Life Sciences 183 (2017) 1–10

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

The effects of chronic FAAH inhibition on myocardial lipid metabolism in normotensive and DOCA-salt hypertensive rats

Agnieszka Polak [a], Ewa Harasim-Symbor [a,*], Barbara Malinowska [b], Irena Kasacka [c], Anna Pędzińska-Betiuk [b], Jolanta Weresa [b], Adrian Chabowski [a]

[a] Department of Physiology, Medical University of Bialystok, Bialystok, Poland
[b] Department of Experimental Physiology and Pathophysiology, Medical University of Białystok, Białystok, Poland
[c] Department of Histology and Cytophysiology, Medical University of Białystok, Białystok, Poland

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form 1 June 2017
Accepted 16 June 2017
Available online 17 June 2017

Keywords:
DOCA
Endocannabinoids
Fatty acid transport
Fat/CD36
Hypertension
URB597

ABSTRACT

Aims: There is significant evidence that the endocannabinoid system (ECS) takes part in the regulation of the cardiovascular system in hypertension. It is quite well established that hypertension causes several changes in the heart metabolism, but it is still unknown whether the ECS affects this process. Therefore, we investigated the influence of prolonged ECS activation on myocardial lipid metabolism in deoxycorticosterone acetate (DOCA)-salt hypertensive rats by chronic fatty acid amide hydrolase (FAAH) inhibition.
Materials and methods: We examined the uptake and oxidation of palmitic acid during the heart perfusion as well as intramyocardial and plasma lipid contents using gas liquid chromatography. Total, plasmalemmal and intracellular expressions of selected proteins were estimated by the Western blot technique. Moreover, the left ventricle's morphology, including myocardial vessels density, was measured using immunohistochemistry.
Key findings: We demonstrated that hypertension induced cardiomyocytes and myocardial blood vessels hypertrophy, followed by a reduction in myocardial palmitate oxidation. Interestingly, prolonged activation of the ECS in the normotensive rats induced cardiomyocyte enlargement and intensified fatty acids metabolism. We have also shown that FAAH inhibition improved morphology of coronary blood vessels and only partially maintained its effect on lipid metabolism in the DOCA-salt hearts (i.e. elevated plasma and intramyocardial TAG contents as well as plasmalemmal FAT/CD36 and total FATP1 expressions).
Significance: This study revealed that chronic FAAH inhibition has no protective effects on the heart lipid metabolism in hypertension.

1. Introduction

Hypertension affects over 25% of the world's adult population [1]. Moreover, it has been predicted that the prevalence of hypertension is going to rise constantly [2]. It should also be noted that in 75% of the cases, hypertension precedes heart failure [3].

In a healthy heart, 70–80% of the produced ATP is derived from long chain fatty acids (LCFA) and 20–30% from glucose [4,5]. Although LCFA can enter the cells by simple diffusion [6], recent studies have indicated that the uptake of LCFA by the myocardium is controlled mostly by plasmalemmal protein transporters [7,8]. So far, three groups of proteins have been identified as LCFA transporters in the heart i.e. fatty acid translocase (FAT/CD36), plasma membrane associated fatty acid binding protein (FABPpm) and fatty acid transport proteins 1, 4 and 6 (FATP1, 4 and 6) [8,9]. Importantly, protein-mediated LCFA transport can adapt to the myocardial energy demands [10] by translocating fatty acid transporters from the intracellular compartments to the plasma membrane [7,8]. Studies on rats revealed that cardiomyocyte's contractile activity can cause translocation of FAT/CD36 from the intracellular pools to the sarcolemma, resulting in the increase in fatty acids uptake [11,12]. It was shown that hypertension greatly contributes to the changes in the heart metabolism. Many studies provide

Abbreviations: AEA, anandamide; ATGL, adipose triglyceride lipase; DAG, diacyloglycerol; DGAT, diglyceride acyltransferase; DMF, *N*,*N*-dimethylformamide; DOCA, 11-deoxycorticoterone acetate; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; FABPpm, plasma membrane associated fatty acid binding protein; FAT/CD36, fatty acid translocase; FATP, fatty acid transport protein; FFA, free fatty acid; GLC, gas-liquid chromatography; KHB, Krebs Henseleit Buffer; LCFA, long chain fatty acid; LDM, low density microsomes; i.p., intraperitoneal; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PL, phospholipids; PM, plasma membrane; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; SHR, spontaneously hypertensive rats; TAG, triacyloglycerol; TLC, thin-layer chromatography; URB597, (30-(aminocarbonyl)[1,10-biphenyl]-3-yl)-cyclohexylcarbamate.

* Corresponding author at: Department of Physiology, Medical University of Bialystok, ul. Mickiewicza 2C, 15-222 Bialystok, Poland.
E-mail address: eharasim@umb.edu.pl (E. Harasim-Symbor).

http://dx.doi.org/10.1016/j.lfs.2017.06.019
0024-3205/

strong evidence that the myocardial metabolism of energy substrates in spontaneously hypertensive rats (SHR) is shifted from LCFA oxidation to glucose utilization [13]. This implies that hypertension may induce alternations in metabolic substrate preference.

A growing body of evidence indicates the role of the endocannabinoid system (ECS) in the regulation of the cardiovascular system in various states such as arrhythmias, hypertension, cardiac hypertrophy, myocardial ischemia or reperfusion [14,15]. Endocannabinoids are endogenous lipid mediators which exert their biological effects mainly via G-protein-coupled CB1 and CB2 receptors (cannabinoid receptors 1 and 2) [16]. The tissue level of the major endocannabinoid, anandamide (AEA), depends on fatty acid amide hydrolase (FAAH) activity [17]. Interestingly, it was shown that plasma level of AEA was elevated in patients with hypertension as well as SHR rats, which indicated that the ECS was up-regulated [18,19]. Furthermore, acute administration of FAAH inhibitors (URB597 or AM3506) normalized blood pressure due to prolonged activation of CB1 receptors [20,21]. On the other hand, Toczek et al. demonstrated that chronic URB597 administration reduced blood pressure and heart rate in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, giving new insights into treatment of hypertension [22]. Nevertheless, it is still unknown how the decreased activity of FAAH would affect the myocardial metabolism. Accordingly, we investigated this phenomenon in the rat model of volume overloaded, low renin and angiotensin II independent DOCA-salt hypertension [23]. In this experimental model we checked out the effects of prolonged activation of the ECS via chronic blockade of AEA degradation (URB597 injections) on the heart lipid metabolism. Therefore, we examined the total expression and subcellular distribution of the key fatty acid transporters (FAT/CD36, FATP1, FATP4 and FABPpm), fatty acid uptake and oxidation in the heart. Additionally, we studied effects of hypertensive state and URB597 treatment on myocardial morphology.

2. Materials and methods

Male Wistar Rats (170-200 g and 6–7 weeks old) were obtained from the Center for Experimental Medicine of the Medical University of Bialystok. The animals were maintained at 22 ± 1 °C on a reverse light-dark cycle in approved animal holding facilities. The rats were fed a standard pelleted rodent chow (Labofeed B, Animal Feed Manufacturer "Morawski", Kcynia, Poland). All the experimental procedures were approved by the Ethical Committee for Animal Experiments at the Medical University of Bialystok.

2.1. Induction of DOCA-salt hypertension and collection of samples

All the animals were anaesthetized by intraperitoneal (i.p.) injection of pentobarbitone sodium in a dose of 300 μmol/kg of body weight before right uninephrectomy. The right kidney was removed after ligation of renal vessels and ureter by silk thread. The next steps were carried out 7 days after animals recovery. Thereafter, the rats were randomized into two groups i.e. hypertensive and normotensive. In the hypertensive group subcutaneous (s.c.) injections of 11-deoxycorticosterone acetate (DOCA, Sigma-Aldrich, 25 mg/kg [22,24–26] in 0.4 ml/kg *N*,*N*-dimethylformamide - DMF) were performed twice a week and salt was added to the diet in a form of 1% NaCl solution for a period of 6 weeks. At the same time the normotensive rats were administrated injections of DOCA solvent (DMF, 0.4 ml/kg, s.c.) and received tap water ad libitum. After 4 weeks, both normotensive and hypertensive rats were again divided into two subgroups. One of the normotensive subgroups and one of the hypertensive subgroups were injected with FAAH inhibitor – URB597 ((30-(aminocarbonyl)[1,10-biphenyl]-3-yl)-cyclohexylcarbamate, Cayman Chemical Company, 1 mg/ml/kg) twice a day for the next 2 weeks [22,27,28]. Simultaneously, rats from the other subgroups (i.e. the normotensive and the hypertensive rats) received only URB597 solvent (1 ml/kg; DMSO, Tween 80 and 0,9% NaCl (1:2:7)). Throughout the experiment hemodynamic parameters, body weight and hypertrophy of selected organs were monitored [22]. Moreover, immunohistochemistry, intramyocardial lipids, subcellular fractionation, protein expression and plasma determinations were performed on normal, not subjected to perfusion rat hearts.

2.2. Heart perfusion

To examine the influence of prolonged ECS activation in the DOCA-salt induced hypertension model on palmitate uptake and oxidation rate we used intact hearts, which were perfused in the Langendorff perfusion system as described previously [29]. Briefly, 12 h after the last injection of URB597 or its solution, the hearts were removed from anaesthetized rats in the presence of heparin (500 IU i.p.) and next perfused for 5 min with Krebs Henseleit Buffer (KHB). Subsequently, the hearts were attached to the reservoir with KHB containing ^{3}H-palmitate (final concentration of palmitate 100 μM, 0.3 palmitate/bovine serum albumin ratio) and continuously gassed for 15 min under normoxic conditions (37 °C, 95% O_2, 5% CO_2). Thereafter, the hearts were removed and immediately freeze – clamped with aluminum tongs precooled in liquid nitrogen and stored at −80 °C until further analysis.

2.3. Immunohistochemical analyses

Fragments of the same heart parts were taken from all animals, fixed in 10% buffered formalin and embedded in paraffin in a routine manner. Sections were cut at 4 μm in thickness, and stained by hematoxylin–eosin (H + E) and an immunohistochemical reaction to find vascular endothelial cells was performed.

Immunohistochemistry was performed using the EnVision Plus-HRP Detection Kit K 4007 (Dako Denmark A/S). In the immunohistochemical study, the EnVision method was used according to Escribano et al. [30].

Immunostaining was carried out using the following protocol. Briefly, paraffin-embedded sections were deparaffinized and hydrated in pure alcohols. For antigen retrieval, the sections were subjected to pretreatment in a pressure chamber heating for 1 min at 21 psi at 125 °C using Target Retrieval Solution S 1699 (Dako Denmark A/S). After being cooled to room temperature, sections were incubated with Peroxidase Blocking Reagent S 2001 (Dako Denmark A/S) for 10 min to block endogenous peroxidase activity.

The sections were incubated overnight in 4 °C in a humidified chamber with the diluted monoclonal mouse anti-human CD31, Endothelial Cells M 0823 (1:1000, Dako Denmark A/S), followed by incubation with secondary antibody (conjugated to horseradish peroxidase-labeled polymer) for 1 h. Bound antibodies were visualized by 1 min incubation with liquid 3,3′-diaminobenzidine substrate chromogen. The sections were finally counterstained in Vector QS hematoxylin, mounted, and evaluated under light microscope. Appropriate washing with Wash Buffer S 3006 (Dako Denmark A/S) was performed between each step, and all incubations were performed in a moist chamber at room temperature.

Negative control was carried out by incubating sections with the Dako Mouse IgG1, code X0931 (Dako Denmark A/S) diluted to the same mouse IgG concentration as the primary antibody in Dako Antibody Diluent, code S0809 (Dako Denmark A/S). All the performed control reactions gave negative results and positive control was conducted for specific tissue recommended by producer; for CD31 is human endothelium in renal glomerular capillaries.

The obtained results of immunohistochemical staining were submitted for evaluation in an Olympus BX41 microscope with Olympus DP12 camera. From each animal five heart sections under the same setting were studied.

From all heart sections, five randomly selected microscopic fields, each field of 0.785 mm^2, in magnification of 200× were documented, subsequently, images were submitted to morphometric evaluation by using NIS-Elements Advanced Research software of Nikon. In each