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Influence of picolinic acid on seizure susceptibility in mice

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ABSTRACT

Background: The mechanism of drug resistance in epilepsy remains unknown. Picolinic acid (PIC) is an endogenous metabolite of the kynurenine pathway and a chelating agent added to dietary supplements. Both inhibitory and excitatory properties of PIC were reported. The aim of this study was to determine the influence of exogenously applied PIC upon the electroconvulsive threshold and the activity of chemical convulsants in eight models of epilepsy in mice.

Methods: All experiments were performed on adult male Swiss albino mice. Electroconvulsions were induced through ear clip electrodes. The electroconvulsive threshold (current strength necessary to induce tonic seizures in 50% of the tested group – CS_{50}) was estimated for control animals and animals pretreated with PIC. To determine the possible convulsant activity of PIC, it was administered subcutaneously or intracerebroventricularly in increasing doses to calculate the CD_{50} values (doses of convulsants necessary to produce seizures in 50% of the animals). Chemical convulsions were induced by challenging the animals with increasing doses of convulsant to calculate the CD_{50} values. The following convulsants were used: 4-aminopyridine, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, bicuculline, N-methyl-D-aspartate, nicotine, pentylenetrazole, pilocarpine hydrochloride and strychnine nitrate.

Results: PIC significantly decreased the electroconvulsive threshold and, after intracerebroventricular injection, but not subcutaneous, produced convulsions. Of the studied convulsants, only the activity of pilocarpine hydrochloride was significantly enhanced by PIC.

Conclusions: PIC enhances seizure activity and potentially may play a role in the pathogenesis of drug resistant epilepsy. Future studies should focus on the interactions between PIC and antiepileptic drugs.

Introduction

Drug resistance in epilepsy is defined as failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom [1]. Despite the introduction of many new antiepileptic drugs the incidence of drug resistant epilepsy remains approximately the same and reaches 30%. The mechanism of drug resistance in epilepsy remains unknown, the proposed mechanisms include increased expression of protein drug transporters, mutations of genes encoding GABA-A receptors or ion channels and interactions of antiepileptic drugs with endo- and exogenous compounds [2].

Picolinic acid (PIC) is an endogenous metabolite of the kynurenine pathway [3]. It has been detected in cell culture supernatants, blood serum, cerebrospinal fluid, human milk, pancreatic juice and intestinal homogenates [3]. Moreover, due to its chelating properties, PIC is added to chromium and iron preparations, that are used in the treatment of diabetes and anemia [4]. Other metabolites of the kynurenine pathway, quinolinic and kynurenic acids, play an important role in the pathogenesis of many neurological disorders, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and epilepsy [5,6]. Literature data concerning PIC are scarce and focus mainly on its chelating properties. There are only a few reports showing the influence of PIC on seizure activity, but the results of these studies are ambiguous. Some studies suggested anticonvulsant properties

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of PIC [7,8]. Others showed no influence of PIC on seizure activity [9,10]. Finally, strong motor excitement was also observed after PIC administration [11]. Additionally, anticonvulsant activity of various PIC benzylamide derivatives was demonstrated [12–15]. Since PIC is both an endogenous and exogenous substance, its influence on seizure susceptibility may be of clinical importance.

Objective

To assess the influence of PIC upon the electroconvulsive threshold. To assess whether exposure to PIC evokes seizures. The effect of picolinic acid on the activity of eight convulsants (pilocarpine hydrochloride (PILO), strychnine (STR), pentetrazole (PTZ), bicuculline (BCC), nicotine (NIC), 4-aminopyridine (4-AP), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA)) was additionally investigated.

Material and methods

The study protocol was accepted by a Local Bioethics Committee. All experiments were performed between 8 a.m. and 3 p.m. on adult male Swiss albino mice weighing 20–26 g. The animals were purchased from a licensed dealer (T. Górzkowska, Warsaw, Poland) and kept under standardized laboratory conditions with free access to food (chow pellets) and tap water, and maintained on a natural light–dark cycle. The experimental groups consisted of 8 animals, each animal was used only once. All experiments were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). All experiments were performed by experimenters, who were blinded to the experimental protocol.

The following substances were used: 4-AP, AMPA, BCC, NMDA, NIC, PTZ, PIC, PILO, scopolamine methyl nitrate (N-SCO), STR. All substances were provided by Sigma-Aldrich, St. Louis, MO, USA.

All solutions and suspensions were prepared just before the experiments. PIC, NIC, PTZ, PILO and STR were dissolved in sterile saline. 4-AP was suspended in a 1% solution of Tween 80 (Sigma-Aldrich, St. Louis, MO, USA). AMPA and NMDA were dissolved in 0.1 N NaOH, supplemented with sterile saline and titrated with 0.2 N HCl to pH 7.35. BIC was dissolved in glacial acetic acid supplemented with sterile saline and titrated with 0.1 N NaOH to pH 5.

All solutions and suspensions were administered in the volume of 0.01 ml/g intraperitoneally (*ip*), 0.005 ml/g subcutaneously (*sc*) or 5 μl intracerebroventricularly (*icv*). *Icv* injections were performed as described by Herman [16]. Control groups received injections of sterile saline.

Electroconvulsions

Electroconvulsions were induced using a Hugo Sachs generator delivering alternate current (500 V, 0.2 s) through ear clip electrodes. Mice were observed for the occurrence of tonic extension of the hind paws. The electroconvulsive threshold (current strength necessary to induce tonic seizures in 50% of the tested group – CS_{50}) was estimated by challenging at least 4 experimental groups with current of various intensity. A dose effect curve was constructed on the basis of the percentage of animals with seizures. PIC or saline was administered *sc* 5 min before the tests. The time interval for PIC was estimated in a pilot study (data not shown). The objective of the electroconvulsive threshold test was to assess the effect of PIC on electroconvulsions and to estimate the subthreshold dose. The subthreshold dose was the highest dose that did not affect the electroconvulsive threshold and was the dose to be used in further experiments – the chemical convulsions.

Chemical convulsions

To determine the possible convulsant activity of PIC, it was administered *sc* or *icv* in increasing doses to calculate the CD_{50} values (doses of convulsant necessary to produce seizures in 50% of the animals). A dose effect curve was constructed on the basis of the percentage of animals with convulsions. The animals were then placed singly in transparent cages and observed for 60 min for the occurrence of clonic seizures.

To determine the possible influence of PIC on chemically induced convulsions at least 4 experimental groups were challenged with increasing doses of convulsant and CD_{50} values were calculated first for animals that received a combination of convulsant and solvent, then for animals challenged with a combination of convulsant and PIC. PIC was used in the subthreshold dose, to avoid any modulation of seizure activity by the convulsive action of PIC itself. Convulsants were administered simultaneously with PIC. The animals were then placed in single transparent cages and observed for 60 min for the occurrence of clonic seizures. 4AP, NIC, PILO and STR were administered *ip*, while BIC and PTZ – *sc*. Moreover, 30 min before administering PILO mice were pretreated *ip* with N-SCO 1 mg/kg to block the peripheral effects of PILO. AMPA and NMDA were administered *icv*. PIC was administered *sc*, simultaneously with convulsants in doses not affecting the electroconvulsive threshold (25 mg/kg and 75 mg/kg) or 100 mg/kg for combinations with AMPA and NMDA. Statistical analysis and CD_{50} values with 95% confidence intervals were calculated according to Litchfield and Wilcoxon [17].

The CS_{50} and CD_{50} values with 95% confidence intervals were estimated using a computer aided probit analysis by Litchfield and Wilcoxon; p values < 0.05 were considered significant.

Results

After *sc* injections of PIC in doses of 50–200 mg/kg no seizures have occurred. *Icv* injections of PIC resulted in a dose dependent occurrence of clonic seizures with the CD_{50} value of 2.48 μmol (1.82–3.38).

PIC (100 and 150 mg/kg) administered *sc* 5 min before electroshock significantly lowered the electroconvulsive threshold in a dose dependent manner, whilst it had no effect on the CD_{50} value at the dose of 75 mg/kg (Table 1).

PIC (75 mg/kg) administered *sc* simultaneously with convulsants significantly increased the convulsant activity of PILO, whilst it did not affect the convulsant activity of STR, PTZ, BCC, NIC and 4-AP (Table 2). PIC (25 mg/kg) administered *sc* simultaneously with PILO did not affect its convulsant activity (Table 2). PIC (100 mg/kg) administered *sc* simultaneously with convulsants did not affect the convulsant activity of *icv* AMPA and NMDA (Table 2).

Discussion

The principal findings of this study are: firstly, PIC administered *icv*, but not *sc*, dose dependently induces seizures; secondly, PIC administered *sc* dose-dependently decreases the electroconvulsive

Table 1
Effect of subcutaneous picolinic acid administration upon electroconvulsive threshold in mice.

Treatment	CS50 with 95% confidence limits (mA)	p
Vehicle	5.9 (5.7–6.3)	–
Picolinic acid 75 mg/kg	5.5 (5.1–5.9)	NS
Picolinic acid 100 mg/kg	5.2 (5.0–5.5)	< 0.05
Picolinic acid 150 mg/kg	5.1 (4.6–5.5)	< 0.05