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Short communication

Protective action of nicotinic acid benzylamide in a variety of chemically-induced seizures in mice

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ABSTRACT

Purpose: The study aims to assess the anticonvulsant effects offered by benzylamide nicotinic acid (Nic-BZA) in many animal models of chemically-induced seizures (i.e., pentylenetetrazole [PTZ], pilocarpine [PILO], bicuculline [BIC], α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], kainic acid [KA], and *N*-methyl-D-aspartic acid [NMDA]). Additionally, it analyses side effects of administering Nic-BZA in the form of loss of co-ordination and memory impairment as evaluated in the rotarod and passive avoidance tests, respectively.

Results: Antiseizure activity of Nic-BZA was reported in numerous models of chemically-induced seizures and its ED_{50} value was 37.1 mg/kg for PTZ, 53.0 mg/kg for AMPA, 81.4 mg/kg for BIC, 86.3 mg/kg for KA, and 182.6 mg/kg for PILO. Moreover, Nic-BZA was totally ineffective (in dosages of up to 200 mg/kg) in mice challenged with NMDA-induced seizures. The evaluation of the side effects present shortly after dosing in the rotarod test has revealed neurotoxicity of Nic-BZA with experimentally determined TD_{50} value of 188.5 mg/kg. Protective index (PI) assessment analysis for Nic-BZA has disclosed a substantial difference between the dosage resulting in acute impairment of co-ordination and the dosage resulting in anticonvulsant effect in various chemically evoked seizures, remaining practically ineffective (in dosages of up to 200 mg/kg) in mice subjected to the NMDA-induced seizure test. Additionally, Nic-BZA (in dosages of up to 100 mg/kg) did not impair long-term memory in mice.

Conclusions: Summing up, Nic-BZA has a wide anticonvulsant effect in different experimental epilepsy models.

Introduction

Epilepsy is a disorder observed in approx. 1% of the population [1]. The treatment involves long-term continuous preventive administration of antiepileptic drugs.

However these substances do not remove the cause of the disorder, but merely prevent the appearance of seizures [2]. In recent years, the methods of treatment for patients with epilepsy have been based on a few new antiepileptic drugs with specific and diverse mechanisms of action [3]. Among the standard drugs of this group the basic action consists in blocking sodium channels or intensifying GABAergic transmission. Among the new antiepileptics, a group of drugs inhibiting glutamate transduction is becoming increasingly popular. However, regardless of the character of the therapeutic action taken, no intended therapeutic effects are achieved in 30% of the patients treated with the use of both modern and traditional antiepileptics [4,5]. This is the reason for constant seeking for more effective medicines from this group which would have a higher clinical effectiveness and no undesirable or significant side effects. This aim is essential in contemporary neuropharmacological research.

Searching for new antiepileptics nowadays, scientists use different methods. The first of them is based on a screen analysis

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BIC, bicuculline; ED50, median effective dose; KA, kainic acid; Nic-BZA, nicotinic acid benzylamide; NMDA, *N*-methyl-D-aspartic acid; PI, protective index; PILO, pilocarpine; PTZ, pentylenetetrazole; TD50, median toxic dose.

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of various synthesized compounds in experimental models of epileptic seizures (i.e., in electrically- and chemically-induced seizure models). The purpose of preclinical research is to select the most effective substances able to provide animals with sufficient protection against tonic–clonic seizures [6]. The second method involves chemical modification of the structure of traditional and novel antiepileptics to create new compounds with a wider spectra of antiepileptic action compared to the parent drugs. Several benzylamide [BZA] derivatives with a cyclic amino group in their structure (including a benzylamide derivative of isonicotinic acid [iso-Nic-BZA], picolinic acid [Pic-BZA] and 2-fluoro-picolinic acid [Pic-2F-BZA] have been described as compounds characterized with antiepileptic action in the maximal electroshock model (MES) and also in other chemically-induced seizure models (i.e., bicuculline [BIC]-, pentylenetetrazole [PTZ]-, pilocarpine [PILO]-, *N*-methyl-D-aspartic acid [NMDA]-, kainic acid [KA]-, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA]-induced seizures) in rodents. This research has confirmed that BZA derivatives have antagonist properties of the AMPA/KA receptor complex [7,8].

Moreover, the high neurotoxicity evaluation of these BZA derivatives has made it possible to calculate their protective index (PI) values as the relationship of applicable TD_{50} and ED_{50} values [9]. As far as PI values for BZA derivatives of nicotinic acid and picolinic acid are concerned, it was determined that the latter BZA derivatives have a strong anticonvulsant effect and a low potential of causing relevant adverse effects in experimental preclinical research [10,11]. Unfortunately, these compounds in rodents undergo rapid transformation to diverse metabolites, which significantly limits their potential clinical application [10,11].

In the study we have assessed anticonvulsant properties of Nic-BZA in a battery of chemically evoked seizures in mice. Additionally, we have tested undesirable side effects (regarding acute neurotoxic symptoms) caused by Nic-BZA in the rotarod test. This test has allowed us to assess undesirable side effects caused by this compound in the form of loss of coordination in animals [12]. Finally, the influence of Nic-BZA on long-term memory in mice was tested using a step-through passive avoidance task.

Materials and methods

Animals

Adult male Swiss albino mice (weighing 20–26 g), housed in colony cages with free access to food (chow pellets) and tap water, were used. Experimental groups consisted of eight mice, the animals were randomly chosen. The described procedures were approved by the Local Ethics Committee at the Medial University of Lublin and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

Nic-BZA as a 1% aqueous solution of Tween 80 (Sigma, St. Louis, MO, USA) was administered intraperitoneally (*ip*) in a volume of 10 ml/kg body weight, at 5 min prior to chemically-induced seizures, passive avoidance task and the rotarod test. Based on our earlier study, the schedule time of Nic-BZA was determined experimentally in the MES test [11]. BIC, PTZ and *N*-methyl-scopolamine (all three from Sigma) were dissolved in distilled water and administered in a volume of 5 ml/kg subcutaneously (*sc*) into a loose fold of skin in the midline of the neck. PILO (Sigma) was dissolved in distilled water and given in a volume of 10 ml/kg *ip* to animals pretreated with *N*-methyl-scopolamine, 30 min earlier. NMDA, AMPA and KA (all three compounds from ICN Biomedicals, Inc., Costa Mesa, CA, USA) were given intracerebroventricularly (*icv*) in a volume of 5 μl, as described earlier [13,14].

Chemically-induced seizures

The animals received BIC (3 mg/kg, *sc*) and PTZ (95 mg/kg, *sc*), being their CD_{97} values (i.e., the dose of BIC and PTZ that evoked clonic seizures in 97% of animal tested). Similarly, NMDA (1.0 nmol/kg, *icv*), AMPA (1.2 nmol/kg, *icv*) and KA (1.4 nmol/kg, *icv*), being their CD_{97} values, were administered into the lateral brain ventricle, according to the method of Lipman and Spencer [15]. After the injection of convulsant agents (BIC, PTZ, NMDA, AMPA and KA) the mice were placed singly into transparent Plexiglas cages (25 × 15 × 10 cm) and observed for 30 min for the occurrence of clonic seizures, as described earlier [8,11]. In our study, clonic seizure activity in mice was defined as clonus of the whole body lasting over 3 s with an accompanying loss of righting reflex.

The mice received also PILO (380 mg/kg, *ip*), being its CD_{97} value (i.e., the dose of PILO that evoked limbic seizures in 97% of animals tested). To alleviate peripheral autonomic adverse effects evoked by PILO, the mice were pretreated with *N*-methyl-scopolamine (1 mg/kg, *ip*, 30 min before PILO administration). After PILO injection, the mice were placed singly into transparent Plexiglas cages (25 × 15 × 10 cm) and observed for 120 min for the occurrence of limbic seizures. PILO-induced limbic seizures are characterized by a sequential development of behavioral patterns, such as hypoactivity, tremor, scratching, head bobbing, and myoclonic movements of the limbs progressing to recurrent myoclonic convulsions with rearing, salivation, falling, and status epilepticus. This experimental procedure has been described in more detail elsewhere [16]. The number of animals displaying convulsions out of the total number of mice studied was noted for each treatment condition. Double-blind observations allowed to assess the anticonvulsant potency for Nic-BZA (expressed as its ED_{50} values in mg/kg, necessary to protect 50% of the animals against clonic seizures). Details are presented elsewhere [13,14]. To assess the ED_{50} values Nic-BZA was administered at increasing doses ranging from 20 to 200 mg/kg.

Rotarod test

The effects of Nic-BZA on motor performance were evaluated in the rotarod test according to Dunham and Miya [17]. In this test, the mice were placed on a bar rotating at a constant speed of 18 rpm (BD, COMT, Białystok, Poland). After administration of Nic-BZA, the mice had to stay on the rotating rod for 3 min. The time the animals spent on the rod was recorded, and the cutoff time of 180 s was used in this test as the main criterion of impairment of motor coordination. Subsequently, the median toxic dose (TD_{50} value) for Nic-BZA, i.e., the dose of Nic-BZA that impaired motor coordination in half of the tested animals was calculated. To assess the TD_{50} value Nic-BZA was administered at increasing doses ranging from 100 to 300 mg/kg.

Light-dark, step-through passive avoidance task

The effects of Nic-BZA on long-term memory were evaluated in the passive avoidance task according to Venault et al. [18]. In this test, the animals were treated with Nic-BZA on the first day before training. Then, the animals were placed in an illuminated box (10 × 13 × 15 cm) connected to a larger dark box (25 × 20 × 15 cm) equipped with an electric grid floor. The animals that entered into the dark box were punished by an electric footshock (0.6 mA for 2 s). On the next day (24 h later), the pre-trained animals (without treatment) were placed again into the illuminated box and observed