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1-Methyl-4-phenylpyridinium and 3-nitropropionic acid diminish cortical synthesis of kynurenic acid via interference with kynurenine aminotransferases in rats

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Abstract

The aim of the present study was to evaluate the effect of mitochondrial inhibitors, 1-methyl-4-phenylpyridinium (MPP^+) and 3-nitropropionic acid (3-NPA), on the brain production of endogenous glutamate antagonist, kynurenic acid (KYNA). MPP^+ and 3-NPA dose-dependently impaired the synthesis of KYNA in rat cortical slices. Enzymatic studies revealed that MPP^+ inhibits in a concentration-dependent manner the activity of kynurenine aminotransferase II (KAT II), but not the activity of kynurenine aminotransferase I (KAT I). 3-NPA impaired the activity of both enzymes, KAT I and KAT II. Thus, MPP^+- and 3-NPA-evoked neurotoxicity may be at least partially associated with the depletion of KYNA.

Keywords: Kynurenic acid; Kynurenine aminotransferase; 1-Methyl-4-phenylpyridinium; 3-Nitropropionic acid; Rat

Increasing evidence suggests that the defects in mitochondrial energy metabolism may play an important role in the pathogenesis of human neurodegenerative diseases and epilepsy [1,16,18]. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), known to contaminate certain 'street drugs', has been described as destroying nigrostriatal dopaminergic neurones in the brain of animals and humans, and producing Parkinson's-like symptoms [3,7]. 1-Methyl-4-phenylpyridinium (MPP^+), the metabolite of MPTP, evokes seizures in rodents following intraventricular application [16]. It acts as the inhibitor of mitochondrial complex I and is formed due to the monoamine oxidase B-mediated oxidation. This process takes place in the glial cells, which suggests an astrocytic contribution to the development of certain neuropathologies [4]. 3-Nitropropionic acid (3-NPA) is the inhibitor of succinic dehydrogenase, a component of mitochondrial complex II. In humans, 3-NPA intoxication is associated with an acute encephalopathy, including convulsions, electroencephalographic abnormalities and late-onset dystonia [8]. In animals, the chronic systemic administration of low doses of 3-NPA induces striatum-selective lesions, followed by motor symptoms resembling Huntington's disease, whereas the acute application, either peripheral or intracerebral, evokes seizures [2,18,19]. It was revealed that cellular events initiated by MPP^+ and 3-NPA might include the secondary activation of excitatory amino acid receptors. The protective effect of glutamate receptor antagonists was demonstrated in the MPTP model of dopaminergic toxicity [15], against seizures caused by MPP^+ [16], and by 3-NPA [19]. These data, together with the fact that the neuropathological pattern following application of MPTP and 3-NPA closely resembles neurodegenerative diseases, coincide with the hypothesis linking energy metabolism failure with the glutamate-mediated neuronal loss [9]. Deranged oxidative phosphorylation associated with the use of mitochondrial toxins may increase the vulnerability of neurones to the endogenous glutamate and thus initiate changes leading to neurodegeneration [1].

Kynurenic acid (KYNA), the only known endogenous antagonist of glutamate receptors [14], is synthesized via an irreversible transamination of its bioprecursor, L-kynurenine, performed by predominantly glial enzymes, kynurenine aminotransferases (KATs) I and II [5]. KAT I moderately contributes to the cerebral KYNA formation under physiological conditions due to its alkaline pH opti-

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mum of 9.5 [5]. KAT II, which has a neutral optimal pH, catalyzes approximately 75% of KYNA synthesis in the brain [5].

Despite certain controversies, KYNA was implicated as a modulator of physiological glutamate neurotransmission, and its altered metabolism has been suggested as one of the factors contributing to the development of seizures and neurodegenerative disorders [14]. Moreover, the interaction between KYNA and glutamate seems to be reciprocal, since the excessive glutamate inhibits KYNA formation in vitro [20]. Therefore, we aimed to evaluate the effect of MPP^+ and 3-NPA on the basic and the glutamate-inhibited brain production of KYNA.

Male Wistar rats (220–250 g), housed under standard laboratory conditions, were used. Experimental procedures have been approved by the Local Ethical Committee in Lublin and are in agreement with the European Communities Council Directive on the use of animals in experimental studies. L-kynurenine (sulphate salt), KYNA, MPP^+, 3-NPA, pyruvate, L-glutamate, glutamine, pyridoxal-5′-phosphate, 2-mercaptoethanol, Trizma base and cellulose membrane dialysis tubing were obtained from Sigma (St. Louis, MO), whereas all high performance liquid chromatography (HPLC) reagents were obtained from Baker. Other chemicals were of the highest available purity. KYNA production in vitro was investigated according to the method of Turski et al. [17]. Briefly, animals were killed by decapitation and their brains were rapidly removed from the skull. Cortical slices (1×1 mm base) were prepared with a McIlwain tissue chopper and placed in culture wells (eight in each well) containing the oxygenated Krebs–Ringer buffer (pH 7.4). The incubation (37 °C, 2 h) was carried out in the presence of 10 μM L-kynurenine and the solutions of tested compounds (added 10 min before L-kynurenine), in a final volume of 1 ml. At least six wells were used for each studied concentration. Upon completion of the incubation period, the media were rapidly separated from the tissue, acidified with 0.1 ml of 1 N HCl and 14 μl of 50% trichloroacetic acid (wt/vol), and centrifuged. Supernatant was applied on the cation-exchange resin (Dowex 50 W^+). Eluted KYNA was subjected to HPLC and quantified fluorimetrically (Varian HPLC system; ESA catecholamine HR-80, 3 μm, C_{18} reverse-phase column), as previously described [20]. The mean control production of KYNA in the presence of 10 μM L-kynurenine was 6.2 ± 0.6 pmol/h per well.

The activities of KAT I and KAT II were assayed according to the method of Guidetti et al. [5], with modification. Briefly, the cortical brain tissue was homogenized (1:10; wt/vol) in 5 mM Tris–acetate buffer (pH 8.0), containing 50 μM pyridoxal-phosphate and 10 mM 2-mercaptoethanol. The resulting homogenate was dialyzed using cellulose membrane dialysis tubing overnight at 8 °C, against 4 l of the buffer composed as above. The enzyme preparation was incubated in the reaction mixture containing 2 μM L-kynurenine, 1 mM pyruvate, 70 μM pyridoxal-5′-phosphate, 150 mM Tris–acetate buffer (all concentrations final), and the solutions of tested compounds, at pH of 7.0 or 9.5, for KAT II or KAT I, respectively. Six replicates were used for each concentration and each experiment was repeated twice. Glutamine (final concentration 2 mM), the inhibitor of KAT I, was added to the samples assaying KAT II activity. Blanks contained the enzyme preparation which was heat-deactivated (100 °C, 10 min). The incubation (37 °C, 2 h) was terminated by the rapid transfer of samples to an ice-bath and by the addition of 50% trichloroacetic acid and 0.1 N HCl. Denaturated protein was removed by centrifugation and the supernatant was applied to a Dowex 50 W^+ column. Further procedures were performed as described above. The mean control activity of KAT I and KAT II in the presence of 2 μM kynurenine was 1.38 ± 0.11 and 0.89 ± 0.08 pmol/mg tissue per h, respectively.

The concentration of a compound necessary to induce the 50% inhibition of KYNA synthesis (IC_{50}), with 95% confidence limits, was calculated using the computerized linear regression analysis of quantal log dose-probit function. The statistical comparisons of results were performed using an ANOVA test, with the adjustment of P value by the method of Bonferroni.

MPP^+ used in the concentration of 2, 3, 5 and 10 mM significantly diminished the synthesis of KYNA in cortical slices to 82.0% ($P < 0.05$), 78.9% ($P < 0.05$), 72.7% ($P < 0.01$) and 60.1% ($P < 0.001$) of control production, respectively (Fig. 1A). MPP^+, in the concentration of 5 mM but not 1 mM, acted synergistically with L-glutamate, enhancing the impairment of KYNA synthesis evoked by 0.25, 1.0 and 3.0 mM L-glutamate ($P < 0.01$, $P < 0.001$, $P < 0.001$, respectively) (Fig. 1B). MPP^+ (up to 10 mM) did not affect the activity of KAT I (Fig. 1C). MPP^+, in concentrations of 3, 5, 7 and 10 mM, reduced the activity of KAT II to 90.1% ($P < 0.05$), 86.4% ($P < 0.01$), 75.3% ($P < 0.01$) and 67.0% ($P < 0.001$) of control, respectively (Fig. 1C).

3-NPA dose-dependently inhibited the production of KYNA in cortical slices reaching the IC_{50} value of 7.9 (6.1–10.4) mM (Fig. 2A). 3-NPA, at the concentration of 5 mM but not 1 mM, significantly augmented the impairment of KYNA production evoked by 0.25, 1.0 and 3.0 mM L-glutamate ($P < 0.001$, $P < 0.001$, $P < 0.001$, respectively) (Fig. 2B). 3-NPA, in the concentration of 3, 5, 7 and 10 mM, inhibited the activity of KAT I to 83.7% ($P < 0.05$), 70.0% ($P < 0.001$), 52.1% ($P < 0.001$) and 40.2% of control (Fig. 2C), with the IC_{50} value of 7.7 (6.7–8.8) mM, and the activity of KAT II to 85.5% ($P < 0.05$), 76.8% ($P < 0.01$), 65.4% ($P < 0.001$) and 55.1% of control, respectively (Fig. 2C).

The above data show that mitochondrial toxins, MPP^+ and 3-NPA, inhibit de novo synthesis of KYNA in rat brain cortical slices. Moreover, MPP^+ inhibits KAT II activity, whereas 3-NPA affects both enzymes known to synthesize KYNA in the rat brain. This varied inhibition pattern might explain relatively weaker inhibition exerted by MPP^+

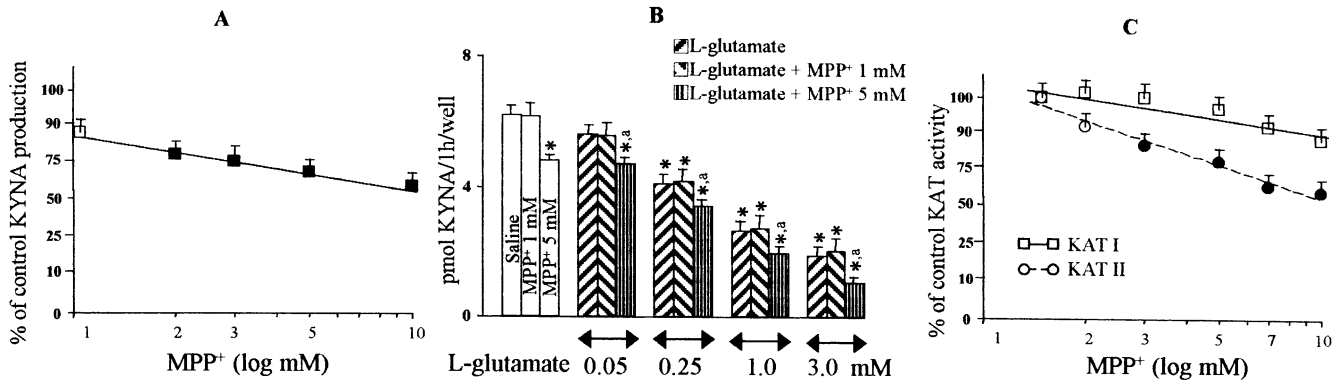

Fig. 1. Effect of MPP^+ on basal (A) and glutamate-inhibited (B) production of KYNA in cerebral cortical slices and (C) on the activity of KATs. All data are mean ± SD values ($n = 6$). (A,B) Slices (1 × 1 mm base) were placed in culture wells (eight per well) containing 1 ml of oxygenated Krebs–Ringer buffer (pH 7.4). Tissue was incubated with 10 μM L-kynurenine and solutions of tested compounds (37 °C, 2 h). Newly synthesized KYNA was separated using Dowex 50 W^+ and quantified fluorimetrically, with HPLC. (C) Dialysate of tissue preparation was incubated with tested compound, 2 μM L-kynurenine, 1 mM pyruvic acid and 70 μM pyridoxal 5′-phosphate in 150 mM Tris–acetate buffer, pH 7.0 for KAT II and pH 9.5 for KAT I analysis (37 °C, 2 h). Glutamine (final concentration 2 mM), the inhibitor of KAT I, was added to samples assaying KAT II activity. Produced KYNA was quantified as above. (A,C) Ordinate was expressed as % of control KYNA production (probit scale), while abscissa was expressed as concentration of MPP^+ (log scale). Filled symbols represent data points which reached statistical significance ($P < 0.05$, ANOVA) vs. control (100%). (B) Ordinate was expressed as KYNA production in pmol/1 h per well (linear scale), while numbers along abscissa indicate concentrations of L-glutamate (mM). $^*P < 0.05$ vs. control and $^aP < 0.05$ vs. L-glutamate (ANOVA). (A) MPP^+: $y = -10.4\ln(x) + 85.1$; $r = -0.99$. (C) KAT I: $y = -10.6\ln(x) + 108.4$; $r = 0.95$. KAT II: $y = -21.9\ln(x) + 10.8$; $r = 0.99$.

in comparison with 3-NPA. The effective milimolar concentration of MPP^+ and 3-NPA here could be viewed as relatively high. However, despite the fact that during prolonged exposure to MPP^+, its extracellular toxic levels are in the micromolar range, MPP^+ undergoes intense, up to 40-fold, accumulation in mitochondria, attaining there a milimolar concentration [10].

Similarly to our findings, it was communicated previously that 3-NPA, studied in a single, 10 mM concentration, strongly inhibits KYNA formation in rat cortical slices [6]. The authors, though, found no effect of 3-NPA on the total KAT activity, assayed as a mixture of non-separated enzymes [6]. This methodological difference between both studies has probably contributed to the observed discrepancy.

The neurotoxic consequences of the impaired mitochondrial respiration were associated with NMDA-mediated excessive neuronal Ca^{2+} accumulation [9]. Although initially controversial, it seems confirmed now that excitatory amino acids are involved in the action of MPP^+ and 3-NPA. Thus, the NMDA receptor antagonists, dizocilpine, 2-amino-7-phosphonoheptanoic acid (APH) and 3-(2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP), were shown to prevent dopaminergic cell loss produced by the intranigral infusion of MPP^+ [13,15]. Similarly, the neurotoxic effects of 3-NPA in striatal and cerebellar cultures were impaired by dizocilpine

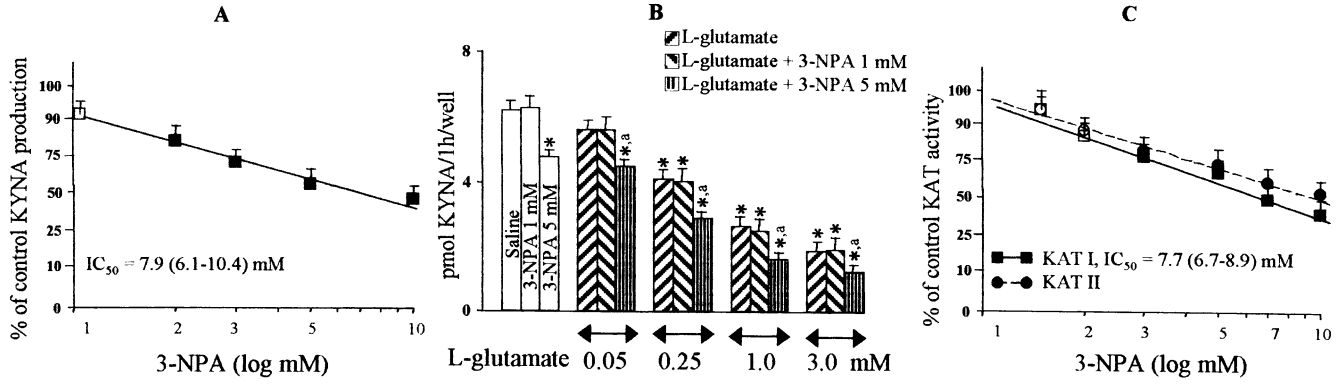

Fig. 2. Effect of 3-NPA on basal (A) and glutamate-inhibited (B) production of KYNA in cerebral cortical slices and (C) on the activity of KATs. Methods as described in Fig. 1. (A,C) Ordinate was expressed as % of control KYNA production (probit scale), while abscissa was expressed as concentration of 3-NPA (log scale). Filled symbols represent data points that have reached statistical significance ($P < 0.05$, ANOVA) vs. control (100%). (B) Ordinate was expressed as KYNA production in pmol/1 h per well (linear scale), while numbers along abscissa indicate concentrations of L-glutamate (mM). $^*P < 0.05$ vs. control and $^aP < 0.05$ vs. L-glutamate (ANOVA). (A) 3-NPA: $y = -17.9\ln(x) + 88.5$; $r = 0.98$. (C) KAT I: $y = -21.2\ln(x) + 94.1$; $r = 1.0$; KAT II: $y = -18.9\ln(x) + 96.2$; $r = 0.99$.

[21,22]. Another NMDA receptor blocker, 7-chlorokynurenic acid, incompletely reversed the depolarization of hippocampal neurones occurring after 3-NPA application [11]. 2-Amino-5-phosphonopentanoic acid (APV) delayed, but did not prevent, 3-NPA-induced toxicity in cultured cerebellar granule cells [21]. In the approaches evaluating the effect of mitochondrial inhibitor on the toxicity of glutamate receptor agonists, it was shown that 3-NPA lowers the threshold for neurodegeneration evoked by intrastriatal NMDA injections [12], and for seizures caused by the intracerebrally applied α-amino-3-hydroxy-5-methyloisoxazolo-4-propionate (AMPA) and kainate, but not by NMDA [19].

The dysfunctional metabolism of brain glutamate antagonist KYNA, the compound possibly modulating NMDA receptor function, was linked with the pathogenesis of seizures and neurodegenerative diseases [14]. Presented data show that both mitochondrial toxins studied here reduce KYNA synthesis, which could additionally contribute to the relative overactivity of glutamate receptors following the inhibition of oxidative phosphorylation. Moreover, the inhibitory concentrations of MPP^+ and 3-NPA act synergistically with L-glutamate, increasing the inhibition of KYNA formation exerted by the latter compound. This could be yet another mechanism by which compromised energy metabolism exacerbates the toxicity of glutamate receptor agonists. Thus, the neurotoxic consequences evoked by MPP^+ and 3-NPA may be at least partially associated with the decrease of KYNA production.

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