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Angiotensin-converting enzyme inhibitors modulate kynurenic acid production in rat brain cortex *in vitro*

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ABSTRACT

It is well established that the renin-angiotensin system (RAS) is present in the brain and that glutamate activates the brain centers responsible for blood pressure control. An antagonist of glutamate, kynurenic acid (KYNA) was shown to decrease blood pressure after intracerebral administration. KYNA is an endogenous metabolite of tryptophan produced from the breakdown of kynurenine by kynurenine aminotransferases (KAT), mainly within astrocytes.

The purpose of this study was to evaluate the influence of three angiotensin-converting enzyme inhibitors (lisinopril, perindopril and ramipril) on KYNA production and KAT activity in the rat brain cortex *in vitro*.

The effect of the angiotensin-converting enzyme inhibitors on KYNA production was examined on rat brain cortical slices incubated for 2 h in the presence of L-kynurenine and the angiotensin-converting enzyme inhibitors. To analyze KAT I and KAT II activity, brain cortical homogenates were incubated for 2 h with L-kynurenine and the tested drugs. KYNA was separated by HPLC and quantified fluorometrically.

Among the examined angiotensin-converting enzyme inhibitors, lisinopril increased KYNA production, perindopril was ineffective, and ramipril decreased KYNA synthesis in rat brain cortical slices. Lisinopril increased KAT I activity and perindopril did not affect it. However, ramipril lowered KAT I activity in rat brain cortex *in vitro*. Neither lisinopril nor perindopril affected KAT II activity, but ramipril decreased KAT II activity in the rat brain cortex *in vitro*.

Our study reveals that angiotensin-converting enzyme inhibitors show various influences on KYNA production in rat brain cortical slices and activity of KATs.

1. Introduction

Arterial hypertension can affect up to 45% of the population and, if not treated appropriately, may lead to life-threatening conditions, such as heart failure, myocardial infarction or stroke (ESH/ESC Task Force for the Management of Arterial Hypertension, 2013). Among antihypertensive agents, the most recommended are renin-angiotensin system (RAS) inhibitors. They not only reduce the blood pressure level but also decrease tissue remodeling in diabetes, kidney failure, metabolic syndrome, cardiac arrhythmias or organ ischemia (Paulis et al., 2016). More than 40 years ago, Ganten et al. (1971) described a renin-like enzyme producing angiotensin in the brain. In addition to its involvement in water and electrolyte balance regulation, the central RAS is involved in the pathogenesis of Alzheimer disease, Parkinson disease (Wright et al., 2013) and epilepsy (Pereira et al., 2010). Within the RAS, angiotensin-converting enzyme plays a crucial role. Angiotensin-converting enzyme inhibitors decrease the conversion of angiotensin I to angiotensin II, as well as the metabolism of other substances, including bradykinin. These actions potentiate their hypotensive (Regulski et al., 2015) and neuroprotective effects (Hirooka and Shiraga, 2007).

The results obtained from animal studies indicate that glutamate is the main neurotransmitter leading to the activation of brain centers responsible for blood pressure control (Reis, 1996).

Kynurenic acid (KYNA) is an endogenous tryptophan metabolite that has been known since 19th century (Liebig, 1853). KYNA is produced from its immediate precursor kynurenine, mainly in astrocytes (Guillemin et al., 2000). Synthesis of KYNA is catalyzed by kynurenine aminotransferases (KAT), of which KAT I and KAT II play the predominant roles (Schwarcz and Pellicciari, 2002). KYNA is a broad spectrum glutamate receptor antagonist (Kemp et al., 1988; Doi et al., 1990). Because of its poor penetration through the

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blood-brain barrier (Fukui et al., 1991), even small changes in KYNA brain production may affect central nervous system function. It has been reported that KYNA takes part in blood pressure control. In animal models of hypertension, intracerebral administration of KYNA decreased blood pressure (Ito et al., 2000) or blocked pressor responses caused by the local injection of glutamate (Araujo et al., 1999).

The goal of the present study was to examine whether three angiotensin-converting enzyme inhibitors (lisinopril, perindopril and ramipril) affect KYNA synthesis and KAT activity in the rat brain cortex *in vitro*.

2. Materials and methods

2.1. Animals

Experiments were conducted on male Wistar rats weighing 150–200 g (purchased from licensed breeder, Brwinow, Poland). Animals were housed under standard laboratory conditions at 20 °C temperature, 12-h light-dark cycles, with food and water available *ad libitum*. Every procedure was performed between 7 a.m. and 1 p.m. All animals were allowed to adapt to the laboratory environment for a minimum of 7 days before tests were carried out. The described procedures were approved by the I Local Ethics Committee for Animal Experiments in Lublin.

2.2. Chemical substances

L-kynurenine (sulfate salt), lisinopril, perindopril erbumine, ramipril, dimethyl sulfoxide (DMSO) and all reagents used to prepare the Krebs-Ringer buffer (sodium chloride, potassium chloride, magnesium sulfate, calcium chloride, sodium phosphate monobasic, sodium phosphate dibasic, glucose, and distilled water), dialysate buffer (Trizma base, acetic acid, pyridoxal 5′-phosphate, 2-mercaptoethanol, and distilled water) and incubation solutions to measure enzyme activity (Trizma base, acetic acid, pyruvate, pyridoxal 5′-phosphate, glutamine, L-kynurenine, distilled water) were purchased from Sigma-Aldrich. Substances used for high-performance liquid chromatography were obtained from J.T. Baker Chemicals and from Sigma-Aldrich.

2.3. Experiments conducted on cortical slices

Tests on brain cortical slices were performed as described previously by Turski et al. (1989). After each rat's decapitation, their brains were removed from their skulls and put immediately on ice. The white matter was dissected from the hemispheres and the cortex was cut with a McIlwain tissue chopper (the Mickle Laboratory Engineering Co Ltd., USA). The obtained cortical slices (size 1 mm x 1 mm) were placed in incubation wells (10 slices in each well), which were filled with 1 ml oxygenated Krebs-Ringer buffer at a pH 7.4. The slices were incubated for 2 h at 37 °C in the presence of 10 μM of L-kynurenine and one of 3 different drug concentrations: 0.01 mM, 0.1 mM and 1 mM. A minimum of 6 wells were used to analyze each drug concentration. The incubation was concluded by placing the wells into an ice cold bath. After incubation, the obtained supernatants were centrifuged and added to an ion exchange resin Dowex 50 W+column. Eluted KYNA was subjected to the high-performance liquid chromatography (Thermo Fisher Scientific HPLC system, ESA catecholamine HR-80, 3 μm, C18 reverse-phase column) and quantified fluorometrically against authentic KYNA.

2.4. Evaluation of kynurenine aminotransferases activity

To analyze KAT I and KAT II activity, the cortical tissue was homogenized in dialysate buffer containing 5 mM Tris-acetate buffer at pH 8.0, 50 μM pyridoxal 5′-phosphate and 10 mM 2-mercaptoethanol. The obtained homogenate was centrifuged, and then the supernatant was dialyzed for 12 h at 8 °C with the use of a cellulose membrane dialysis tubing in 4 l of the dialysate buffer. After dialysis, the enzyme supernatant was incubated in the reaction mixture containing incubation solution, L-kynurenine and solutions of the tested substances (3 concentrations for each drug: 0.01 mM, 0.1 mM and 1 mM). The reaction pH was 9.5 (for KAT I activity) and 7.0 (for KAT II activity). At the end, glutamine was given to decrease KAT I activity and assess the activity of KAT II. Three probes were used for each concentration of drug. The incubation (2 h at 37 °C) was stopped by application of the samples into an ice cold bath. Supernatants were centrifuged and analyzed in the same fashion as the samples from cortical slices.

2.5. Statistical analysis

The data were presented as a percentage of control values. Mean and standard error of the mean (S.E.M.) were calculated. Statistical analysis was performed using one-way analysis of variance (ANOVA) with a post-hoc Tuckey-Kramer test. Statistical significance was set at $P < 0.05$. All calculations were made with GraphPad InStat program, version 3.06.

3. Results

3.1. Evaluation of KYNA production in rat brain cortical slices

Lisinopril increased KYNA production in the rat brain cortical slices *in vitro* at the concentration of 0.01 mM and 0.1 mM to 125% ($P < 0.05$) and 116% ($P < 0.05$) of the control value, respectively (Fig. 1). Perindopril did not affect KYNA production in the rat brain cortex *in vitro* in any of the used concentrations (data not shown), whereas ramipril decreased KYNA production at the 1 mM concentration to 46% of control value ($P < 0.001$) (Fig. 1).

3.2. Evaluation of KAT I activity in rat brain cortical homogenates in vitro

Lisinopril enhanced KAT I activity in the rat brain cortical homogenates *in vitro* at the concentration of 0.01 mM and 0.1 mM to 126% ($P < 0.05$) and 128% ($P < 0.05$) of the control value, respectively (Fig. 2). Perindopril did not have an influence on KAT I activity in the rat brain cortical homogenates *in vitro* at any of the used concentrations (data not shown). Ramipril decreased KAT I activity in the rat brain cortical homogenates *in vitro* at the concentration of 1 mM to 76% ($P < 0.05$) of the control value (Fig. 2).

3.3. Evaluation of KAT II activity in rat brain cortical homogenates in vitro

Lisinopril and perindopril (data not shown) did not affect KAT II activity in the rat brain cortical homogenates *in vitro* at any of the used concentrations, whereas ramipril decreased KAT II activity in the rat brain cortical homogenates *in vitro* at the concentration of 1 mM to 41% ($P < 0.001$) of the control value (Fig. 3).

4. Discussion

In the present study we found that lisinopril enhanced the production of KYNA in rat brain slices. It stimulated KAT I activity,

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