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Group III mGlu receptor agonists potentiate the anticonvulsant effect of AMPA and NMDA receptor block

Giovambattista De Sarro [a], Alba Chimirri [b], Brian S. Meldrum [c,*]

[a] *Cattedra di Farmacologia, Dipartimento di Medicina Sperimentale e Clinica, Università di Catanzaro, Catanzaro, Italy*
[b] *Dipartimento Farmaco Chimico, University of Messina, Messina, Italy*
[c] *GKT School of Biomedical Sciences, Henriette Raphael House, Guy's Campus, London SE1 1UL, UK*

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Abstract

We report the anticonvulsant action in DBA/2 mice of two mGlu Group III receptor agonists: (*R*,*S*)-4-phosphonophenylglycine, (*R*,*S*)-PPG, a compound with moderate mGlu8 selectivity, and of (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid, ACPT-1, a selective agonist for mGlu4α receptors. Both compounds, given intracerebroventricularly at doses which did not show marked anticonvulsant activity, produced a consistent shift to the left of the dose–response curves (i.e. enhanced the anticonvulsant properties) of 1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one hydrochloride, CFM-2, a noncompetitive AMPA receptor antagonist, and 3-((±)-2-carboxypiperazin-4-yl)-1-phosphonic acid, CPPene, a competitive NMDA receptor antagonist, in DBA/2 mice. In addition, (*R*,*S*)-PPG and ACPT-1 administered intracerebroventricularly prolonged the time course of the anticonvulsant properties of CFM-2 (33 μmol/kg, i.p.) and CPPene (3.3 μmol/kg, i.p.) administered intraperitoneally. We conclude that modest reduction of synaptic glutamate release by activation of Group III metabotropic receptors potentiates the anticonvulsant effect of AMPA and NMDA receptor blockade.

Keywords: Epilepsy; mGlu receptor; NMDA receptor antagonist; AMPA receptor antagonist; Antiepileptic drug

1. Introduction

The Group III glutamate metabotropic receptors mGlu4, mGlu7 and mGlu 8 are expressed presynaptically at various glutamatergic and γ-aminobutyric acid-ergic (GABAergic) pathways in the brain (Shigemoto et al., 1996, 1997) where they serve to modulate neurotransmitter release (Conn and Pin, 1997; Cartmell and Schoepp, 2000; Salt and Eaton, 1995). Drugs that act as agonists at these sites show anticonvulsant actions when tested in mice (Tizzano et al., 1995; Ghauri et al., 1996), but also some excitatory and proconvulsant effects (Ghauri et al., 1996). The Group III receptor agonist drugs studied initially L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) and L-serine-*O*-phosphate (L-SOP), although highly selective agonists for Group III vs. Groups I and II metabotropic receptors, may also have some agonist action at NMDA receptors (Contractor et al., 1998). Novel structures that are Group III agonists with some possible preferential action among the Group III receptors have been described, namely (*R*,*S*)-4-phosphonophenylglycine, (*R*,*S*)-PPG, acting potently on mGlu8 (Gasparini et al., 1999) and (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid, ACPT-1, acting preferentially on mGlu4α (Acher et al., 1997). Intracerebroventricular administration of PPG or ACPT-1 produces anticonvulsant effects in a range of mouse models with little or no evidence of an excitatory action (Gasparini et al., 1999; Chapman et al., 1999, 2001). The anticonvulsant effect of ACPT-1 can be reversed by the co-injection of the Group III antagonists, (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid and (*R*,*S*)-α-methylserine-phosphate (Chapman et al., 2001).

In a recent study of the actions of the stereoisomers of (*R*,*S*)-3,4-dicarboxyphenylglycine, we found that (*S*)-3,4-DCPG, which is an mGlu8 receptor agonist, and (*R*)-3,4-DCPG, which is an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist, had much greater anticonvulsant potency in combination than when given singly. We used (*R*,*S*)-PPG in combination with (*R*)-3,4-DCPG to confirm that combining an AMPA receptor antagonist and an mGlu8 receptor agonist poten-

* Corresponding author. Tel.: +44-207-848-6420.
E-mail address: Brian.meldrum@kcl.ac.uk (B.S. Meldrum).

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tiates the anticonvulsant effect of both drugs. In this study, we use (*R*,*S*)-PPG and ACPT-1 to evaluate whether this potentiation is specific for AMPA receptors and mGlu8 receptor agonists, by combining them with an AMPA and with an *N*-methyl-D-aspartate (NMDA) receptor antagonist. We have selected a noncompetitive AMPA receptor antagonist, CFM-2 1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (Chimirri et al., 1997), as hydrochloride and a competitive NMDA antagonist, CPPene (*E*)-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (Herrling et al., 1997).

2. Materials and methods

2.1. Test compounds

PPG ((*R*,*S*)-4-phosphonophenylglycine; MW 231.2) and ACPT-1 ((1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid; MW 217.2) were purchased from Tocris Cookson (Bristol, U.K.). 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. CFM-2 [1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one hydrochloride; MW 345.07] was synthesized by Prof. Alba Chimirri (Dipartimento Farmaco Chimico, University of Messina). CPPene (3-((±)-2-carboxypiperazin-4-yl)-1-phosphonic acid; MW 268.2) was kindly supplied by Dr. P.L. Herrling (Novartis, Berne, Switzerland). Both compounds were dissolved in sterile saline and given i.p. (0.1 ml/10 g of body weight of the mouse) as freshly prepared solution.

2.2. Sound-induced seizures in DBA/2 mice

All animal experiments were carried out in accordance with international and national laws and policies (European Communities Council Directive of 24th November 1986, 86/609EEC).

DBA/2 mice, male and female, aged 22–26 days, 8–12 g, purchased from Harlan Italy (Correzzana, Milano, Italy), were used. They were housed on a 12-h dark/light cycle and were allowed free access to food and water until used experimentally. Test Group III mGlu receptor agonists 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 ether anaesthesia, using a Hamilton syringe and a 25-short-gauge butterfly needle for delivering a 10-μl volume as previously described (De Sarro et al., 1988) whilst CFM-2 or CPPene were administered i.p. Following the different treatments, the mice were maintained at a body temperature of 36–37 °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, 12–16 kHz, 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 20–40 s. The sound stimulus produced 100% wild running, 100% clonic seizures and 80–100% tonic extensions in all the vehicle-treated control groups.

PPG or ACPT-1 were dissolved in distilled water, neutralised and groups of mice ($N = 10$ per group) were injected with PPG (0.5–20 nmol i.c.v.) or ACPT-1 (1–20 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. For assessing possible influences upon anticonvulsant effects of CFM-2 or CPPene injected i.p., (*R*,*S*)-PPG or ACPT-1 were co-injected in groups of DBA/2 mice ($N = 10$ per group) In particular, several doses of CFM-2 or CPPene given i.p. and vehicle, 1 or 2 nmol of PPG or 2 or 4 nmol of ACPT-1 injected in a volume of 10 μl/mouse 30 or 45 min before they were tested for a sound-induced seizure response.

The different routes of administration of CFM-2 or CPPene and Group III mGlu receptor agonists ((*R*,*S*)-PPG and ACPT-1) were used because (*R*,*S*)-PPG and ACPT-1 do not cross the blood–brain barrier and in order to exclude possible pharmacometabolic or pharmacokinetic interactions. In addition, CFM-2 or CPPene plus vehicle (*R*,*S*)-PPG or ACPT-1 were co-injected in various groups of DBA/2 mice ($N = 10$ per group) at various times before they were tested for a sound-induced seizure response in order to evaluate the time course of anticonvulsant action.

2.3. Effects on motor movements

Various doses of CFM-2 or of CPPene alone were administered with vehicle at dose levels up to 100 or 20 μmol/kg, i.p., respectively. When mice were submitted to the combined treatment of CFM-2 + ACPT-1 or PPG or CPPene + ACPT-1 or PPG, the dose of ACPT-1 used were of 2 or 4 nmol/mouse and those of PPG were of 1 or 2 nmol/mouse. Behavioural changes and their onset and duration after the administration of drugs until the time of rotarod test were recorded. In particular, two independent observers followed gross behavioural changes consisting of locomotor activity, ataxia, squatting posture and possible piloerection. These behavioural changes were noted but not statistically analysed. In addition, impaired motor function was evaluated by the rotarod test of Dunham and Miya (1957). Groups of 10 DBA/2 mice, 8–12 g, 22–26 days old, were trained to do coordinated motor movements continuously for 2 min on a rotarod 3-cm diameter 8 rev min^{-1} (U. Basile, Comerio, Varese, Italy). Impairment of coordinated

motor movements was defined as inability of the mice to remain on the rotarod for a 2-min test period. The ability of the mice to remain on the rotarod was tested 30 min after the first i.c.v. administration of ACPT-1 or PPG + vehicle, or after the combined treatment with ACPT-1 or PPG + CFM-2 and + ACPT-1 or PPG + CPPene.

2.4. Statistical analysis

Significant differences between groups of combined treatments of (vehicle + CFM-2 and (*R*,*S*)-PPG + CFM-2 or ACPT-1 + CFM-2 or vehicle + CPPene and (*R*,*S*)-PPG + CPPene or ACPT-1 + CPPene) were estimated using Fisher's exact probability test (incidence of seizures phases) or analysis of variance (ANOVA) and Dunnett's *t*-test (rectal temperature). Values of $P < 0.05$ were considered as statistically significant. The percentage of mice showing 50% of clonic or tonic phase ED_{50} values (± 95% confidence limits) were calculated using a computer program of the method of Litchfield and Wilcoxon (1949) test. The relative anticonvulsant activities were determined by comparison of respective ED_{50} values (+95% confidence limits). TD_{50} values (+95% confidence limits) for each compound were estimated using the method of Litchfield and Wilcoxon (1949).

3. Results

3.1. Effects of (R,S)-PPG on the protective activity of CFM-2 against audiogenic seizures in DBA/2 mice

CFM-2 (1–50 μmol/kg, i.p.) produced a dose-dependent significant protection ($P < 0.05$) against tonic and clonic phases of the audiogenic seizures in DBA/2 mice 30 min after administration (Fig. 1A and B). Low doses of (*R*,*S*)-PPG (1 or 2 nmol/mouse) administered i.c.v. 15 min before auditory stimulation did not significantly reduce the incidence and the severity of audiogenic seizures. However, when (*R*,*S*)-PPG was administered simultaneously with CFM-2, it produced a consistent shift to the left of the dose–response curves (Fig. 1A and B) and a marked reduction of the ED_{50} values against the clonic and tonic phases of the audiogenic seizures (Table 1). In addition, the concomitant administration of (*R*,*S*)-PPG (1 or 2 nmol/mouse) and CFM-2 (33 μmol/kg, i.p.) resulted in reduction of seizure score (Fig. 2A). In particular, following the i.p. administration of saline and CFM-2 (33 μmol/kg, i.p.), maximum protection was

Table 1
Influence of (*R*,*S*)-PPG and ACPT-1 (Group III mGlu agonists) on the anticonvulsant activity of CFM-2 (AMPA antagonist) against audiogenic seizures in DBA/2 mice

Treatment	ED_{50} values	
	Clonus	Tonus
(*R*,*S*)-PPG + saline	3.46 (2.12–5.64)	1.32 (0.67–2.62)
ACPT-1 + saline	5.66 (3.40–9.41)	4.08 (2.51–6.62)
CFM-2 + saline	14.78 (9.59–22.78)	11.71 (7.56–18.12)
CFM-2+(*R*,*S*)-PPG (1)	9.57 (5.91–15.51)[a]	6.1 (4.61–8.10)[a]
CFM-2+(*R*,*S*)-PPG (2)	7.83 (5.00–12.25)[b]	4.72 (3.17–7.03)[b]
CFM-2 + ACPT (2)	11.47 (7.28–18.07)	10.15 (6.65–15.49)
CFM-2 + ACPT (4)	6.99 (4.23–11.25)[b]	6.11 (4.02–9.28)[b]

The data are the dose (μmol/kg, body weight) required for half-maximum effect ED_{50} value (± 95% confidence limits).
[a] $P < 0.05$ vs. respective control group (CFM-2 + saline).
[b] $P < 0.01$ vs. respective control group (CFM-2 + saline).

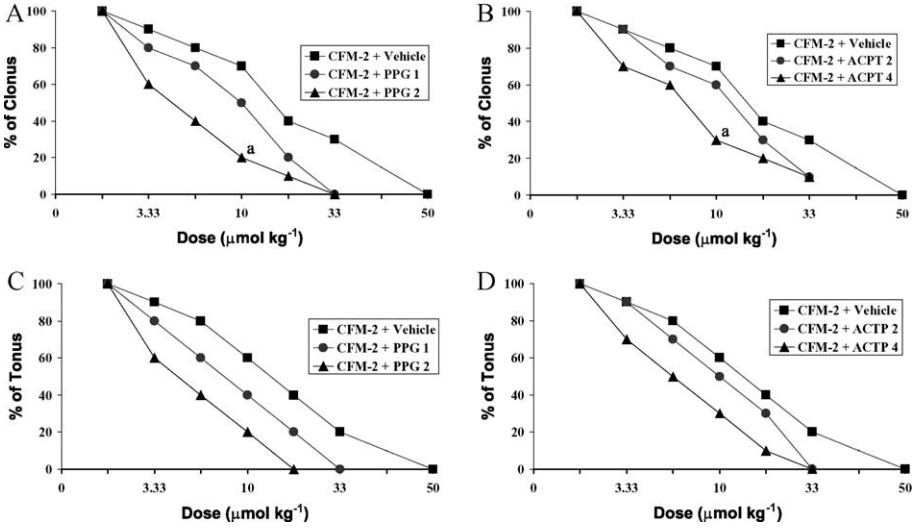

Fig. 1. Influence of (*R*,*S*)-PPG (1 or 2 nmol/mouse) and of ACPT-1(2 or 4 nmol/mouse) on the dose–response curves of the anticonvulsant effect of CFM-2 in DBA/2 mice. Abscissae show the doses; ordinates show (A and B) % of clonic seizures, (C and D) % of tonic seizures. For the determination of each point, 10 animals were used. Significant differences were not observed between CFM-2 + vehicle and CFM-2 + ACPT-1; a significant difference (a = $P < 0.05$) between CFM-2 + vehicle and CFM-2 + PPG2 was found at one dose.

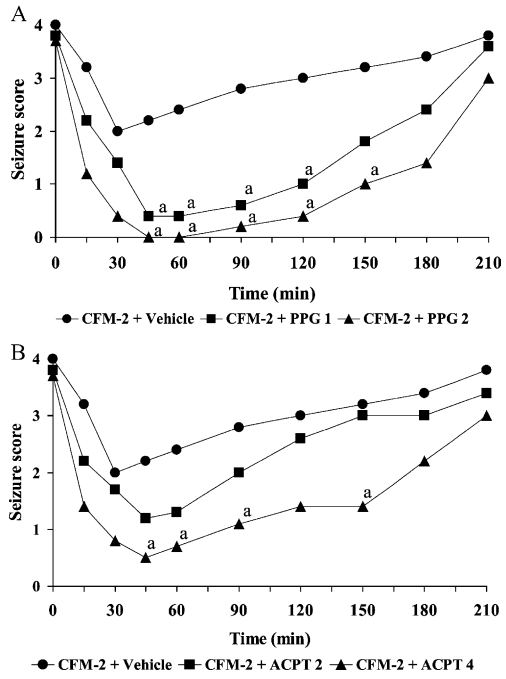

Fig. 2. Influence of (*R*,*S*)-PPG (1 or 2 nmol/mouse) (A) and ACPT-1 (2 or 4 nmol mouse) (B) on the time course of the anticonvulsant effect of CFM-2 (33 μmol/kg, i.p.) in DBA/2 mice. Abscissae show the time after intraperitoneal administration of CFM-2 and (*R*,*S*)-PPG or ACPT-1 in min; ordinates show the seizure score. For the determination of each point, 10 animals were used. Significant differences between CFM-2 + vehicle and CFM-2 + ACPT-1 or PPG are indicated (a = $P < 0.05$).

observed at 30–60 min, with subsequent return to control seizure response at 180 min (Fig. 2A). When DBA/2 mice were pretreated, 15 min before, with (*R*,*S*)-PPG (1 or 2 nmol/mouse) and then received CFM-2 (33 μmol/kg, i.p.), the maximum protection was observed at 15–120 min, with subsequent return to control seizure response at 210 min (Fig. 2A).

3.2. Effects of ACPT-1 on the protective activity of CFM-2 against audiogenic seizures in DBA/2 mice

CFM-2 (1–50 μmol/kg, i.p.) produced a dose-dependent significant protection ($P < 0.05$) against tonic and clonic audiogenic seizures in DBA/2 mice 30 min after administration (Fig. 1C and D). Low doses of ACPT-1 (2 or 4 nmol/mouse) administered i.c.v. 15 min before auditory stimulation did not significantly reduce the incidence and the severity of audiogenic seizures. However, when ACPT-1 was administered simultaneously with CFM-2, it produced a consistent shift to the left of the dose–response curves (Fig. 1C and D) and a marked reduction of the ED_{50} values against the clonic and tonic phases of the audiogenic seizures (Table 2). In addition, the concomitant administration of ACPT-1 (2 or 4 nmol/mouse) and CFM-2 (33 μmol/kg, i.p.) resulted in reduction of seizure score (Fig. 2B). Following the i.p. administration of saline and CFM-2 (33 μmol/kg, i.p.), maximum protection was observed at 30–60 min, with subsequent return to control seizure response at 180 min (Fig. 2B). When DBA/2 mice were pretreated, 15 min before, with ACPT-1 (2 or 4 nmol/mouse) and then received CFM-2 (33 μmol/kg, i.p.), the maximum protection was observed at 15–90 min for ACPT-1 (2 nmol/mouse) or at 15–150 min for ACPT-1 (4 nmol/mouse) with return to control seizure response at 210 min (Fig. 2B).

3.3. Effects of (R,S)-PPG on the protective activity of CPPene against audiogenic seizures in DBA/2 mice

CPPene (0.1–10 μmol/kg, i.p.) produced a dose-dependent significant protection ($P < 0.05$) against tonic and clonic phases of the audiogenic seizures in DBA/2 mice 45 min after administration (Fig. 3A and B). Low doses of (*R*,*S*)-PPG (1 or 2 nmol/mouse) did not significantly reduce the incidence and the severity of audiogenic seizures when administered i.c.v. 15 min before auditory stimulation. However, when it was administered simultaneously with CPPene it produced a consistent shift to the left of the dose–response curves (Fig. 3A and B) and a marked reduction of the ED_{50} values against the clonic and tonic phases of the audiogenic seizures (Table 3). In addition, the concomitant administration of (*R*,*S*)-PPG (1 or 2 nmol/mouse) and CFM-2 (3.3 μmol/kg, i.p.) resulted in reduction of seizure score (Fig. 4A). Following the i.p. administration of saline and CPPene (3.3 μmol/kg, i.p.), maximum protection was observed at 45–180 min, with subsequent return to control seizure response at 300 min (Fig. 4A). When DBA/2 mice were pretreated, 15 min before, with (*R*,*S*)-PPG (1 or 2 nmol/mouse) and then received CPPene (3.3 μmol/kg, i.p.), the maximum protection was observed at 45–240 min, with return to control seizure response at 420 min (Fig. 4A).

Table 2
Influence of (*R*,*S*)-PPG and ACPT-1 (Group III mGlu agonists) on the anticonvulsant activity of CPPene (NMDA antagonist) against audiogenic seizures in DBA/2 mice

Treatment	ED_{50} values	
	Clonus	Tonus
(*R*,*S*)-PPG + saline	3.46 (2.12–5.64)	1.32 (0.67–2.62)
ACPT-1 + saline	5.66 (3.40–9.41)	4.08 (2.51–6.62)
CPPene + saline	1.76 (1.21–2.26)	0.79 (0.44–1.43)
CPPene+(*R*,*S*)-PPG (1)	0.83 (0.50–1.38)[a]	0.49 (0.31–0.79)
CPPene+(*R*,*S*)-PPG (2)	0.43 (0.25–0.73)[b]	0.33 (0.19–0.57)[b]
CPPene + ACPT-1 (2)	1.19 (0.77–1.95)	0.65 (0.39–1.08)
CPPene + ACPT-1 (4)	0.63 (0.37–1.09)[b]	0.43 (0.25–0.73)[b]

The data are the dose (μmol/kg body weight) required for half-maximum effect ED_{50} value (± 95% confidence limits).
[a] $P < 0.05$ vs. respective control group (CPPene + saline).
[b] $P < 0.01$ vs. respective control group (CPPene + saline).

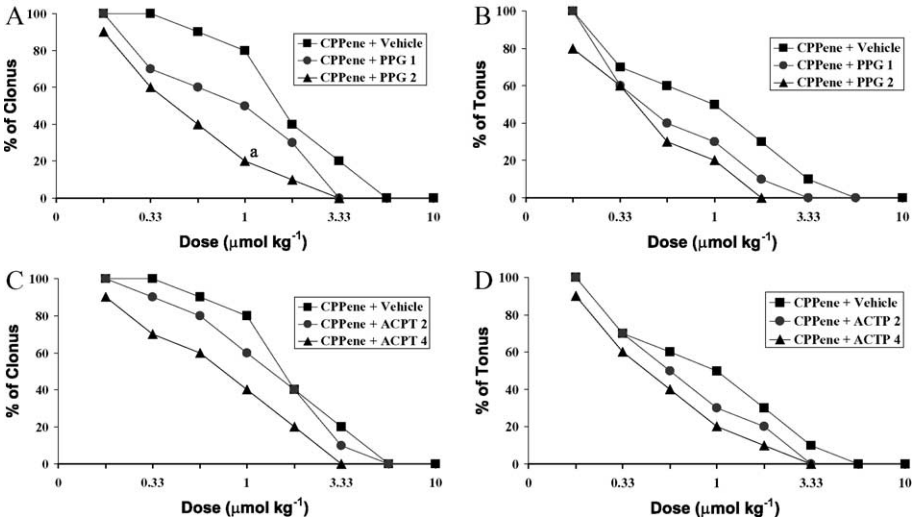

Fig. 3. Influence of (*R*,*S*)-PPG (1 or 2 nmol/mouse) and of ACPT-1 (2 or 4 nmol/mouse) on the dose–response curves of the anticonvulsant effect of CPPene in DBA/2 mice. Abscissae show the doses; ordinates show (A and C) % of clonic seizures, (B and D) % of tonic seizures. For the determination of each point, 10 animals were used. Significant difference between CPPene + vehicle and CPPene + PPG is indicated (a = $P < 0.05$).

3.4. Effects of ACPT-1 on the protective activity of CPPene against audiogenic seizures in DBA/2 mice

CPPene (0.1–10 μmol/kg, i.p.) produced a dose-dependent significant protection ($P < 0.05$) against tonic and clonic phases of the audiogenic seizures in DBA/2 mice 45 min after administration (Fig. 3C and D). Low doses of ACPT-1 (2 or 4 nmol/mouse) administered i.c.v. 15 min before auditory stimulation did not significantly reduce the incidence and the severity of audiogenic seizures. However, when ACPT-1 was administered simultaneously with CPPene, it produced a consistent shift to the left of the dose–response curves (Fig. 3C and D) and a marked reduction of the ED_{50} values against the clonic and tonic phases of the audiogenic seizures. In addition, the concomitant administration of ACPT-1 (2 or 4 nmol/mouse) and CPPene (3.3 μmol/kg, i.p.) resulted in reduction of seizure

Table 3
TD_{50} values (± 95% confidence limits) of vehicle + CFM-2 or CPPene and CFM-2 + ACPT-1 or PPG or CPPene + ACPT-1 or PPG obtained with the rotarod test

Treatment	TD_{50} locomotor deficit	TD_{50}/ED_{50}
Vehicle + CFM-2	56.8 (39.3–82.1)	3.8
CFM-2 + ACPT-1 (2)	55.86 (42.3–74.73)	4.9
CFM-2 + ACPT-1 (4)	39.36 (27.38–56.58)	5.6[a]
CFM-2 + PPG (1)	58.38 (38.53–88.46)	6.1[a]
CFM-2 + PPG (2)	53.39 (39.53–72.11)	6.8[b]
Vehicle + CPPene	7.92 (5.83–10.75)	4.5
CPPene + ACPT-1 (2)	6.55 (5.26–8.16)	5.5
CPPene + ACPT-1 (4)	4.98 (4.02–6.17)	7.9[a]
CPPene + PPG (1)	6.12 (5.05–7.42)	7.37
CPPene + PPG (2)	4.67 (3.62–6.02)	10.86[b]

All data are expressed as μmol/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). TD_{50}/ED_{50} = therapeutic index which represent the ratio between TD_{50} and ED_{50} from the clonic phase of the audiogenic seizures. Significant differences were not observed between CFM-2 + vehicle and CFM-2 ACPT-1 (2) or between CPPene + vehicle and CPPene + ACPT-1 (2) or CPPene + PPG (1) groups.

[a] $P < 0.05$ vs. respective control group.

[b] $P < 0.01$ vs. respective control group.

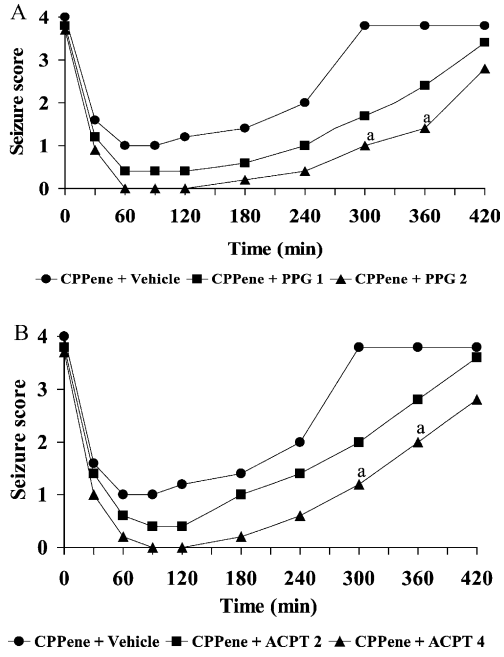

Fig. 4. Influence of (*R*,*S*)-PPG (1 or 2 nmol/mouse) (A) and ACPT-1 (2 or 4 nmol/mouse) (B) on the time course of the anticonvulsant effect of CPPene (3.3 μmol/kg, i.p.) in DBA/2 mice. Abscissae show the time after intraperitoneal administration of CPPene and (*R*,*S*)-PPG or ACPT-1 in min; ordinates show the seizure score. For the determination of each point, 10 animals were used. Significant differences between CPPene + vehicle and CPPene + PPG or ACPT-1 are indicated (a = $P < 0.05$).

score (Fig. 4B). Following the i.p. administration of saline and CPPene (3.3 μmol/kg, i.p.), maximum protection was observed at 45–180 min, with return to control seizure response at 300 min (Fig. 4B). When DBA/2 mice were pretreated, 15 min before, with ACPT-1 (2 or 4 nmol/mouse) and then received CPPene (3.3 μmol/kg, i.p.), the maximum protection was observed at 45–240 min for ACPT-1 (4 nmol/mouse), with return to control seizure response at 420 min (Fig. 4B).

3.5. Influence of PPG and ACPT-1 upon the motor impairment induced by CFM-2 and CPPene

No behavioural changes and neurological deficit were detected at any doses of CFM-2 alone (up to 33 μmol/kg, i.p.) or CPPene (up to 5 μmol/kg, i.p.). When applied at doses equal to those used to assess the anticonvulsant activity of CFM-2 or CPPene against the clonic and tonic phases of audiogenic seizures, ACPT-1 (2 and 4 nmol/mouse) and PPG (1 and 2 nmol/mouse) influenced motor performance of DBA/2. In particular, ACPT-1 (4 nmol/mouse) + CFM-2 treatment was able to produce a marked motor impairment (Table 3). Concomitant treatment with PPG (1) + CFM-2 or PPG (2) + CFM-2 resulted in a weak increase of motor impairment. Similarly, concomitant treatment with PPG (1) + CPPene or PPG (2) + CPPene resulted in a weak increase of motor impairment. In addition, ACPT-1 (4 nmol/mouse) plus CPPene treatment was able to produce a marked motor impairment (Table 3). The therapeutic index of the combination of ACPT-1 or PPG + CFM-2 and those of ACPT-1 or PPG + CPPene were more favourable than that of CFM-2 or CPPene + vehicle (Table 3).

4. Discussion

Moldrich et al. (2001) showed that the anticonvulsant action of the AMPA receptor antagonist (*R*)-3,4-dicarboxyphenylglycine was potentiated by low doses of the mGlu8 selective Group III agonists, (*R*,*S*)-4-phosphonophenylglycine (>10-fold higher affinity for mGlu8 vs. mGlu4) and (*S*)-3,4-dicarboxyphenylglycine(100-fold higher affinity for mGlu8 vs. mGlu4) (Thomas et al., 2001). Competitive and noncompetitive AMPA receptor antagonists have similar anticonvulsant action in DBA/2 mice (Chapman et al., 1991; De Sarro et al., 1994). Our finding that PPG potentiates the anticonvulsant action of the noncompetitive AMPA receptor antagonist CFM-2 confirms the previous observation of AMPA receptor antagonist potentiation by mGlu8 activation. We observe, however, that ACPT-1 produces a comparable potentiation of CFM-2. As ACPT-1 is a preferential agonist for mGlu4α rather than mGlu8, it appears that the effect is not specific to mGlu8 receptor agonists but may apply generally to Group III mGluR receptor agonists. We further find that this potentiation by Group III receptor agonists is not specific to AMPA antagonists, but is also seen with CPPene, a competitive NMDA antagonist that we have previously shown to be anticonvulsant in DBA/2 mice (Chapman et al., 1990). The potentiation of the anticonvulsant action of CPPene by both Group III receptor agonists was clearly similar to that seen with the AMPA receptor antagonists. It is well established that Group I metabotropic receptors show an interaction (potentiation) with NMDA and to a lesser extent AMPA receptors (Bleakman et al., 1992; Fitzjohn et al., 1996; Conn and Pin, 1997). Such an interaction has not been shown for Group III receptors and AMPA or NMDA receptors; apparent interactions can probably be explained in terms of an effect of the Group III agonists on glutamate release (Lafon-Cazal et al., 1999). Thus, the simplest hypothesis to explain the marked potentiation of the anticonvulsant effect of AMPA and NMDA receptor block by Group III receptor agonists is that a partial block of postsynaptic ionotropic receptors at glutamatergic synapses that is insufficient to modify seizure responses becomes significant when there is a simultaneous reduction in synaptic release of glutamate brought about by presynaptic Group III mGluR activation.

Although NMDA and AMPA antagonists are potent anticonvulsants in various animal models of epilepsy, they have not as yet been successful in clinical trial. In a small add-on trial in drug-refractory complex partial seizures, CPPene proved to lack efficacy and to induce toxic side effects. (Sveinbjornsdottir et al., 1993). Initial competitive and noncompetitive AMPA antagonists proved unsuitable for clinical development, but some more recent compounds such as talampanel are undergoing clinical evaluation (Nicolson and Leach, 2001). Earlier preclinical studies using electroshock and kindled seizures have drawn attention to the synergistic anticonvulsant effect of combinations of AMPA and NMDA receptor antagonists (Löscher et al., 1993;Czuczwar et al., 1995; Potschka et al., 1998). The present data suggest that in the search for novel AEDs, there may be an advantage in combining Group III receptor agonist action and AMPA or NMDA antagonist action either by administering compounds of the two classes or by combining the actions in one molecule.

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