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Short communication

Enhanced glutamatergic transmission reduces the anticonvulsant potential of lamotrigine but not of felbamate against tonic-clonic seizures

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Abstract:
The efficacy of lamotrigine and felbamate against maximal electroshock (MES)-induced seizures was assessed under conditions mimicking the pharmacoresistance associated with an increased excitatory neurotransmission. N-methyl-D-aspartate (NMDA), but not kainate applied at subconvulsive dose, reduced the activity of lamotrigine against MES-induced seizures increasing its ED_{50} value from 4.3 (3.2–5.6) to 6.1 (5.2–7.2) mg/kg ($p < 0.001$). This effect was reversed by co-application of an NMDA receptor antagonist D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116) at 0.1 mg/kg [4.5 (3.7–5.6) *vs*. 6.1 (5.2–7.2) mg/kg; $p < 0.001$]. The anticonvulsive action of felbamate was altered by neither NMDA nor kainate. In conclusion, the data presented here indicate that felbamate, but not lamotrigine, effectively prevents generalized tonic-clonic seizures, also when NMDA-mediated neurotransmission is enhanced. The impaired antiepileptic potential of lamotrigine might be restored in such scenario by the co-administration of a very low dose of NMDA receptor antagonist.

Key words:
epilepsy, pharmacoresistance, glutamate receptors, lamotrigine, felbamate

Abbreviations: CGP 40116 – D-(E)-2-amino-4-methyl-5--phosphono-3-pentenoic acid, CS_{50} – electroconvulsive threshold, ED_{50} – dose of a drug preventing 50% of animals from the occurrence of seizures, MES – maximal electroshock, NMDA – N-methyl-D-aspartate

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrently and repeatedly occurring seizures

affecting approximately 1% of world population [12]. Numerous data indicate that an enhanced excitatory amino acid (EAA)-mediated neurotransmission plays a significant role in the development of seizure activity [4, 13, 20]. The experimental application of ionotropic glutamate receptor agonists, either peripheral or intracerebral, induces convulsions in rodents [1, 13, 20]. Conversely, glutamate receptor antagonists display protective action and may prevent epileptogenesis in various experimental seizure models. However, their use is associated with a number of side effects [6, 18, 19, 22]. EAA antagonists may also enhance the anticonvulsant activity of conventional antiepileptic drugs, and such combined treatment seems beneficial in terms of reduced adverse effects [2, 5, 21, 25].

Clinically used classical antiepileptic drugs target various mechanisms, mostly (a) an excessive neuronal firing, e.g. *via* blockade of sodium channels, (b) an augmented excitatory neurotransmission, and (c) the reduced inhibitory neurotransmission [3, 4]. Pharmacoresistance remains, however, a major problem in the therapy of epileptic patients [17]. Approximately one third of patients continue to have seizures despite optimal treatment with traditional antiepileptic drugs. Therefore, during the last two decades, a number of new antiepileptics have been introduced. These novel compounds seem to exhibit a better pharmacological profile and to exert less side-effects in clinical practice [3]. However, the nature of pharmacoresistance still remains obscure. A modification of one or more drug target molecules may be of an importance [17]. Pharmacoresistance can also result from changes in the action of multidrug transporters that control intraparenchymal concentrations of antiepileptic drugs [17]. The literature data suggest a potential role of malfunctioning of glutamatergic transmission in the development of pharmacoresistance. It was demonstrated that refractory epilepsy was associated with a selective loss of hippocampal glutamate receptors type GluR1 and GluR4, accompanied by an increased expression of GluR6 subunits [8]. Changed expression of glutamate transporters, modulating the level of glutamate within brain tissue, was detected in human epileptogenic hippocampus from patients with pharmacoresistant temporal lobe epilepsy [16].

Previously, we have shown that the effectiveness of diazepam and carbamazepine, but not of valproate, phenobarbital or diphenylhydantoin against maximal electroshock (MES)-induced seizures is reduced under conditions mimicking the pharmacoresistance associated with an increased excitatory neurotransmission [23]. Here, we have assessed the efficacy of novel drugs, lamotrigine and felbamate, under similar conditions.

Materials and Methods

Animals

The experiments were performed on male Albino-Swiss mice weighing 20–26 g. The animals were kept under standard laboratory conditions, with natural day/night cycle and food and water available *ad libitum*. Each experimental group included at least ten animals. The Ethics Committee of the Medical University in Lublin approved all animal studies presented here.

Drugs

Lamotrigine (Glaxo Wellcome) and felbamate (Schering-Plough) were suspended in 1% solution of Tween 80 (Sigma) and administered *ip*, 60 min before the electroconvulsions. ED_{50} values (i.e. the doses of drugs preventing 50% of animals from the occurrence of seizures) were established based on the results obtained from at least 3 groups of mice administered different doses of drugs. N-methyl-D-aspartate (NMDA) and kainic acid (both RBI) were dissolved in water, pH was adjusted to 7.2 with 1M NaOH. NMDA and kainate were administered as described before [23], at the non-convulsive doses (equal to 75% of its ED_{16}, and established in preliminary experiments) of 50.0 and 9.0 mg/kg *ip*, respectively. NMDA and kainate were administered at 15 and 30 min before the test, i.e. at the time interval corresponding to the respective seizure latency after the injection of convulsive doses (ED_{97}) of the compounds, established in preliminary experiments, as described earlier [23].

D-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116; RBI) was dissolved in saline and applied *ip*, 120 min before the electroconvulsions. Control animals were given the appropriate amount of solvent. The injection volume was 0.1 ml/10 g of body weight.

Electroconvulsions

Seizures were induced using Hugo Sachs generator (type 21), delivering the alternating current (50 Hz), stimulus duration of 0.2 s, *via* ear-clip electrodes.

The tonic extension of hind limbs was considered to be the end point. The electroconvulsive threshold (CS_{50}) was evaluated as the current strength (in mA) necessary to evoke seizures in 50% of the animals tested. The calculations of CS_{50} values were based on the experiments performed on at least three groups of mice subjected to the electroshock induced by currents of different intensities. The maximal electroshock (MES)-induced seizures were evoked with the current of 25-mA intensity.

Statistics

The calculation of CS_{50} and ED_{50} values together with their respective confidence limits and statistical analysis of the results were performed by fitting the data with the use of computerized probit analysis based on the method of Litchfield and Wilcoxon [11].

Results

NMDA and kainate given at non-convulsive doses of 50.0 and 9.0 mg/kg, respectively, did not affect the CS_{50} value (Tab. 1). CGP 40116 (0.06 and 0.1 mg/kg), also did not alter the CS_{50} value (Tab. 1). NMDA application reduced the activity of lamotrigine against MES-induced seizures increasing its ED_{50} value from 4.4 to 6.1 mg/kg *ip* ($p < 0.001$) (Tab. 2). The anticonvulsant activity of felbamate was not changed by NMDA administration (Tab. 2). Administration of kainate did not influence the anticonvulsant potential of lamotrigine or felbamate, as assessed based on their ED_{50} values (Tab. 2). CGP 40116 at 0.15 mg/kg *ip*, but not at 0.1 mg/kg, enhanced the activity of lamotrigine against MES-induced seizures. Thus, for the studies aiming to reverse the action of NMDA, the dose of CGP 40116, not influencing the activity of the anticonvulsant *per se*, was used. CGP 40116 (0.1 mg/kg *ip*) abolished the NMDA-evoked increase in lamotrigine ED_{50} value from 4.5 to 6.1 mg/kg *ip* ($p < 0.001$) (Tab. 2).

Tab. 1. The effect of kainate, NMDA and CGP 40116 on the electroconvulsive threshold in mice

Treatment (mg/kg)		CS_{50} [95% confidence limits] (mA)
Saline		5.9 [5.1–6.8]
Kainate	(9.0)	5.3 [4.8–5.8]
Saline		5.3 [5.1–5.5]
NMDA	(50.0)	5.4 [5.2–5.6]
Saline		5.9 [5.6–6.2]
CGP 40116	(0.06)	6.1 [5.7–6.5]
	(0.1)	6.0 [5.7–6.3]

CS_{50} is a current strength producing convulsions in 50% of animals tested. Kainate, N-methyl-D-aspartate (NMDA) and D-(E)-2-amino-4--methyl-5-phosphono-3-pentenoic acid (CGP 40116) were administered at 30, 15 and 120 min before the electroconvulsions, respectively

Tab. 2. The effect of kainate, NMDA and CGP 40116 on the anticonvulsant efficacy of lamotrigine and felbamate against MES-induced seizures in mice

Treatment		ED_{50} [95% confidence limits] (mg/kg)
Lamotrigine + solvent		4.4 [3.6–5.4]
Lamotrigine + kainate	(9.0)	4.2 [3.6–5.0]
Felbamate + solvent		42.1 [36.9–48.1]
Felbamate + kainate	(9.0)	43.6 [36.3–52.3]
Lamotrigine + solvent		4.3 [3.2–5.6]
Lamotrigine + CGP 40116	(0.15)	3.3 [2.5–4.2]*
Lamotrigine + CGP 40116	(0.1)	4.2 [3.3–5.3]
Lamotrigine + NMDA	(50)	6.1 [5.2–7.2]***
Lamotrigine + CGP 40116 + NMDA	(0.1) (50)	4.5 [3.7–5.6][a]
Felbamate + solvent		40.1 [34.1–47.9]
Felbamate + NMDA	(50)	41.6 [33.8–50.3]

CS_{50} is a current strength producing convulsions in 50% of animals tested. Kainate, N-methyl-D-aspartate (NMDA) and D-(E)-2-amino-4--methyl-5-phosphono-3-pentenoic acid (CGP 40116) were administered at 30, 15 and 120 min before the maximal electroshock (MES)-induced seizures, respectively. * $p < 0.05$ *vs.* respective drug plus solvent; *** $p < 0.001$ *vs.* respective drug plus solvent; [a] $p < 0.001$ *vs.* respective drug plus NMDA

Discussion

The obtained data indicate that lamotrigine, but not felbamate, is less effective against generalized tonic-clonic seizures when NMDA-mediated neurotransmission is augmented. The anticonvulsive potential of lamotrigine was enhanced by the administration of CGP 40116, a competitive NMDA receptor antagonist. Notably, CGP 40116 given at the very low dose inactive itself, totally reversed the NMDA-evoked action, and restored the anticonvulsive potential of lamotrigine, thus confirming the role of NMDA receptors in the observed action. In contrast, the efficacy of the studied anticonvulsants was not altered by the administration of kainic acid. These data indicate that the anticonvulsant potency of lamotrigine and felbamate is fully preserved also during an excessive activation of kainate receptors.

Our data are in line with the observation indicating that dizocilpine, an NMDA receptor antagonist, extends the anticonvulsant action of lamotrigine in kindled model of seizures [26]. Lamotrigine and felbamate affect various cellular targets [3]. Lamotrigine inhibits the repetitive neuronal firing *via* blockade of voltage-dependent sodium channels, reduces calcium currents, and possibly diminishes the release of glutamate and aspartate [3, 10]. It is not clear whether lamotrigine directly affects the NMDA receptor function. Lamotrigine was shown to potently inhibit NMDA-mediated synaptic responses in rat hippocampal slices and to remain without effect on α-amino-3--hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced postsynaptic depolarization [24]. However, this was not confirmed by others, who have demonstrated that lamotrigine weakly attenuated AMPA- but not NMDA-induced discharges in rat cortical wedge [15]. Felbamate enhances GABA-mediated neurotransmission and blocks voltage-dependent sodium channels [3]. It blocks NMDA receptors as well, especially NR1-2B subtype, acting possibly as an allosteric modulator of glycine site [7, 9]. Felbamate was also suggested to interfere with AMPA receptor-mediated neurotransmission [7]. Thus, felbamate should effectively prevent seizures also under augmented activation of NMDA and kainate receptors, what indeed was observed here. Our data are also in line with the clinical observations demonstrating the value of felbamate in the therapy of a variety of refractory seizures types [14].

In conclusion, the data presented here indicate that felbamate, but not lamotrigine, effectively prevents the occurrence of generalized tonic-clonic seizures, also when NMDA-mediated neurotransmission is enhanced. The impaired antiepileptic potential of lamotrigine might be restored in such scenario by the co-administration of a very low dose of NMDA receptor antagonist. These results further confirm the value of seizure model combining MES with application of low doses of either NMDA or kainate [22] in the evaluation of drugs potentially active against refractory epilepsies.

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