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Short communication

Assessment of the anticonvulsant potency of various benzylamide derivatives in the mouse maximal electroshock-induced seizure threshold model

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ABSTRACT

Purpose: The aim of this study was to assess the anticonvulsant potency of 6 various benzylamide derivatives [i.e., nicotinic acid benzylamide (Nic-BZA), picolinic acid 2-fluoro-benzylamide (2F-Pic-BZA), picolinic acid benzylamide (Pic-BZA), (RS)-methyl-alanine-benzylamide (Me-Ala-BZA), isonicotinic acid benzylamide (Iso-Nic-BZA), and (R)-N-methyl-proline-benzylamide (Me-Pro-BZA)] in the threshold for maximal electroshock (MEST)-induced seizures in mice.
Methods: Electroconvulsions (seizure activity) were produced in mice by means of a current (sine-wave, 50 Hz, 500 V, strength from 4 to 18 mA, ear-clip electrodes, 0.2-s stimulus duration, tonic hindlimb extension taken as the endpoint).
Results: Nic-BZA, 2F-Pic-BZA, Pic-BZA, Me-Ala-BZA, Iso-Nic-BZA, and Me-Pro-BZA administered systemically (*ip*) in a dose-dependent manner increase the threshold for maximal electroconvulsions in mice. Linear regression analysis of Nic-BZA, 2F-Pic-BZA, Pic-BZA, MeAla-BZA, IsoNic-BZA, and Me-Pro-BZA doses and their corresponding threshold increases allowed determining threshold increasing doses by 20% (TID_{20} values) that elevate the threshold in drug-treated animals over the threshold in control animals. The experimentally derived TID_{20} values in the MEST test for Nic-BZA, 2F-Pic-BZA, Pic-BZA, Me-Ala-BZA, Iso-Nic-BZA, and Me-Pro-BZA were 7.45 mg/kg, 7.72 mg/kg, 8.74 mg/kg, 15.11 mg/kg, 21.95 mg/kg and 28.06 mg/kg, respectively.
Conclusion: The studied benzylamide derivatives can be arranged with respect to their anticonvulsant potency in the MEST test as follows: Nic-BZA > 2F-Pic-BZA > Pic-BZA > Me-Ala-BZA > Iso-Nic-BZA > Me-Pro-BZA.

Introduction

Anticonvulsant properties evoked by drugs or agents are usually evaluated in preclinical experiments by documenting efficacy of these compounds in animal models of epilepsy [1,2]. Of these models, the maximal electroshock-induced seizure threshold (MEST) test can readily evaluate whether the examined compounds or drugs elevate the threshold for maximal electroconvulsions in animals. To unequivocally assess the anticonvulsant potential of these agents or drugs in this test, Swinyard et al. [2] have recommended to determine doses of the compounds that increase the electroconvulsive threshold by 20% in drug-treated animals over the threshold in control animals (i.e., TID_{20} values) [1,2]. The TID_{20} values permit researchers to uniformly assess the anticonvulsant potency of drugs or agents in preclinical studies [1,2]. The TID_{20} values are usually calculated using linear regression analysis of drug doses and their corresponding threshold values [1–3].

In our previous studies, we have documented that some second-generation antiepileptic drugs (i.e., gabapentin, levetiracetam, stiripentol, tiagabine, and vigabatrin) increased the threshold for maximal electroconvulsions in mice that allowed the determination of TID_{20} values for these drugs [4–7]. Additionally, we have recently assessed the anticonvulsant potency of

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modafinil, its sulfone and acid metabolites and GBR-12909 (a prototypical dopamine transporter blocker) in the MEST test by calculating the TID_{20} values for these agents [8]. In our pilot studies we have found that 6 benzylamide derivatives (i.e., nicotinic acid benzylamide (Nic-BZA), picolinic acid 2-fluoro-benzylamide (2F-Pic-BZA), picolinic acid benzylamide (Pic-BZA), (RS)-methyl-alanine-benzylamide (Me-Ala-BZA), isonicotinic acid benzylamide (Iso-Nic-BZA), and (R)-N-methyl-proline-benzylamide (Me-Pro-BZA)) exerted the anticonvulsant properties in various experimental models of epilepsy, including the threshold for maximal electroconvulsions in mice [9–12].

Considering the above-mentioned facts, it was of pivotal importance to determine the TID_{20} values for 6 various benzylamide derivatives in the MEST test in mice in order to assess their anticonvulsant potency in this seizure model.

Materials and methods

Animals and experimental conditions

Experiments were performed on adult male albino Swiss mice weighing 22–26 g. The mice were 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 was used only once. All tests were performed between 9.00 a.m. and 2.00 p.m. Procedures involving animals and their care were conducted in conformity with current European Community and Polish legislation on animal experimentation. The experimental protocols and procedures listed below were conformed to the *Guide for the Care and Use of Laboratory Animals* and approved by the Local Ethics Committee in Lublin. All efforts were made to minimize animal suffering as well as the number of animals used in the study.

Drugs

Nic-BZA, 2F-Pic-BZA, Pic-BZA, Me-Ala-BZA, Iso-Nic-BZA and Me-Pro-BZA (synthesized by prof. R. Paruszewski, Department of Drug Chemistry, Medical University of Warsaw, Poland) were suspended in an aqueous 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (*ip*) in a volume of 0.005 ml/g body weight. The studied compounds were injected *ip* at 5 min (Nic-BZA, Iso-Nic-BZA, Pic-BZA and 2F-Pic-BZA) and 15 min (Me-Ala-BZA and Me-Pro-BZA) before the MEST test. The pretreatment times were based on the biological activity of the compounds from the literature and our previous experiments [9–12].

Maximal electroshock seizure threshold (MEST) test

Electroconvulsions (seizure activity) were produced by means of an alternating current (sine-wave, 0.2 s stimulus duration, 50 Hz, 500 V, current strength ranging from 4 to 18 mA) delivered *via* ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension. To evaluate the threshold for maximal electroconvulsions, at least 4 groups of mice, consisting of 8 animals per group, were challenged with electroshocks of various intensities to yield 10–30%, 30–50%, 50–70%, and 70–90% of animals with seizures. Then, a current intensity–response curve was constructed, according to a log-probit method by Litchfield and Wilcoxon [13], from which a median current strength (CS_{50} in mA) was calculated. Each CS_{50} value represents the current intensity required to induce tonic hind limb extension in 50% of the mice challenged. Afterwards, after administration of a single dose of the studied benzylamide derivative to 4 groups of animals, the mice were subjected to electroconvulsions (each group with a constant current intensity). The threshold for maximal electroconvulsions was recorded for 3 different doses of each benzylamide derivative as follows: 5, 10 and 15 mg/kg for Nic-BZA; 5, 10 and 20 mg/kg for 2F-Pic-BZA; 5, 7.5 and 10 mg/kg for Pic-BZA; 10, 20 and 30 mg/kg for Me-Ala-BZA; 15, 30 and 60 mg/kg for Iso-Nic-BZA; and 20, 30 and 40 mg/kg for Me-Pro-BZA. Subsequently, the percentage of increase in CS_{50} values for animals injected with increasing doses of benzylamide derivatives over the control (vehicle-treated animals) was calculated. The doses of benzylamide derivatives and their resultant percentage of threshold increase over the control (vehicle-treated animals) were graphically plotted in rectangular coordinates of the Cartesian plot system and examined with least-squares linear regression analysis [14]. From linear regression equation the TID_{20} values were calculated as recommended by Löscher et al. [1] and Swinyard et al. [2]. The experimental procedure has been described in more detail in our earlier studies [4–8].

Results

Effects of various benzylamide derivatives on the threshold for maximal electroshock-induced seizures

Nic-BZA administered systemically (*ip*) at doses of 5, 10 and 15 mg/kg elevated the threshold for MEST-induced seizures over the control by 11.5%, 26.2% and 57.4%, respectively (Fig. 1 and Table 1). 2F-Pic-BZA administered systemically (*ip*) at doses of 5, 10 and 20 mg/kg increased the threshold for MEST-induced seizures by 17.2%, 20.7% and 45.3%, respectively (Fig. 1 and Table 1). Pic-BZA administered *ip* at doses of 5, 7.5 and 10 mg/kg raised the threshold for MEST-induced seizures by 3%, 14.9% and 25.4%, respectively (Fig. 1 and Table 1). Me-Ala-BZA administered *ip* at doses of 10, 20 and 30 mg/kg elevated the threshold for MEST-induced seizures

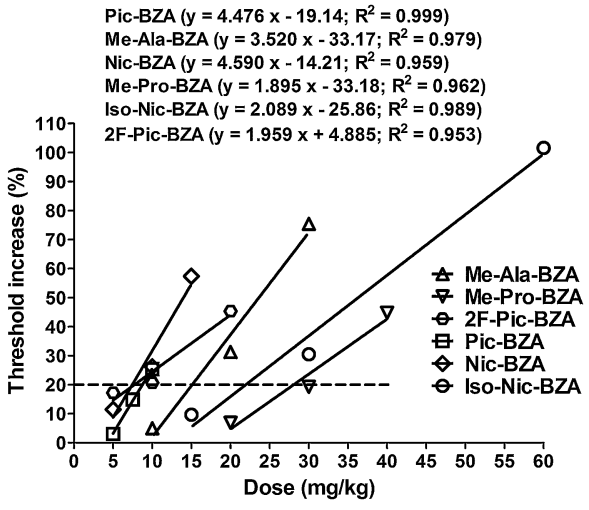

Fig. 1. Dose-threshold increase relation for 6 various benzylamide derivatives in the maximal electroshock seizure threshold (MEST) test in mice. Points placed on the graph represent threshold increasing doses of benzylamide derivatives, which were experimentally denoted in the MEST test in mice. Linear regression analysis allowed for the determination of equations for dose-threshold increase relation for various benzyladmide derivatives (presented on the graph). For each compound, *y*–is the threshold increase in %, *x*–is the dose of the studied benzylamide derivative, and R^2 – coefficient of determination [13]. The dashed line corresponds to the TID_{20} values, (threshold increasing doses by 20%) for the MEST test. From these equations one denotes the TID_{20} values, which were: 7.45 mg/kg for Nic-BZA, 7.72 mg/kg for 2F-Pic-BZA, 8.74 mg/kg for Pic-BZA, 15.11 mg/kg for Me-Ala-BZA, 21.95 mg/kg for Iso-Nic-BZA, and 28.06 mg/kg for Me-Pro-BZA, respectively.