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Anticonvulsant activity of a $mGlu_{4\alpha}$ receptor selective agonist, (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid

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Abstract

The metabotropic Group III agonist, (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid (ACPT-1), selective for the $mGlu_{4\alpha}$ receptor, suppresses sound-induced seizures in DBA/2 mice following its intracerebroventricular (i.c.v.) administration (ED_{50} 5.6 [2.9–10.7], nmol i.c.v., 15 min, clonic phase) and in genetically epilepsy-prone (GEP) rats following focal administration into the inferior colliculus (ED_{50} 0.08 [0.01–0.50], nmol, 60 min, clonic phase). ACPT-1 also protects against clonic seizures induced in DBA/2 mice by the Group I agonist, (*RS*)-3,5-dihydroxyphenylglycine (3,5-DHPG) (ED_{50} 0.60 [0.29–1.2], nmol i.c.v.) and by the Group III antagonist, (*RS*)-α-methylserine-*O*-phosphate (MSOP) (ED_{50} 49.3 [37.9–64.1], nmol i.c.v.). Another Group III agonist, (*RS*)-4-phosphonophenylglycine (PPG), preferentially activating the $mGlu_8$ receptor, previously shown to protect against sound-induced seizures in DBA/2 mice and GEP rats, also protects against seizures induced in DBA/2 by 3,5-DHPG (ED_{50} 3.7 [2.4–5.7], nmol i.c.v.) and by the Group III antagonist, MSOP (ED_{50} 40.2 [21.0–77.0], nmol i.c.v.). At very high doses (500 nmol i.c.v. and above), Group III antagonists have pro-convulsant and convulsant activity. The anticonvulsant protection against sound-induced seizures in DBA/2 mice provided by a fully protective dose (20 nmol, i.c.v.) of the $mGlu_4$ receptor agonist ACPT-1, is partially reversed by the co-administration of the Group III antagonists, MSOP, (*RS*)-α-methyl-4-phosphonophenylglycine (MPPG) or (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4), in the 20–50 nmol dose range. At doses of 50–200 nmol, MPPG and MAP4 cause further reversal of the ACPT-1 anticonvulsant protection, while the MSOP effect on ACPT-1 protection is abolished at higher doses. In contrast, the anticonvulsant protection against sound-induced seizures in DBA/2 mice provided by a fully protective dose (20 nmol, i.c.v.) of the $mGlu_8$ receptor agonist PPG, is not significantly affected by the co-administration of the same Group III antagonists, MSOP, MPPG or MAP4. We conclude that activation of either $mGlu_{4\alpha}$ or $mGlu_8$ receptors confer anticonvulsant protection in DBA/2 mice. Furthermore, the metabotropic Group III receptor antagonists, MSOP, MPPG, and MAP4 appear to be functionally selective for the $mGlu_4$ receptor in this system.

Keywords: Anticonvulsant; Glutamate receptor, metabotropic; $mGlu_4$ receptor; $mGlu_8$ receptor; ACPT-1 ((1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid); PPG (*RS*)-4-phosphonophenyl-glycine); MSOP (*RS*)-α-methylserine-*O*-phosphate); DBA/2 mouse; GEP (genetically epilepsy-prone) rat

1. Introduction

Antagonists acting at Group III (and Group II) metabotropic receptors have previously been shown to suppress seizure activity in several animal epilepsy models (Tizzano et al., 1995; Abdul-Ghani et al., 1997; Tang et al., 1997; Chapman et al., 1999; Gasparini et al., 1999), but it is not established which of the Group III receptors ($mGlu_4$, $mGlu_6$, $mGlu_7$ or $mGlu_8$) are involved in seizure modulation.

It is well established that L-(+)-2-amino-4-phosphonobutyric acid (LAP4) acts presynaptically to block neurotransmission at some but not all glutamatergic pathways (Koerner and Cotman, 1981; Evans et al., 1982), and that LAP4 and the analogous endogenous compound L-serine-*O*-phosphate are broad-spectrum agonists at Group III glutamate metabotropic receptors, but lack action at other mGlu receptors (Okamoto et al., 1994; Pisani et al., 1997; Schoepp et al., 1999). Each of these Group III receptors has a distinctive pattern of expression on glutamatergic pathways in the brain (Conn and Pin, 1997; Saugstad, et al., 1997; Shigemoto et al., 1997; Cartmell and Schoepp, 2000). At certain pathways, there is evidence for a presynaptic action of Group III agonists on γ-amino-butyrate (GABA)ergic transmission, e.g. in ventrobasal thalamus, hippocampus, striatum and somatosensory cortex (Salt and Eaton, 1995; Salt and Turner, 1996), and the synaptic blockade of the lateral perforant path can be linked to

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action of LAP4 and L-serine-*O*-phosphate on $mGlu_8$ receptors.

Studies employing intracerebroventricular (i.c.v.) or focal intracerebral injection in rodents not only demonstrate anticonvulsant, but also some proconvulsant effects of Group III agonists in several epilepsy models, including sound-induced seizures in DBA/2 mice and genetically epilepsy-prone rats (GEP rats). Administration of L-serine-*O*-phosphate into the inferior colliculus of GEP rats produces an immediate excitatory effect followed by a delayed, very prolonged anticonvulsant effect (Tang et al., 1997).

Recently, metabotropic agonists have become available that are reasonably selective for individual receptors within Group III, such as (*R*,*S*)-4-phosphonophenylglycine (PPG) that has a moderate preferential action on $mGlu_8$ receptors (Gasparini et al., 1999), and (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid (ACPT-1) that is a potent, selective agonist for $mGlu_{4\alpha}$ receptors (Acher et al., 1997).

We have earlier shown that PPG has a more powerful and prolonged anticonvulsant action against sound-induced seizures in DBA/2 mice than L-serine-*O*-phosphate and LAP4 (Chapman et al., 1999).

In this study, we show that the $mGlu_{4\alpha}$ receptor agonist, ACPT-1, likewise has potent anticonvulsant activity against sound-induced seizures in rodents. Furthermore, in order to investigate the relative roles played by $mGlu_8$ and $mGlu_{4\alpha}$ receptors in controlling seizures, we have also used three Group III receptor antagonists, (*RS*)-α-methyl-serine-*O*-phosphate (MSOP), (*RS*)-α-methyl-4-phosphonophenylglycine (MPPG), and (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4) in combination with the agonists, PPG and ACPT-1, because there is evidence that these antagonists show some differential action with respect to the four receptors within Group III (Schoepp et al., 1999). We have also compared the anticonvulsant potencies of PPG and ACPT-1 against seizures induced by convulsant metabotropic ligands, (*RS*)-3,5-dihydroxyphenylglycine (3,5-DHPG) and MSOP.

2. Materials and methods

2.1. Test compounds

ACPT-1 ((1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid; MW 217.2), PPG ((*RS*)-4-phosphonophenylglycine; MW 231.2), 3,5-DHPG ((*RS*)-3,5-dihydroxyphenylglycine; MW 183.2), MSOP ((*RS*)-α-methyl-serine-*O*-phosphate; MW 199.1), MPPG ((*RS*)-α-methyl-4-phosphonophenylglycine; MW 245.2) and MAP4 ((*S*)-2-amino-2-methyl-4-phosphonobutanoic acid; MW 197.1) were purchased from Tocris Cookson (Bristol, UK). The compounds were all dissolved in distilled water for the DBA/2 mice experiments, and the final pH values of the resulting solutions were adjusted to approximately 7 using NaOH. For the GEP rats experiments, ACPT-1 was dissolved in phosphate-buffered saline (PBS), pH 7.

2.2. Sound-induced seizures in DBA/2 mice

All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

DBA/2 mice, male and female; age 3–4 weeks (Institute of Psychiatry colony or Harlan and Olac, Bichester, UK) were housed on a 12-h dark, 12-h light cycle and were allowed free access to food and water until used experimentally. Test compounds and vehicle were administered intracerebroventricularly (i.c.v.) (1 mm anterior to the bregma, 1 mm lateral to the midline, to a depth of 3 mm) during brief fluothane anaesthesia, using a Hamilton syringe and a 25-short gauge butterfly needle for delivering a 10-μl volume. Following the drug or vehicle injections, the mice were maintained at a body temperature of 36–38°C by applying heating lamps when required. Mice were observed for abnormal motor behaviour or proconvulsant effects of the drugs prior to testing for sound-induced seizures.

Anticonvulsant testing was carried out on individual mice under a perspex dome (58 cm in diameter) fitted with an electric doorbell at the apex generating a sound stimulus of 109 dB for a period of 60 s or until the onset of clonic convulsions. The sound stimulus produced a sequential seizure response, consisting of a wild running phase, latency 1–4 s, clonic seizures, latency 4–15 s, tonic extension, latency 10–30 s and occasionally respiratory arrest, latency 20–40s. The sound stimulus produced 100% wild running, 100% clonic seizures, and 80–100% tonic extensions in all the vehicle-treated control groups. This study only reports the drug effects on the clonic phase of the sound-induced seizures.

ACPT-1 and PPG were dissolved in distilled water, neutralised, and groups of mice ($n = 10$ per group) were injected with ACPT-1 (2–20 nmol i.c.v.) or PPG (0.5–20 nmol i.c.v.) 15 min before being tested for sound-induced seizure responses. Dose–response curves were constructed from the observed seizure incidence in four to five drug-treated groups. A time course of action of ACPT-1 was determined by testing groups of mice ($n = 10$ per group) for seizure response 15 min to 6 h after ACPT-1 (15 nmol i.c.v.) administration. For assessing possible reversal of the fully anticonvulsant effects of ACPT-1 or PPG by Group III antagonists, groups of DBA/2 mice ($n = 10$ per group) were co-injected i.c.v. with 20 nmol ACPT-1 or 20 nmol PPG mixed with 20–200 nmol of MPPG, MSOP or MAP4 in a total volume of 10 μl per mouse 15 min before they were tested for a sound-induced seizure response.

2.3. Sound-induced seizures in genetically epilepsy-prone (GEP) rats

Adult genetically epilepsy-prone (GEP) rats of either sex were selected from the Institute of Psychiatry colony.

The surgery, audiogenic stimulation and procedures for drug administration were as described by Tang et al. (1997). Briefly, animals under general anaesthesia (0.3 ml immobilon/kg, intramuscularly) were implanted bilaterally with stainless steel guide cannulae (gauge 21) directly above the inferior colliculus using stereotaxic coordinates. Dental acrylic was used to hold the implant in position. At the end of surgery, revivon (0.3 ml/kg, i.m.) was used to reverse the effect of immobilon. The animals were allowed a minimum of 5 days recovery before continuing with the experiment.

Animals implanted with guide cannulae were tested for sound-induced seizures by exposing them individually to a loud sound stimulus for 60 s or until the expression of clonic seizure. Animals which failed to express clonic–tonic seizure with hindlimb extension (seizure score 9, based on the seizure scoring system of Jobe et al., 1973) in all three consecutive stimulations were excluded from the experiment.

An hour after the last of the three stimulations, ACPT-1 (0.01–1 nmol/side) or PBS (control group) was injected into both inferior colliculi simultaneously at a rate of (0.2 μl/min) using a microinfusion pump via injection needles (gauge 27) each connected to a 10-μl Hamilton syringe with a polyethylene tube ($n = 4$–5 per group).

Each animal was observed for abnormal behaviours during and after injection. The animals were exposed to sound stimulation at the following time points after drug administration: 5, 30 min, 1, 2, 4 h, 1, 2, 3, 4 and 5 day.

At the end of the fifth day post-drug, the animals were deeply anaesthetized with pentobarbitone (200 mg/kg), injected with a blue dye using the same method as for drug administration, then decapitated and their brains removed. Coronal brain sections, 30-μm thick, were cut using a cryostat. Absence of blue dye in the inferior colliculi excluded data from that animal from data analysis.

2.4. Seizures induced by 3,5-DHPG administration

DBA/2 mice ($n = 10$ per group) received 1.5 μmol 3,5-DHPG i.c.v. in 10 μl volume under light fluothane anaesthesia at time zero and were observed for 80 min for incidence of clonic seizures. Mice injected with 1.5 μmol i.c.v. 3,5-DHPG alone responded with a 80–90% incidence of clonic seizures (latency to onset 20–65 min). Additional groups of mice ($n = 10$ per group) received a co-administration of 1.5 μmol 3,5-DHPG plus 0.5–5 nmol ACPT-1 or 3–20 nmol PPG in 10 μl volume i.c.v. at time zero and were observed for seizure activity during the subsequent 60–80 min.

2.5. Seizures induced by MSOP administration

Groups of DBA/2 mice ($n = 10$ per group) received 2.5 μmol MSOP i.c.v., alone, or in combination with ACPT-1 or PPG, in a 10-μl volume under light fluothane anaesthesia at time zero and were observed for 30 min for incidence of clonic seizures. In the control group receiving MSOP alone, there was a 100% seizure response. Additional groups received co-administration of MSOP (2.5 μmol, i.c.v.) with 30–70 nmol ACPT-1 or 20–55 nmol PPG and were observed for seizure activity for 30 min.

2.6. Rotarod performance

Drug-induced motor-impairment was assessed using a rotarod. Groups of mice ($n = 9$ per group) were trained prior to drug administration to remain for 2 min on a rotating wooden dowel (diameter = 28 mm, fitted with shallow grooves every 20° and rotating with a speed of 20 rpm. Following the administration of ACPT-1 (5–15 nmol, i.c.v., 15 min) or PPG (10–50 nmol, i.c.v., 15 min), their rotarod performance was again assessed for 2 min by recording the time spent on the rotarod before falling off. The mean rotarod performance for each group was expressed as percent of control and used for calculating an ED_{50} value for motor impairment.

2.7. Statistics

The EC_{50} values with lower and upper confidence values at 95% confidence limit for clonic seizures or rotarod performance were calculated from the dose–response data according to the method of Litchfield and Wilcoxon (1949).

3. Results

3.1. Behavioural effects of ACPT-1 and PPG administration in DBA/2 mice

Following the administration of doses up the 15 nmol ACPT-1 and 10 nmol PPG (i.c.v.) to DBA/2 mice, there were no overt effects on locomotion or exploration or general behaviour. Following the administration of 20 nmol ACPT-1 i.c.v., the mice showed moderate sedation, while 50 nmol ACPT-1 (i.c.v.) caused clonic seizures (approximately 10 min latency and 1 min duration) in 3/10 mice, followed by ataxia and sedation. At doses of 20–55 nmol PPG (i.c.v.), there was an increased level of excitability in the mice (excessive grooming and running) lasting 15–20 min, in agreement with the known proconvulsant activity of high doses (100–500 nmol i.c.v.) of PPG (Chapman et al., 1999).

3.2. Anticonvulsant effect of ACPT-1 and PPG administration on sound-induced seizures in DBA/2 mice, and its reversal by group III antagonists

Following the i.c.v. administration of 2–20 nmol ACPT-1 or 0.5–20 nmol PPG, the incidence of sound-in-

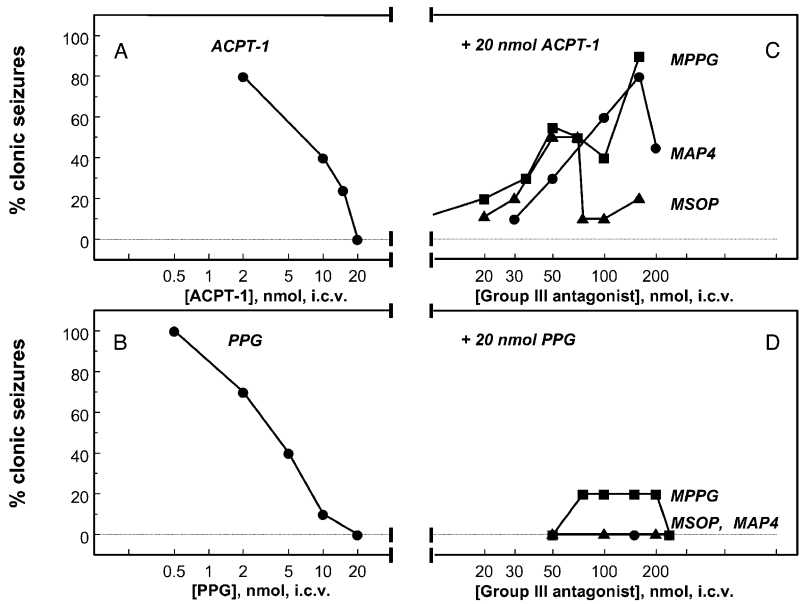

Fig. 1. Dose-dependent inhibition of sound-induced clonic seizures in DBA/2 mice by the $mGlu_{4\alpha}$ agonist, ACPT-1 (A) and the $mGlu_8$ receptor agonist, PPG (B), and the reversal of anticonvulsant protection observed at 20 nmol ACPT-1 by Group III antagonists: MPPG (solid squares), MAP4 (solid circles) or MSOP (solid triangles) (C), and the lack of reversal of the protection observed at 20 nmol PPG by the same Group III antagonists (D). $n = 10$ per group. The results are expressed as the percentage of DBA/2 mice per group responding to the sound-stimulus with clonic seizures. See Materials and methods for further experimental details.

duced clonic seizures in DBA/2 mice, when assessed 15 min after drug-administration, was reduced in a dose-dependent manner from a control value of 100% to complete suppression at 20 nmol of the two Group III agonists (Fig. 1A and B). The corresponding ED_{50} values (nmol i.c.v., 15 min) for the suppression of sound-induced clonic seizures by ACPT-1 and PPG were 5.6 [2.9–10.7] and 3.4 [2.1–5.6], respectively (Table 1).

Table 1
Anticonvulsant efficacies of two metabotropic Group III agonists, ACPT-1 and PPG, against seizures induced in DBA/2 mice (i.c.v.) and GEP rats (focal, IC)

	ACPT-1 ED_{50} (nmol)	PPG ED_{50} (nmol)
Sound-induced seizures, clonic phase, 15 min, DBA/2 mice	5.6 [2.9–10.7]	3.4[a] [2.1–5.6]
MSOP-induced seizures (2.5 μmol i.c.v., 0–30 min), DBA/2 mice	49.3 [37.9–64.1]	40.2 [21.0–77.0]
3,5-DHPG-induced seizures (1.5 μmol i.c.v., 0–60 min), DBA/2 mice	0.60 [0.29–1.2]	3.7 [2.4–5.7]
Impairment of rotarod performance, 15 min, DBA/2 mice	9.3 [5.8–14.8]	33.2 [22.1–49.9]
Sound-induced seizures, clonic phase, 60 min, GEP rats	0.08 [0.01–0.50]	5.4[a] [2.4–8.6]

[a] From Chapman et al. (1999).

When a fully anticonvulsant dose of 20 nmol ACPT-1 was co-administered i.c.v. to groups of DBA/2 mice along with 20–200 nmol doses of MPPG, MAP4 or MSOP, there was a dose-dependent reversal of the anticonvulsant effect of ACPT-1 by 20–160 nmol MPPG or MAP4, and by 20–70 nmol MSOP. At higher doses of MSOP (75–160 nmol i.c.v.), the ability to reverse the anticonvulsant effect of 20 nmol ACPT-1 was abolished (Fig. 1C).

When a fully anticonvulsant dose of 20 nmol PPG was co-administered i.c.v. to groups of DBA/2 mice along with 50–240 nmol doses of MPPG, MAP4 or MSOP, the anticonvulsant activity of PPG was not affected in any significant manner (Fig. 1D).

Fig. 2 shows the duration of the anticonvulsant protection against sound-induced seizures in DBA/2 mice observed after the i.c.v. administration of 15 nmol ACPT-1. The protection was fully established by 15 min and lasted for 2 h. By 4 and 6 h after ACPT-1 administration, the seizure response had been restored to normal.

3.3. Effect of ACPT-1 and PPG on seizures induced by 3,5-DHPG

ACPT-1 (0.5–5 nmol co-injected i.c.v. with 1.5 μmol 3,5-DHPG) and PPG (3–20 nmol co-injected i.c.v. with 1.5 μmol 3,5-DHPG) reduced the incidence of 3,5-DHPG-induced seizures in a dose-dependent manner from control values of 80–90% clonic seizures to complete suppression (Fig. 3A). The corresponding ED_{50} values (Table 1) for the suppression of 3,5-DHPG-induced clonic

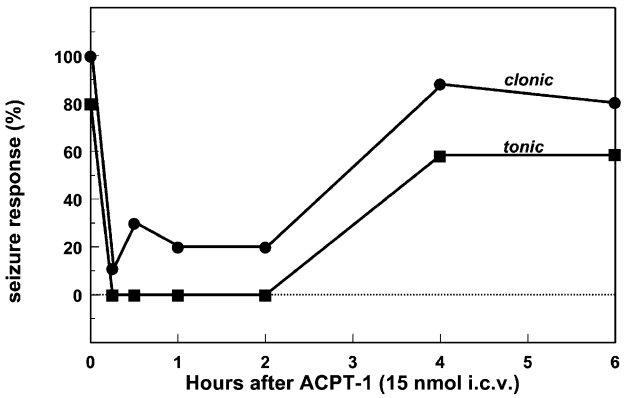

Fig. 2. The time-course of anticonvulsant action of ACPT-1 in DBA/2 mice. Sound-induced clonic ((solid circles) and tonic (solid squares) in groups ($n = 10$ per group) of DBA/2 mice following the i.c.v. administration of 15 nmol ACPT-1. The results are expressed as the percentage of DBA/2 mice per group responding to the sound-stimulus with clonic seizures.

seizures by ACPT-1 and PPG were 0.60 [0.29–1.2] (nmol i.c.v.) and 3.7 [2.4–5.7] (nmol i.c.v.), respectively.

3.4. Effect of ACPT-1 and PPG on seizures induced by MSOP

MSOP (2.5 μmol, i.c.v.) caused clonic seizures in DBA/2 mice within 5–10 s of administration. The seizures were of an average of 184 s (70–325 s) duration. ACPT-1 (30–70 nmol co-injected i.c.v. with 2.5 μmol MSOP) and PPG (20–55 nmol co-injected i.c.v. with 2.5 μmol MSOP) reduced the incidence of MSOP-induced seizures in a dose-dependent manner from control values of 100% clonic seizures to 20% and 40%, respectively, at the highest doses of ACPT-1 and PPG tested (Fig. 3B). The latency to, and duration of, the MSOP-induced seizures were not significantly affected by ACPT-1 and PPG. The corresponding ED_{50} values (Table 1) for the suppression of MSOP-induced clonic seizures by ACPT-1 and PPG were 49.3 [37.9–64.1] (nmol i.c.v.) and 40.2 [21.0–77.0] (nmol i.c.v.), respectively.

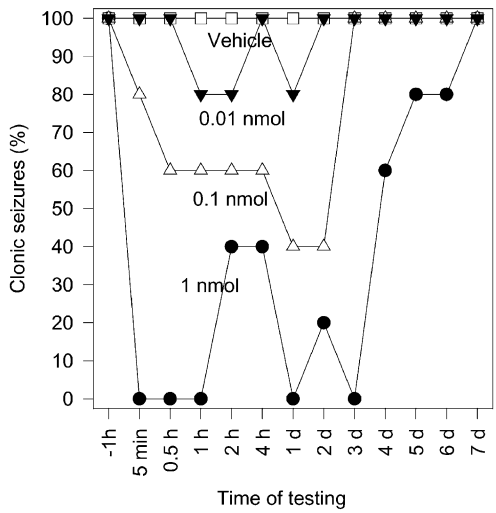

Fig. 4. Protection against sound-induced clonic seizures in GEP rats following bilateral injection of ACPT-1 into the inferior colliculus. Groups ($n = 4$–5 per group) of fully responding (see Materials and methods for details) GEP rats were tested for their control sound-induced seizure response at −1 h; at time zero, they received a focal microinjection of PBS vehicle (open squares), or ACPT-1: 0.01 nmol/side (solid inverted triangles), 0.1 nmol/side (open triangles), and 1 nmol/side (solid circles). The rats were subsequently exposed to a sound-stimulus at 5, 30 min, 1, 2, 4 h, 1, 2, 3, 4, 5, 6 and, 7 day after drug administration. The results are expressed as the percentage of GEP rats per group responding to the sound-stimulus with clonic seizures.

3.5. Effect of ACPT-1 and PPG on rotarod performance

Following i.c.v. administration of ACPT-1 or PPG groups of DBA/2 mice ($n = 9$ per group) showed an impaired rotarod performance when assessed 15-min post injection. Average time spent on the rotarod following doses of 5, 10 and 15 nmol ACPT-1 represents 74%, 49% and 29%, respectively, of control performance (120 s), resulting in an ED_{50} value (Table 1) of 9.3 [5.8–14.8] (nmol i.c.v., 15 min) for the impairment of motor performance.

Average time spent on the rotarod following doses of 10, 20, 30 and 50 nmol PPG represents 90%, 81%, 62 and

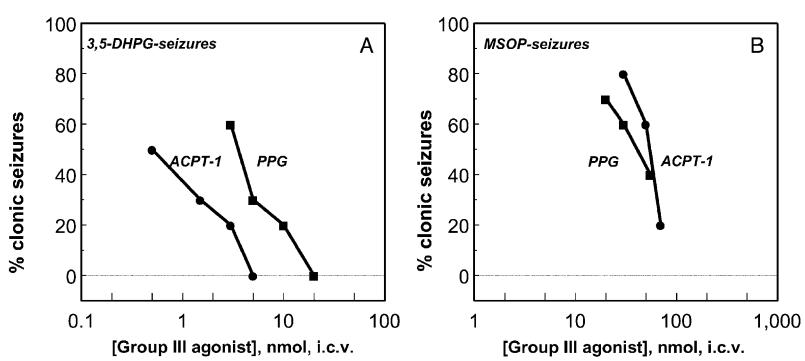

Fig. 3. Dose-dependent suppression of clonic seizures induced by 3,5-DHPG or MSOP, by ACPT-1 and PPG. Seizures were induced in DBA/2 mice by the i.c.v. administration of 1.5 μmol 3,5-DHPG (A), or 2.5 μmol MSOP (B). ACPT-1 (solid circles) and PPG (solid squares), at doses indicated, were co-administered i.c.v. with the convulsants to groups ($n = 10$) of DBA/2 mice. The results are expressed as the percentage of mice per group exhibiting clonic seizures.

Table 2
Anticonvulsant efficacy of ACPT-1 against sound-induced seizures in GEP rats (wild running, clonic and tonic phases) following focal inferior colliculus injections

Time of testing	ED_{50} (nmol, IC)		
	Wild running	Clonic	Tonic
5 min	nd	nd	nd
30 min	nd	nd	nd
1 h	0.08 (0.01–0.5)	0.08 (0.01–0.5)	0.03 (0.004–0.18)
2 h	0.33 (0.016–6.5)	0.33 (0.016–6.5)	0.05 (0.0001–30)
4 h	nd	nd	nd
1 d	0.03 (0.01–0.05)	0.03 (0.01–0.05)	0.03 (0.01–0.05)
2 d	nd	nd	0.08 (0.01–0.6)

IC: inferior colliculus.
nd: unable to determine ED_{50} values by method of Litchfield and Wilcoxon (1949), either due to lack of significant anticonvulsant effect or due to insufficient data points in the appropriate dose range.

23%, respectively, of control performance (120 s), resulting in an ED_{50} value (Table 1) of 33.2 [22.1–49.9 (nmol i.c.v., 15 min).

3.6. Effect of ACPT-1 on sound-induced seizures in GEP rats

Focal administration of ACPT-1 (0.01–1 nmol/side) into the inferior colliculus of GEP rats produced a dose-dependent abolition of sound-induced clonic seizures (Fig. 4). The maximum dose of ACPT-1 (1 nmol/side) produced a biphasic temporal anticonvulsant effect (with an immediate effect at 5–60 min, and a delayed effect at 1–3 day) against sound-induced clonic seizures. The ED_{50} values (nmol/side with 95% confidence limits) against sound-induced clonic seizures were calculated only at 1, 2 h and 1 day (0.08 (0.01–0.5), 0.33 (0.016–6.5) and 0.03 (0.01– 0.05)), respectively). The rest of the ED_{50} values for the anticonvulsant effects of ACPT-1 are shown in Table 2.

No abnormal behavioural activity was observed at any of the doses of ACPT-1 administered into the inferior colliculus of GEP rats.

4. Discussion

We and others have previously shown that Group III metabotropic agonists can have anticonvulsant actions in rodent models of epilepsy (Ghauri et al., 1996; Abdul-Ghani et al., 1997; Tang et al., 1997; Chapman et al., 1999). We now report the novel finding that an agonist acting selectively on $mGlu_4$ receptors is anticonvulsant in mice and rats. $mGlu_4$ receptors are expressed presynaptically on a subset of glutamatergic pathways, where they decrease excitatory transmission (Shigemoto et al., 1997; Cartmell and Schoepp, 2000). Their overall pathophysiological role in epilepsy is as yet not understood. Recently, a striking up-regulation of $mGlu_4$ receptors has been described in hippocampal neurons resected from temporal lobe epilepsy patients with drug-refractory seizures. (Lie et al., 2000). Our data would suggest that this receptor up-regulation may be part of a compensatory or protective process. It has also been previously shown that Group III antagonists such as MSOP, MAP4 and MPPG are convulsant when given at high doses to rodents Ghauri et al., 1996). This suggests that these receptors are normally playing a role in preventing seizures, but says nothing about the specific involvement of $mGlu_4$ receptors in this process.

Studies employing cloned human or rat mGlu receptors show that MSOP, MAP4 and MPPG are all moderately potent antagonists at $mGlu_{4\alpha}$ receptors (Schoepp et al., 1999). Reversal of the anticonvulsant effect of ACPT-1 by these three antagonists is thus readily explained by their blockade of $mGlu_4$ receptor activation over the dose range tested. The lack of effect of these three Group III antagonists over the same dose range against the anticonvulsant effect of PPG is surprising, it may reflect some mismatch in the spectrum of activity of PPG and of the three antagonists with regard to the Group III receptors. ACPT-1 and PPG are however equipotent against MSOP seizures, suggesting that such seizures are dependent on blockade of both $mGlu_4$ and $mGlu_8$ receptors. That MSOP blocks $mGlu_8$ receptor activation is supported by its reversal of LAP4 block of lateral perforant path transmission (Bushell et al., 1996), a site where $mGlu_8$ receptor is uniquely expressed (Shigemoto et al., 1997).

We do not know which glutamatergic pathways are involved in the anticonvulsant effect of ACPT-1 and PPG when these are given i.c.v. in DBA/2 mice. The two compounds are equipotent at suppressing sound-induced seizures in DBA/2 mice, yet ACPT-1 is around sevenfold more potent than PPG at suppressing sound-induced seizures following injection in the inferior colliculus in GEP rats. This suggests that $mGlu_4$ receptors play an important role in the inferior colliculus of the GEP rat. In the absence of experiments with intra-collicular injections in the DBA/2 mice, we cannot say if the rat and mouse differ in this respect.

Earlier experiments with focal intracollicular injections of L-serine-*O*-phosphate showed an early (1–4 h) and a late (1–3 days) phase of anticonvulsant activity; a pattern reproduced by ACPT-1 in the current study (except that the early phase has an earlier onset with ACPT-1, perhaps because the situation is complicated by an early proconvulsant action with L-serine-*O*-phosphate).

We have suggested elsewhere that the early anticonvulsant phase is a direct drug effect on mGlu receptors, but that the delayed phase is indirect and does not depend on the continuing presence of the agonist (Ping et al., submitted). Instead, it appears to depend on protein synthesis and an up-regulation of mGlu receptors, as shown by mRNA ($mGlu_4$ and $mGlu_7$) and protein ($mGlu_7$) changes at 2

days after L-serine-*O*-phosphate injection. It would be interesting to know if such receptor changes occur after ACPT-1 and are confined to $mGlu_4$ receptors.

Both ACPT-1 and PPG differ from L-serine-*O*-phosphate in the relative absence of an early proconvulsant effect. L-serine-*O*-phosphate when given i.c.v. in DBA/2 mice or intracollicularly in the GEP rats produces an early excitant, proconvulsant phase at 0–30 min (at doses similar to those required for anticonvulsant action). In contrast with ACPT-1 and PPG, excitatory effects were much weaker and seen at doses much higher than the anticonvulsant dose. This may reflect the different spectrum of activity on the group III receptors or it may reflect additional sites of action. In particular, agonist effects of LAP4 (and by analogy probably of L-serine-*O*-phosphate) on NMDA receptors have been described (Contractor et al., 1998).

In terms of identifying the most appropriate molecular target for future antiepileptic drugs, the present experiments are not conclusive. The therapeutic index, as assessed by comparing the anticonvulsant ED_{50} in the DBA/2 mice with motor impairment (as measured by the ED_{50} for the rotarod test) suggests that the $mGlu_4$ receptor may not be the best target, since the therapeutic index for ACPT-1 is around 2, whereas that for PPG the therapeutic index is around 10. The therapeutic index may however vary with route of drug administration, species and type of epilepsy.

The further identification of roles for specific mGlu receptors in the pathophysiology will undoubtedly aid the search for novel therapies. In particular, approaches concerned with mechanisms producing long-term changes in the expression or function of these receptors may identify totally novel strategies for preventing or reversing epileptogenesis.

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