ELSEVIER

European Journal of Pharmacology 368 (1999) 17–24

ejp

# Anticonvulsant actions of LY 367385 ((+)-2-methyl-4-carboxyphenylglycine) and AIDA ((*RS*)-1-aminoindan-1,5-dicarboxylic acid)

Astrid G. Chapman [a], Ping K. Yip [a], Joan S. Yap [a], Leann P. Quinn [a], Ellen Tang [a], John R. Harris [b], Brian S. Meldrum [a,*]

[a] *Department of Clinical Neurosciences, Institute of Psychiatry, King's College London, De Crespigny Park, Denmark Hill, London SE5 8AF, UK*
[b] *Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey, UK*

Received 16 July 1998; revised 4 January 1999; accepted 8 January 1999

**Abstract**

We have studied the effects in three rodent models of generalised convulsive or absence epilepsy of two antagonists of group I metabotropic glutamate receptors that are selective for the $mGlu_1$ receptor. LY 367385 ((+)-2-methyl-4-carboxyphenylglycine) and AIDA ((*RS*)-1-aminoindan-1,5-dicarboxylic acid) have been administered intracerebroventricularly (i.c.v.) to DBA/2 mice and lethargic mice (*lh/lh*), and focally into the inferior colliculus of genetically epilepsy prone rats (GEPR). In DBA/2 mice both compounds produce a rapid, transient suppression of sound-induced clonic seizures (LY 367385: $ED_{50} = 12$ nmol, i.c.v., 5 min; AIDA: $ED_{50} = 79$ nmol, i.c.v., 15 min). In lethargic mice both compounds significantly reduce the incidence of spontaneous spike and wave discharges on the electroencephalogram, from $< 30$ to $> 150$ min after the administration of AIDA, 500 nmol, i.c.v., and from 30 to $> 150$ min after the administration of LY 367385, 250 nmol, i.c.v. LY 367385, 50 nmol, suppresses spontaneous spike and wave discharges from 30 to 60 min. In genetically epilepsy prone rats both compounds reduce sound-induced clonic seizures. LY 367385, 160 nmol bilaterally, fully suppresses clonic seizures after 2–4 h. AIDA is fully effective 30 min after 100 nmol bilaterally. It is concluded that antagonists of $mGlu_1$ receptors are potential anticonvulsant agents and that activation of $mGlu_1$ receptors probably contributes to a variety of epileptic syndromes. 

*Keywords:* Epilepsy; Metabotropic glutamate receptor; $mGlu_1$ receptor; AIDA (RS)-1-aminoindan-1,5-dicarboxylic acid; LY 367385; DBA/2 mouse; GEPR (genetically epilepsy prone rat); *lh/lh* mouse

## 1. Introduction

Glutamate is the principal excitatory neurotransmitter in the brain and acts on ionotropic receptors that open cation permeable channels and on metabotropic receptors that are G-protein coupled and have a variety of effects on second messengers and ion channels. Agonists at glutamate ionotropic receptors are convulsant and corresponding antagonists are anticonvulsant in a wide range of animal models of epilepsy. The situation in relation to metabotropic receptors is less clear. The mGlu receptors can be classified into three groups according to their sequence homology, transduction mechanisms and agonist pharmacology (see reviews by Schoepp and Conn, 1993; Nakanishi, 1994; Pin and Duvoisin, 1995; Conn and Pin, 1997). Group I receptors (comprising $mGlu_1$ and $mGlu_5$, with their splice variants) activate phospholipase C, yielding inositol triphosphate and diacylglycerol from phosphoinositides. Groups II and III are negatively coupled to adenylyl cyclase and also act presynaptically to decrease some voltage-sensitive calcium currents.

Convulsant effects of mGlu receptor agonists have been frequently described, particularly for (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (Sacaan and Schoepp, 1992; McDonald et al., 1993; Tizzano et al., 1993, 1995). The similar convulsant action of 3,5-dihydroxyphenylglycine (3,5-DHPG) strongly suggests that group I mGlu receptors are involved in this convulsant effect as 3,5-DHPG is selective for group I relative to groups II and III (Schoepp et al., 1994). The anticonvulsant action of (*S*)-4-carboxy-3-hydroxyphenylglycine has been well docu-

* Corresponding author. Tel.: +44-171-701-2506, +44-171-919-3398; Fax: +44-171-740-5272; E-mail: b.meldrum@iop.bpmf.ac.uk

0014-2999/99/$ - see front matter 
PII: S0014-2999(99)00014-X

mented (Thomsen et al., 1994; Dalby and Thomsen, 1996; Tang et al., 1997). This compound is an antagonist at group I (especially $mGlu_1$), but also an agonist at group II mGlu receptors.

Recently, several more selective agonists and antagonists for $mGlu_1$ and $mGlu_5$ have been described. (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA) is a selective antagonist for $mGlu_{1a}$, with no effect on group II or III metabotropic receptors or ionotropic receptors (Pellicciari et al., 1995). LY 367385, (+)-2-methyl-4-carboxyphenylglycine, is a selective antagonist for $mGlu_{1a}$ receptors vs. $mGlu_{5a}$ receptors when tested for blockade of quisqualate-induced phosphoinositide hydrolysis (Clark et al., 1997), with negligible action on group II or III receptors.

We have used several different rodent models of epilepsy to assess proconvulsant or anticonvulsant actions of these two group I selective antagonists, paying particular attention to the time course of such effects. The models we have used include sound-induced seizures in DBA/2 mice (with intracerebroventricular (i.c.v.) injections) and in genetically epilepsy prone rats (GEPR) (with focal injections into the inferior colliculus). These are models of generalised seizures originating in the hindbrain. We have previously reported on the proconvulsant and anticonvulsant actions of groups II and III agonists and antagonists in these models (Ghauri et al., 1996; Tang et al., 1997). We have also studied the effects of these drugs on a rodent model of absence seizures, the spontaneous spike and wave discharges in lethargic mice (*lh/lh*) which have a mutation involving the β4 subunit of voltage-sensitive calcium channels (Burgess et al., 1997). This model is superior to threshold pentylenetetrazol seizures as a predictor of clinical efficacy of anti-epileptic drugs in childhood absence attacks (Hosford and Wang, 1997).

## 2. Materials and methods

### *2.1. Testing for seizures in seizure-susceptible rodent strains*

#### *2.1.1. DBA/2 mice*

DBA/2 mice, male and female (Institute of Psychiatry (IOP) stock), were housed on a 12-h dark/12-h light cycle (light on 0600–1800 h) and were allowed free access to food and water until used experimentally at the age of 21–28 days (7–13 g weight). They received i.c.v. injection, (1 mm anterior to the bregma, 1 mm lateral to the midline, to a depth of 3 mm) using a Hamilton syringe and a 25-short-gauge butterfly needle, during brief fluothane anaesthesia. By this method, 10 μl of 0.01 M phosphate buffered saline (PBS), at a pH of 7.1 was administered to each mouse in the control groups, or 10 μl of the $mGlu_1$ antagonist solution, at a concentration of 1–500 nmol, was administered to each of the experimental animals ($n = 10$ per group). Log dose and time course response curves were then constructed for the drug-induced suppression of the sound-induced seizures and the $ED_{50}$ values for the anticonvulsant potency of the $mGlu_1$ antagonists were determined.

Following the i.c.v. drug or vehicle injections, the mice were maintained at a body temperature of 36–38°C by

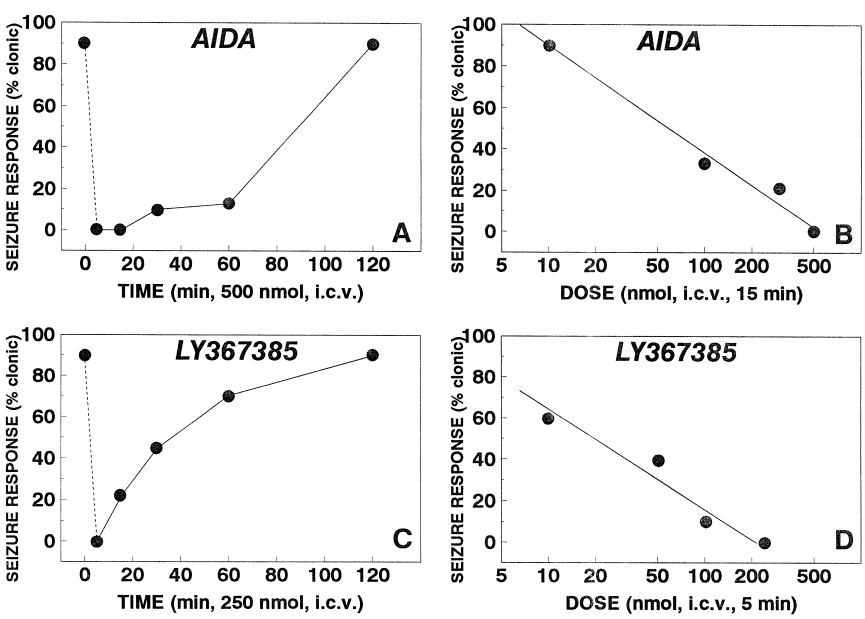


Fig. 1. Time course and dose response curves for LY 367385 and AIDA in DBA/2 mice. Graph shows the time course for the anticonvulsant action of AIDA (500 nmol, i.c.v., $n = 10$ per group; (A)) and LY 367385 (250 nmol, i.c.v., $n = 10$ per group, (C)), and the seizure response (% of mice with a clonic seizure response to sound) at optimal pretreatment times: 15 min following AIDA administration (10–500 nmol, i.c.v., $n = 10$ per group; (B)) and 5 min following LY 367385 administration (10–250 nmol, i.c.v., $n = 10$ per group; (D)).

applying heating lamps when required. Mice were observed for abnormal motor behaviour or convulsant effects of the drugs prior to testing for sound-induced seizures.

Anticonvulsant testing was carried out 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 consistently produced a sequential seizure response, consisting of a wild running phase latency 1–4 s (score 1), clonic seizures latency 4–15 s (score 2), tonic extension latency 10–30 s (score 3) and respiratory arrest latency 20–40 s (score 4). All the mice in the control groups gave seizure response scores of 2 or more.

### 2.1.2. Lethargic (lh/lh) mice

The colonies of *lh/lh* mice in the IOP were maintained by using the $f_1$ progeny (*lh/+*) intercross of co-isogenic, non-epileptic (+/+) and *lh/lh* to produce a ratio of 1:4 (25%), and by pairing female *lh/lh* with a male *lh/+* to give 1:2 (50%) *lh/lh* progeny. By the age of 15 days the *lh/lh* have a recognisable ataxic gait. All animals were given free access to food and water before and after the experiment. The lethargic mice were housed single-sex according to birth dates (12 animals or less per cage (150 mm wide × 400 mm long × 110 mm high), at constant temperature (19–22°C), humidity (54 ± 3%) and light/dark (12/12 h) cycle. Experimental animals were kept singly. A group of *lh/lh* mice of both sexes from age 11–17 weeks were randomly selected for surgery. All mice were anaesthetised with ketamine (7.5 mg/100 g, i.p.) and medetomidine (0.1 mg/100 g, i.p.) given as a single injection for short-term surgical anaesthesia. With the mouse head immobilised in a stereotaxic holder, pairs of bilateral burr holes were drilled over the frontal cortex (1.5 mm anterior to bregma and 1.5 mm lateral to midsagittal suture) and parietal cortex (left: 3 mm and right: 1 mm posterior to lambda, and 1.5 mm lateral to midsagittal sinus) for stainless steel microelectrodes, with another burr hole 1 mm to midsagittal sinus and anterior to lambda for guide cannula implantation. The wires were connected to a female plug used for attachment to the electroencephalogram recorder.

All microelectrodes and guide cannulae (gauge 21) were implanted at 0.8 mm below dura. The implants were fixed with dental acrylic and a layer of epoxy resin.

At least 1 week after surgery each mouse underwent five daily electroencephalogram recordings. The first involved a 2-h baseline recording followed by 3-h recordings for vehicle on even days (2,4) and drug on odd days (3,5). During each 3-h session, mice received i.c.v. either vehicle (0.01 M PBS) or drug (AIDA: 100 or 500 nmol or LY356375: 50 or 250 nmol) per cannula at 30 min after each baseline recording. Injection cannulae (gauge 27) were lowered 2 mm beyond the edge of the guide cannula

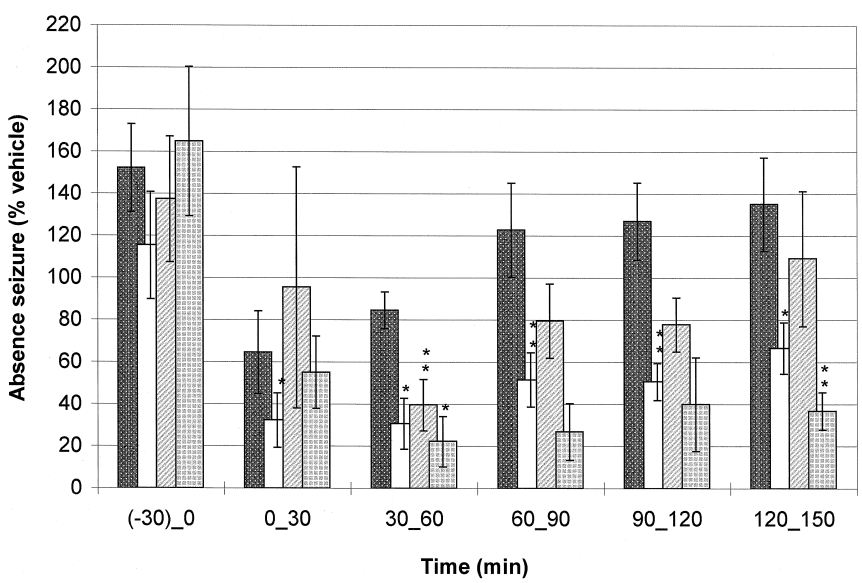


Fig. 2. The anti-absence effects of AIDA (100, 500 nmol, i.c.v., $n = 6$ per group) and LY 367385 (50, 250 nmol, i.c.v., $n = 6$ per group) in *lh/lh* mice. Drugs were administered (at 0 time) after 30 min of baseline electroencephalogram recording. Each animal served as its own control, and the seizure response is expressed as duration of spike and wave discharge during a 30-min observation period as a percentage of the spike and wave discharge in the same animal during the corresponding period following i.c.v. vehicle administration (% vehicle). The 4 bars per set represent: 100 nmol AIDA, black; 500 nmol AIDA, white; 50 nmol LY 367385, dark grey; 250 nmol LY 367385, light grey. Vertical bars represent standard error of mean. Symbols: *: $p < 0.05$ (95% confidence limit) and **: $p < 0.01$ referring to significant differences between testing periods and baseline periods as described in Section 2.2. The overall mean spike and wave discharge (expressed as second of spike and wave discharges during a 30-min period) during pre-drug control conditions were similar in the four groups (AIDA, 100 and 500 nmol; LY 367385, 50 and 250 nmol): 378 ± 52.0, 346 ± 76.7, 361 ± 78.8, 286 ± 62.2 (mean ± S.E.M).

to the ventricles. The vehicle or drug was infused at 2.5 μl/min in a total volume of 10 μl via a Hamilton syringe using a CMA/100 infusion pump. The injection cannula was withdrawn after 1 min of infusion.

During each recording, the behavioural changes after drug treatment in comparison to vehicle were noted. To minimise bias to observations associated with sedation, the mouse was frequently aroused by clapping noise (Hosford et al., 1992). The quantification of absence seizures was based on the duration (s) of electroencephalogram spike wave discharges or polyspikes, as described by Hosford et al. (1992) (i.e., amplitude not less than 60 μV and frequency range of 5–6 Hz, seizures must have a duration no shorter than 0.6 s). The *lh/lh* mice spent on the average around 5 min out of every 30-min test period in absence seizures according to these criteria. Electroencephalogram recordings were at amplification of 200–300 μV/cm and chart speed at 3 mm/s. To assess the pharmacological effect of the compounds over time, each 3 h electroencephalogram recording was divided into six 30-min epochs, the cumulated spike and wave discharges duration (s) per 30 min period after drug administration was calculated, and then normalised by dividing with the corresponding duration for the 30 min period after vehicle injection in each animal. Mean ± S.E.M. of the normalised data gathered from all the animals ($n = 6$) were expressed as the % of vehicle spike and wave discharge duration. This is represented by error bars in Fig. 2. The injection sites were verified by histology following focal injection of Evan's blue dye.

### 2.1.3. Genetically epilepsy-prone rats

Adult GEPR of either sex from our own IOP breeding stock were used in the experiments. The animals were kept in a temperature- and humidity-controlled room with a 12-h light/dark cycle and were given free access to food and water.

The rats were stereotaxically implanted with permanent stainless steel guide cannulae (gauge 21, 11 mm long) bilaterally just above the inferior colliculus under approximately 2% halothane (with a 2:1 mixture of nitrous oxide and oxygen). The coordinates from the interaural were anterior–posterior, +0.7 mm; lateral, +1.5 mm and height; +7.5 mm, according to the atlas of Paxinos and Watson (1986).

After a minimum of 5 days recovery from surgery, the rats were tested for sound-induced seizure on 3 consecutive days of testing before drug injection. Animals which did not score 9 on all these days were excluded from the experiment.

The scoring system was based on Jobe et al. (1973), where briefly: 0–1 = no seizure; 2–5 = clonic seizure after either one or two episodes of wild running; 6–9 = clonic seizure with tonic extension after either one or two episodes of wild running.

Drugs were administered an hour after the third consecutive control testing for sound-induced seizure. Administration was carried out bilaterally using two stainless steel cannulae (gauge 27, 13 mm long) each connected using a polyethylene tubing to a 10 μl Hamilton syringe. The total drug volume administered into the inferior colliculus was 0.5 μl at a rate of 0.2 μl/min using a microinfusion pump. Injection cannulae were left in place after injection for a further 2 min to allow drug to diffuse from cannula tip into the inferior colliculus. After drug administration, each animal was observed for the incidence and latency of abnormal behavioural effects until times of testing, which were at: +5 min, +30 min, +1 h, +2 h, +4 h, +2 days and +3 days.

At the end of the experiment, each rat was culled with an overdose of pentobarbitone given intraperitoneally and

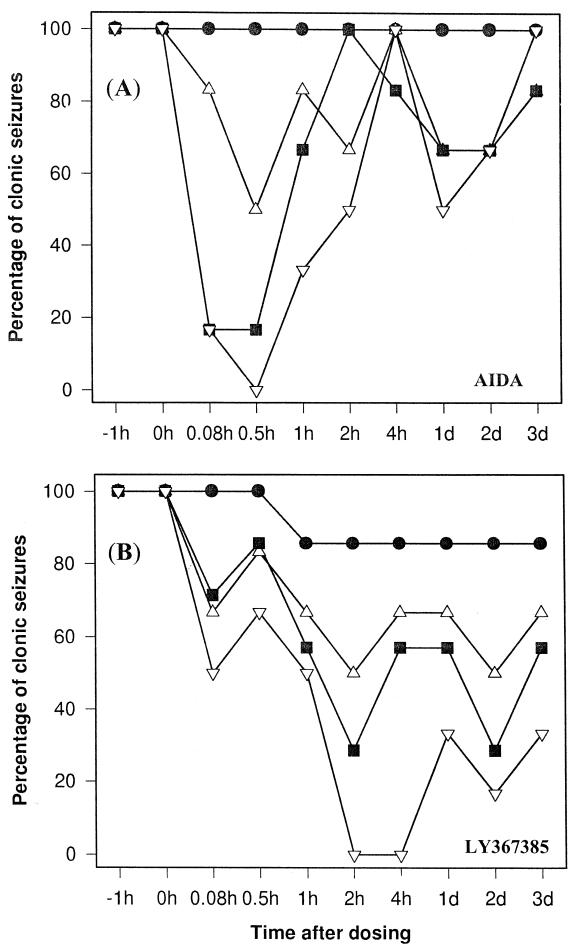


Fig. 3. Time course of the anticonvulsant effects of AIDA (A) and LY 367385 (B) following focal inferior colliculus administration in GEPR. (A) Graph shows % of rats showing clonic seizures in response to loud sound after bilateral injections of 10–100 nmol AIDA (black circles—vehicle; open triangles—10 nmol; black squares—50 nmol; open inverted triangles—100 nmol) into the inferior colliculus in GEPR ($n = 4$–6 per point). (B) Graph shows % of rats showing clonic seizures in response to loud sound at the specified times after the bilateral injection of 20–160 nmol LY 367385 (black circles—vehicle; white triangles—40 nmol; black squares—100 nmol; white inverted triangles—160 nmol) in the inferior colliculus of GEPR ($n = 6$–7 per point).

2% Evan's blue dye was injected into the inferior colliculus using the same method as mentioned above. The brains of the animals were removed and, using a cryostat, cut into 30-μm sections. The presence of Evan's blue dye in the inferior colliculus confirmed the correct site of drug injection. Data from animals with incorrect injection sites were removed from statistical analysis.

### 2.2. Statistics

The $ED_{50}$ values for wild running, clonic seizure and tonic seizure during sound-induced seizures in DBA/2 mice and GEPR were calculated according to the method of Litchfield and Wilcoxon (1949).

In lethargic mice the anti-absence effects of the two compounds during the 3-h testing periods were analysed by calculating the mean spike and wave discharge durations observed following drug administration and comparing it to the corresponding mean spike and wave discharge durations observed following vehicle administration in matching 30-min segments, utilizing a two-tailed paired *t*-test for significant difference between vehicle- and drug-treated groups.

### 2.3. Compounds tested

AIDA was purchased from Tocris Cookson, Bristol. LY 367385 was from Eli Lilly, Windlesham (Dr. J.R. Harris). AIDA and LY 367385 were dissolved in a minimal amount of 1 M NaOH, and brought to volume with 10 mM phosphate-buffered saline. pH was adjusted with HCl to 7.6–7.8 (AIDA) or 7.0–7.4 (LY 367385) immediately prior to testing.

## 3. Results

### 3.1. Effect of AIDA in DBA / 2 mice

The administration of AIDA (10–500 nmol i.c.v.; Fig. 1B) suppressed sound-induced seizures with a maximal effect at 5–15 min (Fig. 1A), and gave significant anticonvulsant protection for $>60$ min. The $ED_{50}$ value at 15 min for clonic seizure suppression was 79 nmol. Toxic effects of this compound observed in the first 40 min following injection included: jumping, ataxia, splayed limbs, circling and hypoactivity, sometimes with short bursts of hyperactivity.

### 3.2. Effect of LY 367385 in DBA / 2 mice

The administration of LY 367385 (10–250 nmol, i.c.v.) suppressed sound-induced seizure response in DBA/2 mice (Fig. 1D); the effect being maximal at 5 min (Fig. 1C). The $ED_{50}$ value for clonic seizure suppression at 5 min was 12 nmol. The anticonvulsant effect of 250 nmol LY 367385 was of very short duration and gradually decreased between 5 and 120 min after i.c.v. administration (Fig. 1C). Toxic effects were mild and predominantly involved intermittent periods of sedation and hypolocomotion sometimes with splayed hind limbs, stretched forelimbs and tremor.

### 3.3. Effect of AIDA in lh / lh mice

The i.c.v. injection ($n=6$) of AIDA in *lh/lh* mice produced a dose-dependant suppression of spike and wave discharge (Fig. 2). The maximum reduction of seizure at

Table 1
$ED_{50}$ values of AIDA and LY 367385 for the anticonvulsant effects against seizures in GEPR induced by a loud sound

| Time after | Drug | $ED_{50}$ values with 95% confidence limits (nmol) | | |
|---|---|---|---|---|
| | | Wild running | Clonic | Tonic |
| 5 min | AIDA | 26 (11–62) | 26 (11–62) | 26 (11–62) |
| | LY 367385 | 186 (59–591) | – | – |
| 0.5 h | AIDA | 26 (9.4–73) | 11 (2.7–45) | 11 (2.7–45) |
| | LY 367385 | – | – | – |
| 1 h | AIDA | 71 (40–126) | 66 (16–272) | 45 (15.5–129) |
| | LY 367385 | – | 168 (21–1328) | 96 (32–288) |
| 2 h | AIDA | – | – | – |
| | LY 367385 | 67 (40–113) | 44 (21–91) | 6.8 (0.7–66) |
| 4 h | AIDA | – | – | – |
| | LY 367385 | 96 (32–288) | 71 (40–127) | 52 (29–93) |
| 1 day | AIDA | 116 (23–579) | – | – |
| | LY 367385 | 90 (51–161) | 96 (32–288) | – |
| 2 days | AIDA | – | – | – |
| | LY 367385 | 90 (51–161) | 41 (16–103) | 41 (16–103) |
| 3 days | AIDA | – | – | – |
| | LY 367385 | 96 (32–288) | 96 (32–288) | 96 (32–288) |

Table 2
Behavioural effects of intracollicular administration of mGluR1 antagonists in GEPR

| Drug | Dose (nmol) | Percentage of behavioural effects[a] | | | |
|---|---|---|---|---|---|
| | | Hypolocomotion | Teeth chattering | Head bobbing | Circling |
| AIDA | 10 | 83 (2) | 50 (3) | 16 (2) | – |
| | 50 | 100 (1) | 33 (2) | 33 (1) | – |
| | 100 | 100 (1) | 33 (2) | 33 (1) | 16 (3) |
| LY 367385 | 40 | 100 (4) | – | – | – |
| | 100 | 90 (3) | 16 (3) | – | 16 (4) |
| | 160 | 100 (1) | – | – | 16 (3) |

[a] Figures in brackets indicate the average time in min to onset from start of drug injection.

100 nmol was at 0–30 min (35%) but the effect was not significant ($p > 0.05$). At 500 nmol, the maximal effect was significant ($p < 0.05$) at 0–60 min (68–70%). This anti-absence effect persisted in a gradually decreasing manner with 50% protection at 60–120 min ($p < 0.01$) and 33% protection at 120–150 min ($p < 0.05$).

The *lh/lh* mice exhibited increased excitability and hyperactivity following administration of AIDA which coincided with seizure-suppression and reduction. At 500 nmol, hyperlocomotion ($n = 6$) occurred immediately after injection and continued around 60 min. The behavioural changes observed were increased mobility and ataxia, face washing, circling, hind-limb scratching, rearing ($n = 6$), tail biting ($n = 3$) and barrel rolling ($n = 2$). The duration of these activities was shorter (around 30 min) following the lower dose (100 nmol) of AIDA.

### 3.4. Effect of LY 367385 in lh / lh mice

LY 367385 (250 nmol, i.c.v) produced a significant ($p < 0.05$) reduction in spike and wave discharge duration at 30–60 min (78%) (Fig. 2). The antiabsence seizure effects were maintained until 150 min (63% protection, $p < 0.01$). The lower dose (50 nmol) showed similar latency to peak effect (60%, $p < 0.01$) but this protection was short-acting and non-significant for the rest of the time course studied.

The administration of LY 367385 (250 nmol) in *lh/lh* mice resulted in hypolocomotion lasting for approximately 30 min after injection, interspersed with temporary limb paralysis in addition to loss of righting reflex, and finally behavioural arrest in semi-prone posture for 4–12 min. These mice also showed mild activities such as rearing, face washing, straub tail, splayed feet, hind-limb scratching and falling due to loss of hind-limb co-ordination. However, the lower dose of LY 367385 (50 nmol) showed no significant behavioural effect at any time point.

### 3.5. Effect of focal injection of AIDA and LY 367385 into the inferior colliculus of GEPR

As shown in Fig. 3A, bilateral administration of AIDA (10–100 nmol/side) into the inferior colliculus of sound-sensitive GEPR ($n = 4–6$) caused a dose-dependent reduction in clonic seizures induced by sound. The peak anticonvulsant effect occurred with 100 nmol/side (the maximum dose used) at 30 min, but the anticonvulsant effect was present at 5 min (which was the earliest time tested), and offset time occurred at 1 h. The $ED_{50}$ values in nmol (with 95% confidence limits) against sound-induced wild running, clonic and tonic seizures at 30 min (when peak anticonvulsant effect occurred) were: 26.1 (9.4–72.6); 10.9 (2.7–44.7) and 10.9 (2.7–144.7), respectively. $ED_{50}$ values for AIDA at other time points are shown in Table 1.

As shown in Fig. 3B, LY 367385 (40–160 nmol/side) produced a dose-dependent reduction in sound-induced clonic seizures when injected into the inferior colliculus of GEPR ($n = 6–7$). The onset and offset of anticonvulsant effect occurred at 1 h and 3 days, respectively after drug administration. The peak anticonvulsant effect against clonic seizures at 2 and 4 h with $ED_{50}$ values in nmol/side (with 95% confidence limits) against sound-induced wild running, clonic and tonic seizures were: 67 (40–113); 44 (21–91); 6.8 (0.7–66) and 96 (32–288); 71 (40–127); 52 (29–93), respectively.

The behavioural effects of the two novel $mGlu_1$ antagonists, AIDA and LY 367385, when injected bilaterally into the inferior colliculus of GEPR are shown in Table 2. Hypolocomotion was the most frequent abnormal behavioural effect, at all doses. Other less frequent abnormal behavioural effects with AIDA were head bobbing (early onset), teeth chattering and circling. Teeth chattering and head bobbing were less evident after LY 367385. The proportion showing circling after the highest dose of LY 367385 was similar to that after AIDA.

## 4. Discussion

The powerful, immediate and relatively transient anticonvulsant action observed in DBA/2 mice after the i.c.v. injection of LY 367385 and AIDA is similar to the effect of (*S*)-4-carboxy-3-hydroxyphenylglycine previously reported (Thomsen et al., 1994; Dalby and Thomsen, 1996; Tang et al., 1997). These two compounds do not show the agonist action at $mGlu_2$ and $mGlu_3$ seen with (*S*)-4-

carboxy-3-hydroxyphenylglycine, and they therefore provide strong evidence that antagonist action at $mGlu_1$ is anticonvulsant.

This is consistent with the immediate convulsant action of group I agonists such as 3,5-DHPG. This immediate convulsant action may be due to direct and indirect effects on ion channels, with only the latter being related to phosphoinositide hydrolysis and the second messenger effects of diacylglycerol and $IP_3$. Thus group I agonists block the normal accommodation to a constant current pulse shown by isolated neurons and potentiate NMDA-receptor-mediated responses, enhance presynaptic glutamate release, and induce a depolarisation of neuronal membranes that depends on decreases in several $K^+$ currents and on enhanced $Na^+/Ca^{2+}$ exchange. These effects may contribute differentially in the various stages of epileptogenesis and in different epilepsy syndromes (Holmes et al., 1996). Nevertheless the powerful anticonvulsant effect of blocking the $mGlu_1$ responses suggests that activation of these receptors is contributing to the epileptic activity both in generalised tonic–clonic seizures and in absence-type attacks. Absence attacks in man and rodents have a pharmacology that differs from that of generalised convulsive seizures in several respects. Thus compounds that increase the extracellular concentration of γ-amino butyric acid (such as vigabatrin and tiagabine) protect against convulsive seizures but exacerbate spike and wave discharges (Hosford and Wang, 1997). $GABA_B$ receptor agonists enhance, and $GABA_B$ receptor antagonists diminish, spike and wave discharges in lethargic (*lh/lh*) mice (Hosford et al., 1992). Thus the similar effects of the $mGlu_1$ receptor antagonists in models of convulsive and non-convulsive epilepsy argue against an action similar to that of $GABA_B$ receptor antagonists.

We have previously reported (Tang et al., 1997) that intra-collicular injection in genetically epilepsy prone rats of (*S*)-4-carboxy-3-hydroxyphenylglycine (an $mGlu_{1a}$ antagonist, but a group II agonist) has a transient anticonvulsant action, similar in onset and duration to that reported here for AIDA. We also found that a group II agonist, (1*S*,3S)-1-aminocyclopentane-1.3-dicarboxylic acid, and a group III agonist, L-serine-*O*-phosphate, have a prolonged anticonvulsant action (2–3 days) with a delayed onset (1–2 h following intra-collicular injection). Thus in this model the effect of LY 367385 resembles that of group II or group III agonists rather than the $mGlu_{1a}$ receptor antagonists, (*S*)-4-carboxy-3-hydroxyphenylglycine and AIDA.

We cannot explain the differences in time course of action of the two $mGlu_1$ receptor antagonists, or their apparent differences in behavioural side effects. It is possible that the compounds differ in their effects on $mGlu_1$ splice variants. Additional effects, such as action on group II and group III receptors (Tang et al., 1997), could explain the delayed actions of LY 367385 in the inferior colliculus, but we have no evidence for such action. Possibly LY 367385 induces long term changes in the expression or function of mGlu receptors or other receptors.

The relative lack of motor side effects with i.c.v. administration in DBA/2 mice suggests that $mGlu_1$ receptor antagonists might have therapeutic potential in epilepsy. The wide range of effects mediated via group I mGlu receptors offers, however, the possibility of many novel side effects of anti-epileptic drugs acting on $mGlu_1$ receptors.

## Acknowledgements

We thank the Epilepsy Research Foundation for financial support.

## References

Burgess, D.L., Jones, J.M., Meisler, M.H., Noebels, J.L., 1997. Mutation of the $Ca^{2+}$ channel β4 subunit gene *Cchb4* is associated with ataxia and seizures in the lethargic (lh) mouse. Cell 88, 385–392.

Clark, B.P., Baker, S.R., Goldsworthy, J., Harris, J.R., Kingston, A.E., 1997. (+)-2-Methyl-4-carboxyphenylglycine (LY 367385) selectively antagonises metabotropic mGluR1 receptors. Bioorg. Med. Chem. Lett. 7, 2777–2780.

Conn, P.J., Pin, J.P., 1997. Pharmacology and functions of metabotropic glutamate receptors. Annu. Rev. Pharmacol. Toxicol. 37, 205–237.

Dalby, N.O., Thomsen, C., 1996. Modulation of seizure activity in mice by metabotropic glutamate receptor ligands. J. Pharmacol. Exp. Ther. 276, 516–522.

Ghauri, M., Chapman, A.G., Meldrum, B.S., 1996. Convulsant and anticonvulsant actions of agonists and antagonists of group III mGluRs. NeuroReport 7, 1469–1474.

Holmes, K.H., Keele, N.B., Shinnick-Gallagher, P., 1996. Loss of metabotropic glutamate receptor (mGluR)-mediated hyperpolarizations and increase in mGluR depolarizations in basolateral amygdala neurons in kindling-induced epilepsy. J. Neurophysiol. 76, 1208–1212.

Hosford, D.A., Wang, Y., 1997. Utility of the lethargic (*lh/lh*) mouse model of absence seizures in predicting the effects of lamotrigine, vigabatrin, tiagabine, gabapentin, and topiramate against human absence seizures. Epilepsia 38, 408–414.

Hosford, D.A., Clark, S., Cao, Z., Wilson, W.A. Jr., Lin, F., Morrisett, R.A., Huin, A., 1992. The role of $GABA_B$ receptor activation in absence seizures of lethargic (*lh/lh*) mice. Science 257, 398–401.

Jobe, P.C., Picchioni, A.L., Chin, L., 1973. Role of brain norepinephrine in audiogenic seizure in the rat. J. Pharmacol. Exp. Ther. 184, 1–10.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

McDonald, J.W., Fix, A.S., Tizzano, J.P., Schoepp, D.D., 1993. Seizures and brain injury in neonatal rats induced by 1*S*,3*R*-ACPD, a metabotropic glutamate receptor agonist. J. Neurosci. 13, 4445–4455.

Nakanishi, S., 1994. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. Neuron 13, 1031–1037.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York, NY.

Pellicciari, R., Luneia, R., Costantino, G., Marinozzi, M., Natalini, B., Jakobsen, P., Kanstrup, A., Lombardi, G., Moroni, F., Thomsen, C., 1995. 1-Aminoindan-1,5-dicarboxylic acid: a novel antagonist at phospholipase C-linked metabotropic glutamate receptors. J. Med. Chem. 38, 3717–3719.

Pin, J.-P., Duvoisin, R., 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34, 1–26.

Sacaan, A.I., Schoepp, D.D., 1992. Activation of hippocampal metabotropic excitatory amino acid receptors leads to seizures and neuronal damage. Neurosci. Lett. 139, 77–82.

Schoepp, D.D., Conn, P.J., 1993. Metabotropic glutamate receptors in brain function and pathology. TIPS 14, 13–20.

Schoepp, D.D., Goldsworthy, J., Johnson, B.G., Salhoff, C.R., Baker, S.R., 1994. 3,5-Dihydroxyphenylglycine is a highly selective agonist for phosphoinositide-linked metabotropic glutamate receptors in the rat hippocampus. J. Neurochem. 63, 769–772.

Tang, E., Yip, P.K., Chapman, A.G., Jane, D.E., Meldrum, B.S., 1997. Prolonged anticonvulsant action of glutamate metabotropic receptor agonists in inferior colliculus of genetically epilepsy-prone rats. Eur. J. Pharmacol. 327, 109–115.

Thomsen, C., Klitgaard, H., Sheardown, M., Jackson, H.C., Eskesen, K., Jacobsen, P., Treppendahl, S., Suzdak, P.D., 1994. (*S*)-4-carboxy-3-hydroxyphenylglycine, an antagonist of metabotropic glutamate receptor (mGluR)1a and an agonist of mGluR2, protects against audiogenic seizures in DBA/2 mice. J. Neurochem. 62, 2492–2495.

Tizzano, J.P., Griffey, K.I., Johnson, J.A., Fix, A.S., Helton, D.R., Schoepp, D.D., 1993. Intracerebral 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) produces limbic seizures that are not blocked by ionotropic glutamate receptor antagonists. Neurosci. Lett. 162, 12–16.

Tizzano, J.P., Griffey, K.I., Schoepp, D.D., 1995. Induction or protection of limbic seizures in mice by mGluR subtype selective agonists. Neuropharmacology 34, 1063–1067.