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Original article

The effect of three angiotensin-converting enzyme inhibitors on kynurenic acid production in rat kidney *in vitro*

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ABSTRACT

Background: The renin-angiotensin system (RAS) is commonly known to regulate blood pressure, water and electrolyte homeostasis, however it also exerts paracrine and autocrine actions on the kidney. Angiotensin-converting enzyme inhibitors (ACE-Is), alongside their hypotensive properties, have been shown to decrease kidney function decline in animal models of nephropathy.
Glutamate (GLU) is the main stimulatory neurotransmitter in the central nervous system, however its importance in the periphery should also be considered. Activation of renal GLU receptors has been linked to normal kidney function and also renal injury. The wide spectrum GLU receptor antagonist kynurenic acid (KYNA) possesses neuroprotective and central hypotensive effects, however its actions outside the brain are less well recognized. KYNA is a tryptophan metabolite synthesized from kynurenine by kynurenine aminotransferases (KATs). The purpose of this study was to examine the influence of three ACE-Is: lisinopril, perindopril and ramipril on KYNA production and KATs activity in rat kidney *in vitro*.
Methods: The effect of ACE-Is on KYNA production and KATs activity was examined in rat kidney homogenates. KYNA was detected by high-performance liquid chromatography (HPLC) and quantified fluorometrically.
Results: All examined ACE-Is: lisinopril, perindopril and ramipril decreased KYNA production in rat kidney *in vitro*. KAT I activity was decreased by lisinopril and ramipril whereas the activity of KAT II was lowered by ramipril.
Conclusion: Our study shows that ACE-Is can decrease KYNA production in rat kidney *in vitro*. Further studies are required to determine the clinical importance of the inhibitory action of ACE-Is on KYNA synthesis in the kidney.
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Introduction

Kidney failure has become a global challenge since it leads to multi-organ damage and an increased risk of premature death [1]. The predominant causes of chronic kidney disease are diabetes mellitus, arterial hypertension and glomerulonephritis [2]. It is widely accepted that hyperactivity of the renin-angiotensin system (RAS) may play a pivotal role in the development of kidney damage due to the promotion of vasoconstriction, oxidative stress, inflammation and fibrosis [3]. Therefore, kidney failure can be efficiently delayed in many patients by using pharmacological agents that target this system. Indeed, the reduction of angiotensin II production by angiotensin-converting enzyme inhibitors (ACE-Is) is the primary mechanism of their renoprotective action [4]. A decrease in oxidative kidney damage [5] and a reduction of albuminuria [6] are the most important advantages of therapy with ACE-Is. Apart from beneficial effects ACE-Is were also shown to reduce glomerular filtration rate with an increase in serum creatinine concentration or hyperkalemia [7].

Glutamate (GLU) is an excitatory neurotransmitter in the central nervous system. However, GLU and its receptors are present in peripheral non-neuronal tissues, including the kidney. Accordingly, the role of GLU in the regulation of acid-base balance, renin secretion or renal blood flow has been described [8]. Moreover, GLU signaling has been shown to be involved in a reduction in the antioxidant status of the kidney after injury [9,10].

In the kidney, ionotropic subtypes of GLU receptors such as the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate receptors were identified on podocytes, glomeruli and mesangial cells [11]. The wide

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distribution of GLU receptors in the juxtaglomerular apparatus and proximal tubules suggests their involvement in electrolyte and water homeostasis [12]. The visualization of anti-GLU receptor immunoreactivity within the granular cells of the afferent arteriole points to their potential involvement in the control of renin release [12]. GLU receptors may also be implicated in kidney pathology. An increased expression of the NR1 subunit of the NMDA receptor was found in an animal model of kidney failure induced by gentamicin [13]. Similarly, an upregulation of NMDA receptor expression was observed in homocysteinemia-induced glomerulosclerosis in rats [14]. In both studies, treatment with the NMDA receptor antagonist MK-801 significantly reduced pathological changes in the kidney [13,14].

Kynurenic acid (KYNA) is a broad spectrum antagonist of ionotropic GLU receptors [15,16]. KYNA is a tryptophan metabolite produced endogenously from kynurenine (KYN) by kynurenine aminotransferases (KATs) [17]. Interestingly, its content in rat kidney is distinctly higher in comparison to other organs and tissues [18]. Unmetabolized KYNA is excreted with urine [19,20]. The involvement of KYNA in the regulation of central nervous system function is well recognized [21–23]. In addition it has been shown that KYNA has hypotensive properties after intracerebral administration in animal models of hypertension [24,25]. Recently, it has been reported that ACE-Is modulate KYNA production in rat brain cortex *in vitro* [26]. Therefore, the aim of this study was to examine whether three ACE-Is, lisinopril, perindopril and ramipril affect KYNA synthesis in rat kidney homogenates and activity of isolated, partly purified KATs *in vitro*.

Material and methods

Animals

Experiments were conducted on male Wistar rats (Brwinów, Poland) weighing 150–200 g. Animals were kept under standard laboratory conditions (20 °C, 12-h light-dark cycles, with food and water available *ad libitum*). Procedures were performed between 7 a.m. and 1 p.m. All animals after arrival were housed in the laboratory conditions for a minimum of 7 days before tests were carried out. Experimental procedures were accepted by the Local Ethics Committee for Animal Experiments and are in agreement with Directive 2010/63/EU for the protection of animals used for scientific purposes.

Chemical substances

L-Kynurenine (sulfate salt), lisinopril, perindopril erbumine, ramipril and dimethyl sulfoxide (DMSO), Krebs-Ringer buffer components (sodium chloride, potassium chloride, magnesium sulfate, calcium chloride, sodium phosphate monobasic, sodium phosphate dibasic, glucose, distilled water), dialysate buffer components (Trizma base, acetic acid, pyridoxal 5′-phosphate, 2-mercaptoethanol) and components of incubation solutions to measure enzyme activity (Trizma base, acetic acid, pyruvate, pyridoxal 5′-phosphate, glutamine) were purchased from Sigma-Aldrich. Reagents used for HPLC: water ($\leq$2.0 ppm residue after evaporation), acetonitrile (>99.99% purity), acetic acid (>99.5% purity), zinc acetate dihydrate (>98% purity), sodium acetate trihydrate (>99.5% purity) were obtained from J.T. Baker Chemicals and from Sigma-Aldrich.

Lisinopril was dissolved in water, whereas perindopril and ramipril were dissolved in DMSO. The final concentration of DMSO in the incubation medium was 5% [27]. DMSO was added to appropriate controls at the same volume.

Evaluation of KYNA production in rat kidney homogenates in vitro

After decapitation the kidneys were removed from the abdomen and put immediately on ice. Kidneys were weighed and homogenized in oxygenated Krebs-Ringer buffer at pH 7.4 (1:4 w/v). Next, the kidney homogenate (50 μl) was added to test tubes, filled with 850 μl of oxygenated Krebs-Ringer buffer at pH 7.4. The incubation lasted 2 h at 37 °C in the presence of 15 μM L-kynurenine (50 μl) and one of 6 different drug concentrations (in mM): 0.001, 0.01, 0.05, 0.1, 0.5 and 1. At least 6 test tubes were used to analyze each drug concentration. The incubation was ended by transporting test tubes into an ice cold bath. 100 μl of 1 M HCl was used to precipitate proteins. All samples were centrifuged (12,000 rpm for 15 min) and subjected to the high-performance liquid chromatography (Thermo Fisher Scientific HPLC system, ESA catecholamine HR-80, 3 μm, C18 reverse-phase column). KYNA content in the samples was quantified fluorometrically against purified KYNA. To calculate de-novo production of KYNA the endogenous content of KYNA found in blanks incubated for 2 h without added L-kynurenine was subtracted from the total amount measured in the sample containing added L-kynurenine. Similar methodology was used in experiments in rat brain slices [26].

Evaluation of the activity of kynurenine aminotransferases in rat kidney homogenates in vitro

To analyze KAT I and KAT II activity, kidneys were homogenized in dialysate buffer with 5 mM Tris-acetate buffer at pH 8.0, 50 μM pyridoxal 5′-phosphate and 10 mM 2-mercaptoethanol. Prepared homogenate was centrifuged (12,000 rpm for 15 min), then obtained supernatant was dialyzed for 12 h at 8 °C by using cellulose membrane dialysis tubing (average flat width 10 mm, Sigma Aldrich) in 4 l of the dialysate buffer prepared as mentioned above. After dialysis the enzyme supernatant was incubated in the reaction mixture containing incubation solution with 2 μM L-kynurenine and solutions of tested substances in 6 concentrations (mM): 0.001, 0.01, 0.05, 0.1, 0.5 and 1. The reaction pH was 9.5 for KAT I activity and 7.0 for KAT II activity. Glutamine was added to the samples to evaluate the activity of KAT II. Three probes were used for each drug concentration. After 2 h at 37 °C the incubation was ended by putting all the samples into an ice cold bath. Supernatants were centrifuged and analyzed using the same methods as samples from kidney homogenates.

Statistical analysis

All presented data are expressed as a percentage of control values. Mean and standard error of the mean (SEM) were calculated. Statistical analysis was performed using one way analysis of variance (ANOVA) with *post-hoc* Tuckey-Kramer test. The $p < 0.05$ was set as statistically significant. All calculations were carried out with GraphPad InStat program, version 3.06.

Results

Evaluation of KYNA production in rat kidney homogenates in vitro

De novo production of KYNA in rat kidney homogenate under standard conditions was 6.13 ± 0.40 pmol/100 μl. Lisinopril at 0.1 mM, 0.5 mM and 1 mM concentration decreased KYNA production in rat kidney homogenates *in vitro* to 81% ($p < 0.01$), 73% ($p < 0.001$) and 65% ($p < 0.001$) of control values respectively (Fig. 1A). Similarly perindopril at 0.1 mM, 0.5 mM and 1 mM concentration lowered KYNA production in rat kidney homogenates *in vitro* to 79% ($p < 0.01$), 78% ($p < 0.01$) and 78% ($p < 0.01$) of control values respectively (Fig. 1 B). Ramipril at the concentration

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