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ORIGINAL ARTICLE

Effect of N-(p-ethoxycarbonylphenylmethyl)-p-isopropoxyphenylsuccinimide on the anticonvulsant action of four classical antiepileptic drugs in the mouse maximal electroshock-induced seizure model

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Abstract

Introduction and objective. The purpose of this study was to determine the effects of N-(p-ethoxycarbonylphenylmethyl)-p-isopropoxyphenylsuccinimide (ECPM-IPPS), a new succinimide derivative, on the protective action of four classical antiepileptic drugs (AEDs): carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT) and valproate (VPA) in the mouse maximal electroshock (MES)-induced tonic seizure model.

Materials and methods. Tonic hind limb extension (seizure activity) was evoked in adult male albino Swiss mice by a current (sine-wave, 25 mA, 500 V, 50 Hz, 0.2 s stimulus duration) delivered via ear-clip electrodes.

Results. ECPM-IPPS administered (i.p.) at a dose of 150 mg/kg significantly elevated the threshold for electroconvulsions in mice ($P<0.05$). Lower doses of ECPM-IPPS (50 and 100 mg/kg) had no significant impact on the threshold for electroconvulsions in mice. Moreover, ECPM-IPPS (100 mg/kg) did not significantly affect the anticonvulsant potency of CBZ, PB, PHT and VPA in the MES test in mice.

Conclusions. ECPM-IPPS elevated the threshold for electroconvulsions in mice in a dose-dependent manner. However, ECPM-IPPS (100 mg/kg) did not affect the anticonvulsant action of various classical AEDs in the mouse MES model, making the combinations of ECPM-IPPS with CBZ, PB, PHT and VPA neutral, from a preclinical point of view.

Key words

antiepileptic drugs, maximal electroshock-induced seizures, N-(p-ethoxycarbonyl-phenylmethyl)-p-isopropoxyphenyl-succinimide

INTRODUCTION

Accumulating evidence indicates that several succinimide derivatives have anticonvulsant properties in animal models of epilepsy [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]. More specifically, it has been reported that N-(anilinomethyl)-p-isopropoxyphenylsuccinimide (AMIPPS) [2], N-pyridyl-substituted succinimides [3], N-(ortho-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide (o-CAMIPPS), N-(meta-carboxyanilino-methyl)-p-iso-propoxyphenylsuccinimide (m-CAMIPPS), and N-(para-carboxyanilinomethyl)-p-isopropoxyphenylsuccinimide (p-CAMIPPS) [4], 3-cyclohexyl-succinimides [5], N-morpholinemethyl derivative of m-bromophenyl-succinimide [6], p-iso-propoxyphenylsuccinimide monohydrate (IPPS) [7], N-hydroxymethyl-p-isopropoxy-phenyl-succinimide (HMIPPS) [8], N-(p-acetylphenyl)-p-isopropoxyphenylsuccinimide (APIPPS) [9], N-morpholinomethyl-p-isopropoxyphenylsuccinimide (MMIPPS) [10], and 3-(N-p-isopropoxy-phenylsuccinimido-methylamino)-cinnamic acid (IPPSMA-CA) [11] exhibited potent anticonvulsant effects in the maximal electroshock-induced seizure (MES) test, recognized as the most widely employed animal seizure model for early identification of candidate anticonvulsant drugs [12, 13].

In our pilot study, we found that N-(p-ethoxycarbonyl-phenylmethyl)-p-isopropoxyphenyl-succinimide (ECPM-IPPS) exerted anticonvulsant properties by suppressing tonic-clonic seizures in the mouse MES test (unpublished data). This was reason enough to continue experiments in order to evaluate the effect of ECPM-IPPS on the threshold for electroconvulsions, and to assess its influence on the protective activity of four classical antiepileptic drugs (AEDs): carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT), and valproate (VPA)) in the mouse MES-induced seizure model. The test evaluating the threshold for electroconvulsions (MEST) and the MES test in mice

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are both thought to be experimental models of tonic-clonic seizures and, to a certain extent, of partial convulsions with or without secondary generalization in humans [13]. In these seizure models the anticonvulsant potential of agents and compounds with anticonvulsant properties can be readily determined. Moreover, in these models one can evaluate the effects of tested compounds on various classical AEDs, which are effective in the suppression of tonic-clonic seizures in humans [13, 14].

MATERIALS AND METHODS

Animals and experimental conditions. Adult male Swiss mice weighing 22–26 g were kept in colony cages with free access to food and tap water, housed under standardized housing conditions with a natural light-dark cycle, at a temperature of 23±1°C with relative humidity of 55±5%), were used. After seven days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups, each comprised of eight mice. Each mouse was used only once and all tests were performed between 08:00–15:00. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in the presented study were approved by the First Local Ethics Committee at the Medical University of Lublin (License No.: 18/2006) and the Second Local Ethics Committee at the University of Life Sciences in Lublin (License Nos.: 79/2009 and 15/2012), and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs. The following drugs were used: N-(p-ethoxycarbonylphenylmethyl)-p-isopropoxyphenyl-succinimide (ECPM-IPPS – $C_{23}H_{26}N_2O_5$ – molecular weight = 410.460 (synthesized by Dr. S. L. Kocharov, Mndjoyan's Institute of Fine Organic Chemistry of the National Academy of Sciences in Yerevan, Armenia); carbamazepine (CBZ – a gift from Polpharma, Starogard Gdański, Poland), phenobarbital (PB – Polfa, Kraków, Poland), phenytoin (PHT – Polfa, Warsaw, Poland) and valproate (VPA – magnesium salt – kindly donated by ICN-Polfa S. A., Rzeszów, Poland).

All drugs, except for VPA, were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water, while VPA was directly dissolved in distilled water. All drugs were administered *intraperitoneally* (*i.p.*), in a volume of 5 ml/kg body weight, as follows: PHT – 120 min, PB and ECPM-IPPS – 60 min, CBZ and VPA – 30 min before electroconvulsions. The pretreatment times before testing of the AEDs were based upon information about their biological activity from the literature and our previous experiments [2, 4, 7–11]. The pretreatment time (60 min) before testing ECPM-IPPS was established in our pilot study as its peak time of maximum anticonvulsant activity (unpublished data).

Maximal electroshock seizure threshold (MEST) test. The MEST test was first used to assess the anticonvulsant effects of ECPM-IPPS administered alone. Electroconvulsions were induced by applying an alternating current (sine-wave, 50 Hz, 500 V) *via* ear-clip electrodes from a rodent shocker generator (type 221; Hugo Sachs Elektronik, Freiburg, Germany). The stimulus duration was 0.2 s and tonic hind limb extension was used as the endpoint. In this test, at least 4 groups of control mice, each consisting of 8 animals, were challenged with currents of varying intensities ranging between 4–8 mA so that 10–30%, 30–50%, 50–70% and 70–90% of animals exhibited the endpoint. After establishing the current intensity-effect curve (i.e., current intensity in mA *vs.* percentage of mice convulsing) for each dose of ECPM-IPPS tested, the electroconvulsive threshold was calculated according to the log-probit method of Litchfield and Wilcoxon [15]. The electroconvulsive threshold was expressed as the median current strength value (CS_{50} in mA) predicted to produce tonic hind limb extension in 50% of the animals tested. This experimental procedure was performed for various increasing doses of ECPM-IPPS (50, 100 and 150 mg/kg), until the threshold for electroconvulsions of ECPM-IPPS-injected animals was statistically different from that of the control animals. Only doses of ECPM-IPPS that did not significantly affect the seizure threshold in the MEST test were selected for testing in combination with four classical AEDs in the MES test (see below). This approach allowed the ruling-out of any contribution of the intrinsic anticonvulsant efficacy of ECPM-IPPS in the effects observed in combination with the AEDs in the MES test.

Maximal electroshock seizure (MES) test. Electroconvulsions were induced by applying an alternating current (sine-wave, 50 Hz, 500 V) *via* ear-clip electrodes from a rodent shocker generator (type 221; Hugo Sachs Elektronik, Freiburg, Germany). The stimulus duration was 0.2 s and tonic hind limb extension was used as the endpoint. In the MES test, mice were challenged with a current of fixed intensity (25 mA) that was 4–5-fold higher than the CS_{50} value in vehicle-treated control mice [13]. These parameters of stimulation (maximal electroshock) typically result in all mice responding with tonic hind limb extension immediately after stimulation. The AEDs administered alone and their combination with ECPM-IPPS were tested for their ability to increase the number of animals not responding with tonus (i.e., protected from tonic hind limb extension) after stimulation. Again, at least 4 groups of mice, each consisting of 8 animals and treated with a different dose of the AEDs alone or in combination with ECPM-IPPS, were challenged with a current of 25 mA to yield 10–30%, 30–50%, 50–70% and 70–90% of animals protected from tonic seizures.

After constructing a dose-effect curve (i.e., dose in mg/kg *vs.* percentage of mice protected), the protective median effective dose (ED_{50}) value of the AED tested was calculated according to a log-probit method [15]. Each ED_{50} value represented a dose of the AED (in mg/kg) predicted to protect 50% of mice tested against MES-induced extension of the hind limbs. ECPM-IPPS was tested for its ability to affect the anticonvulsive potency of AEDs. As mentioned earlier, ECPM-IPPS was administered in a dose of 100 mg/kg that *per se* had no effect on seizure threshold in the MEST test. In this experimental protocol, an increase in the anticonvulsant potency of the AED tested in combination with ECPM-IPPS would be reflected by a lower ED_{50} value of the test AED (i.e., a lower dose of the test drug was necessary to protect 50% of the mice challenged). In the presented study, CBZ and PHT were administered at doses ranging between

8–14 mg/kg, PB at doses ranging between 15–30 mg/kg and VPA at doses ranging between 225–325 mg/kg.

Statistics. Both CS_{50} and ED_{50} values with their 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [15]. Statistical analysis of data from the MEST test was performed with one-way analysis of variance (ANOVA) followed by the *post-hoc* Tukey-Kramer test for multiple comparisons among four CS_{50} values. Statistical analysis of data from the MES test was performed with log-probit analysis according to Litchfield and Wilcoxon [15] for two ED_{50} values. Differences among values were considered statistically significant if $P<0.05$. All statistical tests were performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Influence of N-(p-ethoxycarbonylphenylmethyl)-p-isopropoxyphenylsuccinimide (ECPM-IPPS) on the threshold for electroconvulsions. ECPM-IPPS administered systemically (i.p., 60 min prior to the MEST test) at a dose of 150 mg/kg significantly elevated the threshold for electroconvulsions in mice (by 33%; $P<0.05$; Tab. 1). The experimentally-derived CS_{50} values for animals receiving ECPM-IPPS at doses of 50 and 100 mg/kg did not significantly differ from that for control animals subjected to the MEST test (Tab. 1).

Table 1. Effect of N-(p-ethoxycarbonylphenylmethyl)-p-isopropoxyphenylsuccinimide (ECPM-IPPS) on the threshold for electroconvulsions in mice

Treatment (mg/kg)	CS_{50} (mA)	n
Vehicle	5.62±0.41	24
ECPM-IPPS (50)	5.94±0.43	24
ECPM-IPPS (100)	6.40±0.47	16
ECPM-IPPS (150)	7.45±0.45*	24
F (3;76) = 3.010; P = 0.0353		

Data are presented as median current strengths (CS_{50} values in mA ± S.E.) required to produce tonic hindlimb extension in 50% of animals tested in the maximal electroshock-induced seizure threshold test. ECPM-IPPS was administered *i.p.* 60 min. before the test. Statistical evaluation of data was performed with the log-probit method [15] and one-way ANOVA followed by *post-hoc* Tukey-Kramer test for multiple comparisons.
n – number of animals tested at those current strength intensities, whose seizure effects ranged between 16% and 84%;
F – F-statistics from one-way ANOVA;
P – probability from one-way ANOVA;
* $P<0.05$ *vs.* control (vehicle-treated) animals.

Effects of N-(p-ethoxycarbonylphenylmethyl)-p-isopropoxyphenylsuccinimide (ECPM-IPPS) on the protective action of carbamazepine, phenobarbital, phenytoin and valproate in the mouse maximal electroshock seizure model. All investigated classical AEDs (CBZ, PB, PHT and VPA) administered alone exhibited a definite anticonvulsant activity in the MES test in mice (Tab. 2). When ECPM-IPPS (100 mg/kg) was co-administered with CBZ, PB, PHT and VPA, it did not significantly potentiate the anticonvulsant action of the studied AEDs in the MES test. The experimentally-derived ED_{50} values for the AEDs in combination with ECPM-IPPS (100 mg/kg) did not considerably differ from those ED_{50} values as documented for the AEDs administered separately (Tab. 2).

Table 2. Effect of N-(p-ethoxycarbonylphenylmethyl)-p-isopropoxyphenylsuccinimide (ECPM-IPPS) on the protective activity of four classical antiepileptic drugs against maximal electroshock-induced seizures in mice

Treatment (mg/kg)	ED50 (mg/kg)
CBZ + vehicle	10.55 (8.83–12.60)
CBZ + ECPM-IPPS (100)	9.12 (7.78–10.70)
PB + vehicle	23.90 (20.20–28.26)
PB + ECPM-IPPS (100)	18.17 (14.97–22.07)
PHT + vehicle	11.54 (10.15–13.13)
PHT + ECPM-IPPS (100)	10.55 (8.83–12.60)
VPA + vehicle	283.3 (262.5–305.9)
VPA + ECPM-IPPS (100)	258.2 (237.4–280.8)

Results are presented as median effective doses (ED_{50} in mg/kg, with 95% confidence limits in parentheses) of AEDs, protecting 50% of animals tested against maximal electroshock (MES)-induced seizures. All AEDs were administered *i.p.*: PHT – 120 min., PB – 60 min., CBZ and VPA – 30 min. prior to the MES test. ECPM-IPPS was administered *i.p.* at 60 min. before the MES test. Statistical analysis of data was performed with log-probit method according to Litchfield and Wilcoxon [15].
CBZ – carbamazepine;
PB – phenobarbital;
PHT – phenytoin;
VPA – valproate.

DISCUSSION

Results indicate that ECPM-IPPS dose-dependently elevated the threshold for electroconvulsions in mice. However, ECPM-IPPS at the sub-protective dose of 100 mg/kg (i.e., the dose that by itself did not significantly affect the threshold for electroconvulsions) had no impact on the protective action of the studied AEDs (CBZ, PB, PHT and VPA) against MES-induced tonic seizures in mice, thus indicating neutral interactions between these drugs in the mouse MES model.

Comparing the effects produced by ECPM-IPPS in this study with those reported earlier for AMIPPS, IPPS, o-CAMIPPS, m-CAMIPPS, p-CAMIPPS, HMIPPS, MMIPPS, APIPPS and IPPSMA-CA, it can be ascertained that ECPM-IPPS had no impact on the anticonvulsant properties of the four classical AEDs. Previously, we have documented that p-isopropoxyphenylsuccinimide monohydrate (IPPS) potentiated the anticonvulsant action of PHT and VPA, but not that of CBZ and PB [7]. Moreover, AMIPPS, APIPPS, HMIPPS and MMIPPS significantly enhanced the anticonvulsant action of PB and VPA, but not that of CBZ and PHT in the mouse MES model [2, 8, 9, 10]. On the other hand, o-CAMIPPS attenuated the anticonvulsant action of CBZ and had no significant impact on the protective action of PHT, PB and VPA against MES-induced seizures in mice [4]. With regard to m-CAMIPPS, p-CAMIPPS and IPPSMA-CA, all these succinimide derivatives had no impact on the protective action of classical AEDs in the mouse MES model [4, 11]. Of note, the anticonvulsant profile of ECPM-IPPS when combined with classical AEDs is similar to that reported earlier for m-CAMIPPS, p-CAMIPPS and IPPSMA-CA in the mouse MES model.

Although ECPM-IPPS significantly raised the threshold for maximal electroconvulsions in mice, it did not affect the protective action of the four different classical AEDs in the mouse MES model. Thus, one can ascertain that ECPM-IPPS possesses the anticonvulsant action against electrically-evoked tonic seizures in experimental animals, but this action is too weak to enhance the protective activity

of different classical AEDs in the mouse MES-induced tonic seizure model. Perhaps ECPM-IPPS will be effective in the suppression of clonic or limbic seizures in other experimental models of epilepsy. To confirm this hypothesis, more advanced studies are required.

Finally, based on the results from this study, one can ascertain that the co-administration of ECPM-IPPS with various classical AEDs (CBZ, PB, PHT and VPA) was neutral in the mouse MES model. Additionally, ECPM-IPPS had no impact on acute adverse effect profiles of classical AEDs, as determined in the passive avoidance, grip-strength and chimney tests in mice.

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Disclosure of conflicts of interest

The authors have no disclosures to declare.

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