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Short communication

Metabotropic glutamate receptor agonists inhibit endogenous glutamate release from rat striatal synaptosomes

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Abstract

A striatal synaptosomal preparation was used to assess the action of metabotropic glutamate receptor (mGlu receptor) agonists on 4-aminopyridine (2 mM)-stimulated endogenous glutamate release. 4-Aminopyridine alone increased basal glutamate release by 6.89 ± 0.74 nmol/mg. The mGlu receptor agonists L-2-amino-4-phosphonobutyric acid (L-AP4) ($IC_{50} \approx 0.2$ μM) and (1*S*,3*S*)-1-amino-cyclopentane-1,3-dicarboxylic acid ($IC_{50} \approx 0.2$ μM) inhibited 4-aminopyridine-stimulated release. The putative mGlu receptor antagonist (*S*)-α-methyl-L-AP4, which itself inhibited 4-aminopyridine-stimulated release ($IC_{50} \approx 10$ μM), did not inhibit the effects of the two agonists.

Keywords: Metabotropic glutamate receptor; (Presynaptic); Glutamate release; Striatum; Synaptosome; Parkinson's disease

1. Introduction

The striatum plays a critical role in the processing of information concerned with motor control and other important cognitive functions. The striatum receives major afferents from the substantia nigra pars compacta, thalamus and cerebral cortex. The thalamic and cortical inputs are known to be excitatory, probably utilising glutamate as the transmitter and stimulation of the corticostriatal pathway evokes fast excitatory postsynaptic potentials (EPSPs) mediated by ionotropic glutamate receptors. Glutamate can also activate G protein-linked metabotropic receptors (mGlu receptors), seven subtypes of which have so far been characterised – $mGlu_{1-7}$ receptors (reviewed in Nakanishi, 1992; Saugstad et al., 1994; Okamoto et al., 1994). In striatal neurons, glutamate has been shown to increase inositide phosphate turnover, presumably by activating mGlu receptors coupled to phospholipase C. Indeed, mRNA encoding for $mGlu_1$ receptor and $mGlu_5$ receptor, which couple to phospholipase C, is present within striatal neurons (see Nakanishi, 1992). Additionally, mGlu receptors are thought to reside presynaptically and modulate glutamatergic transmission. Although little is known about the presynaptic mGlu receptors located on glutamatergic terminals in the striatum, it has been shown that the mGlu receptor agonist *trans*-1-amino-cyclopentane-1,3-dicarboxylic acid (*t*-ACPD) reduces glutamate-mediated EPSPs in a corticostriatal slice preparation (Calabresi et al., 1993).

In this study we used a striatal synaptosomal preparation to establish whether activation of presynaptic mGlu receptors modulates glutamate release. Two mGlu receptor agonists, L-2-amino-4-phosphonobutyric acid (L-AP4) and (1*S*,3*S*)-1-amino-cyclopentane-1,3-dicarboxylic acid ((1*S*,3*S*)-ACPD), were chosen because of their reported activity at presynaptic mGlu receptors (Kemp et al., 1994b). Both L-AP4 and (1*S*,3*S*)-ACPD reduce forskolin-stimulated elevations of cAMP in guinea pig cerebrocortical slices (Kemp et al., 1994b). L-AP4 has also been shown to elicit depression of presynaptic responses in a number of glutamatergic pathways, an effect mimicked by (1*S*,3*S*)-ACPD in rat motor neurons (Kemp et al., 1994b). Also, neither of these agonists potently activates phosphoinositide-coupled mGlu receptors, indeed L-AP4 has weak antagonist activity at these receptors (Schoepp et al., 1990).

Using these two agonists (in the absence of suitable antagonists) it may be possible to distinguish actions

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mediated by $mGlu_{2/3}$ receptors and $mGlu_{4/6/7}$ receptors. Thus, from studies of cloned mGlu receptors, it has been demonstrated that L-AP4 is a potent agonist at $mGlu_{4/6/7}$ receptors which couple to the inhibition of adenylyl cyclase via pertussis toxin-sensitive G proteins (Tanabe et al., 1993; Nakajima et al., 1993; Saugstad et al., 1994). However, $mGlu_2$ and $mGlu_3$ receptors (which also couple to inhibition of adenylyl cyclase activity), are not activated by L-AP4 but are sensitive to isomers of *t*-ACPD (Tanabe et al., 1993).

Furthermore, a putative antagonist of L-AP4-sensitive receptors has been developed, (*S*)-α-methyl-L-AP4 (MAP4; Jane et al., 1994), and was used in an attempt to further distinguish between L-AP4 and (1*S*,3*S*)-ACPD responses.

2. Materials and methods

2.1. Synaptosomal preparation

The striata from six adult (250–400 g) male rats were dissected, pooled in 320 mM sucrose (2–3° C) and homogenised by hand in a glass homogeniser using a Teflon-coated pestle. The homogenate was centrifuged at $1000 \times g$ for 10 min and the resulting supernatant centrifuged again at $12000 \times g$ for 25 min. The pellet was resuspended in one volume of 320 mM sucrose, layered over 2 volumes of 800 mM sucrose and centrifuged at $12000 \times g$ for 30 min. The resulting pellet was resuspended in Hepes-buffered medium (HBM; containing (in mM) NaCl (140), KCl (5), Hepes (20), $NaHCO_3$ (5), $MgCl_2$ (1), Na_2SO_4 (0.12), glucose (10)). In each experiment, a sample of resuspended synaptosomes was taken for a Bradford protein assay using bovine serum albumin as standard.

2.2. Glutamate release

Following resuspension in 20 ml HBM (pH 7.4), the synaptosomes were allowed to equlibrate at 37° C. An aliquot of synaptosomes (1 ml) was added to a constantly stirred cuvette with $NADP^+$ (1 mM), 50 U glutamate dehydrogenase and 1.3 mM Ca^{2+} (in experiments involving nominally Ca^{2+}-free conditions, 1.3 mM EGTA replaced the Ca^{2+}). The final volume in the cuvette was 3 ml, the protein concentration being 0.25–0.30 mg/ml.

The fluorescence generated by the conversion of $NADP^+$ to NADPH by glutamate dehydrogenase was monitored by a Perkin-Elmer luminescence spectrometer (model LS-50; excitation at 340 nm and emmission at 460 nm). The glutamate release was monitored over a 5 min period with fluorescence data collected every 2 s. In each experiment a concentration-response curve to exogenous glutamate (1–5 μM) was established and used to convert fluorescence data to the concentration of endogenous glutamate released. In each 5 min period, the first 80 s established basal release and all drugs were added at the 80 s time point. Any change in release was calculated by integrating the fluorescence signal with respect to time and comparing the values before and after drug additions.

Glutamate release was stimulated by the addition of 4-aminopyridine, mGlu receptor agonists were added 5 min prior to 4-aminopyridine and the antagonist added 5 min prior to agonist. The effects of vehicle additions on glutamate release were also quantified and subtracted from the appropriate drug addition.

Significant differences in release between various treatments were calculated using a two-tailed Student's *t*-test.

2.3. Materials

The mGlu receptor agonists L-2-amino-4-phosphonobutyric acid (L-AP4) and (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*S*)-ACPD) were purchased from Tocris Neuramin (Bristol, UK) and (*S*)-α-methyl-L-AP4 (MAP4) was a generous gift from Prof. Jeff Watkins. The Coomassie Blue reagent used in the potein assay was from BioRad (Munich, Germany). 4-Aminopyridine was bought from Sigma (Poole, UK), other chemicals were of the highest quality commercially available.

3. Results

3.1. Glutamate release

When synaptosomes were incubated in buffer containing 1.3 mM Ca^{2+}, 4-aminopyridine (2 mM) increased basal release by 6.89 ± 0.74 nmol glutamate/mg protein ($n = 24$). Where Ca^{2+} was replaced by EGTA, the mean release elicited by 4-aminopyridine (2 mM) was 0.34 nmol glutamate/mg protein ($n = 2$) below basal release. This negative value arose because vehicle injections caused apparently larger release than 4-aminopyridine in Ca^{2+}-free conditions.

4-Aminopyridine-stimulated glutamate release from striatal synaptosomes was inhibited by both L-AP4 and (1*S*,3*S*)-ACPD in a concentration-dependent fashion. As shown in Fig. 1a, 3 μM L-AP4 inhibited glutamate release by approximately 60% ($IC_{50} \approx 0.2$ μM); however, higher concentrations were less effective at inhibiting release. Similarly, 0.3 μM (1*S*,3*S*)-ACPD inhibited 4-aminopyridine-stimulated release by about 70% ($IC_{50} \approx 0.2$ μM; Fig. 1b) with a comparable inhibition being maintained at concentrations up to 10 μM. In order to further characterise the receptors

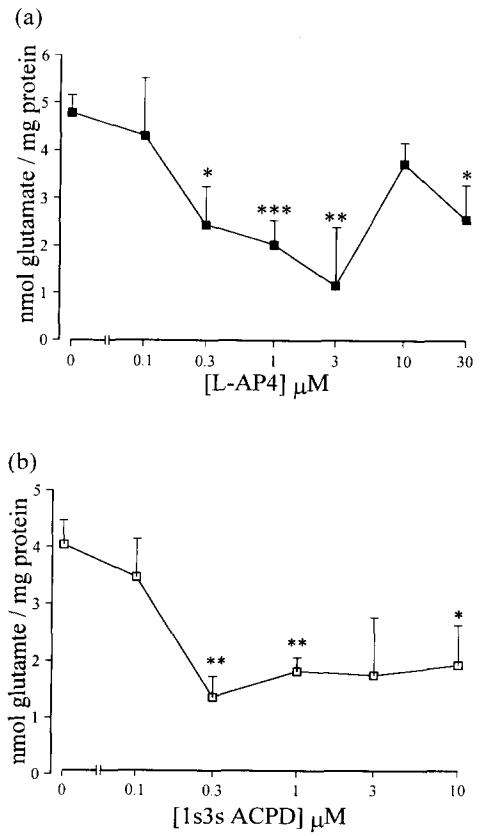

Fig. 1. Inhibition of 4-aminopyridine-stimulated glutamate release from striatal synaptosomes by the mGlu receptor agonists L-AP4 (a) and (1*S*,3*S*)-ACPD (b). Agonists was applied to the synaptosomes 5 min prior to the addition of 2 mM 4-aminopyridine. Significant inhibition was observed both with L-AP4 (a: $^{***}P < 0.002$, $^{**}P < 0.005$, $^{*}P < 0.02$ compared to 4-aminopyridine alone; $n = 3$–7 experiments) and (1*S*,3*S*)-ACPD (b: $^{**}P < 0.005$, $^{*}P < 0.05$ compared to 4-aminopyridine alone; data shown are the mean ± S.E.M., $n = 3$–4 experiments).

responsible for these effects, a putative antagonist of the L-AP4 receptor (MAP4) was employed.

Prior application of MAP4 (3 or 30 μM) failed to antagonise the inhibitory effects of L-AP4 (1 μM; Fig. 2a) or (1*S*,3*S*)-ACPD (0.3 μM; Fig. 2b). Indeed, 30 μM MAP4 alone inhibited 4-aminopyridine-stimulated release by about 45% ($IC_{50} \approx 10$ μM; Fig. 2c).

4. Discussion

4.1. Which mGlu receptors mediate the actions of L-AP4 and (1S,3S)-ACPD?

We have demonstrated that activation of mGlu receptors can inhibit glutamate release from terminals within the striatum of the rat. An important question to address is the type of mGlu receptor(s) that mediate this presynaptic action of L-AP4 and (1*S*,3*S*)-ACPD. L-AP4 is known to be a selective agonist at $mGlu_{4/6/7}$ receptors (Tanabe et al., 1993; Nakajima et al., 1993; Saugstad et al., 1994) and it is probable that one of these receptors underlies the action of L-AP4 on glutamate release. However, a contribution by $mGlu_6$ re-

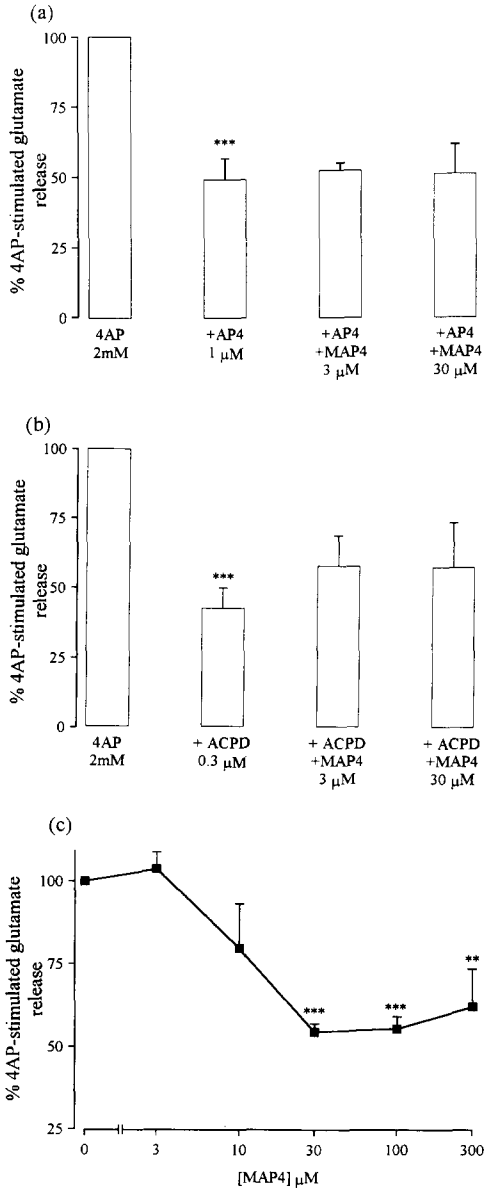

Fig. 2. The putative mGlu receptor antagonist MAP4 (3 and 30 μM) does not block the inhibitory effects of 1 μM L-AP4 (a: $^{***}P < 0.001$ compared to 4-aminopyridine (4AP) alone; $n = 4$–8) or 0.3 μM (1*S*,3*S*)-ACPD (b: $^{***}P < 0.001$ compared to 4-aminopyridine; $n = 3$–8 experiments) on 4-aminopyridine-stimulated glutamate release. No significant differences in release were observed between agonist alone or in the presence of MAP4, applied to the synaptosomes 5 min prior to agonist. The control 4-aminopyridine (2 mM)-stimulated release in (a) and (b) was 7.78 ± 1.20 nmol glutamate/mg protein. MAP4 (3–300 μM) alone significantly inhibited 4-aminopyridine-stimulated glutamate release (c: $^{***}P < 0.0001$, $^{**}P < 0.02$ compared to 4-AP alone; $n = 3$–5 experiments). The control 4-aminopyridine (2 mM)-stimulated release in (c) was 8.26 ± 1.35 nmol glutamate/mg protein.

ceptors is unlikely as expression of mRNA for this receptor is restricted to the inner nuclear layer of the retina (Nakajima et al., 1993; although one cannot discount the presence of undetectable levels of mRNA which may encode receptors in other regions). On the other hand, mRNA encoding for $mGlu_7$ receptors is abundantly expressed in the cerebral cortex (Saugstad et al., 1994), which forms the major excitatory input to the striatum. Thus, presynaptic $mGlu_7$ receptors on corticostriatal terminals may mediate the action of L-AP4 on glutamate release. Similarly, $mGlu_4$ receptors may be present within a striatal synaptosomal preparation because mRNA encoding for $mGlu_4$ receptors is present in the thalamus (Tanabe et al., 1993) which also projects to the striatum.

We tentatively suggest (in the absence of specific antagonists) that the action of (1*S*,3*S*)-ACPD is mediated by $mGlu_2$ receptors and/or $mGlu_3$ receptors which are sensitive to ACPD but insensitive to L-AP4 (Tanabe et al., 1993). Indeed, mRNA encoding for $mGlu_2$ receptors and $mGlu_3$ receptors has been found in the cortex and thalamus of the rat (Ohishi et al., 1993; Tanabe et al., 1993) from which glutamatergic striatal afferents originate.

It is possible that (1*S*,3*S*)-ACPD and L-AP4 act at the same receptor to inhibit glutamate release. To try and resolve this issue we used MAP4 which has been reported to antagonise presynaptic depression caused by L-AP4 in rat spinal cord (apparent K_D of 22 μM) but not affect the depression caused by (1*S*,3*S*)-ACPD (Jane et al., 1994). This differential activity of MAP4 suggests that L-AP4 and (1*S*,3*S*)-ACPD activate different receptors, at least in the spinal cord. However, no antagonism of L-AP4 or (1*S*,3*S*)-ACPD-induced reduction of glutamate release was seen in our study (Fig. 2a and b) and MAP4 alone inhibited glutamate release (Fig. 2c). This, firstly, indicates that L-AP4-sensitive receptors in the striatum are different from those in the spinal cord in terms of their sensitivity to MAP4 and, secondly, does not allow us to conclude whether (1*S*,3*S*)-ACPD and L-AP4 act at different receptors in the striatum to inhibit glutamate release. To date there are no reports on the affinity of MAP4 for cloned mGlu receptor subtypes but it is possible that this compound possesses agonist activity which may underlie its ability to inhibit glutamate release. Agonist activity of MAP4 has also been noted in rat forebrain slices where it reduced forskolin-stimulated cAMP production (Kemp et al., 1994a). Finally, the possibility also exists that the actions of L-AP4 and (1*S*,3*S*)-ACPD are mediated by as yet unknown mGlu receptors.

4.2. Consequences for striatal functioning

Application of L-AP4 can reduce evoked EPSPs in striatal slices without affecting postsynaptic responses to exogenous glutamate (Calabresi et al., 1993). Our findings suggest that reduction of glutamate release may underlie this presynaptic action of L-AP4. Similarly, activation of presynaptic mGlu receptors and reduced glutamate release may be responsible for high-frequency stimulation-induced depression of synaptic transmission observed in the striatum (Lovinger et al., 1993) although some forms of striatal synaptic depression seem to have a postsynaptic locus (Calabresi et al., 1993). A number of transmitters have been proposed to regulate glutamate release within the striatum including dopamine (Maura et al., 1988) and nitric oxide (Guevara-Guzman et al., 1994). Our results indicate that glutamate may also inhibit its own release via activation of mGlu receptors on terminals within the striatum. It remains to be established under what levels of stimulation presynaptic mGlu receptors are activated.

In certain disease states, e.g. Parkinson's disease, there is a functional overactivity of the corticostriatal pathway and in animal models it has been demonstrated that modulating postsynaptic ionotropic Glu receptors can produce anti-parkinsonian effects (Carroll et al., 1995). Our results suggest that activating presynaptic mGlu receptors may also prove beneficial in Parkinson's disease by reducing the overactivity of the corticostriatal pathway. Studies are ongoing to address this issue.

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