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Original research article

Propafenone enhances the anticonvulsant action of classical antiepileptic drugs in the mouse maximal electroshock model

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ABSTRACT

Background: Antiarrhythmic and antiepileptic drugs share some mechanisms of actions. Therefore, possibility of interactions between these in epileptic patients with cardiac arrhythmias is quite considerable. Herein, we attempted to assess interactions between propafenone and four conventional antiepileptic drugs: carbamazepine, valproate, phenytoin and phenobarbital.

Methods: Effects of propafenone on seizures were determined in the electroconvulsive threshold test in mice. Interactions between propafenone and antiepileptic drugs were estimated in the model of maximal electroshock. Motor coordination was evaluated in the chimney test, while long-term memory in the passive-avoidance task. Brain concentrations of antiepileptics were determined by fluorescence polarization immunoassay.

Results: Propafenone up to 50 mg/kg did not affect the electroconvulsive threshold, significantly enhancing this parameter at doses of 60–90 mg/kg. Applied at its subthreshold doses, propafenone potentiated the antielectroshock action of all four tested classical antiepileptics: carbamazepine, valproate, phenytoin, and phenobarbital. Propafenone alone and in combinations with antiepileptics impaired neither motor performance nor long-term memory in mice. Propafenone did not change brain concentration of phenytoin and phenobarbital; however, it significantly decreased brain levels of carbamazepine and increased those of valproate.

Conclusions: Propafenone exhibits its own anticonvulsant effect and enhances the action of classical antiepileptic drugs against electrically induced convulsions in mice. Further investigations are required to determine the effect of propafenone on antiepileptic therapy in humans.

Introduction

Propafenone, widely known as a representative of class 1 C antiarrhythmic drugs, presents local anesthetic and membrane stabilizing effects on myocardiocytes. From the electrophysiological point of view, propafenone reduces the fast inward sodium current, thereby inhibiting phase 0 of the monophasic action potential. Additionally, both experimental and clinical studies showed beta-sympatholytic activity of propafenone at about 1/50 the potency of propranolol [1]. All these mechanisms of action may mediate also antiseizure effects of propafenone. In fact, more than 30 years ago, the antiarrhythmic drug was reported to reduce maximal electroshock-induced seizures in rats [2]. On the other hand, propafenone overdose (1350 mg) was described in literature as resulting in tonic-clonic seizures and a widened QRS complex. However, convulsions are considered as a rare manifestation of propafenone overdose [3].

The relationship between antiepileptic and antiarrhythmic drugs seems to be bidirectional. Some antiepileptics, like phenytoin, have confirmed antiarrhythmic properties. Additionally, antiarrhythmic drugs can affect seizure threshold and susceptibility [4]. This phenomenon can be explained by similarities between cardiac and neural action potentials closely related to the sodium inward currents [5,6]. Antiarrhythmic and antiepileptic drugs may have the same or similar targets. In details, propafenone blocks sodium channel protein type 5 subunit alpha [7]. The same subunit of voltage-dependent sodium channels is also blocked by carbamazepine and phenytoin. Phenytoin additionally binds to sodium channel protein type 1 subunit alpha [8]. On the other hand, valproate inhibits sodium channel protein type 1–11 subunit alpha and type 1–4 subunit beta [9].

Furthermore, seizures were reported to cause disturbances in cardiac rhythm in the mechanism affecting the function of the central autonomic control centers. On the other hand, cardiac

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dysrhythmias may result in hemodynamic changes leading finally to seizures [10]. Thereby, probability of concomitant antiepileptic and antiarrhythmic therapy is quite considerable.

This assumption prompted us to evaluate the influence of propafenone, an antiarrhythmic used in the treatment of supraventricular arrhythmias, on the anticonvulsant action of conventional antiepileptic drugs in the mouse model of tonic-clonic convulsions. Despite many limitations, the maximal electroshock model remains the most useful screening test for potential antiepileptic drugs [11].

Materials and methods

Animals

The experiments were carried out on 20–25 g male Swiss mice, which were kept in colony cages with free access to tap water and food. Standard laboratory conditions with a natural dark-light cycle were maintained during experiments. All procedures were conducted between 9 a.m. and 3 p.m. and experimental groups comprised of 8–10 animals. All the investigations undertaken in this study were approved by the I Local Ethical Committee at the Medical University of Lublin.

Drugs

Propafenone (PROP), a representative of antiarrhythmic drugs, and four antiepileptic drugs were used in the study: phenobarbital (PB), valproate magnesium (VPA), carbamazepine (CBZ;) and phenytoin (PHT). Phenobarbital was purchased from UNIA Pharmaceutical Department, Warszawa, Poland, while all remaining medications from Sigma, St. Louis, MO, USA. Valproate was dissolved in sterile saline, whereas phenobarbital, phenytoin, carbamazepine and propafenone were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in saline. All drugs were administered in a volume of 10 ml/kg intraperitoneally (*ip*) phenytoin, 120 min, phenobarbital and propafenone, 60 min, valproate, and carbamazepine, 30 min before the tests.

Maximal electroshock seizure test in mice

Maximal electroshock (MES) is recognized as a standard preclinical model to evaluate anticonvulsant effects in tonic-clonic seizures [11]. Experimental procedures were performed previously and described by Borowicz et al. [12]. A dose–response curve was constructed based on the percentage of mice protected [13]. The anticonvulsant activity of the drugs was determined as their ability to protect 50% of the mice against MES-induced electroconvulsions. Tonic hindlimb extension was taken as the endpoint. The respective median effective doses (ED_{50} values) were evaluated (mg/kg), according to Litchfield and Wilcoxon [13].

Chimney test

In order to determine the effects of the combinations of propafenone with various antiepileptic drugs on motor coordination impairment, the chimney test of Boissier et al. [14] was used. The test was thoroughly described by Borowicz et al. [12]. Drugs were administered alone, at doses equal to their ED_{50} values, or in combinations with propafenone (50 mg/kg).

Step-through passive avoidance task

The step-through passive avoidance test is regarded as a measure of long-term memory and is based on natural aversion of rodents to bright places. Mice were tested according to description provided by Borowicz et al. [12]. Control animals did not enter the dark compartment within 180 s. Similarly to the chimney test, antiepileptic drugs were administered alone at their ED_{50}s and in combinations with propafenone (50 mg/kg).

Measurement of brain concentrations of antiepileptic drugs

Total brain concentrations of phenobarbital, carbamazepine, valproate and phenytoin were determined by fluorescence polarization immunoassay. Control animals were administered with one of the conventional antiepileptic drugs and saline. The combination groups were administered with the respective antiepileptic drug and propafenone at the dose of 50 mg/kg. The mice were killed by decapitation, at times scheduled for the MES test, and the brains were removed, weighed and homogenized (Ultra Turax T8 homogenizer, IKA, Staufen, Germany) with Abbott buffer (2:1 vol/weight). Homogenates were centrifuged at 10 000 g for 15 min and supernatants (75 μl) were analyzed for AED content using an ARCHITECT PLUS C 4000 immunoassay analyzer (Abbott Laboratories, Warszawa, Poland) and Abbott reagents. Concentrations were automatically calculated by the and expressed in micrograms per milliliter. Data were subsequently computed as means ± SD of at least eight determinations.

Statistics

ED_{50} values with their respective 95% confidence limits were determined using computer log-probit analysis according to Litchfield and Wilcoxon [13]. Subsequently, standard error (SEM) of the mean values were calculated on the basis of confidence limits and ED_{50} values were compared with the Student's *t* test.

Qualitative variables from the chimney test were analyzed by the Fisher's exact probability test.

Total brain concentrations of antiepileptic drugs were evaluated by the use of the unpaired Student's *t* test. The significance level was set at $p \leq 0.05$.

Results

Electroconvulsive threshold

Effects of propafenone applied at the wide dose range of 5–90 mg/kg were assessed in the electroconvulsive threshold test. The antiarrhythmic drug at doses 5–50 mg/kg did not affect the threshold for electroconvulsions in mice. Propafenone administered at 60, 70 and 90 mg/kg significantly increased this parameter from 5.8 ± 0.39 mA to, respectively, 7.8 ± 0.52, 7.3 ± 0.48 and 8.2 ± 0.54 mA (Table 1).

Maximal electroshock test

Propafenone was combined with classical antiepileptic drugs at its subthreshold doses ineffective in the electroconvulsive threshold test. The antiarrhythmic drug applied at 2.5, 5, 30, and 50 mg/kg potentiated the antielectroshock of carbamazepine, decreasing its ED_{50} value from 17.9 ± 0.61 to 14.2 ± 0.86, 13.3 ± 0.73, 11.3 ± 0.72, and 7.9 ± 0.77 mg/kg (Fig. 1). Moreover, propafenone given at 20, 30 and 50 mg/kg decreased ED_{50} values of valproate from 294.5 ± 11.11 to 246.9 ± 15,54, 237,1 ± 14,94 and 197.1 ± 9.55 mg/kg, respectively (Fig. 2). The action of phenytoin was enhanced only by propafenone administered at the highest subthreshold dose of 50 mg/kg. The ED_{50} value for phenytoin was decreased from 12.7 ± 1.44 to 6.7 ± 1.07 mg/kg (Fig. 3). Finally, enhancement of the anticonvulsive effect of phenobarbital was reflected by decrement of its ED_{50} from