mGlu1 and mGlu5 receptor antagonists lack anticonvulsant efficacy in rodent models of difficult-to-treat partial epilepsy

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Received 7 November 2005; received in revised form 26 January 2006; accepted 1 February 2006

Abstract

Modulation of metabotropic glutamate (mGlu) receptors represents an interesting new approach for the treatment of a range of neurological and psychiatric disorders. Several lines of evidence suggest that functional blockade of group I (mGlu1 and mGlu5) receptors may be beneficial for treatment of epileptic seizures. This study was conducted to investigate whether mGlu1 or mGlu5 receptor antagonists have the potential to block partial or secondarily generalized seizures as occurring in partial epilepsy, the most common and difficult-to-treat type of epilepsy in patients. For this purpose, we systemically administered novel highly selective and brain penetrable group I mGlu receptor antagonists, i.e., the mGlu1 receptor antagonist EMQCM [3-ethyl-2-methyl-quinolin-6-yl-(4-methoxy-cyclohexyl)-methanone methanesulfonate] and the mGlu5 receptor antagonist MTEP ([(2-methyl-1,3-thiazol-4-yl) ethynyl] pyridine), at doses appropriate for mGlu1 or mGlu5 receptor-mediated effects in rodent models of partial seizures. Two models were used: the 6-Hz electroshock model of partial seizures in mice and the amygdala-kindling model in rats. Clinically established antiepileptic drugs were included in the experiments for comparison. Antiepileptic drugs exerted significant anticonvulsant effects in both models, while EMQCM and MTEP were ineffective in this regard, although both compounds were administered up to doses associated with essentially full receptor occupancy and with typical mGlu receptor-mediated effects in rodent models of anxiety or pain. Brain microdialysis for determining extracellular levels of MTEP following i.p. administration in rats substantiated that effective brain concentrations were reached at times of our experiments in seizure models. The present results do not support a significant anticonvulsant potential of group I mGlu receptor antagonists in rodent models of difficult-to-treat partial epilepsy.

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Keywords: Metabotropic glutamate receptors; Epilepsy; Antiepileptic drugs; Kindling

1. Introduction

The effectiveness of epilepsy therapy is limited by resistance to drug treatment in at least 30% of all patients, resulting in persistent seizures despite trials of multiple antiepileptic medications (Kwan and Brodie, 2000). Resistance to antiepileptic drugs (AEDs) is one of the most serious clinical problems in epilepsy, resulting in shortened lifespan, excessive bodily injury, neuropsychological and psychiatric impairment, and social disability (Sperling, 2004). Thus, there is a pressing need to develop new therapies for patients not controlled by current medications (Löschner and Schmidt, 2004).

Based on a variety of laboratory studies, metabotropic glutamate (mGlu) receptors appear to be interesting new targets for drug therapy in epilepsy (Meldrum et al., 1999; Doherty and Dingledine, 2002; Moldrich et al., 2003; Ritzen et al., 2005). mGlu receptors are a family of G-protein-coupled receptors comprising eight members, referred to as mGlu1—8, which are subclassified into three groups (Ritzen et al., 2005). Group I (mGlu1 and 5) receptors are located primarily post-synaptically, are positively coupled to inositol phosphate hydrolysis, and increase neuronal excitation, making these
receptors an an attractive drug target in diverse diseases, including epilepsy (Ritzen et al., 2005). Agonists acting on mGlu1 or mGlu5 receptors are convulsant, while mGlu1 and mGlu5 receptor antagonists are anticonvulsant in rodent models of generalized seizures and absence seizures (Moldrich et al., 2003). However, the latter seizure types are commonly easy to treat in patients with epilepsy, while partial (focal) seizures, such as occurring in temporal lobe epilepsy (TLE), the most common type of epilepsy in humans, are frequently resistant to existing AEDs (Browne and Holmes, 2001). Thus, in terms of eventual clinical utility, it would be important to study mGlu1 or mGlu5 receptor antagonists in rodent models of partial seizures to assess their potential efficacy in difficult-to-treat types of epilepsy.

The amygdala-kindling model of TLE in rats is an established model of difficult-to-treat partial and secondarily generalized seizures, which is widely used in the preclinical evaluation of novel AEDs (White, 2003). The kindling model correctly predicted the antiepileptic efficacy of most AEDs against partial seizures in patients with epilepsy (Löscher, 1998a). However, as in patients, partial and secondarily generalized kindled seizures are more difficult to suppress by AEDs than primary generalized seizures (Löscher, 1997). With respect to mGlu receptors, an upregulation of mGlu1 and mGlu5 receptor expression has been reported in brain regions of kindled rats (Akbar et al., 1996; Al Ghoul et al., 1998; Nagaraja et al., 2004) as well as in humans with TLE (Blümcke et al., 2000), indicating that group I mGlu receptors may contribute to seizure susceptibility. In a recent study in amygdala-kindled rats, the selective mGlu1 receptor antagonist LY456236 [(4-methoxy-phenyl)-(6-methoxy-quinazolin-4-yl)-amine HCl] was shown to exert dose-related anticonvulsant effects at oral doses of 10–60 mg/kg, suggesting that blockade of mGlu1 receptors may form a clinically useful approach to the treatment of TLE (Shannon et al., 2005). To our knowledge, mGlu5 receptor antagonists have not yet been studied in the amygdala-kindling model.

Another potentially useful model in the search of new AEDs against difficult-to-treat partial or limbic epilepsy is the 6-Hz “psychomotor” seizure model in mice (Brown et al., 1953; Barton et al., 2001; Kaminski et al., 2004). In contrast to kindled rats, in which chronic brain hyperexcitability is produced by repeated application of initially subconvulsive electrical stimuli, the 6-Hz electroshock model is an acute seizure model using a low-frequency, long-duration stimulation paradigm in mice to induce partial seizures that are resistant to several AEDs (Barton et al., 2001). Barton et al. (2003) reported that the mGlu1 receptor antagonist LY456236 and the prototypical mGlu5 receptor antagonist MTEP [2-methyl-6-(phenylethynyl)pyridine HCl] produced dose-dependent protection in the 6-Hz electroshock model at doses below those inducing neurotoxicity.

The aim of the present study was to further characterize the anticonvulsive effects of selective, systemically active mGlu1 and mGlu5 receptor antagonists in the kindling and 6-Hz electroshock models of partial seizures. For this purpose, the mGlu1 receptor antagonist EMQMCM [3-ethyl-2-methyl-quinolin-6-yl-(4-methoxy-cyclohexyl)-methanone methanesulfonate] (Lesage et al., 2002) and the mGlu5 receptor antagonist MTEP [(2-methyl-1,3-thiazol-4-yl) ethynyl] pyridine] (Cosford et al., 2003) were used. We have recently evaluated the activity of these compounds in animal models of anxiety (Pietraszek et al., 2005a), of learning (Gravius et al., 2005) and in a prepulse inhibition model and locomotor activity tests (Pietraszek et al., 2005b), but, to our knowledge, the anticonvulsant activity of these potent and highly selective group I metabotropic receptor antagonists has not been previously studied. In addition to testing these compounds in seizure models at doses appropriate for mGlu1 or mGlu5 receptor-mediated effects, we also investigated their brain penetration in rats, using brain microdialysis.

2. Materials and methods

2.1. 6-Hz psychomotor seizures in mice

2.1.1. Animals

Adult male NMRI mice (Elevage-Janvier, Strasbourg, France) weighing between 20 and 30 g were used in the experiments. All animals were allowed free access to food (chow pellets) and tap water and were housed in a temperature-, humidity-, and light-controlled environment (12-h on/12-h off). In the studies each animal was employed only once. The experiments were approved by the Ethical Committee (Regierungspraesidium Darmstadt, Hessen, Germany) and performed in accordance with the recommendations and policies of the US National Institutes of Health Guidelines for the Use of Animals.

2.1.2. Drugs

Carbamazepine (Sigma, Munich, Germany) was suspended in 1% solution of Tween 80 in distilled water and administered in a dose range of 5–40 mg/kg, 15 min before the experiment. A commercial solution of diazepam (Diazepam-Ratiopharm, Ratiopharm, Ulm, Germany) was diluted to the final concentration with 1% Tween and administered at 0.5–5 mg/kg, 30 min before the electrical stimulation. Sodium valproate (Sigma, Munich, Germany) was dissolved in physiological saline and injected at 100–175 mg/kg, 15 min before the test. EMQMCM (synthesized by Merz Pharmaceuticals, Frankfurt, Germany) was dissolved in distilled water containing 10% Tween 80 and administered at 5–40 mg/kg, 30 min prior to the experiment. MTEP (synthesized by Merz) was dissolved in 10% Tween 80 solution in distilled water and injected in a dose range of 10–40 mg/kg, 30 min before the test. All the drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg.

2.1.3. Induction of seizures and drug testing

‘Psychomotor’ seizures were induced via corneal stimulation (6 Hz, 0.2 ms rectangular pulse width, 3 s duration) using an ECT Unit 57800 (Ugo Basile, Italy). A drop of 0.9% saline was placed on the eyes prior to the placement of corneal electrodes. Animals were manually restrained and released immediately following the stimulation and observed for the presence or absence of seizure activity. The seizure was characterized by: stum, forelimb clonus, twitching of the vibrissae, and Straub-tail. Protection against seizures was defined as the absence of at least three of the features listed above. Before starting the drug experiments, the median convulsive current (CC50, a current intensity necessary to induce seizures in 50% of the population) was determined using different shock intensities ranging from 8 to 32 mA.

Pharmacological studies were conducted at a fixed stimulus intensity of 32 mA, identical with the current used in previous studies (Barton et al., 2003; Shannon et al., 2005). The model was first validated using established AEDs shown to be effective in TLE and in the amygdala kindling model, i.e. diazepam, carbamazepine and valproic acid (Löscher, 1997, 1998b).

2.1.4. Statistical analysis

For the determination of the CC50 value, a minimum of 9–11 mice was used per dose. The CC50 was calculated by means of probit analysis (Litchfield...
and Wilcoxon, 1949). In the pharmacological experiments, a minimum of eight animals per dose was used to calculate the median effective dose (ED₅₀) and corresponding 95% confidence intervals with probit analysis (Litchfield and Wilcoxon, 1949). The effect of each of the drug was quantified by varying the dose between the limits of 0% protection and 100% protection. Where the calculation of the ED₅₀ was not possible (i.e., in cases when even at the highest dosing level the stimulation-induced seizures were not suppressed by more than 50% of animals in the experimental group), the Fisher’s exact test was used. With respect to the use of this test with group sizes of eight mice, it should be noted that at least 60% of the animals have to be protected by drug treatment to obtain a significant difference to vehicle control.

2.2. Amygdala-kindled seizures in rats

2.2.1. Animals

Female Wistar rats were purchased at a body weight of 200–220 g (Harlan Winkelmann Versuchstierzucht, Borchern, Germany) and were then kept under controlled environmental conditions (24–25 °C, 50–60% humidity, 12 h light/dark cycle) with free access to standard laboratory chow (Altromin 1324 standard diet) and tap water. All experiments were performed at the same time of day, i.e., in the morning to minimize possible effects of circadian variation. During the period of experiments animals had a body weight between 232 g and 378 g. All animal experiments were carried out in accordance with the European Communities Council Directive of 24. November 1986 (86/609/ECC) and were formally approved by the animal subjects review board of our institution. All efforts were made to minimize the number of animals used and their suffering.

2.2.2. Electrode implantation

For implantation of kindling electrodes rats were anaesthetized with chloral hydrate (360 mg/kg, i.p.), the skull surface was exposed, and a bipolar electrode was implanted into the right hemisphere aimed at the basolateral amygdala using the following stereotaxic coordinates according to the atlas of Paxinos and Watson (1986): 2.2 mm caudal, 4.8 mm lateral, 8.5 mm ventral (all with respect to bregma). The electrodes consisted of two twisted Teflon-coated stainless steel wires (250 μm diameter) separated by 0.5 mm at the tip. A screw, which served as grounding electrode, was positioned over the left parietal cortex. Bipolar and ground electrodes were connected to plugs, and the electrode assembly and anchor screws were held in place with dental acrylic cement applied to the exposed skull surface. After surgery, the rats were treated with antibiotics for 1 week to prevent infection.

2.2.3. Kindling procedure and experiments in fully kindled animals

Following a post-operative recovery period of 2 weeks, constant current stimulations (330 μA, 1 ms, monophasic square-wave pulses, 50 Hz for 1 s) were delivered to the amygdala once daily (five times per week) until at least 10 sequential fully kindled stage-5 seizures were elicited. For drug testing in these fully kindled rats, seizures were either induced by fixed, suprathreshold stimulation with 400 μA or by individual determination of the threshold for after-discharges (ADT) recorded from the basolateral amygdala before and after drug administration. The ADT was determined by administering a series of stimulations at intervals of 1 min increasing in steps of about 20% of the previously applied current. The ADT was defined as the lowest current intensity producing after-discharges with a duration of at least 5 s. Determination of afterdischarge threshold was repeated at least two times to prove reproducibility before animals were used for drug testing. If only focal seizure activity was induced at the ADT current, the procedure was continued with serial stimulation at intervals of 1 min to determine the generalized seizure threshold (GST). The generalized seizure threshold was defined as the lowest current intensity producing generalized seizure activity lasting at least 5 s. In all experiments, seizure duration and afterdischarge duration were recorded in addition to seizure severity and afterdischarge threshold.

Seizure severity was scored according to Racine (1972): 1, immobility, eye closure, ear twitching, twitching of vibrissae, sniffing, facial clonus; 2, head nodding associated with more severe facial clonus; 3, clonus of one forelimb; 3.5, bilateral clonus without rearing; 4, bilateral clonus accompanied by rearing; 4.5, generalized clonic seizures without rearing and falling (e.g. because of direct loss of balance); 5, rearing and falling accompanied by generalized clonic seizures. Seizure duration was the time period of limbic and/or motor seizures. Limbic seizure activity which sometimes occurred after termination of secondarily generalized seizures was not included in seizure duration. Afterdischarge duration was defined as the period of high amplitude spiking (at least 1 Hz frequency and twice the pre-stimulation amplitude) in the electroencephalogram (EEG) of the amygdala electrode, including the time of stimulation.

2.2.4. Drug experiments

Drug experiments were performed in a total of 17 fully kindled rats. Eight of these rats (age 14–20 months; “group 1”) had been used previously to test other compounds. The period between the previous and current studies was long enough (at least 1 month), so that there was ample time for complete washout from the previously tested drug. Experiments in group 1 were repeated in a second group of nine rats (age 6–9 months; “group 2”) in order to exclude that the negative data obtained in group 1 were a result of the high age or previous drug history of this group.

In a first series of experiments in group 1, a fixed (suprathreshold) stimulus of 400 μA was used for drug testing. In a second series, individual ADTs and GSTs were determined in each rat after drug administration. In the second group of rats, determination of drug effects on ADT and GST was repeated as done in group 1. Pretreatment time was 60 min for carbamazepine and 30 min for the other compounds. Control recordings were done 3–4 days before and after each drug experiment with i.p. injection of vehicle. Carbamazepine was dissolved in 30% polyethylene glycol 400 in distilled water. EMQMCM and MTEP were dissolved in 10% Tween 80 in distilled water. The pH was adjusted to 6–7 for EMQMCM and to 3–4 for MTEP. Injection volume was 2 ml/kg bodyweight. Dose selection of carbamazepine was based on previous experiments in kindled rats, showing that a dose of 20 mg/kg i.p. exerts significant anticonvulsant activity in this model (Loesch, 1998b). Selection of doses of EMQMCM and MTEP was based on previous experiments with these compounds in different rat models (Gravius et al., 2005; Pietraszek et al., 2005a,b).

The kindled rats were allowed to adapt to the laboratory environment, then body temperature was measured and animals were put into open cages for constant observation. Fifteen and 28 min (EMQMCM and MTEP) or 28 and 58 min (carbamazepine) following drug or vehicle administration, behavioural alterations and body temperature were determined. Adverse effects were scored during observation in open cages and in an open field. In addition, rats were subjected to the rota rod test (polypropylene, foam-coated rod, 5 cm in diameter, 8 rpm) as described previously (Hönack and Lösch, 1995). Animals were considered to have failed this test, when they fell from the rod in each of three consecutive 1-min attempts.

Ataxia and sedation were scored as follows (Hönack and Lösch, 1995). Ataxia: 0, absent; 1, slight ataxia in hindlegs; 2, more pronounced ataxia with dragging of hindlegs; 3, further increase of ataxia and more pronounced dragging of hindlegs; 4, marked ataxia and loss of balance during forward locomotion; 5, very marked ataxia with frequent loss of balance; and 6, permanent loss of righting reflex. Sedation: 0, absent; 1, slightly reduced forward locomotion; 2, reduced locomotion with rest periods between periods of locomotion; 3, reduced locomotion with more frequent rest periods; and 4, no forward locomotion and animal sits quietly with closed eyes. Other adverse effects (reduced righting reflex, flat body posture, circling, Straub tail, piloerection, hyperlocomotion) were scored by 0 (absent), 1 (equivocal), 2 (present) and 3 (intense).

2.2.5. Statistical analysis

Statistical significance of seizure data was calculated by Wilcoxon signed rank test for paired replicates.

2.3. In vivo microdialysis in freely moving animals

2.3.1. Animals

Experimentally naive adult male Sprague–Dawley rats (230–250 g) were kept four or five per cage, in a room with controlled temperature (21 ± 1 °C) and humidity. Food and water were available ad libitum and the animals were...
kept under an alternating 12 h/12 h day–night cycle (lights on at 07:00) for at least 5 days before surgery. After surgery animals were housed individually in modified Macrolon® type III cages. Each animal was used once only. The experiments were approved by the Ethical Committee (Regierungspräsidium Darmstadt, Hessen; Germany) and performed in accordance with the recommendations and policies of the US National Institutes of Health Guidelines for the Use of Animals.

2.3.2. Surgery

Using standard stereotaxic procedures, siliconized guide cannulas (MAB 6.14, MAB, Stockholm, Sweden) were implanted unilaterally in anaesthetized animals (50 mg/kg pentobarbital, i.p., pre-treatment with 0.1 ml atropine sulphate i.p.) aiming at the caudate-putamen (CPu; AP, +0.2 mm; L, +2.6 or −2.6 mm; DV, −3.2 mm relative to bregma) with the incisor bar set to 3.3 mm below the interaural line according to the atlas of Paxinos and Watson (1986). Each rat was given at least 2 days to recover from the surgery before starting microdialysis experiments.

2.3.3. Microdialysis

Microdialysis experiments were performed in the home cage of the animal with the lid replaced by an acrylic frame bearing a counter-balanced arm with the swivel assembly. A microdialysis probe (MAB 6.14, 4 mm exposed membrane length, PES membrane MAB, Stockholm, Sweden) was lowered through the guide cannula to the CPu (ventral position of probe tip with reference to the skull: −7.2 mm) up to 18 h before the onset of microdialysis experiments. Food pellets were available throughout the experiment.

The probes were perfused with artificial cerebrospinal fluid (aCSF) at a flow rate of 2 μl/min using a CMA 102 or a CMA 100 perfusion pump (CMA, Solna, Sweden). The composition of the aCSF was 147 mM Na⁺, 2.7 mM K⁺, 1.2 mM Ca²⁺, 0.85 mM Mg²⁺. The sample collection was started 1 h after start of perfusion. Three 20-min fractions were taken to obtain baseline values. Thereafter MTEP was applied i.p. followed by sample collection for 180 min (nine samples). The samples (40 μl) were collected systematically with a fraction collector (CMA 142 or CMA 140; CMA, Solna, Sweden) and stored frozen (−20°C) until analysis. The animals were connected by a head block tether system (Instech, Plymouth Meeting, USA or self-made) to a dual channel liquid swivel 375/D/22QM (Instech, Plymouth Meeting, USA) or a collar with connector to a dual channel liquid swivel 375/D/22QM (Instech, Plymouth Meeting, USA). FEP tubing and tubing adapters (CMA, Solna, Sweden or MAB, Stockholm, Sweden) were used.

All data were corrected for in vitro recovery. The average in vitro recovery of MTEP using MAB 6.14.4 probes was 16.7% under the applied dialysis conditions.

MTEP in dialysate was analyzed by means of high performance liquid chromatography (HPLC) in combination with atmospheric pressure ionization tandem mass spectrometry (API-MS/MS). The instrument setup consisted of a HPLC (model 1100, Agilent Technologies, Waldbronn, Germany) coupled to API 4000 Q Trap (tripole quadrupole, Applied Biosystems/MD Sciex, Darmstadt, Germany) equipped with a Turbolonspray source (ESI) operating in MRM (Multiple Reaction Monitoring) detection mode. A Synergi Polar-RP 80A column (100 × 4.6 mm, 4 μm, Phenomenex, Aschaffenburg, Germany) was used. The detection limit for MTEP was 5 nM. We also performed microdialysis experiments with EMQMCM, but data were not further evaluated (see Section 3).

3. Results

3.1. 6-Hz psychomotor seizures in mice

The CC50 value for induction of seizures in this model was found to be 17.6 mA (95% confidence interval 14.9–20.8 mA, r = 0.98), which is within the range of values determined recently by Barton et al. (2001) and Kaminski et al. (2004). At a fixed current intensity of 32 mA, drugs with established antiepileptic efficacy, i.e., diazepam, carbamazepine and valproic acid, displayed protective effects in the 6-Hz electroshock model. Diazepam exerted a dose-dependent anticonvulsant effect with an ED50 value of 1.6 mg/kg (Table 1). Likewise, carbamazepine dose-dependently protected the mice against seizures produced by the 6-Hz electrical stimulation. The calculated ED50 of carbamazepine was found to be 19.5 mg/kg (Table 1). Also, the administration of valproic acid afforded a dose-dependent protection in this model of partial seizures. The ED50 value of valproic acid was 127 mg/kg (Table 1).

In contrast to clinically established AEDs, the mGlu1 receptor ligand, EMQMCM, exerted only a modest protective effect in the 6-Hz seizure model. Even at the highest dose employed in the experiments, i.e., 40 mg/kg, EMQMCM afforded protection in only 50% of the group population (Table 2). The effect failed to reach statistical significance as assessed by means of the Fisher’s exact test (p = 0.0769; Table 2). Higher doses were not tested, as they would markedly exceed the range expected to cause complete in vivo mGlu receptor occupancy (Lavreysen et al., 2004) and produce motor impairment (Pietraszek et al., 2005a).

Similar to EMQMCM, the mGlu5 receptor antagonist MTEP exerted only weak anticonvulsant effects in the 6-Hz electroshock model (Table 1). All doses of MTEP used in the present experiments produced very weak, insignificant protective effects in up to 20% of mice stimulated with 6-Hz current of 32-mA intensity (Table 2).

The mice used in 6-Hz electroshock test did not show any overt toxicity signs at any dose level of the mGlu receptor antagonists. However, a separate study in mice, aimed specifically at assessing overall behavioral toxicity, revealed that ataxia (rotarod test) and sedation for these agents start to be seen at 12–36 mg/kg (data not shown).

3.2. Amygdala-kindled seizures in rats

3.2.1. Suprathreshold stimulation in group 1

Administration of 20 mg/kg carbamazepine resulted in a modest but statistically significant decrease of seizure severity when using fixed, suprathreshold stimulation (Table 3). Furthermore, the duration of seizures and after-discharges tended to be reduced by carbamazepine, although this did not lead to statistically significant changes.

Table 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED50 (mg/kg)</th>
<th>95% confidence interval (mg/kg)</th>
<th>r value</th>
</tr>
</thead>
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<tr>
<td>Diazepam</td>
<td>1.6</td>
<td>0.8–3.3</td>
<td>0.98</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>19.5</td>
<td>13.1–29.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>127</td>
<td>107–151</td>
<td>0.98</td>
</tr>
<tr>
<td>MTEP*</td>
<td>&gt;40</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>EMQMCM*</td>
<td>&gt;40</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

ED50 values (expressed in mg/kg) were determined, where applicable, using probit analysis (Litchfield and Wilcoxon, 1949). n.a., not applicable.

* Even at the highest dose used in this experiment, i.e., 40 mg/kg, protection against behavioural seizures did not exceed 50% (see Table 2).
Administration of EMQMCM, 5 mg/kg i.p., did not induce any significant alteration in ADT or GST (Figs. 3 and 4). Furthermore, seizure parameters recorded at ADT or GST currents were not changed by EMQMCM. Similarly, administration of MTEP, 5 mg/kg i.p., did not result in any increase of ADT or GST, or alterations in seizure parameters recorded at ADT or GST currents (Figs. 3 and 4).

3.2.4. Adverse effects in kindled rats

Carbamazepine administration resulted in moderate ataxia and sedation (Table 4), and a slight reduction of the righting reflex. All rats passed the rotarod test (not illustrated). Following EMQMCM or MTEP, only slight signs of ataxia, sedation, and reduced righting reflex were observed (Table 4). All rats treated with the mGlu receptor antagonists passed the rotarod test. Body temperature was significantly decreased by all compounds, both compared to individual predrug values and to respective vehicle control values (Table 4).

3.3. In vivo microdialysis in rats

Following i.p. administration of MTEP, 5 mg/kg, in rats, peak concentrations in brain extracellular fluid of 4600 nM on average were determined in the dialysate sample collected from 20 to 40 min after drug injection (Fig. 5). A similar study with EMQMCM failed to provide reliable results (probably due to substance binding to the microdialysis probe and/or tubing), therefore these data are not presented here. However, preliminary data on plasma and brain homogenate levels of EMQMCM in rats are provided in the discussion.

4. Discussion

In view of previous reports on other mGlu1 and mGlu5 receptor antagonists (Barton et al., 2003; Shannon et al., 2005), the results of the present study were quite unexpected, because neither of the highly selective, potent and systemically active mGlu1 or mGlu5 antagonists exerted any robust anticonvulsant effect in the rodent models of partial seizures at doses appropriate for mGlu1 or mGlu5 receptor-mediated effects. In order to avoid non-selective drug actions, we restricted the dose range examined in these seizure models to doses that (1) lead to up to complete receptor occupancy in vivo (Anderson et al., 2003; Lavreysen et al., 2004), and (2) exert mGlu receptor-associated effects in other rodent models not reach statistical significance. Neither EMQMCM (5 mg/kg) nor MTEP (5 mg/kg) exerted any significant effect on seizure severity (Table 3). However, afterdischarge duration was significantly increased by MTEP (Table 3). Because significant anticonvulsant effects can be missed when using a fixed, suprathreshold current in all rats, we undertook a second series of experiments in which we determined the anticonvulsant activity of carbamazepine and MTEP on individual seizure thresholds (ADT/GST).

3.2.2. ADT/GST determination in group 1

Carbamazepine, 20 mg/kg i.p., produced a significant increase (300% of control) of the ADT (Fig. 1). In addition, GST was significantly increased (154% of control; Fig. 2). Seizure parameters recorded at ADT and GST currents were not changed significantly by carbamazepine.

MTEP, 5 mg/kg i.p., did not produce a significant increase of ADT or GST (Figs. 1 and 2). Seizure parameters at ADT and GST current were not changed in a significant manner.

In order to exclude that the lack of any anticonvulsant activity of MTEP and EMQMCM was due to the high age of rats used in group 1, we repeated the experiment in a second group of younger rats.

3.2.3. ADT/GST determination in group 2

Carbamazepine, 20 mg/kg i.p., produced a significant increase (242% of control) of the ADT (Fig. 3). GST was significantly increased (229% of control), too (Fig. 4). Seizure parameters at ADT and GST current were not changed significantly by carbamazepine.
(Busse et al., 2004; Gravius et al., 2005; Klodzinska et al., 2004; Pietraszek et al., 2005a, b; Varty et al., 2005). As shown by the present microdialysis experiments with the mGlu5 receptor antagonist MTEP (5 mg/kg i.p.), this drug penetrates well into the brain, reaching extracellular concentrations of up to 5 μM within 40 min (based on in vitro recovery). For comparison, in vitro binding affinity of MTEP for mGlu5 receptors is about 15–30 nM (Cosford et al., 2003; C.G.}

**Fig. 1.** Effect of carbamazepine (20 mg/kg) and MTEP (5 mg/kg) on afterdischarge threshold (ADT) and seizure parameters recorded at ADT in amygdala-kindled rats of group 1 (age 14–20 months). Data are mean values ± SEM of eight rats. Individual graphs give results on ADT, seizure severity (SS), seizure duration (SD), and afterdischarge duration (ADD). Significant differences between control and drug experiments are marked by asterisk (p < 0.05).

**Fig. 2.** Effect of carbamazepine (20 mg/kg) and MTEP (5 mg/kg) on generalized seizure threshold (GST) and seizure parameters recorded at GST in amygdala-kindled rats of group 1 (age 14–20 months). Data are mean values ± SEM of eight rats. Individual graphs give results on GST, seizure severity (SS), seizure duration (SD), and afterdischarge duration (ADD). Significant differences between control and drug experiments are marked by asterisk (p < 0.05).
Parsons, unpublished data) with over 5000 fold selectivity vs. mGlu1 receptors. Following i.p. administration of 3 mg/kg MTEP, full (100%) occupancy of mGlu5 receptors was rapidly achieved for at least 1 h in rats, and MTEP rapidly reached 75% receptor occupancy at this dose in mice (Anderson et al., 2003). At 5 mg/kg in rats, MTEP causes impairment of radial maze learning (Gravius et al., 2004), inhibition of fear potentiated startle, enhancement of locomotor activity induced by the N-methyl-D-aspartate (NMDA) antagonist MK-801 (dizocilpine), and enhancement of disruption of prepulse inhibition by MK-801 (Pietraszek et al., 2005a,b).

The mGlu1 receptor antagonist EMQMCM was also effective in several of these and other paradigms in rats at 5 mg/kg or below (Gravius et al., 2005; Pietraszek et al., 2005a,b). Our microdialysis study with EMQMCM in rats failed to provide reliable results due to technical problems (likely binding of this agent to the microdialysis probe or tubing). However, preliminary experiments of our group in rats indicate that 20–30 min after i.p. administration of EMQMCM at 5 mg/kg i.p., drug levels are about 450 nM in plasma and 700 nM in brain homogenates. These values are far above the in vitro binding affinity of this agent for mGlu1 receptors (about 1 nM as assessed in inositol triphosphate assay in cerebellar granule cells; C.G. Parsons, unpublished data). In vitro binding assays indicated an over 100-fold selectivity of EMQMCM for mGlu1 vs. mGlu2, mGlu4, mGlu5, mGlu6 and mGlu8 receptors, respectively (C.G. Parsons, unpublished data).

Furthermore, as to the selectivity of EMQMCM, we tested this compound at 10 µM concentration (over 1000-fold higher concentration than affinity at mGluR1) on 147 targets (so called Panlab Screen) and only three targets showed any activity at this concentration, i.e., 5-HT2B (58% inhibition), sigma1 (60% inhibition), and monoamine transporter (73% inhibition), respectively (C.G. Parsons, unpublished data).

Based on these data with EMQMCM and MTEP, we used 5 mg/kg of these compounds as a maximal necessary dose to address the involvement of mGlu1 and mGlu5 receptors in the kindling model. Neither compound exerted any anticonvulsant effect, but both compounds significantly decreased body temperature. Higher doses, exceeding those associated with complete receptor occupancy, were not tested in rats, because such doses are likely to be associated with non-selective actions. Furthermore, EMQMCM at 10 mg/kg, and MTEP at 20 mg/kg produce ataxia in the rotarod test in rats (Pietraszek et al., 2005a). In addition, MTEP, at 10 mg/kg, and EMQMCM, at 5 and 10 mg/kg, given 30 min before training, impaired acquisition of the passive avoidance response in rats (Gravius et al., 2005).

In the 6-Hz seizure model in mice, we tested doses of these antagonists of group I metabotropic glutamate receptors of up to 40 mg/kg without observing any significant anticonvulsant activity. Although EMQMCM blocked seizures in 50% of mice at the highest dose (40 mg/kg) tested, this dose is high above the dose range (0.6–10 mg/kg) associated with
significant effects in other rodent models (Gravius et al., 2005; Pietraszek et al., 2005a,b), indicating that any effect at 40 mg/kg does not reflect actions at mGlu1 receptors. The lack of any significant anticonvulsant effects clearly contrasted EMQMCM and MTEP from clinically established AEDs, which exerted significant anticonvulsant effects in both the 6-Hz and kindling models.

To our knowledge, neither EMQMCM nor MTEP have been tested previously in models of seizures or epilepsy. However, the non-competitive mGlu1 receptor antagonist LY456236 was recently reported to exert anticonvulsant activity in the 6-Hz electroshock and kindling models (Barton et al., 2003; Shannon et al., 2005). Using the same stimulus strength (32 mA) as in the present study in the 6-Hz mouse model, ED50 values of 16 (Barton et al., 2003) or 29 mg/kg (Shannon et al., 2005) were reported for LY456236, which is below the neurotoxic ED50 of this compound (59 mg/kg) determined in the rotarod test in mice (Barton et al., 2003). In amygdala-kindled rats, LY456236 significantly increased ADT only at a high dose of 30 mg/kg, but decreased severity and duration of seizures (recorded at ADT current) already at 10 mg/kg (Shannon et al., 2005). Thus, these data with LY456236 are in apparent contrast to the present lack of any significant anticonvulsant effects of the mGlu1 receptor antagonist EMQMCM. One possible explanation is that the anticonvulsant effects associated with high doses of CBZ MTEP

Table 4
Adverse effects of carbamazepine, EMQMCM and MTEP in kindled rats. Ataxia and sedation were scored by a rating system (see Section 2).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ataxia (score)</th>
<th>Sedation (score)</th>
<th>Body temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before drug</td>
<td>After drug</td>
<td></td>
</tr>
<tr>
<td>Vehicle control</td>
<td>0</td>
<td>0</td>
<td>38.29 ± 0.36</td>
</tr>
<tr>
<td>Carbamazepine (20 mg/kg i.p.)</td>
<td>1.7 ± 0.16</td>
<td>1.5 ± 0.12</td>
<td>38.33 ± 0.11</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>0</td>
<td>0</td>
<td>38.38 ± 0.09</td>
</tr>
<tr>
<td>EMQMCM (5 mg/kg i.p.)</td>
<td>1.22 ± 0.15</td>
<td>1 ± 0</td>
<td>38.66 ± 0.07</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>0</td>
<td>0</td>
<td>38.37 ± 0.08</td>
</tr>
<tr>
<td>MTEP (5 mg/kg i.p.)</td>
<td>0.59 ± 0.12</td>
<td>1 ± 0</td>
<td>38.34 ± 0.08</td>
</tr>
</tbody>
</table>

Rectal body temperature was determined before and after drug administration. Recordings after drug administration were done shortly before amygdala stimulation, i.e., 58 min after injection of carbamazepine or 28 min after injection of the mGlu receptor antagonists. For comparison, vehicle control data are shown. Data are shown as means ± SEM of 17 rats, respectively. For body temperature, significant difference to predrug control is indicated by asterisk (p < 0.05). Furthermore, significant difference between body temperature after drug or vehicle treatment is indicated (p < 0.05).
LY456236 in mice and rats do not reflect actions at mGlu1 receptors.

With respect to mGlu5 receptor antagonists, Barton et al. (2003) reported that MPEP blocks seizures in the 6-Hz electroshock model in mice with an ED50 of 17.4 mg/kg i.p., which was clearly below its neurotoxic ED50 of 145 mg/kg in the rotenoid test. However, it is important to note that the ED50 of MPEP determined by Barton et al. (2003) in the 6-Hz model is well above the doses of MPEP needed to obtain full (100%) mGlu5 receptor occupancy in mice (Anderson et al., 2003). Thus, the anticonvulsant effect of MPEP in the 6-Hz electroshock model is likely to be due to other, non-specific effects of MPEP, which are not related to mGlu5 receptors, as recently shown for the neuroprotective effect of MPEP (Lea et al., 2005). Although MPEP shows excellent selectivity as an antagonist for mGlu5 receptors (IC50 2–30 nM, depending on the preparation) over all other mGlu receptor subtypes, this compound does have a weak allosteric modulatory action at mGlu4 receptors (Ritzen et al., 2005). It also has also been reported to have moderate affinity for NMDA receptors and is furthermore an inhibitor of the noradrenaline transporter with an IC50 of 2.8 μM (Ritzen et al., 2005), although our own unpublished data do not support these findings.

In apparent contrast to the results obtained by Barton et al. (2003) with MPEP, no significant anticonvulsant activity was observed with the highly selective mGlu5 receptor antagonist MTEP in the present experiments in the 6-Hz electroshock model, although doses of up to 40 mg/kg, i.e., doses well above those needed to achieve full mGlu5 receptor occupancy in mice, were tested. MTEP is as potent and selective as MPEP at Glu5 receptors but is claimed to be more selective than MPEP at NMDA receptors (Cosford et al., 2003), which may explain the different anticonvulsant effect of MPEP and MTEP at high doses, because antagonism at NMDA receptors is known to be associated with anticonvulsant effects in electroshock models, including the 6-Hz model (Barton et al., 2003). MTEP is a widely used, highly selective mGlu5 receptor antagonist in the experimental modulation of pain and anxiety (Cosford et al., 2003; Busse et al., 2004; Klodzinska et al., 2004; Zhu et al., 2004; Pietraszek et al., 2005a; Varty et al., 2005), but it has not yet been tested in seizure models. Furthermore, no data are available on any mGlu5 receptor antagonist in the kindling model of TLE. The present data with MTEP do not suggest that antagonism of mGlu5 receptors is an efficacious strategy to block partial seizures in the 6-Hz electroschock or kindling models.

In conclusion, in contrast to clinically established AEDs, both mGlu1 and mGlu5 antagonists failed to exert any significant anticonvulsant effects in two rodent models of difficult-to-treat partial seizures. This result is similar to previous studies with antagonists at the NMDA subtype of glutamate receptors, which failed to suppress partial seizures in the kindling model and patients with epilepsy (Lösch, 1998b). In contrast, antagonists at non-NMDA ionotropic glutamate receptors proved to be potent anticonvulsants in the kindling model (Lösch, 1998b; Lösch and Rogawski, 2002) and such compounds are currently in clinical trials in patients with AED-resistant partial epilepsy (Bialer et al., 2004). Our data substantiate that antagonists at different types of metabotropic or ionotropic glutamate receptors strikingly differ in their anticonvulsant efficacy in rodent models of partial seizures. Based on our disappointing results with EMQMCM and MTEP in rodent models of difficult-to-treat partial seizures, the present experiments do not support the hypothesis that mGlu1 or mGlu5 receptors are interesting targets for development of novel AEDs, at least not for patients with partial epilepsy. However, there is growing evidence supporting the potential utility of mGlu1 and mGlu5 receptor antagonists for a variety of neurological or psychiatric diseases, including chronic pain, anxiety and Parkinson’s disease (Ritzen et al., 2005; Shipe et al., 2005; Swanson et al., 2005).

Acknowledgements

We thank Mrs Doris Pieper-Matriciani and Mrs Nicole Ernst for skilful technical assistance in the kindling experiments, Bernd Eilbacher and Sergio Greco for the analysis of the microdialysis samples, and Dr Rafał M. Kaminski (Epilepsy Research Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA) for advice on the 6-Hz model.

References


