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Prolonged anticonvulsant action of glutamate metabotropic receptor agonists in inferior colliculus of genetically epilepsy-prone rats

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Abstract

The anticonvulsant activity of (*S*)-4-carboxy-3-hydroxyphenylglycine ((*S*)-4C3HPG) (an antagonist of Group I and an agonist of Group II metabotropic glutamate (mGlu) receptors), of (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*S*)-ACPD) (an agonist of Group II mGlu receptors), and of L-serine-*O*-phosphate (an agonist of Group III mGlu receptors) was studied against sound-induced seizures in genetically epilepsy-prone (GEP) rats following bilateral microinjection into the inferior colliculus. All 3 drugs produce dose-dependent suppression of all phases of sound-induced seizures (wild running, clonic and tonic). (*S*)-4C3HPG produces an immediate and short-lasting (< 2 h) protection against sound-induced seizures with an ED_{50} value of 4.3 (3.2–5.7) nmol, at 5 min. The preferential agonists of Group II and Group III mGlu receptors produce an immediate, transient (< 10 min) proconvulsant effect followed by a prolonged (> 1 day) anticonvulsant effect against sound-induced seizures. The anticonvulsant ED_{50} value for (1*S*,3*S*)-ACPD is 9 (5–18) nmol at 2 h, and for L-serine-*O*-phosphate is 36 (6.5–199) nmol at 2 days. It is concluded that mGlu receptor activation potently modifies seizure threshold.

Keywords: Epilepsy; Glutamate; Genetically epilepsy-prone rat; Metabotropic glutamate receptor; (*S*)-4C3HPG ((*S*)-4-carboxy-3-hydroxyphenylglycine); (1*S*,3*S*)-ACPD ((1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid); L-Serine-*O*-phosphate

1. Introduction

A proconvulsant effect of glutamate and related agonists acting on glutamate ionotropic receptors has long been recognised (Hayashi, 1952; Meldrum, 1991). Effects of glutamate and related agonists acting via metabotropic (mGlu) receptors are less clear. This is partly because of the diversity of mGlu receptors (Pin and Duvoisin, 1995; Schoepp and Conn, 1993). Eight mGlu receptors have been cloned from the mammalian central nervous system (Nakanishi, 1992; Nakanishi and Masu, 1994). In terms of sequence homology, functionality and pharmacology they fall into three groups. Group I, comprising mGlu receptors 1a,b,c and 5a,b, activates phospholipase C and is non-selectively activated by (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) but selectively activated by (*S*)- or (*RS*)-3,5-dihydroxyphenylglycine (3,5-DHPG) and selectively blocked by (*S*)-4-carboxy-3-hydroxyphenylglycine ((*S*)-4C3HPG). Group II receptors, consisting of mGlu2 and mGlu3, negatively modulate adenylate cyclase and are preferentially activated by (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (L-CCG-I), (2*S*,1′*R*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV), and (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*S*)-ACPD). Group III receptors also negatively modulate adenylate cyclase and are selectively activated by L-serine-*O*-phosphate and L-2-amino-4-phosphonobutyrate.

The functional effects of metabotropic receptor activation are extremely diverse (Pin and Duvoisin, 1995). However, the non-specific mGlu receptor agonist (1*S*,3*R*)-ACPD and the Group I mGlu receptor-selective agonist 3,5-DHPG are convulsant when injected focally into the striatum of rats or the thalamus of mice or i.c.v. into mice (Sacaan et al., 1991; Sacaan and Schoepp, 1992; Tizzano et al., 1993; Ghauri et al., 1996). Data concerning agonists at Group II and III mGlu receptors are discordant, some reports indicate an anticonvulsant effect following focal (Tizzano et al., 1995a,b; Attwell et al., 1995) or i.c.v.

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injection (Dalby and Thomsen, 1996), whereas others report a convulsant effect of these agonists given i.c.v. in mice (Ghauri et al., 1996). Studies with i.c.v. injections have reported acute effects (5–90 min) (Thomsen et al., 1994; Dalby and Thomsen, 1996; Ghauri et al., 1996) whereas some studies with focal injections have reported exceptionally long-lasting effects (Suzuki et al., 1996).

We have sought to resolve these problems by studying the pro- and anti-convulsant effects of focal injections in genetically epilepsy-prone (GEP) rats in which generalised seizures can be provoked by exposure to a loud sound. Seizures in this model are initiated in the inferior colliculus; we have therefore injected this structure bilaterally with a Group I antagonist, (*S*)-4C3HPG, a Group II agonist, (1*S*,3*S*)-ACPD, and a Group III agonist, L-serine-*O*-phosphate. This model has the advantage that the time-course of agonist action can be studied, at both short and long time intervals.

2. Materials and methods

2.1. Surgery in GEP rats

GEP rats, of either sex (taken from the Institute of Psychiatry GEP rat-9 colony) aged 8–16 weeks, were used in this study. The animals were housed in groups of 3–7 in PVC cages (35 cm wide × 53 cm long × 18 cm high) under a 14 h light (light on from 06:00 to 20:00 h) and 10 h dark cycle in a maintained environment of 19–22°C and relative humidity of 55 ± 3%. Food and water were continuously available. All animals were anaesthetized with 2% halothane (in 70% N_2O, 30% O_2) and bilateral stainless steel guide cannulae (21 gauge, 11 mm in length) were stereotaxically implanted 0.5 mm above the inferior colliculus (from interaural: AP = +0.7, L = +2.0, H = +7.5) according to the atlas coordinates of Paxinos and Watson (1982). The cannulae were secured, along with stainless steel screws placed on the cranium with dental acrylate. Animals were allowed a minimum of 5 days to recover from surgery and were then tested for sound-induced seizures for three consecutive days before the start of the experiment.

2.2. Sound-induced seizures in GEP rats

The animals were individually placed in an enclosed metal cylinder (50 cm in diameter) and exposed to a 110 dB sound stimulus (emitted from an electric doorbell) for a maximum of 60 s, or until the beginning of convulsions. The resulting behavioural response was scored on an audiogenic response score scale of 0–9 where: 0 = no response; 1 = wild running only; 2–5 = one or two episodes of wild running followed by a mild or severe clonic seizure; 6–9 = one or two episodes of wild running followed by a severe whole body clonus and partial extension or full tonic extension of the hindlimbs (Smith et al., 1996). Only those animals with a seizure score of 9 for three consecutive days were used in the study. On the third day, the animals received (*S*)-4C3HPG, (1*S*,3*S*)-ACPD, L-serine-*O*-phosphate or vehicle 1 h after the third pre-test audiogenic stimulation.

2.3. Administration of drugs in GEP rats

For the administration of (*S*)-4C3HPG (3–12 nmol/side), (1*S*,3*S*)-ACPD (5–40 nmol/side), L-serine-*O*-phosphate (80–500 nmol/side) or vehicle (10 mM phosphate-buffered saline) the animals were gently hand-restrained and injections were made bilaterally into the inferior colliculus using stainless steel injector cannulae (27 gauge, 13 mm in length) each connected by a polyethylene tube to a 10 μl Hamilton syringe. Injections were made in a volume of 0.5 μl at a rate of 0.2 μl/min using a CMA 100 (Biotech Instrument, UK) infusion pump, and the cannulae were left in place for a further 2 min. The anticonvulsant activity of the drugs were tested in GEP rats at the time points of 5 min and 0.5 h, 1 h, 2 h, 4 h, 24 h, 2 days, 3 days, and if necessary, 4 days and 5 days after injection. Any abnormal behavioural effects during the experiment were recorded. Animals were used once, and at the end of the experiment, 0.5 μl of 2% Evans blue was injected into the inferior colliculus using the same method as above. The brains were removed and sectioned on a cryostat; the location of the Evans blue dye (examined microscopically) determined whether the placement was correct, and only those which were correct were included in the study.

2.4. Drugs

(*S*)-4-Carboxy-3-hydroxyphenylglycine ((*S*)-4C3HPG) (molecular weight: 211.17) was a gift from Dr. C. Thomsen (Novo Nordisk, Denmark). (1*S*,3*S*)-1-Aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*S*)-ACPD) (molecular weight: 173.17) was a gift from Prof. J.C. Watkins (Bristol University, Bristol, UK) and L-serine-*O*-phosphate (molecular weight: 185.07) was obtained from Sigma Chemicals (Poole, UK).

All compounds were dissolved in 10 mM of phosphate-buffered saline (vehicle) and the pH adjusted, using NaOH, to 7.1–7.4 for (*S*)-4C3HPG and (1*S*,3*S*)-ACPD and 6.8–8.5 for L-serine-*O*-phosphate.

2.5. Statistical analysis

The ED_{50} values (with 95% confidence limits) for the suppression of each phase of the seizure response were determined according to the method of Litchfield and Wilcoxon (1949).

3. Results

3.1. Anticonvulsant effect of (S)-4C3HPG

Bilateral microinjection of (*S*)-4C3HPG (3–12 nmol/side) into the inferior colliculus of GEP rats resulted in a dose-dependent suppression of sound-induced seizures (Fig. 1). The maximal protective dose was 12 nmol/side with peak effect occurring at 5 min (earliest time point tested with this drug) and with an offset time of 2 h. The ED_{50} values with 95% confidence limits against sound-induced wild running, clonic, and tonic seizures at peak effect (5 min) were: 4.7 (2.9–7.5) nmol, 4.3 (3.2–5.7) nmol and 4.3 (3.2–5.7) nmol, respectively. Anticonvulsant ED_{50} values determined at 5 min and at 0.5 h are presented in Table 1.

3.2. Anticonvulsant effect of (1S,3S)-ACPD

Dose-dependent suppression of sound-induced seizures in GEP rats was observed following bilateral injection of (1*S*,3*S*)-ACPD (5–40 nmol/side) into the inferior colliculus (Fig. 2). The time of onset for full anticonvulsant effect with 40 nmol/side was 1 h and lasted up to 3 days with

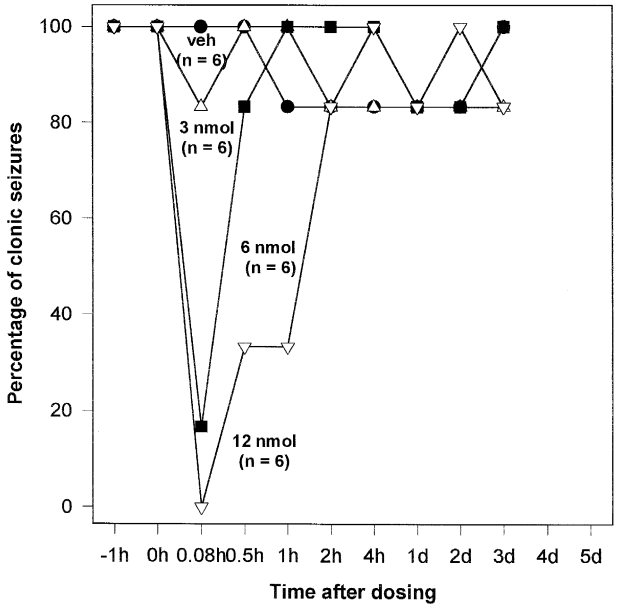

Fig. 1. Anticonvulsant action of (*S*)-4C3HPG in the sound-sensitive GEP rats. Groups of 6 animals were microinjected bilaterally into the inferior colliculus at time 0 with 10 mM phosphate-buffered saline (vehicle; filled circles) and (*S*)-4C3HPG at doses of: 3 nmol/side (open triangles); 6 nmol/side (filled squares); and 12 nmol/side (open inverted triangles). Sound sensitivity was assessed at the times, and the responses graded, as outlined in Section 2.

Table 1
Anticonvulsant efficacy of (*S*)-4C3HPG, (1*S*,3*S*)-ACPD, and L-serine-*O*-phosphate against audiogenic seizures in GEP rats

Time after injection	Drug	ED_{50} values (nmol)		
		Wild running	Clonic	Tonic
5 min	(*S*)-4C3HPG	4.7 (2.9–7.5)	4.3 (3.2–5.7)	4.3 (3.2–5.7)
	(1*S*,3*S*)-ACPD	–	–	–
	L-Serine-*O*-phosphate	–	–	–
0.5 h	(*S*)-4C3HPG	11 (5.9–23)	9.7 (5.9–16)	6.6 (3.8–12)
	(1*S*,3*S*)-ACPD	23 (8–68)	15 (7–34)	11 (5–25)
	L-Serine-*O*-phosphate	–	–	400 (139–1154)
1 h	(*S*)-4C3HPG	–	–	–
	(1*S*,3*S*)-ACPD	19 (9–40)	–	9 (5–18)
	L-Serine-*O*-phosphate	–	343 (195–602)	–
2 h	(*S*)-4C3HPG	–	–	–
	(1*S*,3*S*)-ACPD	11 (5–24)	9 (5–18)	9 (5–18)
	L-Serine-*O*-phosphate	178 (66–475)	94 (23–396)	80 (23–277)
4 h	(*S*)-4C3HPG	–	–	–
	(1*S*,3*S*)-ACPD	10 (4–28)	10 (4–28)	–
	L-Serine-*O*-phosphate	80 (23–277)	73 (29–187)	73 (29–187)
1 day	(*S*)-4C3HPG	–	–	–
	(1*S*,3*S*)-ACPD	11 (5–24)	11 (5–24)	–
	L-Serine-*O*-phosphate	73 (29–187)	72 (30–176)	–
2 days	(*S*)-4C3HPG	–	–	–
	(1*S*,3*S*)-ACPD	14 (5–38)	11 (5–24)	–
	L-Serine-*O*-phosphate	53 (13–222)	36 (6.5–199)	36 (6.5–199)
3 days	(*S*)-4C3HPG	–	–	–
	(1*S*,3*S*)–ACPD	–	–	9 (5–18)
	L-Serine-*O*-phosphate	847 (222-3233)	797 (146–4365)	400 (139–1154)
4 days	(*S*)-4C3HPG	–	–	–
	(1*S*,3*S*)-ACPD	26 (13–50)	26 (13–50)	23 (13-41)
	L-Serine-*O*-phosphate	–	–	–

The drugs were injected bilaterally into the inferior colliculus of the animals and tested at times specified. Table depicts the ED_{50} values (and their 95% confidence limits) for the suppression of wild running, clonic and tonic seizures using a method based on Litchfield and Wilcoxon (1949). –: data unavailable.

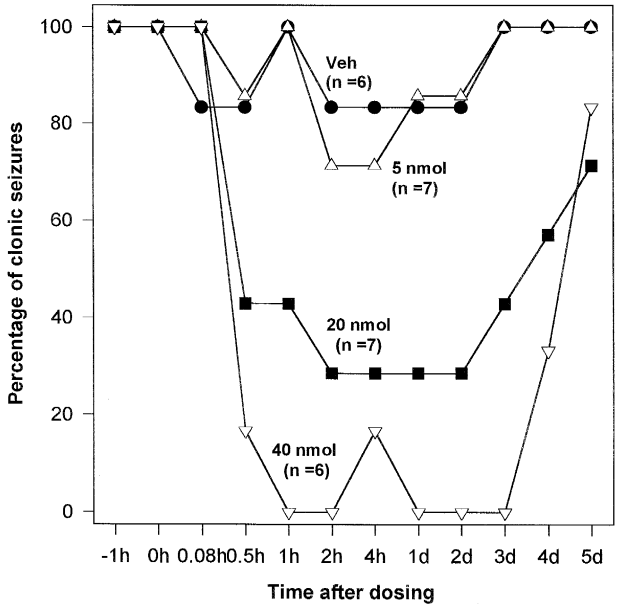

Fig. 2. Suppression by (1*S*,3*S*)-ACPD of sound-induced clonic seizures in groups of 6–7 GEP rats following focal, bilateral injection into the inferior colliculus. Each group of animals received either 10 mM phosphate-buffered saline (vehicle; filled circles) or (1*S*,3*S*)-ACPD at doses of: 5 nmol/side (open triangles); 20 nmol/side (filled squares); and 40 nmol/side (open inverted triangles) at time 0.

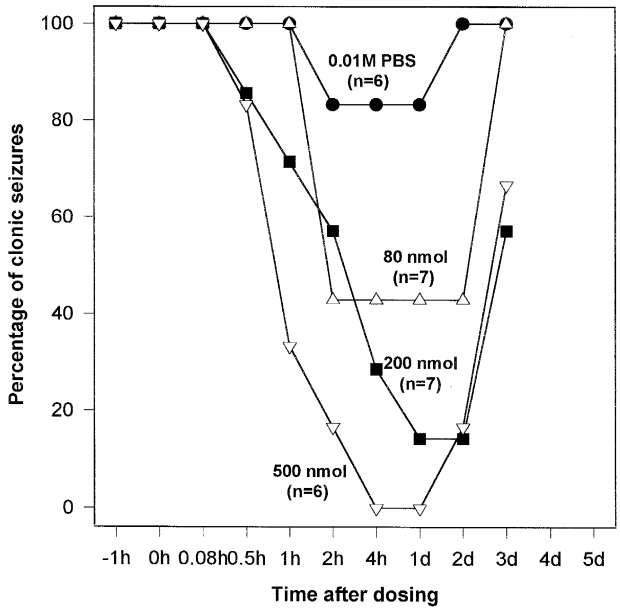

Fig. 3. Suppression of sound-induced clonic seizure response in GEP rats, tested at the various time points after the bilateral injection of L-serine-*O*-phosphate (80 nmol/side (open triangles); 200 nmol/side (filled squares); and 500 nmol/side (open inverted triangles) or vehicle (10 mM phosphate-buffered saline (vehicle; filled circles)). Results are expressed as mean clonic seizure response ($n = 6–7$ per group).

peak effect at 2 h against clonic seizures. The ED_{50} values (nmol) against sound-induced seizures at 2 h were 11 (5–24) for wild running, 9 (5–18) for clonic and 9 (5–18) for tonic seizures. ED_{50} values determined during the full anticonvulsant time-course (0.5 h–4 days) are presented in Table 1.

3.3. Anticonvulsant effect of L-serine-O-phosphate

L-Serine-*O*-phosphate (80–500 nmol/side) produced a dose-dependent inhibition of sound-induced seizures in GEP rats (Fig. 3). The highest dose tested (500 nmol/side) produced prolonged anticonvulsant effect which lasted 0.5 h–3 days, with peak effect at 4 h–1 day. The ED_{50} values at 2 days were 53 (13–222), 36 (6.5–199) and 36 (6.5–199) nmol for wild running, clonic and tonic seizures, respectively. ED_{50} values determined during the full anticonvulsant time-course (0.5 h–3 days) are presented in Table 1.

3.4. Behavioural effects of (S)-4C3HPG, (1S,3S)-ACPD and L-serine-O-phosphate

The behaviour exhibited in GEP rats, following focal administration of the drugs into the inferior colliculus, is described in Table 2. Typical behaviour included hyperlocomotion, teeth chattering, circling and clonic-tonic seizures in the absence of a sound stimulus. Microinjection of (*S*)-4C3HPG (3–12 nmol/side) caused teeth chattering and circling with the fully anticonvulsant dose. All doses

Table 2
Behavioural effects of focal (*S*)-4C3HPG, (1*S*,3*S*)-ACPD, and L-serine-*O*-phosphate administration in GEP rats

Drug (nmol)		Behavioural activity (% of animals exhibiting behaviours)			
		Hyperlocomotion	Teeth chattering	Circling	Non-audiogenic clonic-tonic seizures
(*S*)-4C3HPG	3	–	17	–	–
	6	–	17	–	–
	12	–	33	17	–
(1*S*,3*S*)-ACPD	5	14	29	–	–
	20	14	14	29	14
	40	33	–	–	67
L-Serine-*O*-phosphate	80	–	–	–	–
	200	–	–	14	43
	500	–	–	17	83

Any abnormal behavioural activities in the rats ($n = 6–7$) during and after bilateral microinjection of drug into inferior colliculus was recorded. –: no apparent behavioural abnormalities.

of (1*S*,3*S*)-ACPD and L-serine-*O*-phosphate caused a shortening of the latent period to seizures when tested 5 min after injection. During or within 5 min after injection, the higher doses of (1*S*,3*S*)-ACPD (20–40 nmol/side) and L-serine-*O*-phosphate (200–500 nmol/side) caused circling and in some of the animals clonic-tonic seizures, which at the fully anticonvulsant doses lasted 5–15 min.

4. Discussion

The acute anticonvulsant action of (*S*)-4C3HPG is closely parallel to the anticonvulsant action of this compound against sound-induced seizures in DBA/2 mice, with i.c.v. injection. Thus in the mouse there is suppression of sound-induced seizures by (*S*)-4C3HPG, 100–500 nmol, with onset 5–15 min following i.c.v. injection (Thomsen et al., 1994; Dalby and Thomsen, 1996). Although (*S*)-4C3HPG is both an agonist at Group II mGlu receptors and an antagonist at Group I mGlu receptors (Hayashi et al., 1994), evidence of various sorts suggests that the acute anticonvulsant action of (*S*)-4C3HPG is probably predominantly mediated by antagonism at Group I mGlu receptors. Firstly, the Group I-selective agonist (*R*,*S*)-3,5-DHPG produces convulsions with a similar acute time-course when injected i.c.v. in mice. Secondly, (*S*)-4CPG is slightly less potent than (*S*)-4C3HPG as a Group I antagonist, but much less potent as a Group II agonist (Hayashi et al., 1994). It is modestly less potent than (*S*)-4C3HPG as an anticonvulsant when given i.c.v. to mice. Thirdly, we find that partially selective Group II agonists tend to be acutely proconvulsant rather than anticonvulsant.

The acute proconvulsant action of (1*S*,3*S*)-ACPD and of L-serine-*O*-phosphate following intracollicular injection is closely similar to the acute convulsant action we observe in DBA/2 mice following the i.c.v. injection of these compounds (Ghauri et al., 1996; Meldrum et al., 1996). Not unexpectedly the effect of focal intracerebral injection (in the rat inferior colliculus) is slightly more immediate than that of i.c.v. injection in the mouse, presumably because the time required for diffusion to the receptors involved is less. Although (1*S*,3*S*)-ACPD acts preferentially as an agonist at Group II mGlu receptors, it can act as an agonist at Group I mGlu receptors at high doses, thus it is possible that the acute convulsant effect is due to an agonist action on Group I mGlu receptors, similar to the effect of (*R*,*S*)-3,5-DHPG. (1*S*,3*S*)-ACPD may contain 1–5% of (1*R*,3*R*)-ACPD which is a potent NMDA receptor agonist (Sunter et al., 1991). The high concentrations of (1*S*,3*S*)-ACPD used in the present study may be sufficient to activate NMDA receptors leading to proconvulsant effects. However, the similarity of this acute convulsant effect to that of L-serine-*O*-phosphate and L-2-amino-4-phosphonobutyrate (unpublished observations) when injected into the inferior colliculus and the similar acute convulsant effects of i.c.v. injection in DBA/2 mice of (1*S*,3*S*)-ACPD, L-serine-*O*-phosphate and L-2-amino-4-phosphonobutyrate, suggests that they have a common mechanism of action. The common mechanism of action of the Group II and Group III agonists in biochemical terms is the negative modulation of adenylyl cyclase but in terms of synaptic action it is a presynaptic action reducing the release of glutamate and γ-aminobutyric acid (GABA) (that probably results from suppression of a voltage-activated P/Q-type calcium conductance (Takahashi et al., 1996).

A presynaptic action involving predominantly GABA release or glutamate release onto GABAergic neurons could explain the proconvulsant effect of (1*S*,3*S*)-ACPD, L-serine-*O*-phosphate and L-2-amino-4-phosphonobutyrate. An effect of this kind appears to explain the disinhibitory action of L-2-amino-4-phosphonobutyrate and L-serine-*O*-phosphate in the rat cortex (Wan and Cahusac, 1995). There is evidence that the expression of particular mGlu receptors in presynaptic terminals varies according to the postsynaptic target cell (Shigemoto et al., 1996). A study of picrotoxin-induced bursts in guinea pig hippocampal slices (Merlin et al., 1995) using (1*S*,3*R*)-ACPD, L-CCG-I, (*RS*)-4C3HPG and (*S*)-4-carboxyphenyl-glycine (*S*-4CPG) has concluded that agonists acting on Group II mGlu receptors increase the frequency of epileptiform bursts. This evidence supports a mechanism for the proconvulsant action for group II mGlu receptor agonists involving the reduction of GABA release. NMDA receptor involvement cannot however be totally excluded as it is known that high concentrations of L-2-amino-4-phosphonobutyrate produce a depolarisation of frog spinal motorneurones that is antagonised by NMDA receptor antagonists (Evans et al., 1982).

The most striking novel finding in this study is the delayed, prolonged anticonvulsant action of (1*S*,3*S*)-ACPD and of L-serine-*O*-phosphate. A comparable finding has very recently been reported with intra-amygdala injections in amygdala-kindled rats (Suzuki et al., 1996). Suppression of kindled seizures is modest at 6 h but maximal at 1–3 days after the intra-amygdala injection of L-2-amino-4-phosphonobutyrate (200 nmol). (In preliminary experiments we find that the delayed prolonged anticonvulsant effect of L-serine-*O*-phosphate following inferior colliculus injection is replicated by L-2-amino-4-phosphonobutyrate but with lower potency.) There is ample evidence that (1*S*,3*S*)-ACPD, L-2-amino-4-phosphonobutyrate and L-serine-*O*-phosphate can suppress excitatory transmission at certain glutamatergic synapses, with the lateral perforant path (Johansen et al., 1995; Bushell et al., 1996) and the monosynaptic path in the neonatal rat spinal cord providing the most detailed studies (Jane et al., 1994). These presynaptic effects are, however, seen immediately either with local application in vitro or iontophoresis in vivo. We have no evidence that they occur in an appropriately delayed and prolonged way in vivo to account for the

anticonvulsant effect observed here. The involvement of long-term cAMP cascade effects altering neuronal excitability possibly through changes in glutamatergic effects needs to be investigated.

L-Serine-*O*-phosphate is an endogenous compound, generated from phospholipids, that is potentially a neurotransmitter for Group III mGlu receptors. At mGlu4 receptors expressed in baby hamster kidney cells L-serine-*O*-phosphate is equipotent with potential i.e. it is a weaker presynaptic subgroup III mGlu receptor agonist as an agonist (EC_{50} = 4.0 μM; Thomsen and Suzdak, 1993). In the neonatal rat spinal cord L-serine-*O*-phosphate is less potent than L-2-amino-4-phosphonobutyrate at depressing the monosynaptic component of the dorsal root evoked-ventral root (Jane, D.E., Thomas, N.K. and Watkins, J.C., unpublished observations). EC_{50} measurements for reduction in cAMP formation using expressed mGlu receptors also tend to show a lower potency for L-serine-*O*-phosphate (4.0 μmol vs. 0.5 μmol for L-2-amino-4-phosphonobutyrate) (Knöpfel et al., 1995). Thus we cannot at present explain the greater anticonvulsant potency of L-serine-*O*-phosphate (relative to L-2-amino-4-phosphonobutyrate) following their focal administration in the inferior colliculus.

In conclusion, we would emphasize that the actions of mGlu receptor agonists and antagonists on seizure threshold are complex and clearly involve several mechanisms. Their study requires close attention to the time-course of the effects on seizures in relation to the time course of mGlu receptor effects, as well as attention to dose-effect relationships and comparisons between compounds with similar effects. Agents that are selectively active at one mGlu receptor are likely to be the most informative. Delayed but prolonged anticonvulsant actions, of the kind reported here, may be the best indicator of therapeutic potential.

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