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Anticonvulsant activity of a metabotropic glutamate receptor 8 preferential agonist, (*R*,*S*)-4-phosphonophenylglycine

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Abstract

Agonists at group III glutamate metabotropic receptors, such as L-serine-*O*-phosphate, have pro- and anti-convulsant activities in rodent models. We have used intracerebroventricular administration to test a novel group III agonist, (*R*,*S*)-4-phosphonophenylglycine (PPG), that is preferential for $mglu_8$, against sound-induced seizures in DBA/2 mice. Tonic and clonic seizures are abolished at 15 min (ED_{50s} 0.14 [0.04–0.4] nmol, and 3.4 [2.1–5.6] nmol, respectively). The protection against tonic and clonic seizures by 20 nmol PPG is complete for 4 h, diminished by 6 h, and absent by 10 h. In contrast, L-Serine-*O*-phosphate gives only partial protection against sound-induced clonic seizures for 15–30 min (ED_{50} 79 [45–139] nmol) in DBA/2 mice. In genetically epilepsy prone rats sound-induced seizures were blocked 5–60 min after the bilateral administration of PPG, 5–10 nmol, into the inferior colliculus. These data suggest that the $mglu_8$ receptor is a potential target for novel antiepileptic drugs.

Keywords: Epilepsy; $mglu_8$ receptor; L-Serine-*O*-phosphate; Glutamate receptor metabotropic; (DBA/2 mice); Anticonvulsant

1. Introduction

The classic metabotropic group III agonists, L(+)-2-amino-4-phosphonobutyric acid (L-AP4) and L-serine-*O*-phosphate (L-SOP) are thought to act presynaptically on $mglu_4$, $mglu_7$ and $mglu_8$ receptors to reduce glutamate release (Pin and Duvoisin, 1995; Conn and Pin, 1997). In rodent models of epilepsy they can have early transient proconvulsant actions and more prolonged anticonvulsant actions (Tang et al., 1997). Seizures induced by the Group I metabotropic agonist, 3,5-dihydroxyphenylglycine are dose-dependently suppressed by high doses of L-AP4 (0.4–1 μmol i.c.v.) or L-SOP (1.6–3.2 μmol i.c.v.) (Tizzano et al., 1995). A wide dose-range of these two group III agonists (0.01 nmol–3 μmol) has minimal activity against sound-induced seizures in DBA/2 mice (tested 30 min after their i.c.v. administration), but doses above 0.5 μmol cause convulsions (Ghauri et al., 1996).

(*R*,*S*)-4-phosphonophenylglycine (PPG) is a cyclic analogue of L-AP4 and L-SOP, which inhibits forskolin-stimulated cAMP formation in recombinant cell lines expressing group III human mGluRs with a preferential agonist action at mGluR8a (Flor et al., 1998; Gasparini et al., 1999). We have tested PPG as an anticonvulsant against sound-induced seizures in DBA/2 mice and in genetically epilepsy-prone (GEP) rats, and compared its anticonvulsant properties to those of L-SOP.

2. Materials and methods

2.1. Sound-induced seizures in DBA/2 mice

DBA/2 mice, male and female; age 21 to 28 days; 7 to 13 g wt. (Institute of Psychiatry colony), were housed on a 12-h dark, 12-h light cycle and were allowed free access to food and water until used experimentally. For i.c.v. drug administration, the drugs were dissolved in distilled water, pH adjusted to 7.4–7.6, and 10 μl were injected i.c.v. into DBA/2 mice under light fluothane anaesthesia as previously described (Chapman et al., 1999).

Following the drug or vehicle injections, the mice were maintained at a body temperature of 36–38°C by applying heating lamps when required, and observed for possible abnormal motor behaviour or proconvulsant effects of the drugs prior to testing for sound-induced seizures.

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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 sec or until the onset of tonic convulsions ($n = 10$ per group). 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; all phases attaining close to 100% incidence in the control groups, followed occasionally by respiratory arrest, latency 20–40 s.

PPG and L-SOP were dissolved in distilled water and administered i.c.v. 15 min before determining the dose-dependency (0.1–500 nmol PPG; 20–200 nmol L-SOP) of the sound-induced seizure responses. The time courses of action of PPG (20 nmol) and L-SOP (120 nmol) were determined by testing groups of mice ($n = 8$–10 per group) at various intervals after drug administration.

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

Adult female GEP rats 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, (*R,S*) PPG (1–10 nmol/side) or distilled water (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.

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 min, 30 min, 1 h, 2 h, 4 h, 1 day, 2 day, 3 day and 4 day.

At the end of the fourth 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.

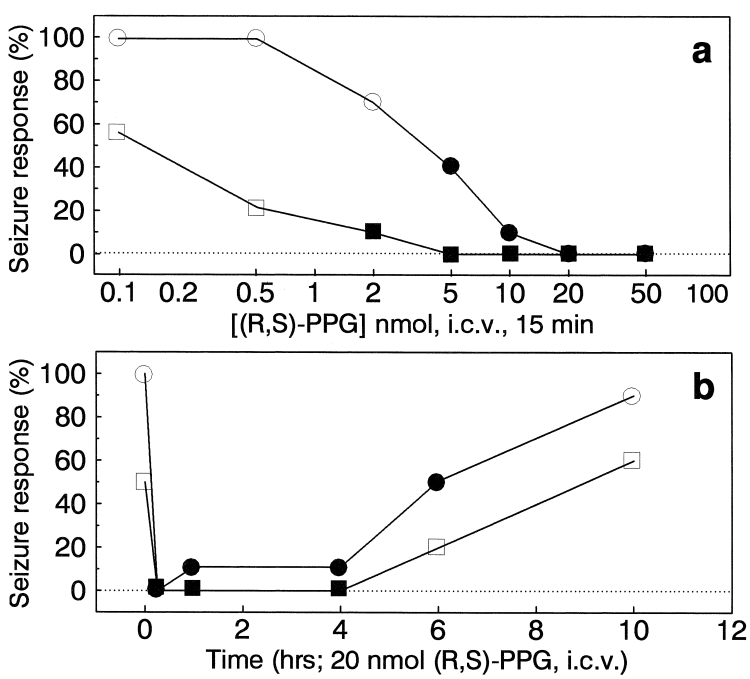

Fig. 1. Effect of PPG on sound-induced clonic and tonic seizures in DBA/2 mice. (a) Percent incidence of sound-induced clonic (circles) and tonic (squares) seizures in groups ($n = 9$–10 per group) of DBA/2 mice 15 min after the i.c.v. administration of 0.1–50 nmol PPG. The corresponding pooled control group ($n = 25$) had a 96% incidence of clonic, and 60% incidence of tonic seizures. (b) Time-course for the anticonvulsant action of PPG (20 nml i.c.v.; $n = 9$–10 per group) against sound-induced clonic (circles) and tonic (squares) seizures in DBA/2 mice. Filled symbols indicate statistically significant ($p < 0.05$; fisher's exact probability test) suppression of seizures.

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.

2.3. Compounds tested

(*R*,*S*)-4-phosphonophenylglycine (PPG; M.W. = 249.2) and L-serine-*O*-phosphate (L-SOP; M.W. = 185.1) were purchased from Tocris Cookson, Bristol, U.K.

2.4. Statistics

The ED_{50} values with lower and upper confidence values at 95% confidence limit for clonic and tonic seizures were calculated from the dose–response data according to the method of Litchfield and Wilcoxon (1949). The significance of the drug-induced suppression of sound-induced clonic and tonic seizures was assessed by Fisher's exact probability test.

3. Results

3.1. Effect of PPG on sound-induced seizures in DBA/2 mice

The i.c.v. administration of 0.1–50 nmol PPG to groups ($n = 9$–10 per group) of DBA/2 mice, produced a dose-dependent reduction in the incidence of clonic and tonic seizures evoked by a sound-stimulus 15 min later (Fig. 1a). Clonic and tonic sound-induced seizures were significantly ($p < 0.005$) suppressed by 5–50 nmol PPG, and tonic seizures were also significantly ($p < 0.05$) suppressed by 2 nmol PPG (i.c.v., 15 min). Tonic seizures were suppressed with an ED_{50} value of 0.14 [0.04–0.4] nmol, and clonic seizures were suppressed with an ED_{50} value of 3.4 [2.1–5.6] nmol, respectively, at 15 min. post-i.c.v.-administration.

When fully anticonvulsant doses (20 nmol) of PPG were administered to groups of DBA/2 mice ($n = 9$–10 per group), there was a complete protection against the tonic phase ($p < 0.05$), and a virtually complete protection against the clonic phase ($p < 0.005$) of the sound-induced seizures when tested between 15 min and 4 h post i.c.v.-administration. The anticonvulsant protection was diminished by 6 h ($p < 0.01$ for clonic seizures, non-significant for tonic seizures), and absent by 10 h, after PPG administration (Fig. 1b).

In the dose range of 0.1 to 10 nmol PPG there were no overt behavioural side-effects associated with the i.c.v. administration of PPG. Following the administration of 20 and 50 nmol PPG, there was a transiently enhanced level of excitability (increased grooming, running and jumping) lasting 15–20 min. At higher doses of PPG (100–500 nmol, i.c.v.) there were severe behavioural side effects. After 100, 150 and 200 nmol PPG there was a 22%, 50% and 11% incidence of brief episodes of spontaneous clonic seizures, respectively (latency 25–60 min). After 200–500 nmol PPG i.c.v. the mice became heavily sedated or comatose around 20–30 min and after 500 nmol 50% suffered respiratory arrest.

3.2. Effect of L-SOP on sound-induced seizures in DBA/2 mice

L-SOP provided short-lasting, partial protection against sound-induced clonic and tonic seizures for 15–30 min following the i.c.v. administration of 20–200 nmol L-SOP (Fig. 2a,b, significance values as indicated). Clonic sound-induced seizures were significantly ($p < 0.05$) suppressed by 50, 100 and 120 nmol L-SOP (i.c.v., 15 min), with estimated ED_{50} values of 79 [45–139] nmol for clonic and 34 [4.9–235] nmol for tonic seizures. However, protection was abolished with further increases in the dose of L-SOP to 150 and 200 nmol when tested at 15 min post i.c.v. administration (Fig. 2a). At a dose of 120 nmol L-SOP, there was significant protection ($p < 0.05$) against clonic and tonic seizures when tested 15 and 30 min after drug administration, but at 60 min post i.c.v. administration of 120 nmol L-SOP, the seizure response had returned to

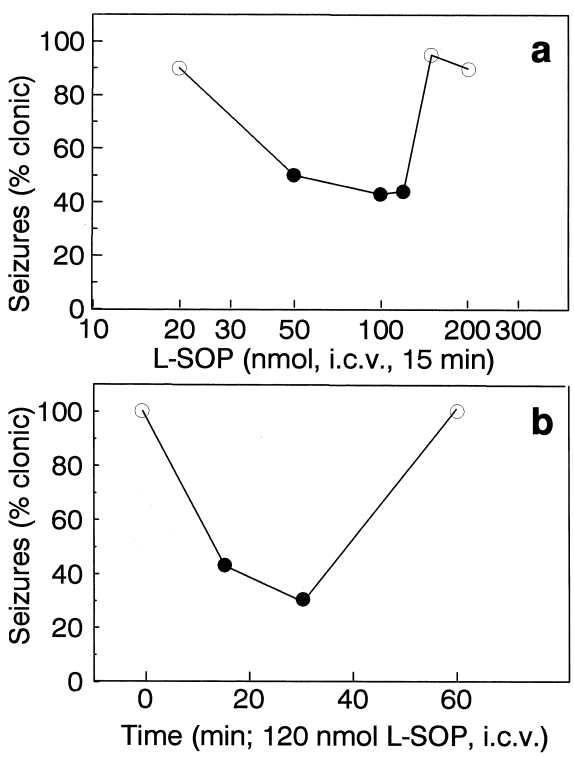

Fig. 2. Effect of L-SOP on sound-induced clonic seizures in DBA/2 mice. (a) Percent incidence of sound-induced clonic seizures in groups ($n = 8$–10 per group, except for $n = 18$–19 at 100 nmol and 150 nmol L-SOP) of DBA/2 mice 15 min after the i.c.v. administration of 20–200 nmol L-SOP. (b) Time-course for the anticonvulsant action of L-SOP (120 nml i.c.v.; $n = 8$–9 per group) against sound-induced clonic seizures in DBA/2 mice. Filled symbols as in Fig. 1.

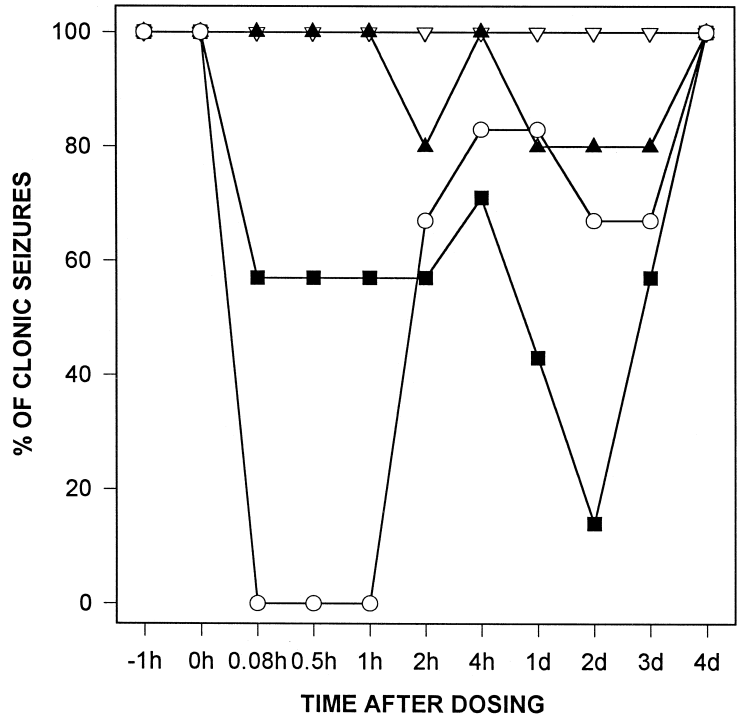

Fig. 3. Protection against sound-induced clonic seizures in GEP rats following bilateral microinjection of distilled water (inverted white triangle) or PPG: 1 nmol/side (black triangle), 5 nmol/side (black square) and 10 nmol/side (white circle) into the inferior colliculus. An hour before drug or vehicle administration, all animals were tested for sound-induced seizures. Only the animals which expressed full clonic-tonic seizures with tonic extension were used in the experiment. After drug or vehicle administration at time 0, the animals were exposed to a sound stimulus at 5 min, 30 min, 1 h, 2 h, 4 h, 1 day, 2 day, 3 day and 4 day. The results are expressed as a percentage of GEP rats ($n = 4–7$ per time point) responding with a clonic seizure.

control values (Fig. 2b). No behavioural side-effects were observed following the administration of 20–200 nmol L-SOP.

3.3. PPG in inferior colliculus of GEP rats

Bilateral administration of PPG, 1–10 nmol/side, into the inferior colliculus in GEP rats ($n = 5–7$) caused a dose-dependent suppression of clonic seizures induced by sound (Fig. 3). The maximal anticonvulsant effect occurred after 10 nmol/side at 5 min to 1 h. The ED_{50} values in nmol/side (with 95% confidence limits) against sound-induced wild running, clonic and tonic seizures for PPG at time points up to 24 h are shown in Table 1. However, 2 days after the intracollicular injection of PPG, 5 nmol/side, in GEP rats, there was a reduction in sound-induced clonic seizures. No abnormal behaviours were observed after PPG, 1–10 nmol/side, compared to control animals.

4. Discussion

This study shows that PPG, a group III agonist acting preferentially on $mglu_8$, has more potent and sustained anticonvulsant activity against sound-induced seizures in

Table 1
The ED_{50} (nmol/side) for the suppression of wild running, clonic and tonic seizures induced by a sound stimulus in GEP rats following intracollicular injection of PPG. The ED_{50} values were calculated according to Litchfield and Wilcoxon (1949)
n.s = Not significant.

Time point	Wild running	Clonic	Tonic
5 min	6.1 (3.7–9.9)	5.4 (2.4–8.6)	5.4 (2.4–8.6)
30 min	6.4 (3.4–9.4)	5.4 (2.4–8.6)	2.4 (1.1–4.8)
1 h	n.s	5.4 (2.4–8.6)	2.0 (0.8–4.9)
2 h	n.s	n.s	n.s
4 h	n.s	n.s	n.s
1 d	n.s	n.s	n.s

Table 2
Affinities of PPG, L-AP4 and L-SOP to group III metabotropic receptors Values are the ED_{50} values in μM for inhibition of forskolin-stimulated cyclic AMP accumulation for human metabotropic glutamate receptors expressed in HEK293 cells and rat metabotropic glutamate receptors expressed in chinese hamster ovary cells (taken from Nakajima et al., 1993; Okamoto et al., 1994; Pin and Duvoisin 1995; Gasparini et al., 1999).

PPG	L-AP4	L-SOP
$hmglu_{4a} = 5.2$	$rmglu_4 = 0.9$	$rmglu_4 = 4.2$
$hmglu_6 = 4.7$	$rmglu_6 = 0.9$	$rmglu_6 = 2.7$
$hmglu_{7b} = 185$	$rmglu_7 = 160$	$rmglu_7 = 160$
$hmglu_8 = 0.2$	$rmglu_8 = 0.6$	

DBA/2 mice and GEP rats than the classic group III agonist, L-SOP. In an earlier study we found that L-SOP 500 nmol was required intracollicularly to suppress sound-induced seizures in GEP rats (Tang et al., 1997). This result is also in agreement with the recent report of Gasparini et al., 1999, showing that PPG is anticonvulsant in the mouse maximal electroshock model, with an ED_{50} of 78 nmol, i.c.v., whereas L-SOP and L-AP4, 60–220 nmol i.c.v., were not anticonvulsant at 15 min in the mouse maximal electroshock model. Furthermore, PPG shows much less of the transient proconvulsant activity associated with the central or focal administration of L-SOP or L-AP4 (Tang et al., 1997). In the case of L-SOP this proconvulsant effect gives rise to spontaneous seizures at 500–1000 nmol i.c.v. in DBA/2 mice (Ghauri et al., 1996), but also appears to curtail the anticonvulsant effect at 100–200 nmol (Fig. 2). Similarly, Gasparini et al., 1999 saw no proconvulsant effects of PPG, 10–2200 nmol i.c.v., but saw clonic/clonic-tonic seizures in 40–60 % of the mice 5–10 min after L-SOP or L-AP4, 2200 nmol i.c.v.

An explanation for the difference between the effect of PPG and L-SOP is presumably to be sought in (a) their differing potencies as agonists at $mglu_4$, $mglu_7$ and $mglu_8$ receptors and (b) the different sites of expression and function of these three receptor subtypes. All three receptors are expressed presynaptically at glutamatergic synapses. Immunocytochemical studies, however, show marked differences in their expression on specific pathways (Shigemoto et al., 1997), with for example $mglu_{7a}$ being widely expressed in the hippocampus but $mglu_8$ being confined to lateral perforant path terminations in the outer molecular layer of the dentate gyrus. There is evidence that group III mGluRs are also expressed presynaptically on GABAergic pathways, e.g., reticular nucleus to relay nuclei in thalamus (Salt and Turner, 1996; Saugstad et al., 1997). Our data do not allow conclusions about the possible role of presynaptic actions on GABAergic pathways in the proconvulsant effects of L-AP4 and L-SOP. We cannot exclude that the proconvulsant actions of L-AP4 and L-SOP relate to effects at other sites, such as potentiation of NMDA receptors.

Table 2 compares the affinity of PPG for human group III receptors with those for L-AP4 and L-SOP for rat group III receptors. These data are compatible with the superior acute anticonvulsant effect of PPG being related to its greater relative activity at $mglu_8$ but they do not give any decisive indication of the likely mechanism for the acute proconvulsant effect of L-AP4 and L-SOP, or the delayed anticonvulsant effect of L-SOP and PPG following intracollicular injection.

It is clear, however, that agonists with a selective action on mGluR8 merit investigation as potential anticonvulsant agents. PPG itself lacks activity when given systemically. Clinical trial will become possible once compounds are developed with similar specificity and potency but more appropriate pharmacokinetics.

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