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Long-term administration of fatty acid amide hydrolase inhibitor (URB597) to rats with spontaneous hypertension disturbs liver redox balance and phospholipid metabolism

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ABSTRACT

Purpose: The effect of chronic administration of [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate (URB597), inhibitor of fatty acid amide hydrolase (FAAH) that hydrolyzes anandamide, on cross-talk between endocannabinoid system, oxidative status and pro-inflammatory factors in the liver of spontaneously hypertensive rats (SHRs) was investigated.

Materials/methods: Experiments were conducted using SHRs and normotensive control Wistar-Kyoto rats treated by intraperitoneal injection with URB597 for 14 days. The biochemical parameters were assayed in the rat's livers.

Results: In the liver of SHRs an increase in endocannabinoids level, the activity of enzymes degrading them and expression of the cannabinoid receptor type 2 (CB_2) receptor as well as a decrease in the expression of the CB_1 and vanilloid 1 receptor (TRPV1) were shown. These changes were related to inflammatory conditions as well as oxidative stress resulting from increased reactive oxygen species (ROS) generation due to enhanced activity of enzymes generating ROS accompanied by decrease in the effectiveness of transcription activity of nuclear factor erythroid 2 and the activity of antioxidant enzymes, as well as level of glutathione and vitamins. Chronic administration of URB597 to SHRs caused a decrease in FAAH activity and an increase in anandamide and *N*-arachidonoyl-dopamine level as well as a decrease in CB_2 and an increase in TRPV1 receptor expression. The levels/activities of pro- and antioxidant and inflammatory factors tended to normalize, but phospholipid peroxidation and DNA modifications were increased.

Conclusion: In conclusion, long-term chronic administration of URB597 to SHRs by altering interactions between endocannabinoid and redox systems enhances some liver metabolic disturbances observed in hypertension.

1. Introduction

The liver is a highly complex vascular organ and therefore may be characterized by impaired functioning in vascular diseases such as hypertension [1]. It plays a fundamental role in detoxification of endogenous and exogenous compounds including nitric oxide and reactive oxygen species (ROS) and thus can protect the body from oxidative disorders [2]. However it was observed that in hypertensive rats the hepatic antioxidants status is reduced [3]. Therefore in the development of hypertension, high level of ROS may affect multiple tissues metabolism either directly or through reaction of superoxide with nitric oxide: a critical endogenous vasodilator [4]. Although ROS are needed in normal cellular functions, overproduction of ROS may lead to increased inflammatory response through activation of pro-inflammatory molecules such as tumor necrosis factor alpha (TNFα), which in turn aggravates oxidative stress and initiates a chain of deleterious events eventually culminating in cellular dysfunction and death [5,6]. Oxidative stress occurs when there is an imbalance between ROS level and antioxidant defense [2] that results in oxidative modifications of cells components including proteins and lipids what have been found in human hypertension in the blood vessels, heart and kidney, as well as liver tissue of hypertensive rats [4,7,8].

The development of hypertension is also strongly associated with functioning of endocannabinoid system including endocannabinoids –

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phospholipid metabolites, their receptors and the enzymes degrading endocannabinoids [9,10]. It was shown that the level of the main endocannabinoid – anandamide was increased in the plasma of patients with hypertension, and in different tissues of rats with primary and secondary hypertension including liver of rats with secondary hypertension [9–12]. Endocannabinoids are agonists of cannabinoid receptors that are present in most tissues including the liver and whose activation modulates oxidative and inflammatory conditions [13–15]. It has been shown that activation of cannabinoid receptor type 1 (CB_1) increases, whereas cannabinoid receptor type 2 (CB_2) and vanilloid receptor 1 (TRPV1) activation diminishes the level of ROS and inflammatory mediators [16]. It was also indicated that CB_2 agonists suppressed pro-inflammatory cytokines level and oxidative stress as well as cell death in the liver, while inhibition of CB_1 prevented liver injury in a model of hepatic fibrosis [14,15,17]. However, it was also shown that anandamide promotes hepatocytes apoptosis by a mechanism unrelated to CB receptors [18]. The third elements of endocannabinoid system are enzymes degrading endocannabinoids and as a result are responsible for level of endocannabinoids.

Endocannabinoids, including anandamide, have been shown to be involved in the regulation of blood pressure [19] via cannabinoid receptors, mainly CB_1, non-cannabinoid receptors (including TRPV1 and peroxisome proliferator-activated receptor (PPARγ)) as well as their metabolites with the generation of vasodilatory compounds, including prostacyclins [20]. Because anandamide elevation promotes blood pressure lowering, it has been suggested that the use of anandamide metabolism inhibitors should reduce blood pressure. Moreover, it was found that in males the gene modification of FAAH 129T is accompanied by reduced blood pressure [21]. Therefore, inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for degradation of anandamide, have been suggested as potential antihypertensive compounds. It was also shown that single administration of two different FAAH inhibitors, [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate (URB597) [9] and 5-(4-hydroxyphenyl) pentanesulfonyl fluoride (AM3506) [10], to primary hypertensive rats (SHR) normalized blood pressure. In contrast, chronic URB597 administration to rats with secondary hypertension reduced blood pressure in a manner dependent on age [22].

In spite of the above, anandamide by increasing the expression of receptors it can modify the level of ROS and consequently redox balance as well as other ROS-dependent processes [16]. Considering this, as well as the fact that FAAH activity in rat liver belongs to the highest [23] one should analyze the effect of changes in FAAH activity on the cellular consequences of the redox balance. It was shown e.g. that enhanced endocannabinoid level results in the activation of the CB_1 hepatic receptor, causing an increase in liver lipogenesis [24]. Therefore, we suggest that URB597 through modulation the action of endocannabinoid system may change liver redox balance responsible for this organ functioning.

Considering that the chronic influence of the FAAH inhibitor on the endocannabinoid system and redox balance in the liver has not been studied, we decided to evaluate the interactions between these systems in the liver of rats with primary hypertension.

2. Materials and methods

2.1. Ethical approval

All procedures and experimental protocols were approved by the local Animal Ethics Committee in Białystok, Poland (resolution No. 4/2012 of 25.01.2012).

2.2. Animals

Experiments were conducted using male rats with primary hypertension (SHR/NHsd Inbred, Harlan Laboratories, USA) and normotensive control Wistar-Kyoto rats (WKY/NCrl, Charles River Laboratories, Germany), aged 8–10 weeks and weighing 270–350 g. Rats were kept under standard conditions (12-h light/12-h dark cycles) and fed a pelleted rat chow (Labofeed B – maintenance; feed producer 'Morawski', Poland). The diet formula was based on the recommendations of the National Research Council in the field of the Nutrient Requirements of Laboratory Animals (67%-carbohydrates, 25%-proteins, 8%-fats). More details can be found on the website https://www.sukces.info.pl/labofeed-b.

The rats were divided into 4 groups of six rats each:

group 1 [WKY]: WKY rats were treated by intraperitoneal injection with URB597 solvent (1 mL mixture of DMSO, Tween 80 and saline (0.9% NaCl) [1:2:7; v:v:v]) every 12 h for 14 days.

group 2 [WKY + URB597]: WKY rats were treated by intraperitoneal injection with URB597 (1 mg/kg body weight in 1 mL of URB597 solvent) every 12 h, for 14 days.

group 3 [SHR]: SHRs were treated by intraperitoneal injection with URB597 solvent (1 mL) every 12 h for 14 days.

group 4 [SHR + URB597]: SHRs were treated by intraperitoneal injection with URB597 (1 mg/kg body weight in 1 mL of URB597 solvent) every 12 h for 14 days.

Systolic blood pressure (SBP) was measured in conscious rats using the tail-cuff method before and after URB597 (or solvent) treatment. Rats with SBP values $\geq$ 150 mmHg were considered hypertensive. URB597 treatment for 2 weeks did not modify SBP in SHR (187 $\pm$ 15 mmHg and 191 $\pm$ 49 mmHg) or WKY (117 $\pm$ 18 mmHg and 101 $\pm$ 10 mmHg) rats (before the first and after the final dose, respectively). The solvent for URB597 did not modify SBP both in SHR (184 $\pm$ 34 and 205 $\pm$ 43 mmHg) and in WKY (114 $\pm$ 18 and 110 $\pm$ 13 mmHg) before the first and the final injection.

2.3. Tissue preparation

At the end of the experimental period, rats were anesthetized with an intraperitoneal injection of pentobarbital (70 mg/kg body weight) and sacrificed. The livers were rapidly removed and prepared in three different ways: fresh tissue samples were used immediately for the determination of total ROS generation; part of the fresh tissue samples were frozen in liquid nitrogen and pulverized for determination of endocannabinoids, fatty acids and their metabolites as well as glutathione level and monoacylglycerol lipase and FAAH activity; the rest of liver was homogenized in 0.9% NaCl solution – 10% homogenates were centrifuged at 20,000 $\times$ *g* for 15 min at 4 °C and aliquots of the supernatants were taken for the measurement of other biochemical parameters.

2.4. Determination of ROS

Total ROS generation was measured by the production of a stable nitroxide CM-radical ($t_{1/2}$ = 4 h), using electron spin resonance (ESR) and the reaction between ROS and the spin probe CMH (1-hydroxy-3-methoxy-carbonyl-2,2,5,5-tetrame-thylpyrrolidine) [25].

2.5. Determination of prooxidant enzyme activity

NADPH oxidase (NOX – EC 1.6.3.1) activity was assayed using luminescence assay according to the method of Griendling [26]. Enzyme specific activity was expressed in RLU (Relative Luminescence Units) per milligram of protein.

Xanthine oxidase (XO – EC1.17.3.2) activity was measured spectrophotometrically at 290 nm by determination of uric acid generated from xanthine [27]. One unit of XO activity was defined as the amount of the enzyme which was required to release 1 μmol of uric acid per minute. Enzyme specific activity was expressed in U per milligram of protein.