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Presynaptic $mGlu_1$ type receptors potentiate transmitter output in the rat cortex

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Abstract

In the present study we used freely moving rats with a microdialysis probe placed in their parietal cortex to study the effects of local application of agonists and antagonists of metabotropic glutamate (mGlu) receptors on glutamate release. (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD; 0.1–1 mM), a non-selective agonist of metabotropic glutamate (mGlu) receptors, increased glutamate concentration in the dialysate up to 3-fold. A significant increase in glutamate output in cortical dialysates was also obtained with (*RS*)-3,5-dihydroxyphenylglycine (DHPG; 0.5–1 mM), a group 1-selective mGlu receptor agonist, suggesting the involvement of group 1 mGlu receptors in 1*S*,3*R*-ACPD effects. *S*-4-carboxyphenylglycine (*S*-4CPG; 0.3 μM), a $mGlu_1$ receptor antagonist with a mild agonist action on $mGlu_2$ receptors, antagonised, in a surmountable manner, the effects of 1*S*,3*R*-ACPD. Similarly, 1-aminoindan-1,5-dicarboxylic acid (AIDA; 0.03–1 mM) a selective group 1 antagonist with a preferential action on $mGlu_1$ type receptors, antagonised the effects of 1*S*,3*R*-ACPD. Finally, (*S*)-(+)-2-(3′-Carboxybicyclo[1.1.1]pentyl)-glycine (UPF596; 30–300 μM), a potent $mGlu_1$ antagonist with modest agonist activity on $mGlu_5$, antagonised 1*S*,3*R*-ACPD-induced glutamate release. In conclusion, our data showed that 1*S*,3*R*-ACPD-induced glutamate release in the parietal cortex is mediated by $mGlu_1$ receptors and that, under basal conditions, these receptors are not tonically activated.

Keywords: Glutamate release; Microdialysis; 1*S*,3*R*-ACPD ((1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid); Metabotropic glutamate (mGlu) receptors; L-CCG1 ((2*S*,3*S*,4*S*)-α-carboxycyclopropyl-glycine); *S*-4CPG ((*S*)-4-carboxy-phenylglycine); AIDA (aminoindan-1,5-dicarboxylic acid); DHPG ((*RS*)-3,5-dihydroxyphenylglycine)

1. Introduction

(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD, 30–300 μM), a non-specific agonist of metabotropic glutamate (mGlu) receptors (Schoepp and Conn, 1993), potentiates the depolarization-induced glutamate release in cortical synaptosomes (Herrero et al., 1992) and in cortex slices (Lombardi et al., 1994, 1996), while in caudate synaptosomes and in caudate slices, it causes a significant inhibition of depolarization-induced transmitter release (Lombardi et al., 1993; East et al., 1995). Anatomical (Neki et al., 1996), physiological (Lovinger, 1991; Calabresi et al., 1992) and pharmacological (Lombardi et al., 1993) data suggest that the inhibitory actions observed in the caudate nucleus are largely mediated by type 2 mGlu receptors. Using selective antagonists in vivo, it has also been shown that this inhibitory effect is tonically active and contributes significantly to overall striatal function (Cozzi et al., 1997). While the potentiation of transmitter release in cortical preparations seems to implicate mGlu receptors able to activate phospholipase C and the protein kinase C-mediated phosphorylation of a K^+ channel located in the presynaptic terminals (see Sanchez-Prieto et al., 1996 for a review), the mGlu receptor subtype(s) responsible for this increase remains to be characterized. One of the difficulties in achieving this task has been the lack of potent and selective antagonists for $mGlu_1$ receptor subtypes. New tools for purpose are now becoming available (Watkins and Collingridge, 1994; Pellicciari et al., 1996b; Monn et al., 1997) and in view of the importance of glutamate mediated neurotransmission in physiology (including synaptic plasticity) and in pathology, we thought it interesting to characterise the receptors

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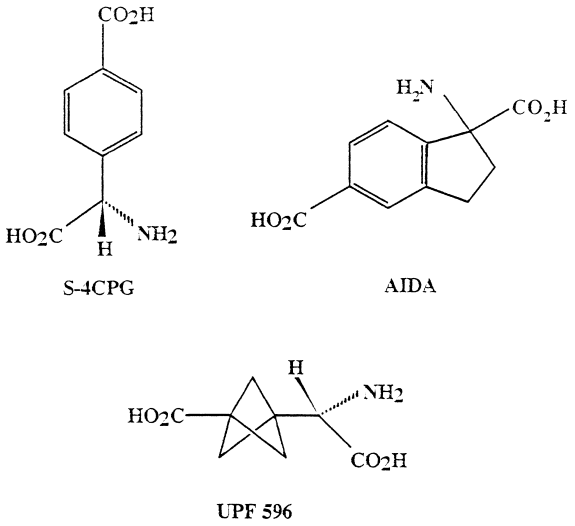

Fig. 1. Chemical structures of the $mGlu_1$ receptor antagonists mentioned. (*S*)-4C-PG is (*S*)-4-carboxyphenylglycine; AIDA is 1-aminoindan-1,5-dicarboxylic acid; UPF596 is (*S*)-(+)-2-(3′-Carboxybicyclo[1.1.1]pentyl)-glycine.

potentiating transmitter release in cortical preparations. We took advantage of the properties of three group 1 receptor antagonists: (1) (*S*)-4-carboxy-phenylglycine (*S*-4CPG), a member of the widely studied family of the phenylglycines that antagonise $mGlu_1$ and act as a partial agonists on

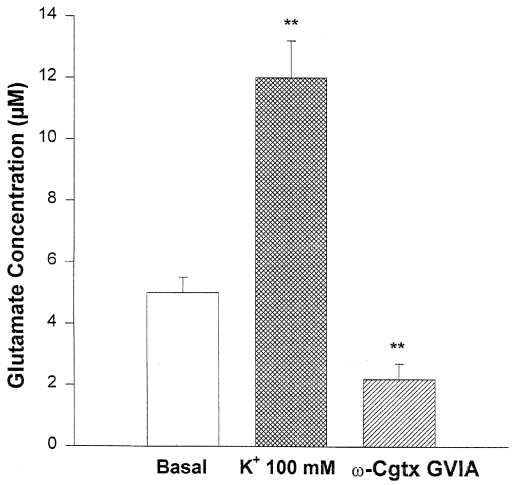

Fig. 2. Effects of depolarisation or of N type calcium channel antagonism on glutamate concentration in the extracellular fluid of the rat cortex. Each column represents the mean (±S.E.) glutamate concentration in cortical dialysates. The amino acid was measured in fractions collected every 5 min (17.5 μl) beginning 15–18 h after the implantation of the dialysis cannula. The mean value obtained for each animal from the 3 samples collected just before treatment with pharmacological agents was defined as basal spontaneous output (100%) and was used for the calculations. ω-conotoxin G VIA (1 μM) was applied for 20 min in 7 rats. The iso-osmotic solution containing KCl 100 mM was applied for 5 min at the end of each experiment. The basal concentration reported here is the mean obtained from at least 40 rats. Glutamate recovery in the probes was 55±5% ($n=6$). Open column: basal concentration; Black column: KCl 100 mM; Hatched column: ω-conotoxin G VIA. $**P<0.01$ vs. control.

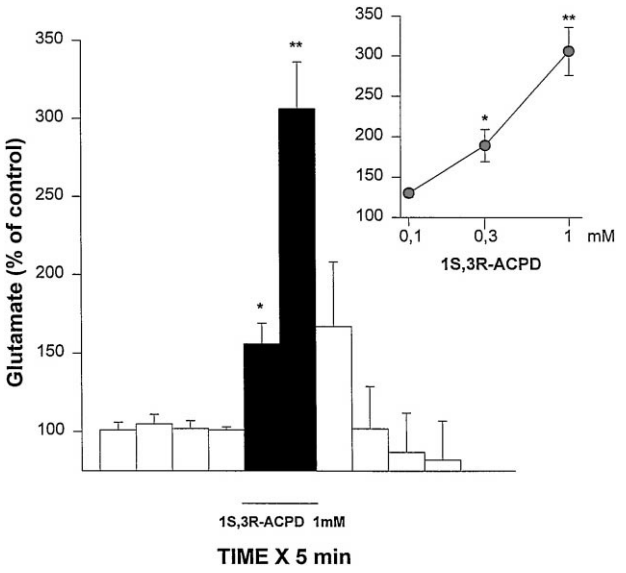

Fig. 3. Time-course and concentration–response curve (inset) of 1*S*,3*R*-ACPD effects on glutamate content in cortical dialysates. Basal glutamate value (100% in the ordinate scale) was evaluated as reported in Fig. 2. At least 6 animals were used for each concentration of 1*S*,3*R*-ACPD. Each column or point (insert) represents the mean±S.E. The hatched columns indicate the addition of 1*S*,3*R*-ACPD to the perfusion fluid. $*P<0.01$; $**P<0.001$ vs. control.

$mGlu_2$ receptors (Watkins and Collingridge, 1994; Brabet et al., 1995), (2) 1-aminoindan-1,5-dicarboxylic acid (AIDA), a preferential type 1 mGlu receptor antagonist (Pellicciari et al., 1995; Moroni et al., 1997) and (3) the newly described, (*S*)-(+)-2-(3′-Carboxybicyclo-[1.1.1]pentil)-glycine (UPF596), a potent $mGlu_1$ receptor antagonist (IC_{50} of 25 μM) with modest agonist activity (EC_{50} = 150 μM) on type 5 mGlu receptors (Pellicciari et al., 1996b) (see Fig. 1).

Using transverse microdialysis in the parietal cortex of freely moving rats and appropriate concentrations of these new antagonists, we attempted to characterise the mGlu subtype involved in the 1*S*,3*R*-ACPD-induced release of glutamate. The results of our studies suggest strongly that $mGlu_1$ receptor stimulation causes a significant glutamate release in the rat parietal cortex.

2. Materials and methods

2.1. Microdialysis

The experiments were approved by the Ethical Committee of the Department of Pharmacology of the University of Florence and were performed in compliance with E.U. recommendations (86/609/CEE). Male Wistar rats (Harlan-Nossan, Monza, Italy, 200–250 g body weight) were anaesthetized with chloral hydrate (300 mg/kg) and placed in a stereotaxic frame. Transcerebral microdialysis tubing (internal diameter 220 μm, external diameter 310

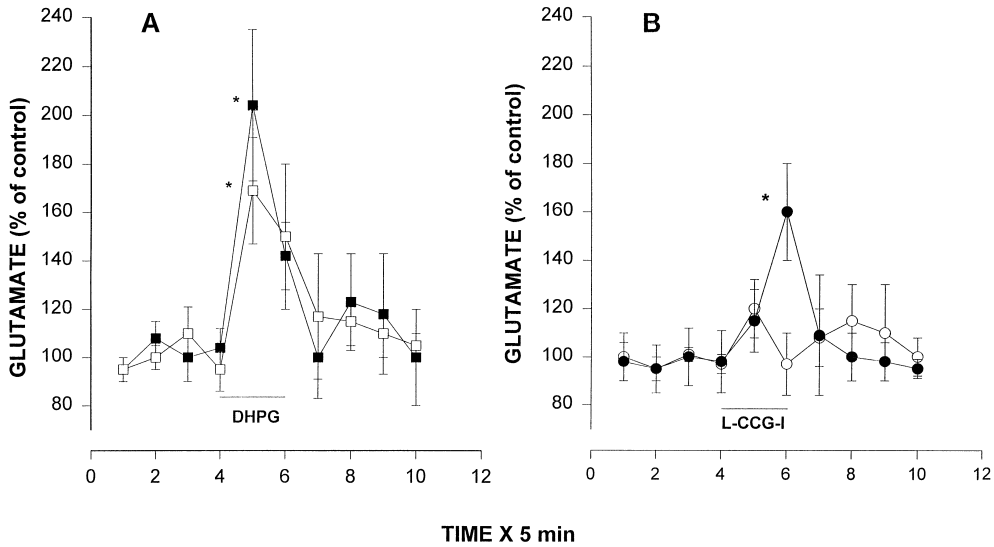

Fig. 4. Time-course of DHPG (500–1000 μM) or L-CCG-1 (100–1000 μM) effects on glutamate content in cortical dialysates. Basal glutamate value (100% in the ordinate scale) was obtained as reported in Fig. 2. At least 6 animals were used for each concentration of the mGlu receptor agonists. Each point represents the mean ± S.E. (A) DHPG 500 μM (□); 1000 μM (■). (B) L-CCG-I (○) 100 μM (●) 1000 μM. $* P < 0.01$ vs. control.

μm, molecular cut off $> 15\,000$) was prepared according to Ungerstedt (1984). Dialysis fibres were implanted through small burr holes drilled in the skull at the following co-ordinates (for fibre inlet and outlet): (i) from the bregma (A-P) −0.2 mm; (ii) from the skull surface (H): 2.2 mm. Both ends of the tubing were kept in place with screws and dental cement. The length of the exposed membrane surface was 4 mm and the remaining portions of the membrane were coated with epoxy resin. Approximately 18 h after surgery, the membranes were perfused at a flow rate of 3.5 μl/min with an iso-osmotic solution (NaCl 155, KCl 5.5 and $CaCl_2$ 2.3 mM) by means of a Carnegie Medicine microperfusion pump (model CMA/100). After a washout period of approximately 2 h, several 5 min fractions were collected to determine the basal output. The membranes were then perfused for 10 min with solutions containing the indicated concentrations of mGlu receptor agents and again with the control solution to monitor the recovery of the basal output. At the end of the experiment (usually 240 min after the beginning of perfusion), a solution containing 100 mM K^+ was injected through the dialysis fibre to assess the function of the preparation. Experiments were accepted for analysis only when this solution increased basal glutamate output at least 2-fold. Correct placement of the tubing and the absence of gross histological lesions were verified post-mortem in each rat from coronal sections cut through and on either side of the probe path. The recovery of L-glutamate in the probes perfused at a flow rate of 3.5 μl/min was 55 ± 5%.

2.2. Glutamate measurements

High pressure liquid chromatography separation and fluorometric detection was used for quantitative measurements of glutamate as previously described (Lombardi and Moroni, 1992; Blandina et al., 1995). Particular care was

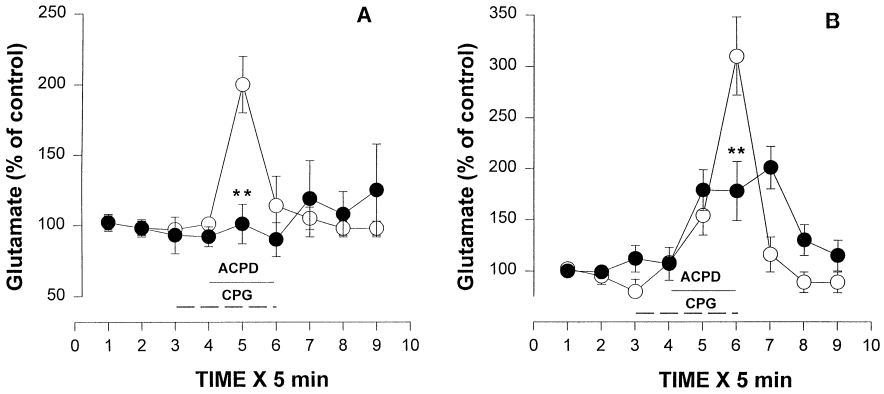

Fig. 5. Time-course of the effects of simultaneous administration of 1*S*,3*R*-ACPD and *S*-4CPG on glutamate output in cortical dialysates. *S*-4CPG 300 μM (● both in A and B) was added to the perfusion fluid 10 min before 1*S*,3*R*-ACPD. In A (○) 1*S*,3*R*-ACPD was used at 300 μM and in B (○) at 1 mM. Each point is the mean ± S.E. obtained from at least 6 freely moving animals. $** P < 0.01$ vs. (1*S*,3*R*)-ACPD.

necessary in separation of the peaks of mGlu receptor agents (L-CCG-1, 1*S*,3*R*-ACPD, DHPG, AIDA, *S*-4CPG and UPF 596) and those of glutamate or its internal standard (cysteic acid). This was achieved by appropriate modifications of the routinely used gradient program for amino acid elution.

2.3. Drugs

(*S*)-(+)-2-(3′-Carboxybicyclo[1.1.1]pentyl)-glycine (UPF596) and 1-aminoindan-1,5-dicarboxylic acid (AIDA) were synthesized as previously described (Pellicciari et al., 1995, 1996b). (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD); (*RS*)-3,5-dihydroxyphenylglycine (DHPG); (2*S*,3*S*,4*S*)-α-carboxycyclopropylglycine (L-CCG-I); (*S*)-4-carboxyphenylglycine (*S*-4CPG) and (+)-α-methyl-4-carboxyphenylglycine ((+)MCPG) were obtained from Tocris Cookson (Bristol, UK). ω-Conotoxin G VIA was obtained from Alomone Labs (Jerusalem, Israel). Concentrated solutions (100 mM) of mGlu receptor agents were prepared in NaOH (0.1 M). A portion of these solutions was neutralised with 0.1 M HCl and finally diluted with the dialysis fluid.

3. Results

3.1. Glutamate concentrations in the dialysates

The experimental conditions selected (namely implantation of the dialysis membrane approximately 18 h before the experiments and a stabilisation period of at least 2 h of perfusion before collection of the 'basal' samples) allowed for measurement of either a decrease or an increase in brain extracellular concentration of glutamate. In preliminary experiments, we showed that an iso-osmotic solution containing 100 mM of KCl (applied at the end of the experiments) increased glutamate concentrations approximately 3-fold. On the other hand, the addition of ω-conotoxin GVIA, an inhibitor of the N type Ca^{2+} channels (Olivera et al., 1985), to the perfusion fluid reduced basal glutamate concentrations by almost 50% (see Fig. 2).

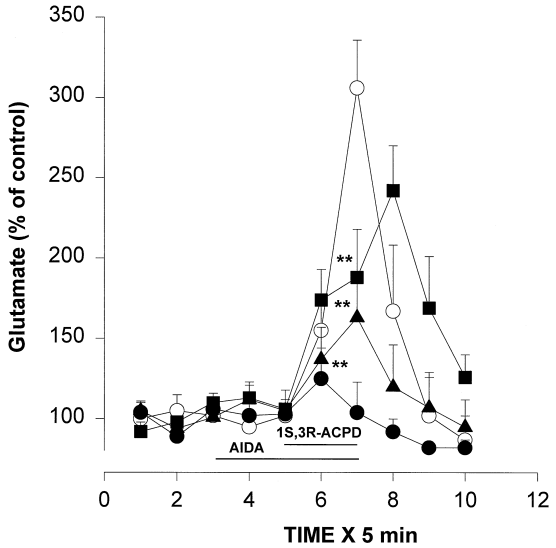

Fig. 6. Time-course and dose-dependent antagonism by AIDA of the effects of 1*S*,3*R*-ACPD on glutamate output in cortical dialysates. AIDA 0.03 (■); 0.3 (▲) or 1 mM (●) was added to the perfusion fluid 10 min before 1*S*,3*R*-ACPD. 1*S*,3*R*-ACPD 1 mM (m) without antagonist. Each point is the mean ± S.E. obtained from at least 6 freely moving animals. ** $P < 0.01$ vs. (1*S*,3*R*)-ACPD.

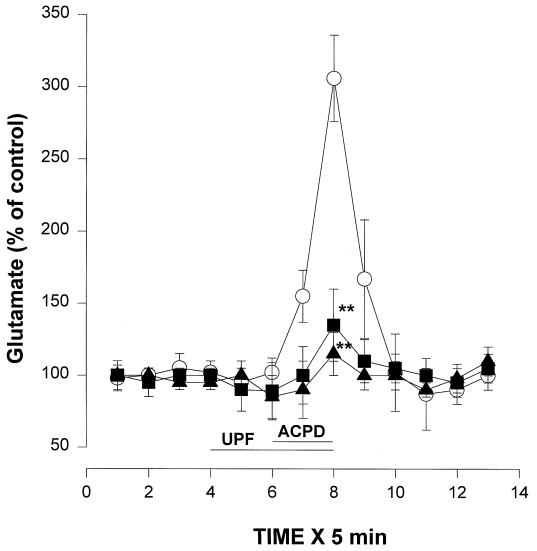

Fig. 7. Time-course and dose-dependent antagonism by UPF 596 of the effects of 1 mM 1*S*,3*R*-ACPD (○) on glutamate output in cortical dialysates. A 0.03-mM UPF 596 (■) or 0.1 mM (▲) was added to the perfusion fluid 10 min before 1*S*,3*R*-ACPD. Each point is the mean ± S.E. obtained from at least 6 freely moving animals. ** $P < 0.01$ vs. (1*S*,3*R*)-ACPD.

3.2. Effects of 1S,3R-ACPD, DHPG and L-CCG-1 on glutamate output from the parietal cortex

We previously showed that group 1 mGlu receptor agonists potentiate depolarisation-induced transmitter release in rat cortex slices in vitro (Lombardi et al., 1996). In the present experiments, we found that suitable concentrations of 1*S*,3*R*-ACPD (100–1000 μM) applied to the rat cortex through the perfusion fluid caused a gradual increase of glutamate concentration in the perfusates. This increase reached its maximum at the end of 10 min of 1*S*,3*R*-ACPD perfusion, when glutamate concentrations had increased 3-fold over the basal levels (see Fig. 3). Similar results were obtained with DHPG (500–1000 μM), but the maximal increase in dialysate glutamate concentration was lower (approximately 2-fold; see Fig. 4). When L-CCG-1 (100 μM), a preferential group 2 mGlu receptor agonist, was used, the glutamate concentration in the dialysates did not increase significantly. A modest increase of glutamate content in the dialysis fluid was found when a very high concentration of L-CCG-1 (1 mM) was used (see

Fig. 4B). It is interesting to note that in separate experiments, with the dialysis probe inserted in the striatum, we observed that 1*S*,3*R*-ACPD and L-CCG-1 significantly decreased glutamate output (Cozzi et al., 1997).

3.3. Effects of S-4CPG, AIDA and UPF596 on glutamate output from the striatum

The basal glutamate concentrations in cortical dialysates were not affected by the addition to the dialysis fluid of any of the three $mGlu_1$ receptor antagonists [*S*-4CPG (0.3 mM), AIDA (0.03–1 mM) or UPF 596 (0.03–0.1 mM)] studied. However, the simultaneous presence of *S*-4CPG (0.3 mM) and 1*S*,3*R*-ACPD (0.3 mM) in the dialysis fluid completely prevented the agonist-induced glutamate release. A higher concentration of 1*S*,3*R*-ACPD (1 mM) applied together with *S*-4CPG (0.3 mM) was still able to cause a modest increase in glutamate concentration in the dialysates, thus suggesting strongly that the antagonist of $mGlu_1$ receptors is responsible for the action of *S*-4CPG (see Fig. 5). To confirm this suggestion, we tested the effects of AIDA and UPF 596. As previously mentioned, AIDA preferentially antagonised $mGlu_1$ but at higher concentrations, it is also a $mGlu_5$ receptor antagonist, while UPF 596 is an antagonist at $mGlu_1$ receptors and is a low-affinity agonist at $mGlu_5$ receptors. Figs. 6 and 7 show that both compounds antagonised the 1*S*,3*R*-ACPD-induced increase of glutamate concentration in the dialysates. AIDA was active when used at 300–1000 μM and UPF 596 at 30–100 μM.

4. Discussion

The present results showed that group 1 mGlu receptor agonists cause transmitter release in rats with a dialysis probe placed in their cortex, and expand on previous findings from work on perfused cortex slices in vitro (Lombardi et al., 1996) or forebrain synaptosomes (Herrero et al., 1992). Group 1 mGlu receptors are usually subdivided into $mGlu_1$ and $mGlu_5$ subtypes (Nakanishi, 1994). In order to identify the receptor subtype involved in the 1*S*,3*R*-ACPD-induced glutamate release we used appropriate concentrations of three different $mGlu_1$ receptor antagonists. The results obtained suggest strongly that $mGlu_1$ receptor stimulation positively modulates transmitter output.

Using electrophysiological approaches, several groups have reported that $mGlu_1$ receptor agonists may have excitatory actions in rat cortical synapses (see Schoepp and Conn, 1993, Pin and Duvoisin, 1995 for reviews). Using anatomical approaches, it has been demonstrated that type 1 mGlu receptor proteins are abundant in the cortical neuropil, apparently reflecting terminal patterns that may arise from the thalamus, where the $mGlu_1$ receptor mRNA level is high (Fotuhi et al., 1993). Thus it is reasonable to assume that one of the excitatory actions $mGlu_1$ receptor agonists have in the cortex is a result of transmitter release from thalamo–cortical neuronal terminals. Unfortunately, the inherent limitations of methods we used do not allow us to rule out the possibility that the potentiation of release we observed was mediated through mechanisms other than positive presynaptic modulation. Thus it is difficult to rule out $mGlu_1$ receptor-mediated activation of local circuits or changes in carrier function. The direct presynaptic action of $mGlu_1$ receptor agents is apparently not consistent with results of an elegant series of studies with electron microscopy associated with immunogold reactions, demonstrating that $mGlu_1$ receptors are mostly located on postsynaptic neurones near the active site of glutamatergic synapses (Nusser et al., 1994; Lujan et al., 1996). While these studies carefully identify $mGlu_1$ receptor location in the hippocampus and in the cerebellum, similar morphological studies in different layers of the rat cortex are not available. It should be mentioned, however, that 1*S*,3*R*-ACPD significantly increases glutamate release as found not only in microdialysis studies (see Fig. 2) and in cortex slices (Lombardi et al., 1996) but also in isolated cortical neuronal terminals, thus strongly suggesting a presynaptic location of protein kinase C-associated mGlu receptors able to facilitate transmitter release (Coffey et al., 1994). Results of the present pharmacological studies with the local application of antagonists are in line with the proposal that, in the parietal cortex, presynaptic type 1 mGlu receptors are positively associated with transmitter release.

1*S*,3*R*-ACPD-mediated transmitter release in cortex slices and in synaptosomes requires the presence of arachidonate or other unsaturated fatty acids (Herrero et al., 1992; Lombardi et al., 1996). In microdialysis studies, it is difficult to completely eliminate fatty acids from brain tissue, thus the cortical release of glutamate found in vivo may certainly be based on mechanisms completely different from those studied in vitro. The receptors involved, however, seem to have identical pharmacological properties and to be of the $mGlu_1$ type. Moreover, experiments performed in vitro with a series of $mGlu_1$ antagonists such as MCPG, *S*-4CPG and AIDA have shown a competitive antagonism against 1*S*,3*R*-ACPD facilitatory effects of the depolarisation-induced release (Moroni et al., 1997). In the present experiments, we again used *S*-4CPG, a molecule capable of antagonising $mGlu_1$ but not $mGlu_5$ receptors (Brabet et al., 1995). Unfortunately, large concentrations of *S*-4CPG also stimulate the inhibitory $mGlu_2$ receptors and this stimulation could result indirectly in a diminished 1*S*,3*R*-ACPD effect. This possibility was ruled out by the results reported in Fig. 4B, showing that inhibitory actions of *S*-4CPG could be surmounted by an increased agonist concentration. Furthermore, suitable concentrations of AIDA, a preferential $mGlu_1$ receptor antagonist with very modest activity on $mGlu_5$ and a very low affinity (≥ 1 mM) for $mGlu_2$ receptors (Moroni et al., 1997), antagonised 1*S*,3*R*-ACPD releasing actions. We also tested UPF

596, a newly described compound able to antagonise 1*S*,3*R*-ACPD effects in $mGlu_1$ transfected cells with a IC_{50} of 25 μM. Higher concentrations of UPF 596 have partial agonist activity in cells transfected with $mGlu_5$ receptors (EC_{50} 150 μM). No activity has been observed (up to 500 μM) on other mGlu receptor subtypes, on ionotropic receptors or on glutamate carriers (Pellicciari et al., 1996a). The compound therefore seems to be a quite specific $mGlu_1$ receptor antagonist and is certainly suitable for separating actions mediated by $mGlu_1$ from those mediated by $mGlu_5$ receptors. Adding various concentrations of UPF 596 (which should be considered selectively active on $mGlu_1$ receptors) to the dialysis fluid, prevented 1*S*,3*R*-ACPD-induced glutamate release, supporting the proposal that $mGlu_1$ receptors are responsible for the positive modulation of transmitter release.

As mentioned in the Introduction, when the dialysis probe was placed in the caudate nucleus, concentrations of 1*S*,3*R*-ACPD in the range of those used in the present studies, caused a significant inhibition of glutamate release. Selective antagonists of $mGlu_2$ receptors not only prevented 1*S*,3*R*-ACPD effects, but also caused a large increase of glutamate concentration in brain extracellular spaces, suggesting that these inhibitory receptors are tonically activated (Cozzi et al., 1997). Group 3 mGlu receptors have been shown to be particularly important in the modulation of transmitter release in other brain areas (Koerner and Cotman, 1982; Jane et al., 1994). The control of transmitter release at excitatory synapses therefore seems controlled by a significant number of receptors with different organisation. In addition to glutamate receptors (autoreceptors) of the ionotropic (Roberts and Mcbean, 1981; Chittajallu et al., 1996) and metabotropic type, adenosine (Corradetti et al., 1984; Vazquez et al., 1995) or $GABA_B$ receptors (Bowery, 1993) appear to be of particular importance for powerful modulation of synaptic function.

A significant percentage of the glutamate we measured in the in vivo microdialysis model is likely to have originate in non-neuronal pools (Butcher and Hamberger, 1987). In spite of this, our experimental conditions allowed detection not only of depolarisation-induced increase of glutamate in extracellular spaces, but also inhibition of the Ca^{2+}-dependent output (see Fig. 2). Since the basal glutamate output was not affected by $mGlu_1$ receptor antagonists, it is reasonable to assume that these receptors are not tonically activated. Their functional state therefore seems significantly different from that of $mGlu_2$ receptors in the caudate nucleus (Cozzi et al., 1997). A $mGlu_1$-mediated facilitation of transmitter release may, however, become important in particular situations (for instance during the learning process; Klein et al., 1997) and could be one of the mechanisms leading to synaptic plasticity and synaptic potentiation (Sanchez-Prieto et al., 1996). Furthermore, a marked increase of glutamate in the extracellular spaces occurs under ischemic conditions or after trauma (Benveniste et al., 1984; Lombardi and Moroni, 1992). Under such conditions, the positive modulation of transmitter output described here may become operative and contribute to the development of the processes leading to ischemic neuronal death. Along these lines, it should be mentioned that $mGlu_1$ receptor antagonists or antisense oligodeoxynucleotides against $mGlu_1$ receptors have been shown to reduce neuronal death in vitro and in vivo after trauma (Mukhin et al., 1996) or after transient brain ischemia in gerbils (manuscript in preparation).

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