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Fatty acid amide hydrolase inhibitor (URB597) as a regulator of myocardial lipid metabolism in spontaneously hypertensive rats

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ABSTRACT

Pressure overload, which is typical of hypertension, is known to evoke alterations not only in the morphology of the heart but also in the preference of myocardial energetic substrates usage. Nowadays, the endocannabinoid system (ECS) serves as a potential therapeutic target for cardiovascular disorders and, simultaneously, affects whole body metabolism homeostasis. Therefore, an open question is whether ECS, apart from decreasing blood pressure, also affects cardiac muscle metabolism in hypertensive conditions. All experiments were conducted on a genetic model of primary hypertension i.e. spontaneously hypertensive rats (SHRs) and Wistar Kyoto rats (WKY) served as a normotensive control. ECS was chronically activated by 2-weeks intraperitoneal injections of fatty acid amide hydrolase (FAAH) inhibitor – URB597. Lipid analyses in the left ventricle and serum were based on ex vivo heart perfusion in Langendorff perfusion system, thin layer chromatography, and gas liquid chromatography. The total expression of selected proteins was determined using Western blot as well as immunohistochemical techniques. As expected, URB597 markedly reduced systolic as well as mean blood pressures in SHRs. Moreover, prolonged FAAH inhibition resulted in stimulation of ^{3}H-palmitate uptake and incorporation into different lipid fractions in cardiomyocytes in the hypertensive as well as normotensive conditions. An increase in fatty acid oxidation caused by URB597 treatment was observed only in WKY rats, but not SHRs, and was accompanied by an elevation in peroxisome proliferator-activated receptor alpha (PPARα) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) expressions. Chronic activation of ECS significantly upregulates palmitate uptake and its esterification but not oxidation in the SHR's myocardium.

1. Introduction

Hypertension is constantly spreading worldwide and when untreated, it can be a serious risk factor for heart failure and cardiovascular morbidity. Left ventricle hypertrophy is commonly observed in hypertensive states as a result of increased pressure overload. Metabolic remodelling, which is closely related to myocardial hypertrophy, has been recently investigated extensively. It was shown that cardiac muscle is effectively able to change the type of energetic substrate in order to maintain constant level of myocardial ATP, which has to be adjusted to the current contractile function of the heart. Long chain fatty acids (LCFAs) and glucose which provide 70–80% and 20–30% of energy respectively, are two main substrates for energy production in a healthy cardiac muscle (Stanley et al., 2005). It is known that LCFA flux into cardiomyocytes predominantly depends on fatty acid transporters and facilitated diffusion (van der Vusse et al., 2000; Bonen et al., 2002). Three types of fatty acid transporters, i.e. fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABPpm) and fatty acid transport proteins (FATP1,4,6) have been identified in the heart (Glatz et al., 2010). Increasing body of evidence indicates that FAT/CD36 is the major LCFA uptake regulator in metabolically active tissues. It was revealed that in FAT/CD36 knockout mice fatty acid uptake was reduced up to 80% in adipose tissue, skeletal and cardiac muscles (Coburn et al., 2000). Moreover, the rate of fatty acid oxidation

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was also markedly diminished (40–60%) in perfused hearts of FAT/CD36 null mice (Kuang et al., 2004). The data from previous studies indicate that increased pressure overload and hypertension predispose the myocardium to shift its metabolism towards enhanced glucose utilization (Coprean et al., 1993; Allard et al., 1994). However, this mechanism also depends on additional factors, e.g. cardiac hypertrophy degree as well as age (Hernandez et al., 2013).

Nowadays, the endocannabinoid system (ECS) is a new therapeutic approach for hypertension treatment, although there is only little information of its influence on lipid metabolism in the cardiac muscle. ECS is an endogenous signalling system which includes fatty acid-derived ligands, i.e. anandamide (AEA) and 2-arachidonoylglycerol (2-AG), G-protein coupled cannabinoid receptors 1 and 2 (CB_1 and CB_2) as well as enzymes responsible for the ligand biosynthesis and degradation, i.e. fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). All the above mentioned ECS compounds exhibit their expression in the heart (Pędzińska-Betiuk et al., 2017). In the present study we conducted experiments on the animal model of primary hypertension, i.e. spontaneously hypertensive rats (SHR) in which ECS was activated by chronic injections of FAAH inhibitor – URB597 thereby increasing mainly AEA concentration. However, it was shown that FAAH apart from AEA and 2-AG also breaks down the endocannabinoid-like substances, i.e. N-oleoylethanolamine (OEA) and N-palmitoylethanolamine (PEA) (Saghatelian et al., 2004). Recent data have confirmed effectiveness of URB597 in the reduction of blood pressure by affecting both blood vessels and myocardium (Pędzińska-Betiuk et al., 2017; Toczek et al., 2016; Bátkai et al., 2004). Previously we have shown that chronic FAAH inhibition (by URB597) significantly affects myocardial lipid metabolism in the secondary (DOCA-salt model) hypertension (Polak et al., 2017). However, there are no studies demonstrating the influence of URB597, as a potential therapeutic agent against elevated blood pressure, on myocardial lipid metabolism as well as fatty acid transporters expression in a genetic model of primary hypertension. Therefore, we evaluated plasma lipids, myocardial rate of radiolabelled palmitate uptake, its oxidation and incorporation into different lipid pools along with expression of proteins involved in fatty acids transport and metabolism after activation of ECS by URB597 injections in spontaneously hypertensive rats.

2. Materials and methods

2.1. Animals

Six to seven weeks old (170–200 g) rats were purchased from the Center for Experimental Medicine of the Medical University of Bialystok, Poland. The animals were housed with unrestricted access to standard pellet chow (Labofeed B, Animal Feed Manufacturer "Morawski", Kcynia, Poland) and tap water. Rats were maintained under a 12-h light-dark cycle. All procedures were approved by the Ethical Comitee for Animal Experiments at the Medical University of Bialystok, Poland.

2.2. Experimental protocol

In our experiment we used normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats. After a time of acclimatization the rats (8–9 weeks old) were divided into four groups: WKY – normotensive, control rats, WKY + URB597 – normotensive rats treated with URB597, SHR – hypertensive rats and SHR + URB597 – hypertensive rats treated with URB597 (each group included 10 rats). We applied the same dosage regimen of URB597 as reported previously in the DOCA-salt model of hypertension (Toczek et al., 2016; Polak et al., 2017), in which 2-week URB597 injections effectively decreased blood pressure (BP) value. Briefly, URB597 (1 mg/kg) or its solvent (mixture of DMSO, Tween 80 and 0.9% NaCl (1:2:7), 1 ml/kg) were injected intraperitoneally (i.p.) twice a day for the consecutive 2 weeks for the normotensive and hypertensive rats. Throughout the experiment body weight, hemodynamic parameters and hypertrophy of selected organs were monitored. Experimental procedures were completed when the rats from all the groups reached the 10–11th week of age.

2.3. ^{3}H-palmitate oxidation and incorporation into different lipid fractions

To examine the effects of prolonged ECS activation on palmitate metabolism, we used intact hearts, which were perfused in Langendorff perfusion system as reported previously (Pędzińska-Betiuk et al., 2017; Polak et al., 2017; Kalinowska et al., 2009). For this purpose, 12 h after the last injection of URB597 or its solvent, the hearts were removed from rats under pentobarbital anaesthesia (70 mg/kg body weight, i.p.) combined with heparin (500 IU, i.p.). Then, hearts were perfused for 5 min with Krebs Henseleit buffer (KHB) and switched to KHB containing ^{3}H-palmitate (100 μmol/l; 0.3 palmitate/bovine serum albumin (BSA) ratio) and continuously gassed for 15 min under normoxic conditions (95% O_2, 5% CO_2, 37 °C). Subsequently, the hearts were removed and freeze-clamped in liquid nitrogen. The samples were stored at −80 °C until further analysis. Myocardial lipids were extracted according to Folch method (Folch et al., 1957). Lipids from chloroform phase were separated by thin-layer chromatography (TLC). After identification of lipid fractions, selected lipid bands were scrapped off, dissolved in hexane and ^{3}H-palmitate incorporation in each fraction was determined. Oxidation rate of ^{3}H-palmitate was measured in water-soluble layer from lipids extraction as well as in the buffer at the end of the heart perfusion, which was described in details by Kalinowska et al. (Kalinowska et al., 2009). Finally, palmitate oxidation was counted as a sum of the ^3H_2O and water-soluble intermediates present in the cardiac muscle tissue as well as in the perfusion eluent at the end of the Langendorff perfusion. Total palmitate uptake was estimated as a sum of oxidation and esterification.

2.4. Lipid analyses in serum

Serum lipids were extracted in chloroform-methanol solution according to Folch method (Folch et al., 1957). Selected fractions (free fatty acids – FFA and triacylglycorols – TAG) were separated using TLC. Identification and quantification of individual fatty acid methyl esters were based on the retention times of standards using gas-liquid chromatography (GLC; Hawlett-Packard 5890 Series II gas chromatograph, HP-INNOWax capillary column). Total amount of FFA and TAG was estimated as the sum of the particular fatty acid species in each assessed fraction. Lipids content was expressed in nanomoles per mililiter of the serum.

2.5. Immunoblotting analyses

The expression of selected proteins was determined in the left ventricle of normal, not subjected to perfusion hearts, using Western Blot procedure, as described previously (Konstantynowicz-Nowicka et al., 2015). After protein concentration determination (bicinchoninic acid method), samples were subjected to sodium dodecyl sulphate (SDS) poliacrylamid gel electrophoresis and transfer. Thereafter, the membranes were incubated with primary antibodies: β-HAD – β-hydroxyacyl-CoA dehydrogenase (1:500, Santa Cruz Biotechnology, USA), CPT I – carnitine palmitoyltransferase I (1:500, Santa Cruz Biotechnology, USA), FABPpm (1:8000, Abcam, UK), FAT/CD36 (1:1000, Novus Biological, USA), FATP1 (1:500, Santa Cruz Biotechnology, USA), FATP4 (1:500, Santa Cruz Biotechnology, USA), AMPK – AMP-activated kinase (1:1000; Cell Signaling), PPARα – peroxisome proliferator-activated receptor alpha (1:200; Santa Cruz Biotechnology) and GAPDH – glyceraldehyde 3-phosphate dehydrogenase (1:1000; Santa Cruz Biotechnology). Then, membranes were blocked (bovine serum albumin or 5% non-fat milk) and incubated with appropriate secondary antibody conjugated with horseradish peroxidise (HRP)

(Santa Cruz Biotechnology, USA). After visualization with enhanced chemiluminescence substrate, the obtained signals were quantified densitometrically using ChemiDoc visualization system (Bio Rad, Poland). Ponceau S staining was used to confirm equal protein concentration loading on each line (30 µg). Moreover, protein expression was standardized to GAPDH expression and the control group was set as 100%.

2.6. Immunohistochemical analysis

Immunohistochemistry was performed on formalin fixed paraffin-embedded LV tissue from hearts not subjected to perfusion using the EnVision Plus-HRP Detection Kit for Rabbit antibodies (K 4011; Dako Denmark A/S, Denmark) (Herman and Elfont, 1991). In brief, the paraffin blocks were cut into 4-µm sections and attached to positively charged glass slides. Then, paraffin-embedded sections were deparaffinized and hydrated in 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 pH 9.0 S 2367 (Dako Denmark A/S, Denmark). After being cooled to room temperature, sections were incubated with Peroxidase Blocking Reagent for 10 min to block endogenous peroxidase activity. The sections were incubated 1 h at room temperature in a humidified chamber with the diluted FAT/CD36 (1:1000; Novus Biological, USA) and FABPpm (1:500; Anti-GOT-2 antibody, ab180162, Abcam, UK) antibodies followed by incubation with secondary antibody conjugated with HRP-labelled polymer for 30 min. Bound antibodies were visualized by 1 min incubation with liquid 3,3′-diaminobenzidine substrate chromogen. The sections were finally counterstained in Vector QS hematoxylin (H-3404; Vector Laboratories Inc., USA), mounted, and evaluated under light microscope. Appropriate washing with Wash Buffer S 3006 (Dako Denmark A/S, Denmark) was performed between each step.

2.7. Statistical analysis

All data are expressed as mean values ± SD or percentage of the control group based on ten independent determinations. Statistical differences between groups were tested with two-way analyses of variance (ANOVA) and appropriate post-hoc test using Graph Pad Prism 5 (GraphPad Software., La Jolla, USA). Results were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. General characteristic of the experimental model

As expected, regardless of the URB597 treatment, mean and systolic blood pressure were both substantially elevated in rats with primary hypertension in comparison with the normotensive rats (SHR, +95.9% and +90.7%; SHR + URB597, +71.3% and 67.4%, $P < 0.05$; Table 1, respectively). Nonetheless, prolonged activation of ECS in SHR rats significantly diminished the values of mean blood pressure (MBP) together with systolic blood pressure (SBP) compared to hypertensive group alone (-12.5% and -12.2%, $P < 0.05$; Table 1, respectively). Additionally, it was surprising that heart rate was increased not only in all hypertensive groups but also in the normotensive rats chronically treated with URB597 compared to WKY normotensive rats (SHR, +27.4%; SHR + URB597, +24.6% and WKY, +13.4%, $P < 0.05$; Table 1). Independently of URB597 treatment all hypertensive rats displayed substantially reduced body mass compared to the normotensive WKY rats (SHR, -14% and SHR + URB597, -12.8%, $P < 0.05$; Table 1). Whereas in the same experimental hypertensive groups heart weight/body weight ratio as well as heart weight/tibia length ratio were markedly elevated compared to the normotensive rats (SHR, +16.9% and +12.8%; SHR + URB597, +23.1% and +19.6%, $P < 0.05$; Table 1, respectively) and concomitantly the second above mentioned ratio was even further increased in the SHR group by URB597 (SHR + URB597, +6%, $P < 0.05$; Table 1 vs SHR group). Moreover, we noticed simultaneous significant rise in the value of the left ventricle/heart weight ratio only in the hypertensive group not treated with FAAH inhibitor compared to the normotensive rats (SHR, +18.5%, $P < 0.05$; Table 1).

Furthermore, plasma measurements revealed significant rise in TAG fraction in the WKY rats chronically treated with FAAH inhibitor (+73.2%, $P < 0.05$; Table 1) and simultaneous decline in all hypertensive groups (SHR, -59.4% and SHR + URB597, -56.4%; $P < 0.05$, Table 1) compared to the WKY group alone while FFA fraction remained relatively unchanged throughout the experiment ($P > 0.05$, Table 1).

3.2. Effect of prolonged activation of the ECS on the myocardial ^{3}H-palmitate uptake, oxidation and its incorporation into different lipid fractions (PL, FFA, TAG and cholesterol) in the normotensive state and primary hypertension

We noticed significant increase in ^{3}H-palmitate uptake after chronic URB597 administration in both WKY (+61.1%, $P < 0.05$; Fig. 3a vs control group) and SHR (+84% and +374%, $P < 0.05$; Fig. 1a vs control and hypertensive groups, respectively) in the left ventricle. Moreover, FAAH inhibition resulted in marked elevation of the myocardial oxidation rate only in the WKY group in comparison with the normotensive WKY group (+26.6%, $P < 0.05$; Fig. 1b).

We observed that chronic URB597 treatment caused alternations in palmitic acid incorporation in all examined intramyocardial lipid fractions except for PL. There was a parallel significant increase in the content of FFA (+77.6%, $P < 0.05$; Fig. 3d), TAG (+64.9%, $P < 0.05$; Fig. 1e) and Cholesterol (+30.9%, $P < 0.05$; Fig. 1f) fractions in the normotensive WKY rats after chronic activation of ECS compared to the control normotensive group. Similar effects of URB597 treatment were seen in the hypertensive SHR rats, in which

Table 1
Hemodynamic, body/heart weight parameters as well plasma free fatty acids (FFA) and triacylglycerols (TAGs) concentrations after chronic URB597 treatment in spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY, control group) at the age of 10–11 weeks. The data are expressed as the mean ± SD, n = 10 in each group.

Parameter	WKY	WKY + URB597	SHR	SHR + URB597
Body mass (g)	334 ± 31	343 ± 33	287 ± 16[a]	291 ± 17[a]
Heart weight/body weight (mg/g)	4.76 ± 0.5	4.79 ± 0.63	5.56 ± 0.42[a]	5.85 ± 0.41[a]
Heart weight/tibia lenght (mg/mm)	41.5 ± 2.7	43.6 ± 6.6	46.8 ± 2.9[a]	49.6 ± 2.5[ac]
Left ventricle/heart weight (mg/mg)	0.42 ± 0.05	0.41 ± 0.04	0.5 ± 0.05[a]	0.47 ± 0.04[c]
MBP (mmHg)	94 ± 16	82 ± 13	184 ± 24[a]	161 ± 14[ac]
HR (beats/min)	280 ± 28	317 ± 37[a]	358 ± 25[a]	349 ± 23[a]
SBP (mmHg)	105 ± 19	92 ± 13	200 ± 28[a]	176 ± 13[ac]
FFA (nmol/ml)	384 ± 22	409 ± 32	339 ± 24	335 ± 40
TAG (nmol/ml)	732 ± 76	1268 ± 26[a]	297 ± 17[a]	317 ± 27[a]

[a]$P < 0.05$ significant difference: control vs examined group, [c]$P < 0.05$ significant difference: SHR + URB597 vs SHR.

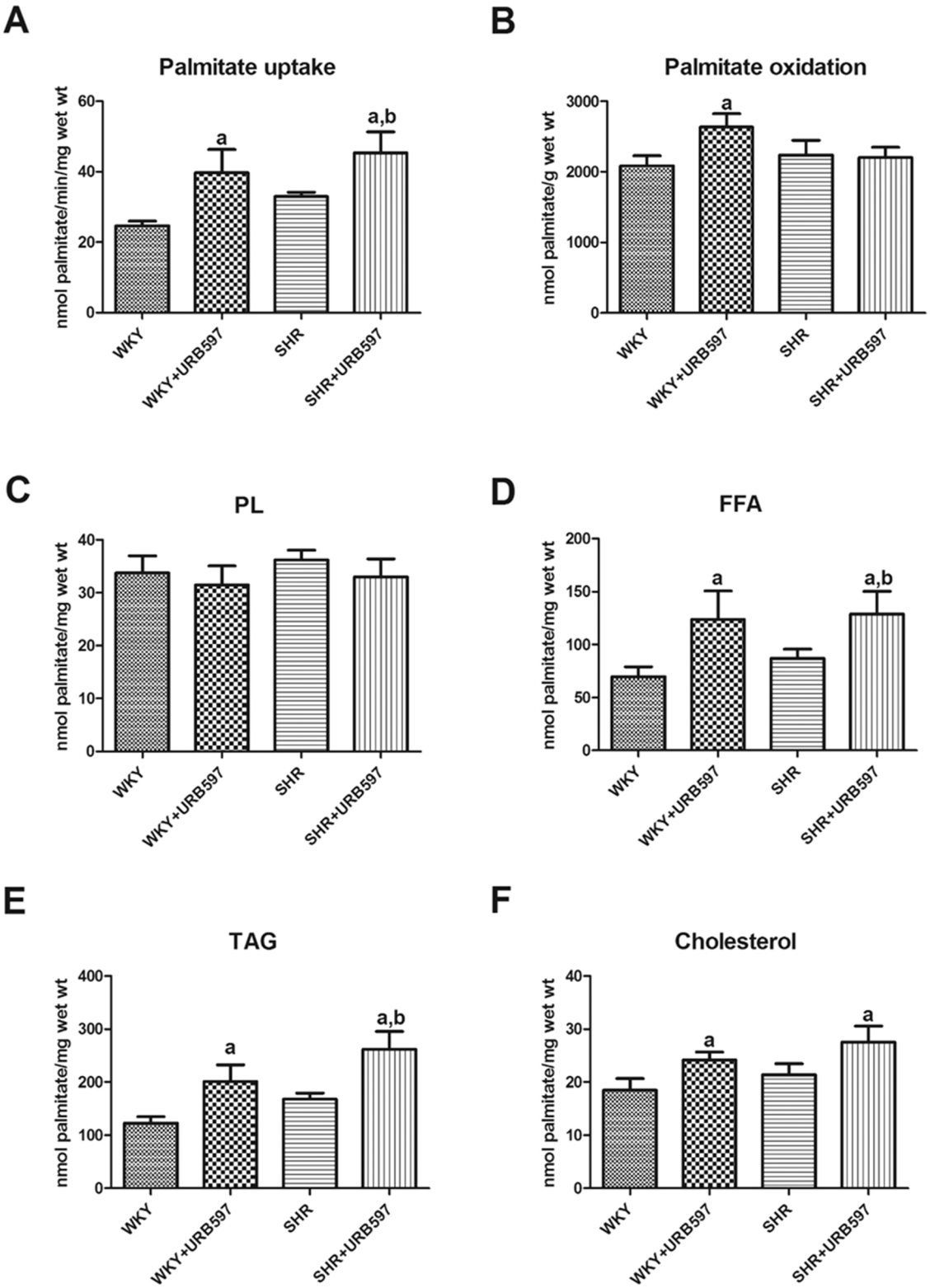

Fig. 1. Myocardial ^{3}H-palmitate uptake (A) and oxidation (B) as well as its incorporation into different lipid fractions i.e. phospholipids – PL (C), free fatty acids – FFA (D), triacylglycerols – TAG (E) and cholesterol (F) in the left ventricle after chronic URB597 treatment in spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY, control group). The data are expressed as the mean ± SD, n = 10 in each group. $^{a}P < 0.05$ significant difference: control vs examined group.

intramyocardial content of FFA (+84.9% and +48.1%, $P < 0.05$; Fig. 1d vs WKY and SHR group, respectively), TAG (+114.5% and +56%, $P < 0.05$; Fig. 1e vs WKY and SHR group, respectively) as well as Cholesterol (+49.2%, $P < 0.05$; Fig. 1f vs WKY) was substantially augmented compared to the normotensive and hypertensive rats.

3.3. Effect of prolonged activation of the ECS on the total myocardial expression of fatty acid transport proteins in the normotensive state and primary hypertension

We noticed that total myocardial expression of major fatty acid

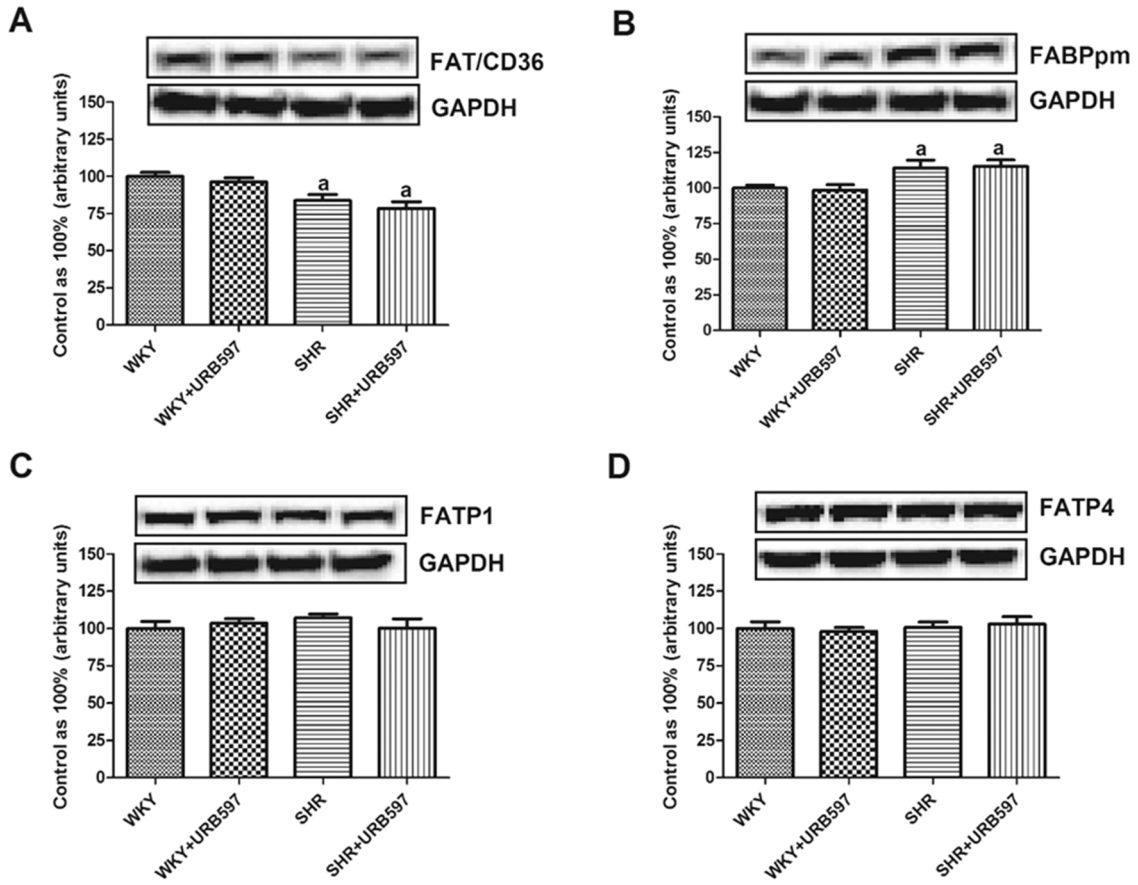

Fig. 2. Total expression of FAT/CD36 (A), FABPpm (B), FATP1 (C) and FATP4 (D) in the left ventricle after chronic URB597 treatment in spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY, control group). The data are expressed as the mean ± SD, n = 10 in each group. $^{a}P < 0.05$ significant difference: control vs examined group.

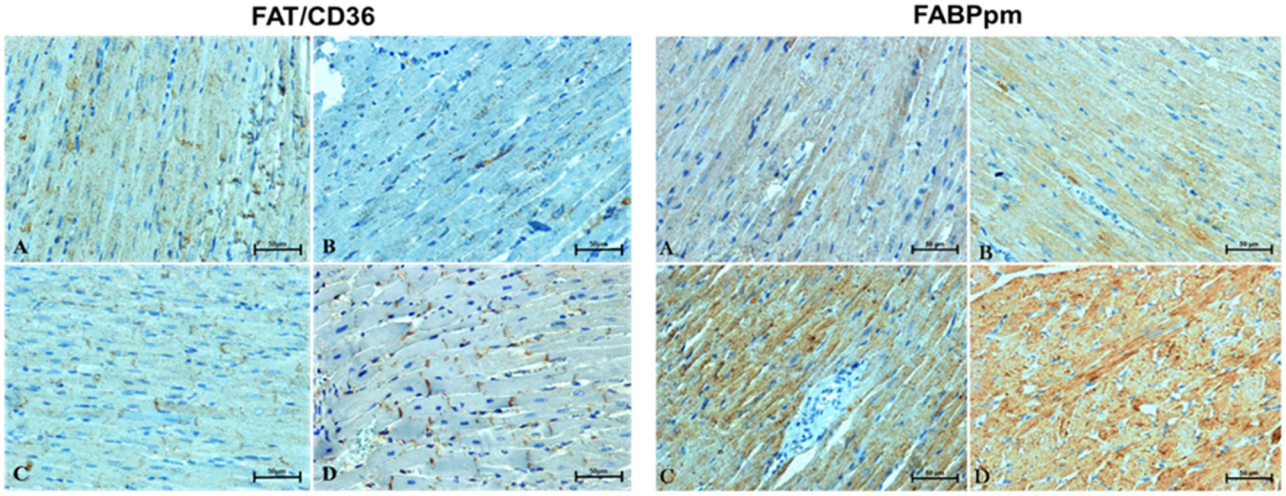

Fig. 3. Representative images of FAT/CD36 and FABPpm expression in the left ventricle cardiomyocytes after chronic URB597 treatment in spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY, control group), where (A) WKY group, (B) WKY + URB597 group, (C) SHR group and (D) SHR + URB597.

transporter FAT/CD36 decreased markedly in both hypertensive SHR groups in comparison with the normotensive control group (SHR, -13.6% and SHR + URB597, -11.9%; $P < 0.05$, Figs. 2 and Fig. 3a). In addition, in the untreated SHR group there was a significant increase in the total expression of FABPpm compared to the WKY group (+14.1%, $P < 0.05$; Figs. 2b and 3) and a trend towards the enhancement of its expression in treated with URB597 hypertensive rats (+11.3%, $P = 0.07$; Figs. 2b and 3). Whereas, total expression of other examined protein fatty acids transporters i.e. FATP1 and FATP4 were not affected neither by chronic activation of ECS nor primary hypertension ($P > 0.05$, Fig. 2c, d).

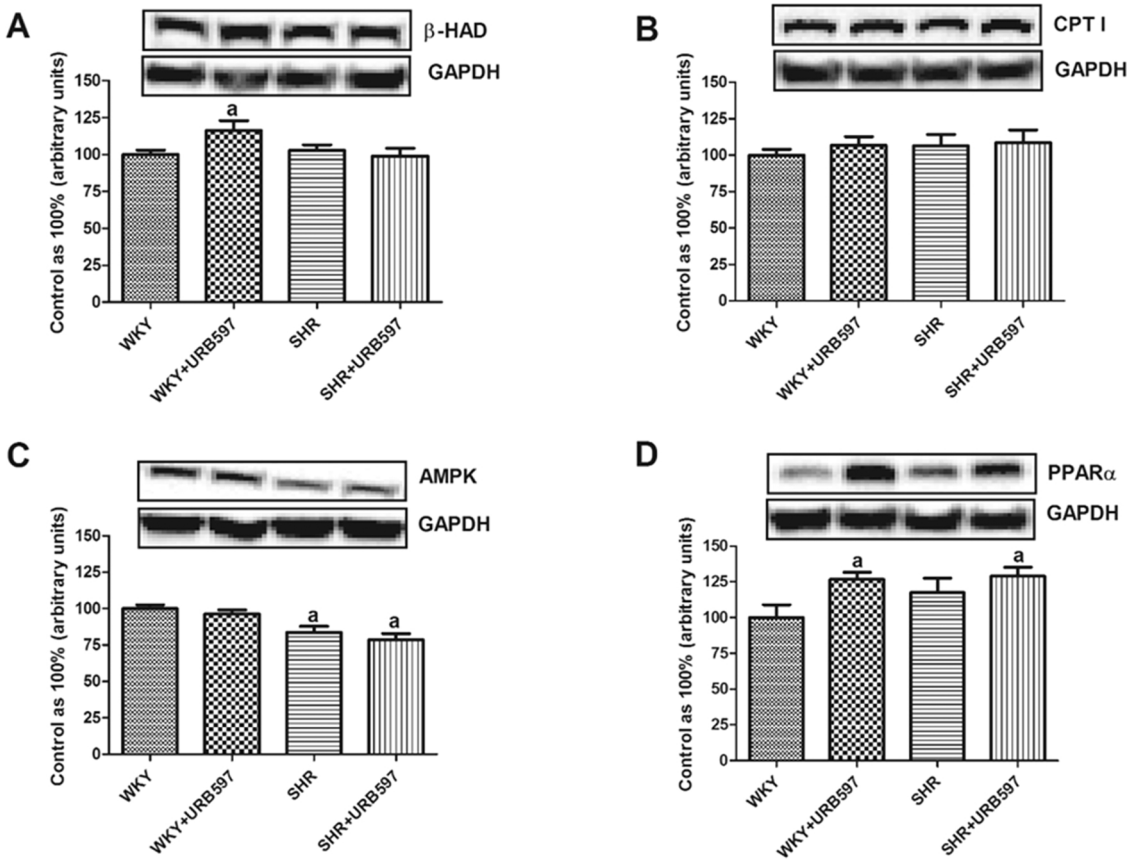

Fig. 4. Total expression of β-HAD (A), CPT I (B), AMPK (C) and PPARα (D) in the left ventricle after chronic URB597 treatment in spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY, control group). The data are expressed as the mean ± SD, n = 10 in each group. [a]P < 0.05 significant difference: control vs examined group.

3.4. Effect of prolonged activation of the ECS on the total expression of proteins involved in the myocardial lipids metabolism in the normotensive state and primary hypertension

Interestingly, in the experimental model of primary hypertension we observed, regardless of prolonged inhibition of FAAH, substantial diminishment of total myocardial expression of AMPK compared to the normotensive WKY rats (SHR, -16.3% as well as SHR + URB597 -21.5%, P < 0.05, Fig. 4c). Furthermore, 2-week treatment with URB597 caused marked elevation in PPARα's myocardial expression in the normotensive and hypertensive rats compared to the control group (WKY + URB597, +26.6% and SHR + URB597, +29%; P < 0.05, Fig. 4d). Amongst examined mitochondrial enzymes we noticed an increase only in the expression of β-HAD in the normotensive rats chronically treated with URB597 in comparison with the control WKY rats (+16.2%, P < 0.05; Fig. 4a).

4. Discussion

In the present study we have shown that chronic activation of ECS significantly affected myocardial lipid metabolism in a genetic model of primary hypertension, i.e. spontaneously hypertensive rats. As expected, 2-weeks URB597 treatment have reduced basic hemodynamic parameters that are systolic and mean blood pressures, which were initially increased compared to the control WKY rats (Table 1). Moreover, SHRs developed basic manifestation of hypertensive state, i.e. hypertrophy of the left ventricle, which was assessed by three different ratios (Table 1). According to the previous studies one of the probable causes of the above mentioned hypertrophy in SHRs is reduced LCFAs uptake and consequently diminished ATP production from fatty acids oxidation in cardiomyocytes (Hajri et al., 2001). The most likely explanation for impaired fatty acids influx as well as their intramyocardial availability in SHRs is declined expression of predominant cardiac LCFA protein transporter i.e. FAT/CD36 (Hajri et al., 2001; Bonen et al., 2009; Lauzier et al., 2011). In our experiment we also observed substantially decreased total expression of FAT/CD36 in the left ventricle of rat model with genetic hypertension. Furthermore, our observations revealed that plasma TAG concentration in SHR was lower compared to the Wistar Kyoto rats which together with defective FAT/CD36 expression could weaken LCFA uptake in the cardiomyocytes as well as intensify the left ventricle's hypertrophy development. Although, chronic inhibition of FAAH decreased pressure overload (i.e. MBP and SBP) in SHRs, it did not improve the degree of cardiac muscle hypertrophy, which is in line with our previous study conducted on DOCA-salt rats (Polak et al., 2017). In both models of hypertension, i.e. primary (SHR) as well as secondary (DOCA-salt), rats were administered the same URB597 dosage for 2 weeks, which was satisfactory to reduce pressure overload and provoke the cardiac muscle metabolism alterations in both cases.

Moreover, our research has shown that presumably myocardial substrate preference in hypertensive state was shifted towards enhancement of glucose utilisation rather than long chain fatty acids, as it was indicated in other studies (Hajri et al., 2001; Klevstig et al., 2011). As a consequence, in the SHR group we did not observe any changes in the plasma FFA, palmitate acid uptake and oxidation as well as its incorporation into different lipid fractions in the left ventricle's cardiomyocytes. The lack of alternations in the lipid metabolism probably resulted from substantial reduction in the total myocardial expression of FAT/CD36 and AMPK, and no simultaneous changes in the expression of mitochondrial enzymes (β-HAD and CPT I) in our model of

primary hypertension. Even though diminished expression of FAT/CD36 in the SHR group alone was compensated by an increase in the total myocardial expression of FABPpm, there was no change in ^{3}H-palmitate uptake in the cardiomyocytes. Hajri et al. (Hajri et al., 2001) also confirmed that due to decreased myocardial expression of FAT/CD36 in SHRs, long chain fatty acid transport is impaired, unlike short chain. Nevertheless, when 2-week URB597 treatment was introduced in SHRs, alternations in the left ventricle lipid metabolism were significant on various levels. Undoubtedly, the most pronounced change, which was triggered by chronic ECS activation, was the substantial enhancement in radiolabelled palmitate uptake during ex vivo Langendorff heart perfusion in both examined groups, i.e. SHR and WKY rats. An increase in ^{3}H-palmitate transport into cardiomyocytes in the left ventricle, which was induced by URB597 in the normotensive conditions (WKY rats), is in agreement with our recent study (Polak et al., 2017). It is likely that URB597 through increasing AEA level (Biernacki et al., 2016) and CB_1 receptor activation (Pędzińska-Betiuk et al., 2017) in the cardiomyocytes, stimulates signalling pathways therefore promoting translocation of LCFA protein transporters from an intracellular depots to the plasma membrane, as it was underlined in our previous study carried out on secondary hypertension rat model (Polak et al., 2017). Indeed, it is known that increased plasmalemmal expression of LCFA transporters, especially FAT/CD36 (Habets et al., 2007) but also FABPpm (Chabowski et al., 2007, 2006), FATP1,4,6 (Chiu et al., 2005; Gimeno et al., 2003), is the most potent factor, which stimulates fatty acids uptake in metabolically active tissues, including the cardiac muscle (Luiken et al., 2002). Therefore, it is reasonable explanation of the above mentioned elevation in the rate of LCFA uptake in SHR was induction by URB597 recruitment of protein transporters from intracellular compartments to the sarcolemma and consequential stimulation of protein mediated fatty acid transport. Additionally, there are several other lines of evidence to indicate that by inhibiting FAAH, URB597 is a potential regulator of myocardial lipid metabolism along with energy substrate preference and homeostasis in the normotensive as well hypertensive conditions. In the present study, augmented radiolabelled palmitate influx into the cardiomyocytes resulted in its enhanced incorporation into different intramyocardial lipid pools, i.e. FFA, TAG and cholesterol in both WKY and spontaneously hypertensive rats. What is interesting is that the aforementioned incorporation after chronic ECS activation was greater in the case of SHRs than normotensive controls, probably, due to lack of changes in the palmitate β-oxidation level when the workload is increased. It is known that FAT/CD36 resides also in the mitochondria (Campbell et al., 2004) and SHRs exhibit diminished expression of this LCFA transporter as well as its deficiency in mice revealed substantially reduced fatty acid uptake and β-oxidation in the heart (Hajri et al., 2002). Consequently, it is not surprising that in our hypertension model we did not observe any changes in the oxidation rate of ^{3}H-palmitate. Furthermore, chronic URB597 treatment did not affect the level of myocardial β-oxidation in SHRs either, which was probably associated with reduced mitochondrial expression of FAT/CD36 as well as diminished total expression of AMP-activated kinase in this model of hypertension. Presumably, concomitant reduction in myocardial expression of AMPK and no change in CPT I, as major regulators of LCFA oxidative metabolism (Hardie and Pan, 2002), were involved in the lack of alterations in the β-oxidation rate in both SHR groups. Moreover, studies conducted on 24-week old SHRs revealed deterioration of mitochondrial enzymes activity as well as mitochondria remodelling malfunction in the left ventricle (Tang et al., 2014). Additionally, Dodd et al. demonstrated that in SHR model ATP production in cardiac muscle is based on glucose oxidation rather than fatty acids (Dodd et al., 2012).

However, a primary transcriptional regulator of fatty acid metabolism i.e. PPARα was upregulated in the left ventricle after chronic URB597 administration irrespective of afterload conditions. Recent studies demonstrated that reduced PPARα expression represents a switch in substrate preference from fatty acid towards glucose utilisation (Karbowska et al., 2003). Previously, showed that chronic in vivo PPARα activation in rats (by WY14643) elevated myocardial palmitate uptake and oxidation, which was accompanied by redistribution of FAT/CD36 from intracellular compartments to the sarcolemma (Kalinowska et al., 2009). Furthermore, it was shown that FAAH inhibitors, including URB597, by increasing endocannabinoid-like substances' levels (OEA and PEA) were associated with induction of PPARα translocation from the cytosol to the nucleus (Wollank et al., 2015). Taken altogether, it appears that prolonged URB597 treatment in a genetic model of hypertension indirectly, via PPARα activation, contributes to the stimulation of LCFAs protein mediated transport in the heart.

5. Conclusions

In conclusion, we can speculate that the ECS modulation by chronic URB597 treatment can serve as a potential and effective therapeutic strategy for elevated arterial blood pressure treatment rather than the left ventricle hypertrophy. Undoubtedly, FAAH inhibitor is an indirect myocardial metabolism modulator which ameliorates fatty acid utilisation in the SHR's cardiac muscle. We found that URB597 in a genetic model of hypertension enhances both LCFAs influx in the cardiomyocytes and their incorporation into intramyocardial lipid pools. Moreover, it seems that long-term effects of the ECS activation in the heart partially restores energetic balance in the cardiac muscle substrate preference in hypertensive conditions.

Author contributions

B.M. and A.Ch. conception and design of the research; E.H.S., A.P., A.L., I.K., A.P.B. and J.W. performed experiments; E.H.S. and A.P. analysed data; E.H.S. and A.Ch. interpreted results of experiments; E.H.S. and A.P. prepared figures and drafted manuscript; E.H.S., A.P., I.K. and A.Ch. edited and revised the manuscript; E.H.S., A.P., B.M., I.K., A.P.B., J.W. and A.Ch. approved final version of manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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