD, H.F. (1998). Anticonvulsant and glutamate release-inhibiting properties of the highly potent metabotropic glutamate receptor agonist (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV). *Brain Res.,* **805,** 138–143.

BABA, A., LEE, E., TATSUNO, T. & IWATA, H. (1982). Cysteine sulphinic acid in the central nervous system: antagonistic effect of taurine on cysteine sulphinic acid-stimulated formation of cyclic AMP in guinea-pig hippocampal slices. *J. Neurochem.,* **38,** 1280–1285.

BARNES, J.M., DEV, K.K. & HENLEY, J.M. (1994). Cyclothiazide unmasks AMPA-evoked stimulation of [^{3}H]L-glutamate release from rat hippocampal synaptosomes. *Br. J. Pharmacol.,* **113,** 339–342.

BOSS, V., NUTT, K.M. & CONN, P.J. (1994). L-Cysteine sulfinic acid as an endogenous agonist of a novel metabotropic receptor coupled to stimulation of phospholipase D activity. *Mol. Pharmacol.,* **45,** 1177–1182.

BUTCHER, S.P., ROBERTS, P.J. & COLLINS, J.F. (1987). DL-2-[3,4-^{3}H]amino-4-phosphonobutyrate binding sites in the rat hippocampus: distribution and possible physiological role. *Brain Res.,* **419,** 294–300.

CLARK, B.P., BAKER, S.R., GOLDSWORTHY, J., HARRIS, J.R. & KINGSTON, A.E. (1997). (+)-2-Methyl-4-carboxyphenylglycine (LY367385) selectively antagonises metabotropic glutamate mGluR1 receptors. *Bioorg. Med. Chem. Lett.,* **7,** 2777–2780.

COFFEY, E.T., HERRERO, I., SIHRA, T.S., SÁNCHEZ-PRIETO, J. & NICHOLLS, D.G. (1994). Glutamate exocytosis and MARKS phosphorylation are enhanced by a metabotropic glutamate receptor coupled to a protein kinase C synergistically activated by diacylglycerol and arachidonic acid. *J. Neurochem.,* **63,** 1303–1310.

CROUCHER, M.J., AHMADI, H. & THOMAS, L.S. (2000b). Metabotropic glutamate receptor-mediated responses to sulphur-containing amino acids: pharmacological characterisation and role of arachidonic acid. *Eur. J. Neurosci.,* **12** (Suppl. 11), 213.

CROUCHER, M.J. & BRADFORD, H.F. (1989). Kindling of full limbic seizures by repeated microinjections of excitatory amino acids into the rat amygdala. *Brain Res.,* **501,** 58–65.

CROUCHER, M.J., JAMES, S. & THOMAS, L.S. (1998). A distinct antagonist pharmacological profile of AMPA-type glutamate autoreceptors in the rat forebrain. *Naunyn-Schmied. Arch. Pharmacol.,* **358** (Suppl. 1), 1127.

CROUCHER, M.J., LAWRENCE, V. & THOMAS, L.S. (2000a). Agonist activity of sulphur-containing excitatory amino acids at presynaptic metabotropic glutamate autoreceptors. *Br. J. Pharmacol.,* **129,** 107P.

CROUCHER, M.J., MORANT, R., THOMAS, L.S. & JANE, D.E. (1997). Evidence for a role of group I metabotropic glutamate receptors in the positive feedback control of synaptic glutamate release *in vitro*. *The Pharmacologist,* **39,** 215.

CURTIS, D.R., PHYLLIS, J.W. & WATKINS, J.C. (1961). Actions of amino acids on the isolated hemisected spinal cord of the toad. *Br. J. Pharmacol.,* **16,** 262–283.

CURTIS, D.R. & WATKINS, J.C. (1960). The excitation and depression of spinal neurones by structurally related amino acids. *J. Neurochem.,* **6,** 117–141.

CURTIS, D.R. & WATKINS, J.C. (1963). Acidic amino acids with strong excitatory actions on mammalian neurones. *J. Physiol. (Lond.),* **166,** 1–4.

DO, K.Q., MATTENBERGER, M., STREIT, P. & CUENOD, M. (1986). *In vitro* release of endogenous excitatory sulphur-containing amino acids from various rat brain regions. *J. Neurochem.,* **46,** 779–786.

DUNLOP, J., MASON, H.V., GRIEVE, A. & GRIFFITHS, R. (1989). Excitatory sulfur amino acid-evoked neurotransmitter release from rat brain synaptosome fractions. *J. Neural. Transm.,* **78,** 195–208.

FOLBERGROVÁ, J., HAUGVICOVÁ, R. & MARES, P. (2000). Behavioural and metabolic changes in immature rats during seizures induced by homocysteic acid: The protective effect of NMDA and non-NMDA receptor antagonists. *Exp. Neurol.,* **161,** 336–345.

FRANCIS, P.T., POYNTON, A., LOWE, S.L., NAJLERAHIM, A., BRIDGES, P.K., BARTLETT, J.R., PROCTER, A.W., BURTON, C.J. & BOWEN, D.M. (1989). Brain amino acid concentrations and Ca^{2+}-dependent release in intractable depression assessed antemortem. *Brain Res.,* **494,** 315–324.

GASPARINI, F., LINGENHOHL, K., STOEHR, N. FLOR, P.J., HEINRICH, M., VRANESIC, I., BIOLLAZ, M., ALLGEIER, H., HECKENDORN, R., URWYLER, S., VARNEY, M.A., JOHNSON, E.C., HESS, S.D., RAO, S.P., SACAAN, A.I., SANTORI, E.M., VELIÇELEBI, G. & KUHN, R. (1999). 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology,* **38,** 1493–1503.

GORMAN, A. & GRIFFITHS, R. (1994). Sulphur-containing excitatory amino acid-stimulated inositol phosphate formation in primary cultures of cerebellar granule cells is mediated predominantly by N-methyl-D-aspartate receptors. *Neuroscience,* **59,** 299–308.

GRIFFITHS, R. (1990). Cysteine sulphinate (CSA) as an excitatory amino acid transmitter candidate in the mammalian central nervous system. *Prog. Neurobiol.,* **35,** 313–323.

GRIFFITHS, R. & DUNLOP, J. (1991). Cysteine sulphinic acid as a candidate endogenous EAA ligand. *Trends Pharmacol. Sci.,* **12,** 94–96.

HAYASHI, Y., SEKIYAMA, N., NAKANISHI, S., JANE, D.E., SUNTER, D.C., BIRSE, E.F., UDVARHELYI, P.M. & WATKINS, J.C. (1994). Analysis of agonist and antagonist activites of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. *J. Neurosci.,* **14,** 3370–3377.

HEAFIELD, M.T.E., FEARN, S., STEVENTON, G.B., WARING, R.H., WILLIAMS, A.C. & STURMAN, S.G. (1990). Plasma cysteine and sulphate levels in patients with motoneurone, Parkinson's and Alzheimer's disease. *Neurosci. Lett.,* **110,** 216–220.

HERRERO, I., MIRAS-PORTUGAL, M.T. & SÁNCHEZ-PRIETO, J. (1992). Positive feedback of glutamate exocytosis by metabotropic presynaptic receptor stimulation. *Nature,* **360,** 163–166.

HERRERO, I., MIRAS-PORTUGAL, M.T. & SÁNCHEZ-PRIETO, J. (1994). Rapid desensitization of the metabotropic glutamate receptor facilitates glutamate release in rat cerebrocortical nerve terminals. *Eur. J. Neurosci.,* **6,** 115–120.

IWATA, H., YAMAGAMI, S. & BABA, A. (1982b). Cysteine sulfinic acid in the central nervous system: specific binding of [^{35}S] cysteic acid to cortical synaptic membranes - an investigation of possible binding sites for cysteine sulfinic acid. *J. Neurochem.,* **38,** 1275–1279.

IWATA, H., YAMAGAMI, S., MIZUO, H. & BABA, A. (1982a). Cysteine sulphinic acid in the CNS - uptake and release of cysteine sulphinic acid by a rat brain preparation. *J. Neurochem.,* **38,** 1268–1274.

KILPATRICK, I.C. & MOZLEY, L.S. (1986). An initial analysis of the regional distribution of excitatory sulphur-containing amino acids in the rat brain. *Neurosci. Lett.,* **72,** 189–193.

KIM, J.P., KOH, J. & CHOI, D.W. (1987). L-Homocysteate is a potent neurotoxin on cultured cortical neurones. *Brain Res.,* **437,** 103–110.

KINGSTON, A.E., LOWNDES, J., EVANS, N., CLARK, B., TOMLINSON, R., BURNETT, J.P., MAYNE, N.G., COCKERHAM, S.L. & LODGE, D. (1998). Sulphur-containing amino acids are agonists for group I metabotropic receptors expressed in clonal RGT cell lines. *Neuropharmacology,* **37,** 277–287.

KLANCNIK, J.M., CUENOD, M., GAHWILER, B.H., JIANG, Z.P. & DO, K.Q. (1992). Release of endogenous amino acids, including homocysteic acid and cysteine sulphinic acid, from rat hippocampal slices evoked by electrical stimulation of Schaffer collateral-commissural fibres. *Neuroscience,* **49,** 557–570.

LIPTON, S.A. & ROSENBERG, P.A. (1994). Excitatory amino acids as a final common pathway for neurological disorders. *New Eng. J. Med.,* **330,** 613–622.

LOMBARDI, G., LEONARDI, P. & MORONI, F. (1996). Metabotropic glutamate receptors, transmitter output and fatty acids: studies in rat brain slices. *Br. J. Pharmacol.,* **117,** 189–195.

LOMBARDI, G., PELLEGRINI-GIAMPIETRO, D.E., LEONARDI, P., CHERICI, G., PELLICCIARI, R. & MORONI, F. (1994). The depolarization-induced outflow of D-[^{3}H]aspartate from rat brain slices is modulated by metabotropic glutamate receptors. *Neurochem. Int.,* **24,** 525–532.

MARTIN, D., BUSTOS, G.A., BOWE, M.A., BRAY, S.D. & NADLER, J.V. (1991). Autoreceptor regulation of glutamate and aspartate release from slices of the hippocampal CA1 area. *J. Neurochem.,* **56,** 1647–1655.

MEWETT, K.N., OAKES, D.J., OLVERMAN, H.J., SMITH, D.A.S. & WATKINS, J.C. (1983). Pharmacology of the excitatory actions of sulphonic and sulphinic amino acids. In *CNS Receptors–From Molecular Pharmacology to Behaviour*. ed. Mandel, P. & De Feudis, F.V. pp. 163–174. New York: Raven Press.

MINC-GOLOMB, D., EIMERL, S. & SCHRAMM, M. (1989). Cysteine sulfinic acid-induced release of D-[^{3}H]aspartate and [^{14}C]GABA in hippocampus slices: the role of sodium channels and cAMP. *Brain Res.,* **490,** 205–211.

MISRA, C.H. & OLNEY, J.W. (1975). Cysteine oxidase in brain. *Brain Res.,* **97,** 117–126.

MORONI, F., LOMBARDI, G., THOMSEN, C., LEONARDI, D., ATTUCCI, S., PERUGINELLI, F., TORREGROSSA, S.A., PELLEGRINI-GIAMPIETRO, D.E., LUNEIA, R. & PELLICCIARI, R. (1997). Pharmacological characterisation of 1-aminoindan-1,5-dicarboxylic acid, a potent mGluR1 antagonist. *J. Pharmacol. Exp. Ther.,* **281,** 721–729.

MURPHY, D.E. & WILLIAMS, M. (1987). Interaction of sulfur-containing amino acids with quisqualate and kainate excitatory amino acid receptors in rat brain. In *Excitatory Amino Acid Transmission*. ed. Hicks, T.P., Lodge, D. & McLennon, H. pp. 63–66. New York: Alan R. Liss.

OLNEY, J.W., HO, O.L. & RHEE, V. (1971). Cytotoxic effects of acidic and sulphur-containing amino acids on the infant mouse central nervous system. *Expl. Brain Res.,* **14,** 61–76.

PALMER, A.M. (1999). Excitatory amino acids and neurodegeneration. In *Amino Acid Neurotransmission*. ed. Stephenson, F.A. & Turner, A.J. pp. 195–226. London: Portland Press.

PALMER, A.M. & REITER, C.T. (1994). Comparison of the superfused efflux of preaccumulated D-[^{3}H]aspartate and endogenous L-aspartate and L-glutamate from rat cerebrocortical minislices. *Neurochem. Int.,* **25,** 441–450.

PATEL, D.R. & CROUCHER, M.J. (1997). Evidence for a role of presynaptic AMPA receptors in the control of neuronal glutamate release in the rat brain. *Eur. J. Pharmacol.,* **332,** 143–151.

PATEL, D.R. & CROUCHER, M.J. (1998). A role for presynaptic group I metabotropic glutamate receptors in the control of glutamate release in the rat striatum: an *in vivo* microdialysis study. *Br. J. Pharmacol.,* **123,** 207P.

PELLEGRINI-GIAMPIETRO, D.E., TORREGROSSA, S.A. & MORONI, F. (1996). Pharmacological characterisation of metabotropic glutamate receptors coupled to phospholipase D in the rat hippocampus. *Br. J. Pharmacol.,* **118,** 1035–1043.

PENDE, M., LANZA, M., BONANNO, G. & RAITERI, M. (1993). Release of endogenous glutamic and aspartic acids from cerebrocortex synaptosomes and its modulation through activation of a γ-aminobutyric acid$_B$ ($GABA_B$) receptor subtype. *Brain Res.,* **604,** 325–330.

PIN, J.-P. & DUVOISIN, R. (1995). The metabotropic glutamate receptors: structure and functions. *Neuropharmacology,* **34,** 1–26.

PORTER, R.H.P. & ROBERTS, P.J. (1993). Glutamate metabotropic receptor activation in neonatal rat cerebral cortex by sulphur-containing excitatory amino acids. *Neurosci. Lett.,* **154,** 78–80.

PULLAN, L.M., OLNEY, J.W., PRICE, M.T., COMPTON, R.P., HOOD, W.F., MICHEL, J. & MONAHAN, J.B. (1987). Excitatory amino acid receptor potency and subclass specificity of sulfur-containing amino acids. *J. Neurochem.,* **49,** 1301–1307.

RECASENS, M., FAGNI, L., BAUDRY, M. & LYNCH, G. (1984a). Potassium and veratridine stimulated L-[^{3}H]-cysteine sulphinate and L-[^{3}H]-glutamate release from rat brain slices. *Neurochem. Int.,* **6,** 325–332.

RECASENS, M., SAADOUN, F., BAUDRY, M., MAITRE, M. & LYNCH, G. (1984b). A comparative study of L-[^{3}H]glutamate and L-[^{3}H]cysteine sulfinate binding sites in subcellular fractions of rat brain. *J. Neurosci. Res.,* **11,** 157–169.

RECASENS, M., SAADOUN, F., MAITRE, M., BAUDRY, M., LYNCH, G., MANDEL, P. & VINCENDON, G. (1983). Cysteine sulfinate receptors in the rat CNS. In *CNS Receptors–From Molecular Pharmacology to Behaviour*. ed. Mandel, P. & De Feudis, F.V. pp. 211–219. New York: Raven Press.

RECASENS, M., VARGA, V., NANOPOULOS, D., SAADOUN, F., VINCENDON, G. & BENAVIDES, J. (1982). Evidence for cysteine sulfinate as a neurotransmitter. *Brain Res.,* **239,** 153–173.

SCHOEPP, D.D., GOLDSWORTHY, J., JOHNSON, B.G., SALHOFF, C.R. & BAKER, S.R. (1994). 3,5-Dihydrophenylglycine is a highly selective agonist for phosphonoinositide-linked metabotropic glutamate receptors in the rat hippocampus. *J. Neurochem.,* **63,** 769–772.

SHARP, R.L., MAYNE, N.G. & BURNETT, J.P. (1994). Cyclothiazide differentially modulates human metabotropic glutamate receptors linked to phosphonoinositide hydrolysis stimulation in oocytes. *Eur. J. Pharmacol.,* **269,** R5–R7.

THOMAS, L.S., JANE, D.E. & CROUCHER, M.J. (1997). Relative activities of halogenated willardiine derivatives at presynaptic AMPA receptors: Comparison with postsynaptic potencies. *Brain Res. Assoc. Abstr.,* **14,** 36.

THOMAS, L.S., JANE, D.E., HARRIS, J.R. & CROUCHER, M.J. (2000). Metabotropic glutamate autoreceptors of the mGlu5 subtype positively modulate neuronal glutamate release in the rat forebrain *in vitro*. *Neuropharmacology,* **39,** 1554–1566.

THOMPSON, G.A., JONES, P.L. ST. J. & KILPATRICK, I.C. (1995). The actions of a range of excitatory amino acids at (1S, 3R)-1-aminocyclopentane-1,3-dicarboxylic acid-depolarising receptors on neonatal rat motoneurones. *Neuropharmacology,* **34,** 857–863.

THOMPSON, G.A. & KILPATRICK, I.C. (1996). The neurotransmitter candidature of sulphur-containing excitatory amino acids in the mammalian central nervous system. *Pharmacol. Ther.,* **72**(1), 25–36.

TURSKI, W.A. (1989). Homocysteic acid: convulsant action of stereoisomers in mice. *Brain Res.,* **479,** 371–373.

VÁZQUEZ, E., HERRERO, I., MIRAS-PORTUGAL, M.T. & SÁNCHEZ-PRIETO, J. (1994). Role of arachidonic acid in the facilitation of glutamate release from rat cerebrocortical synaptosomes independent of metabotropic glutamate receptor responses. *Neurosci. Lett.,* **174,** 9–13.

WALDMEIER, P.C., WICKI, P., FELDTRAUER, J., MICKEL, S.J., BITTIGER, H. & BAUMANN, P.A. (1994). GABA and glutamate release affected by $GABA_B$ receptor antagonists with similar potency: No evidence for pharmacologically different presynaptic receptors. *Br. J. Pharmacol.,* **113,** 1515–1521.

WALLER, S.J., KILPATRICK, I.C., CHAN, M.W.J. & EVANS, R.H. (1991). The influence of assay conditions on measurement of excitatory dibasic sulphinic and sulphonic alpha-amino acids in nervous tissue. *J. Neurosci. Meth.,* **36,** 167–176.

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