Prostaglandins and Other Lipid Mediators 138 (2018) 54–63

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

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

Original Research Article

Hypertension and chronic inhibition of endocannabinoid degradation modify the endocannabinoid system and redox balance in rat heart and plasma

Michał Biernacki[a], Barbara Malinowska[b,*], Magdalena Timoszuk[a], Martek Toczek[b], Anna Jastrząb[a], Patryk Remiszewski[b], Elżbieta Skrzydlewska[a]

[a] Department of Analytical Chemistry, Medical University of Bialystok, Mickiewicza 2D, 15-222 Bialystok, Poland
[b] Department of Experimental Physiology and Pathophysiology, Medical University of Bialystok, Mickiewicza 2A, 15-222 Bialystok, Poland

ARTICLE INFO

Keywords:
DOCA-salt
Endocannabinoids
FAAH inhibitor
Hypertension
SHR
URB597

ABSTRACT

The interaction between the endocannabinoid and ROS signaling systems has been demonstrated in different organs. Inhibitors of fatty acid amide hydrolase (FAAH), the key enzyme responsible for degradation of the endocannabinoid anandamide, are postulated to possess anti-hypertensive potential. Here, we compared the effects of hypertension and chronic FAAH inhibition by URB597 on the endocannabinoid system and redox balance in spontaneously hypertensive rats (SHR) and hypertensive deoxycorticosterone acetate (DOCA)-salt rats. Enhanced oxidative stress and lipid peroxidation were found in both hypertension models. Hypertension affected cardiac and plasma endocannabinoid systems in a model-dependent manner: anandamide and 2-arachidonoylglycerol levels decreased in SHR and increased in DOCA-salt. Cardiac CB_1 receptor expression increased in both models while higher CB_2 receptor expression was only in DOCA-salt. URB597 increased endocannabinoid levels in both models but produced the partial reduction of oxidative stress in DOCA-salt but not in SHR. Notably, URB597 decreased antioxidant defense and increased lipid peroxidation products in normotension. Therefore, the therapeutic potential of FAAH inhibitors should be interpreted cautiously.

1. Introduction

Hypertension is the most common cardiovascular risk factor contributing to widespread morbidity and mortality worldwide. The endocannabinoid system is suggested to buffer increases in blood pressure (BP) in hypertension and inhibitors of fatty acid amide hydrolase (FAAH), the key enzyme responsible for degradation of the endocannabinoid anandamide (AEA) [1], are postulated to be potential antihypertensive agents [2–5]. Correspondingly, the plasma AEA level was higher in hypertensive patients and in rats with primary (spontaneously hypertensive rats; SHR) and some models of secondary hypertension [3,6,7]. In SHR anandamide induced much stronger CB_1 receptor-mediated hypotension as compared to normotensive controls [5,8] and the acute FAAH inhibition by URB597 [5] and AM3506 [4] normalized BP. The CB_1 receptor-mediated vascular responses [9,10] were enhanced in deoxycorticosterone acetate (DOCA)-salt hypertensive rats, suggesting a protective role for these receptors. The hypotension and cardio-protection following chronic URB597 administration in DOCA-salt rats [11,12] (not observed in SHR [12]), can be partially dependent on its beneficial influence on vessels [9], cardiac performance [12] and the coronary blood vessel morphology [13].

Oxidative stress is one of the key factors in the pathogenesis of hypertension or its consequences. It results from the overproduction of reactive oxygen species (ROS) and a decrease in antioxidant defense [14–17]. One of its consequences is disturbed ROS-mediated phospholipid metabolism related peroxidation of polyunsaturated fatty acids (PUFAs), including arachidonic (AA), linoleic (LA) and docosahexaenoic (DHA) acid [18,19]. The existence of interaction between ROS signaling systems and endocannabinoids, products of enzymatic phospholipid metabolism, has been proven in different organs [17,20,21] including the cardiovascular system. Activation of peripheral CB_1 receptors promotes oxidative stress in experimental cardiomyopathy [22,23], in human cardiomyocytes [24] and coronary artery [25]. In contrast, activation of CB_2 receptors reduced oxidative stress in a rat [26] and mouse [27] model of myocardial ischemia/reperfusion. The stimulation of sensitive to cannabinoids GPR55 receptors reduced the ROS production stimulated by another endocannabinoid, 2-arachidonoylglycerol (2-AG) in human neutrophils [28] and TRPV1 receptors

* Corresponding author.
E-mail address: bmalin@umb.edu.pl (B. Malinowska).

https://doi.org/10.1016/j.prostaglandins.2018.09.001
Received 5 June 2018; Received in revised form 25 July 2018; Accepted 5 September 2018
Available online 07 September 2018

prevented cardiac damage induced by oxidative stress [29]. FAAH was suggested to play a key role in controlling the tissue injury associated with acute oxidative/nitrative stress since cardiac injury and enhanced oxidative/nitrative stress was greater in $FAAH^{-/-}$ compared to $FAAH^{+/+}$ mice [24]. Its inhibitor URB597 exerted protective and antioxidative effects in rat cardiomyocytes [26].

Chronic administration of URB597 in DOCA-salt disrupted the liver and kidney endocannabinoid system and caused an imbalance in redox status [20,21] and of a nonspecific cannabinoid receptor agonist Δ^9-tetrahydrocannabinol exerted the cardioprotective effect in diabetic rats connected with a decrease in serum markers of oxidative stress and lipid peroxidation [30].

The aim of our study was to examine: (1) whether the endocannabinoid system is changed in two different models of hypertension in relation to oxidative stress and lipid peroxidation; and (2) whether the cardiovascular effects of chronic FAAH inhibition by URB597 in rats with primary and secondary hypertension and their normotensive controls correlates with the cardiac and plasma oxidative stress and changes in the endocannabinoid system.

2. Materials and methods

2.1. Reagents

Reagents were obtained from the following suppliers (in parentheses are given abbreviations and the catalogue numbers): dimethyl sulphoxide (DMSO; 276855), N,*N*-dimethylformamide, (DMF; 227056), 11-deoxycorticosterone acetate, (DOCA; D7000), HCl (H1758), NaCl (S7653), KCl (P9333), Na_2HPO4 (71640), KH_2PO4 (60230), hydrogen peroxide, H_2O_2 (H1009), Tween-80 (P1754), Tween-20 (P1379), chloro-2,4-dinitro benzene (CDNB; 237329), butylated hydroxytoluene (BHT; W218405), 5,5′-dithiobis (2-dinitrobenzoic acid) (DTNB; D8130), m-nitroaniline (m-NA; N9829), β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH; N7505), sodium azide (NaN_3; S8032), boron trifluoride (BF_3, B1252), chloroform (372978), L-glutathione reduced (GSH; PHR1359), L-glutathione oxidized (GSSG; G4376), glutathione peroxidase (GSH-Px; 77332), acetonitrile LC–MS (SZBF253MV), methanol LC–MS (SZBF225MV), ethanol (46139), hexane (439177), n-heptane (650536), n-hexane (34859), tris(hydroxymethyl)aminomethane (TRIS; 252859), glycerol (G9012), 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol, TRITON X-100 (X100), disodium ethylenediaminetetraacetate dehydrate (EDTA; E1644), sucrose (S9378), α-tocopherol (T3251), cis-4,7,10,13,16,19-docosahexaenoic acid (DHA; 53171), nonadecanoic acid (N5252), anti-GPR55 (SAB2900461), Na^+/K^+ ATPase (A3979), secondary antibodies against rabbit (A3687), secondary antibodies against mouse (A3562), 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate system (BCIP/NBT; B3804), laemmli buffer (S3401), 2-mercaptoethanol (M6250), fluorescein (46955-100G-F), methyl nonadecanoate (N5377), supelco 37 Component FAME Mix (CRM47885), 1,1,3,3-tetraethoxypropane (108383), benzaldehyde-d6 (485551), N,O-bis(trimethylsilyl)trifluoroacetamide, (BSTFA:TMCS (99:1); 15238), o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (76735), 1,4-piperazinediethanesulfonic acid (PIPES; P8203) from Sigma-Aldrich (Steinheim, Germany); diisopropyl ether (1009211000), glacial acetic acid (1000562500) from Merck (Darmstadt, Germany); n-pentane (1.00882 EMD) from Avantor (Poland); 1-hydroxy-3-methoxycarbonyl -2,2,5,5-tetramethylpyrrolidine, CMH (NOX-02.1–50 mg), electron spin resonance (ESR)-Krebs HEPES Buffer (NOX-07.6.1–0.5 L), ESR Grade Water 500 ml (NOX-07.7.1–0.5 L) from Noxygen (Germany); pentobarbital sodium from Biowet (Puławy, Poland); 3-(3-carbamoylphenyl) phenyl N-cyclohexylcarbamate, (URB597; 10046), anandamide-d8 (AEA-d8; 390050), 2-arachidonoyl glycerol-d8 (2-AG-d8; 362160), N-arachidonoyl dopamine-d8 (NADA-d8; 10007431), 8-iso Prostaglandin F2α-d4, (8-isoPGF2α–d4; 316350) decanoyl m-nitroaniline (90349), arachidonoyl-1-thio-glycerol (A-1-TG; 10007904) from Cayman Chemical Company (Ann Arbor, MI, USA); anti-CB_1 (sc-20754), anti-CB_2 (sc-25494), anti-TRPV1 (sc-12498), cell lysate (sc-2414) from Santa Cruz Biotechnology (Santa Cruz, CA, USA); skim milk (170-6404), tris buffered saline (TBS; 170-6435) from BioRad (Hercules, California, USA). Drugs were dissolved with the following way: DOCA in DMF; URB597 in a mixture of DMSO and Tween 80 (1:2) and then diluted in saline (3:7) immediately before the injection.

2.2. Animals

All procedures and experimental protocols were performed in accordance with the European Directive (2010/63/EU) and were approved by the local Animal Ethics Committee in Białystok (Poland). Rats were obtained from the Center for Experimental Medicine of the Medical University of Białystok (Poland). They had free access to food pellets and water and were maintained under a 12/12 h light/dark cycle. Experiments were performed on male rats with primary (SHR) and secondary DOCA-salt hypertension (see below) in order to distinguish changes induced by hypertension from those related to any one particular hypertension model. We applied DOCA-salt model since a salt-rich diet is one of the main lifestyle factors leading to hypertension and SHR – the most commonly used animal model of hypertension.

2.3. Experimental groups and protocol

DOCA-salt hypertension was induced in Wistar rats (4–5 weeks old; initially weighing 100–140 g) as described previously [11]. All rats were anaesthetized intraperitoneally (i.p.) with pentobarbital sodium (70 mg/kg) and unilaterally nephrectomised. After one week recovery period, hypertension was induced by 6 weeks by subcutaneous (s.c.) injections of DOCA (25 mg/kg b.w. in 0.4 ml DMF/kg b.w.) twice weekly and replacement of drinking water with 1% NaCl solution. Control normotensive sham-operated (SHAM) Wistar rats received s.c. DMF (the vehicle for DOCA) twice weekly for 6 weeks and drank tap water.

2.4. Chronic URB597 administration

We applied the same protocol and dose of URB597 as reported previously [11–13,20,21]. Rats were divided randomly to the following experimental groups: (1) hypertensive DOCA-salt (4 weeks after the onset of DOCA-salt administration) and (2) their respective normotensive control age-matched SHAM rats (4 weeks after the onset of DOCA-salt or its vehicle, respectively); (3) 8–10 week old male SHR (270–350 g) and (4) their respective normotensive age-matched Wistar-Kyoto rats (WKY; 290–390 g).

One part of particular hypertensive and normotensive rats were injected i.p. with URB597 (1 mg/kg in 1 ml of URB597 solvent) every 12 h for 14 days, whereas the second part of each groups received solvent for URB597.

2.5. Determination of blood pressure in conscious rats

Systolic blood pressure (SBP) was measured in conscious rats by the non-invasive tail-cuff method using the Rat Tail Blood Pressure Monitor from Hugo Sachs Elektronik-Harvard Apparatus (March–Hugstetten, Germany) before the first dose of URB597 or its vehicle and 12 h after the final dose of URB597 (or its solvent).

2.6. Tissues preparation for biochemical examinations

Twelve hours after the final dose of URB597 (or its vehicle) rats were anesthetized with pentobarbital sodium (70 mg/kg b.w.) to collect blood and heart. Blood samples were drawn by puncture of the left ventricle and collected into heparinized (ROS determination) and EDTA tubes (for other determinations). Plasma from EDTA tubes was