

Effect of IEM 1460 – a selective antagonist of GluR2-lacking AMPA receptors – on the action of conventional antiepileptic drugs against maximal electroshock in mice

Kinga K. Borowicz, Monika Banach

Experimental Neuropathophysiology Unit, Department of Pathophysiology, Medical University, Lublin, Poland

Abstract: The objective of the study was to determine the effect of combined treatment with IEM-1460 (a selective GluR2-lacking, Ca²⁺ permeable AMPA receptors) and conventional antiepileptic drugs: CBZ (carbamazepine), valproate (VPA), phenobarbital (PB), and phenytoin (PHT) against maximal electroshock-induced convulsions in mice. IEM-1460 (up to 30 mg/kg) did not show anticonvulsant action in the test for electroconvulsive threshold. The substance did not influence the antiseizure effectiveness of antiepileptic drugs. This may indicate that not all AMPA receptor antagonists may be useful as additive drugs in the complex therapy of epilepsy. On the other hand, the GluR2-lacking, Ca²⁺ permeable subtype of AMPA receptors may be not crucial for seizure phenomena.

Key words: IEM-1460, glutamatergic metabotropic receptors, antiepileptic drugs

INTRODUCTION

IEM-1460, a dicationic compound [1] is a selective blocker of GluR2-lacking, Ca²⁺ permeable α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. The substance also blocks NMDA receptor-mediated currents [2]. Both AMPA and NMDA receptors are ionotropic complexes belonging to the excitatory glutamatergic family. Antagonizing neurotransmission by blockade of AMPA-type receptors is a promising pharmacological strategy for neuroprotection in neurodegenerative diseases, epilepsy, and acute treatment of stroke [3]. Several AMPA receptor antagonists proved antiepileptic activity in a variety of animal models of epilepsy and potentiated the protective action of anticonvulsant drugs in these models. An example may be GYKI 52466 [1] or LY 300164 [4, 5]. The latter substance is known as a new generation antiepileptic drug named Talampanel® [5].

The aim of the present study was to evaluate the effect of IEM-1460 on the protective action of phenobarbital, carbamazepine, phenytoin, and valproate against the maximal electroshock-induced convulsions in mice.

MATERIALS AND METHODS

Animals. All experiments were performed on male Swiss mice, kept in colony cages with free access to food and tap water, under standardized housing conditions. The animals were randomly assigned to experimental groups consisting of 8 mice. Each mouse participated in only in 1 experiment. All tests were performed between 09:00-14:00 to minimize

confounding effects of circadian rhythms. All experimental procedures were approved by the Local Ethics Committee of the Medical University in Lublin.

Drugs. IEM-1460 (Tocris Cookson Ltd, UK) and valproate (Polfa, Rzeszów, Poland) were dissolved in a sterile saline, while carbamazepine, diphenylhydantoin (both drugs obtained from Sigma, St. Louis, MO, USA), and phenobarbital (Polfa, Warsaw, Poland) were suspended in a 1% solution of Tween 80 (also from Sigma St. Louis, MO, USA). All drugs were administered *i.p.*, at times of their maximal anticonvulsant action against maximal electroshock-induced seizures.

Electroconvulsions. Electroconvulsions were induced with the use of alternating current (50 Hz) produced by a Rodent Shocker Generator (Ataner, Lublin, Poland), and delivered by ear-clip electrodes. The stimulus duration was 0.2 s. The electroconvulsive threshold (CS₅₀) was strength of current (in mA) necessary to produce tonic hind limb extension in 50% of the animals tested. The median effective dose (ED₅₀) was a drug dose (in mg/kg) that provided a protective effect in 50% of the animals tested against maximal electroshock (25 mA). In order to evaluate CS₅₀s and ED₅₀s at least 4 groups of 8 mice were used. Subsequently, intensity-response and dose-response curves, respectively, were constructed.

Statistics. Statistical analysis of the data obtained in the electroconvulsive test was performed by computer probit analysis, according to Litchfield and Wilcoxon [6].

RESULTS

Effect of IEM-1460 on the electroconvulsive threshold. IEM-1460 (up to 30 mg/kg) did not affect the electroconvulsive threshold (Table 1).

Corresponding author: Prof. Kinga K. Borowicz, Department of Pathophysiology, Medical University, Jaczewskiego 8, 20-090 Lublin, Poland.
E-mail: kinga.borowicz@am.lublin.pl

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Table 1 Effect of IEM 1460 on the electroconvulsive threshold in mice. Results are presented as CS_{50} values (in mA), the strength of current strength which produced convulsions in 50% of animals. IEM 1460 was administered 15 min before the test

Treatment (mg/kg)	CS_{50} (mA)
Control	5.9 (5.4-6.4)
IEM 1460 (10)	7.2 (6.5-8.1)
IEM 1460 (20)	7.5 (6.5-8.6)
IEM 1460 (30)	7.6 (6.7-8.5)

Effect of IEM-1460 on the protective action of conventional antiepileptic drugs. IEM-1460 (up to 20 mg/kg) did not influence the anticonvulsant action of phenobarbital, carbamazepine, diphenylhydantoin or valproate (Table 2).

Table 2 Effect of IEM 1460 on the protective action of conventional antiepileptic drugs. Results are presented as ED_{50} values (in mg/kg), median effective doses that produced protection against seizures in 50% of animals. IEM 1460 was administered 15 min, carbamazepine (CBZ) and valproate (VPA) 30 min, phenobarbital (PB) 60 min and phenytoin (PHT) 120 min before the test

Treatment (mg/kg)	CS_{50} (mA)
PB + saline	18.8 (15.8 - 22.1)
PB + IEM (10)	18.6 (15.1 - 22.4)
PB + IEM (20)	19.2 (15.6 - 23.0)
CBZ + saline	11.3 (9.4 - 13.1)
CBZ + IEM (10)	10.8 (8.1 - 12.6)
CBZ + IEM (20)	10.6 (8.0 - 12.4)
PHT + saline	11.2 (9.4 - 13.5)
PHT + IEM (10)	10.5 (8.6 - 12.3)
PHT + IEM (20)	10.3 (8.2 - 11.9)
VPA + saline	246.2 (220.4 - 272.7)
VPA + IEM (10)	240.4 (218.5 - 269.6)
VPA + IEM (20)	236.5 (211.9 - 264.8)

DISCUSSION

The results presented demonstrate that IEM-1460 (up to 30 mg/kg) was ineffective against the electroconvulsive threshold test. The AMPA receptor antagonist (up to 20 mg/kg) did not affect the anticonvulsant action of phenobarbital, carbamazepine, diphenylhydantoin, and valproate against maximal electroshock-induced seizures in mice. This test is widely accepted as the animal model of generalized tonic-clonic convulsions. IEM-1460 was not used to combinations at 30 mg/kg, because at this dose it produced several toxic effects (ataxia, motor impairment).

Lack of anticonvulsant effects was not expected in the case of IEM-1460. Previous studies reported that other AMPA receptor antagonists presented significant antiseizure properties [7,5]. Nevertheless, it should be borne in mind that IEM-1460 is selective towards GluR2-lacking, Ca^{2+} permeable receptor subtypes. This may indicate that this receptor subtype does not play an essential role in seizure phenomena. However, further investigations are needed to confirm this assumption.

CONCLUSIONS

1. IEM-1460 is not effective against electroconvulsions in mice.
2. As far as experimental data may be transferred into clinical conditions, the concomitant treatment with IEM-1460 and conventional antiepileptic drugs is not advisable.
3. GluR2-lacking, Ca^{2+} permeable AMPA receptors are probably not crucial for the process of seizure generation and propagation.

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