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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

Table 2
Effect of subcutaneous picolinic acid administration upon the activity of convulsants.

Treatment	CD50 with 95% confidence limits	*p*
PILO + vehicle	330.8 mg/kg (309.2–353.8)	–
PILO + PIC (25 mg/kg)	317.7 mg/kg (296.0–340.6)	NS
PILO + PIC (75 mg/kg)	300.8 mg/kg (262.0–344.4)	$p < 0.05$
STR + vehicle	1.21 mg/kg (1.14–1.29)	–
STR + PIC (75 mg/kg)	1.15 mg/kg (1.05–1.27)	NS
PTZ + vehicle	68.8 mg/kg (62.9–75.22)	–
PTZ + PIC (75 mg/kg)	67.9 mg/kg (60.3–76.4)	NS
BCC + vehicle	2.2 mg/kg (2.0–2.4)	–
BCC + PIC (75 mg/kg)	2.2 mg/kg (2.0–2.5)	NS
NIC + vehicle	8.9 mg/kg (7.3–10.9)	–
NIC + PIC (75 mg/kg)	8.4 mg/kg (7.6–9.4)	NS
4-AP + vehicle	6.9 mg/kg (6.1–7.8)	–
4-AP + PIC (75 mg/kg)	6.8 mg/kg (6.1–7.7)	NS
AMPA + vehicle	0.251 nmol (0.199–0.316)	–
AMPA + PIC (100 mg/kg)	0.248 nmol (0.186–0.351)	NS
NMDA + vehicle	0.345 nmol (0.294–0.404)	–
NMDA + PIC (100 mg/kg)	0.343 nmol (0.297–0.397)	NS

NS – not significant.

threshold; and finally, PIC enhanced the convulsant activity of PILO, but not of the other convulsants studied.

The fact that after *icv* injection of PIC dose-dependently induced clonic seizures (CD50–2.48 μmol), but not after *sc* injections (up to 200 mg/kg), might suggest that PIC has a limited ability to cross the blood-brain barrier or is rapidly eliminated after systemic administration. On the other hand, PIC administered *sc* at the dose of 100 mg/kg significantly decreased the electroconvulsive threshold, which seems to point at its ability to cross the blood-brain barrier.

PIC (75 mg/kg) administered *sc* did not affect the convulsant activity of STR, PTZ, BCC, NIC, 4-AP, AMPA and NMDA. It therefore seems that PIC does not affect the basic mechanisms of action of these convulsants, which are as follows: 4-AP – voltage activated potassium channel type Kv1 blocker; BCC – GABA-A ionotropic receptor antagonist; AMPA – AMPA subtype glutamate receptor agonist; NMDA – NMDA subtype glutamate receptor agonist; NIC – nicotinic cholinergic receptor agonist; PTZ – GABA-A ionotropic receptor antagonist; STR – glycine receptor antagonist [18].

We have found, that PIC enhanced the activity of PILO. To the best of our knowledge this is the first paper investigating the interactions of PIC and PILO. Taking into consideration the mechanism of action of PILO it would be reasonable to assume, that this action of PIC may be explained by stimulation of cholinergic muscarinic receptors [18]. However, both in our study and in previous reports [11] exposure to high doses of PIC did not result in systemic cholinergic effects, and that is why direct action of PIC on the cholinergic muscarinic receptors seems rather unlikely. It may be speculated that the enhancement of convulsant activity of PILO was caused by sensitization of muscarinic receptors to its action. Involvement of other mechanisms, such as pharmacokinetic interactions, cannot be ruled out.

Available data on the influence of PIC on seizure activity are scarce and ambiguous. Lapin observed strong motor excitement in half of the studied mice after *icv* injection of 50 μg of PIC, which confirms its stimulatory effect [11]. The dose used by Lapin was 6 times lower than evoked seizures in our study. Moreover, Lapin administered PIC *ip* in doses of 200–1000 mg/kg: at 200 mg/kg no visible effect occurred, at 400–800 mg/kg tremor in most animals was observed, while at 1000 mg/kg all animals rapidly died [11]. These findings are in concordance with our results, that systemic administration of PIC does not induce seizures. This might suggest that the ability of PIC to cross the blood brain barrier is limited, or that the systemic effects result in death before PIC reaches sufficient level in the brain to evoke seizures. Moreover, PIC plasma levels are at concentrations 3–15 times higher than those in the brain, which further confirms that the flux of PIC through the blood brain barrier is likely to be limited [19]. Therefore, the pro-convulsive effect of PIC observed in the present study may have been indirect: either *via* a PIC metabolite that enters the brain or by a brain-penetrant effector molecule that is stimulated after PIC administration in the periphery.

In another study Lapin assessed the influence of PIC on the activity of various convulsants in mice [7]. PIC after systemic or *icv* administration prolonged the latency of seizures induced by PTZ and STR, but did not affect the percentage of animals with seizures. PIC did not have any effect on seizure activity induced by thiosemicarbazide, caffeine and quinolinic acid. This study was however intended to investigate the possible anticonvulsant activity of PIC and the doses of convulsants used evoked seizures in 80–90% of mice, therefore a possible convulsant effect of PIC might have been overlooked. Moreover, the suggested anticonvulsant activity was limited in most cases to prolonging the latency of seizures, which should not be interpreted as true anticonvulsant activity.

On the other hand, PIC after systemic (*ip* and *po*) administration dose-dependently blocked seizures induced by *icv* administration of kynurenine sulfate [7]. This is the only case of fully documented anticonvulsant action of PIC. Both PIC and kynurenine are metabolites of the kynurenine pathway, and, as discussed above the metabolites of this pathway may affect seizure activity. Complex interactions, both pharmacokinetic and pharmacodynamic, may be suspected between various metabolites of the kynurenine pathway and these could explain the anticonvulsant action of PIC in this seizure model. Interestingly, the authors observed that PIC blocked kynurenine-induced seizures also after *icv* injection, but in a reverse dose-response relationship [7], that might be related to the previously described excitatory effect of PIC [11]. These results also suggest, that PIC after systemic administration crosses the blood brain barrier in a sufficient amount to interact with other pharmacologically active substances.

Neuroprotective effects of PIC have also been reported. The finding that PIC influences the excitotoxic effect of kainic acid and quinolinic acid (which require an intact glutamatergic afferent input for their neurotoxic effect) led to raising a hypothesis, that the protective effect of PIC may involve an interaction with the glutamatergic input [20]. The authors also suggested that the effect on quinolinic acid induced neurotoxicity may result from the fact, that both compounds derive from the kynurenine pathway. In another study the authors further investigated the neuroprotective influence of PIC on kainic and quinolinic acid induced neurotoxicity and did not confirm the involvement of glutamatergic afferent input in this interaction [21]. The authors concluded that the mechanism of action of PIC remains unknown and that influencing the release of another neurotransmiter or neuromodulator cannot be excluded. Surprisingly, in the same study a stimulatory effect of PIC administered alone was also reported [21]. Finally, it was found that PIC decreased quinolinic acid induced neurotoxicity without significantly influencing quinolinic acid induced excitation [22]. These findings additionally confirm the extremely complex spectrum of effects of PIC in the central nervous system.

Interestingly, more is known about the effect of PIC derivatives on seizure susceptibility than in the case of the parent compound. Many PIC benzylamide analogs were obtained and screened for anticonvulsant properties and picolinic acid-2-fluorobenzylamide (PIC-2F-BZA) appeared to be the most effective of these compounds [12]. Therefore, PIC-2F-BZA was further tested in numerous experimental seizure models: MES, BIC, PTZ, PILO, AMPA, kainic acid (KA) and NMDA-induced seizures, producing clear-cut anti-seizure effects in all of these models [13]. Continuing

the project of screening for new antoconvulsants, 12 new benzylamides, including 5 PIC derivatives were obtained [14]. However, taking onto consideration the PIC derivatives, only picolinic acid 2-chlorobenzylamide and picolinic acid phenylethylamide demonstrated a potent anticonvulsant activity, unfortunately together with an neurotoxic effect and thus were not qualified for further testing [14]. Finally, the anticonvulsant potency of various benzylamide derivatives was compared and PIC-2F-BZA and PIC benzylamide were among the three most potent derivatives [15]. The anticonvulsant effect of PIC benzylamide derivatives is especially interesting when confronted with the convulsive activity of the parent compound shown in the present study.

In conclusion, 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.

Conflict of interest

The authors declare no conflict of interest.

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