Prostaglandins & other Lipid Mediators 98 (2012) 133–142

Contents lists available at SciVerse ScienceDirect

Prostaglandins and Other Lipid Mediators

EET agonist prevents adiposity and vascular dysfunction in rats fed a high fat diet via a decrease in Bach 1 and an increase in HO-1 levels

Komal Sodhi[a,1], Nitin Puri[a,1], Kazuyoshi Inoue[a], John R. Falck[c], Michal L. Schwartzman[b], Nader G. Abraham[a,*]

[a] Department of Physiology and Pharmacology, The University of Toledo College of Medicine, Health Education Building, 3000 Arlington Avenue, Toledo, OH 43614-2598, USA
[b] Department of Pharmacology, New York Medical College, Valhalla, NY 10595, USA
[c] Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390, USA

ARTICLE INFO

Article history:
Received 21 July 2011
Received in revised form 9 December 2011
Accepted 15 December 2011
Available online 24 December 2011

Keywords:
Cytochrome P450
HO-1
Metabolic dysfunction
Obesity
Adiponectin

ABSTRACT

Recent reports have shown interplay between EETs (epoxides) and the heme oxygenase (HO) system in attenuating adipogenesis in cell culture models; prompting an examination of the effectiveness of EET agonist on obesity and associated cardio-metabolic dysfunction. Patho-physiological effects of an EET agonist (NUDSA) were contrasted in the absence and in the presence of stannous mesoporphyrin (an HO inhibitor) in SD rats fed a high fat (58%, HF) for 16 weeks. Animals on HF diet exhibited enhanced oxidative stress, increased levels of inflammatory cytokines and decreased levels of adiponectin along with reduced vascular and adipose tissue levels of EETs, HO-1; as compared to control rats (11% dietary fat). Treatment with NUDSA not only reversed serum adiponectin and vascular and adipose tissue levels of EETs and HO-1, but also, decreased blood pressure, subcutaneous and visceral fat content and serum TNFα and IL-6 levels in rats on HF diet. Aortic endothelial function, peNOS expression and adipose tissue markers of energy homeostasis i.e. pAMPK, Sirt1 and FAS, impaired in rats fed a HF diet, were restored in animals treated with this EET agonist. That NUDSA enhanced HO-1 expression, was accompanied by increase in p-GSK-3β and pAKT levels along with attenuation of adipose tissue levels of Bach 1 – the transcriptional suppresser of HO-1 expression. Prevention of these beneficial effects of NUDSA, in animals on HF diet and concurrently exposed to NUDSA and SnMP, supports the role of EET-HO interaction in mediating such effects. Taken together, our findings suggest that the EETs stimulate HO-1 expression via suppression of Bach 1 and interplay of these two systems affords vascular and metabolic protection in diet induced obesity.

1. Introduction

Epoxyeicosatrienoic acids (EETs) are catalyzed from arachidonic acid (AA) by a family of enzymes belonging to the Cytochrome P450 (CYP) super family [1,2]. Upon formation, EETs are rapidly hydrolyzed by soluble epoxide hydrolase (sEH) to their respective dihydroxyepoxytrienoic acids (DHETs) as well as to esterification products primarily to glycerophospholipids [3]. EETs exhibit potent biological effects including vasodilation, stimulation of ion transport, inhibition of inflammatory response and stimulation of epithelial cell growth [4–6]. Studies that demonstrate that the induction of CYP2C23 [7] and inhibition of sEH provide vascular protection [4,5] further highlight the role of EETs as modulators of vascular function. In addition, emerging studies indicate a role of this ubiquitous lipid mediator in the regulation of metabolic homeostasis [8]; thereby warranting further examination of its biological role in conditions such as obesity.

The heme oxygenase (HO) isoforms, HO-1 (inducible) and HO-2 (constitutive), gene expression is known to increase signaling molecules – mediated antioxidant and anti-inflammatory properties [9]. HO-1 is a stress response protein whose expression is principally regulated by transcription factors NRF2 and Bach 1. Where electrophiles activate NRF2-depndent HO-1 expression, molecules such as heme bind to and inhibit Bach 1 which is an inhibitor of HO-1 expression in quiescent cells. Increased HO-1 expression and activity is associated with an increase in adiponectin secretion and downstream signaling resulting in the stimulation of NO bioavailability [9–11]. HO-1 derived carbon monoxide (CO) regulates vascular tone in part by decreasing

Abbreviations: CYP, cytochrome P450; EETs, epoxyeicosatrienoic acids; NUDSA, EET agonist; HO, heme oxygenase; SnMP, stannous mesoporphyrin; ROS, reactive oxygen species; IR, insulin receptor; O_2^-, superoxide; sEH, soluble epoxide hydrolase.

* Corresponding author. Tel.: +1 419 383 4144; fax: +1 419 383 4262.
E-mail address: nader.abraham@utoledo.edu (N.G. Abraham).

[1] Both the authors contributed equally to this work.

1098-8823/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.prostaglandins.2011.12.004

CYP450-derived vasoconstriction [12]. Furthermore, activation of the heme-HO system could contribute towards the vasodilatory effects of lipid mediators such as EETs [13,14].

Obesity, and associated metabolic syndrome, is a systemic inflection characterized by increased oxidative stress (ROS) along with inhibition of the HO-adiponectin system while increasing levels of inflammatory cytokines and insulin resistance [15,16]. We have recently reported that epoxides attenuate the metabolic syndrome like phenotype in the HO-2 null mice via the activation of HO-1 dependent pathways [8]. In addition, EETs attenuate adipocyte hypertrophy and improve adipocyte function in bone marrow derived mesenchymal stem cells (MSCs) via an increase in HO-1 expression [17]. In light of this evidence, the present study aims to explore the effectiveness of an EET agonist on the prevention of adiposity and a possible interaction between the HO and epoxide systems, in vivo, where metabolic pathologies have been induced in rats fed a HF diet. This study corroborates the existence of an epoxide-HO axis whose stimulation via an exogenous EET agonist (NUDSA) induces Bach 1-dependent HO-1 expression and abates obesity associated vascular dysfunction and improves metabolic homeostatic markers in visceral adipose tissues, in SD rats. Prevention of these beneficial effects in HF fed rats concurrently exposed to SnMP and NUDSA exposes interplay of epoxide-HO systems in affording aforementioned vasculo-metabolic protections.

2. Materials and methods

2.1. Materials

The EET analog, 11-(nonyloxy)undec-8(Z)-enoic acid (NUDA), is a potent vasorelaxant in mesenteric and renal arteries [18,19]. For in vivo studies, NUDA was conjugated with L-aspartic acid to form (S)-2-(11-(nonyloxy) undec-8(Z)-enamido)succinic acid (NUDSA) to minimize β-oxidation and improve solubility in aqueous milieu.

2.2. Animal experimentation

All animal experiments followed an institutionally approved protocol in accordance with the NIH Guidelines. Forty, 8-week-old Sprague-Dawley (SD) rats were used in the studies. Rats were divided into four groups (10 rats/group): (A) Control, (B) HF + vehicle, (C) HF + NUDSA, (D) HF + NUDSA + SnMP. Control rats (group A) were fed ad libitum a normal diet containing 11% fat, 62% carbohydrate, and 27.0% protein with a total calories of 12.6 kJ/g. The remaining animals (groups B, C, D) were fed a high-fat diet containing 58% fat (from lard), 25.6% carbohydrate, and 16.4% protein with total calories of 23.4 kJ/g (Bio-SERV, Frenchtown, NJ) for 16 weeks [20,21]. After 14 weeks of HF diet, NUDSA was injected, intraperitoneally, for 2 weeks daily at a dose of 1.5 mg/100 gm of body weight. Similarly after 14 weeks of HF diet, SnMP was injected intraperitoneally, 3 times a week at a dose of 20 mg/kg of body weight, for 2 weeks. After a 6-h fast, rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and blood was obtained from a tail vein for glucose measurement using a glucometer (Lifescan Inc., Miligitas, CA). Blood pressure was measured by the tail cuff method immediately prior to NUDSA administration and every 7 days thereafter. Body weights of SD rats at the beginning of the experiment were 269 ± 12 g. At the time of sacrifice the body weight, visceral and subcutaneous fat content of all rats was measured. Blood samples were collected in K_3EDTA tubes at sacrifice and the plasma was separated. Samples were flash frozen in liquid nitrogen and maintained at −80 °C until needed.

2.3. Assessment of vascular reactivity

The aorta was removed, cleaned of fat and loose connective tissue, placed in cold Krebs-bicarbonate solution, and sectioned into 3-mm-long rings. Vasorelaxation responses of phenylephrine-constricted arteries to cumulative increments in acetylcholine (10^{-9} to 10^{-4} mol/L) were examined in the presence of indomethacin (10 μmol/L) as described [8].

2.4. Measurement of EETs and DHETs

Aorta was homogenized in 66% methanol containing a 500 pg mixture of internal standards (PGE2-d4; 8(9)-EET-d11; 11(12)-EET-d8; 12-HETE-d8; 20-HETE-d6, and 11,12-DHET-d11). EETs concentration was calculated as described [8].

2.5. Western blot analysis of HO-1, Bach 1, GSK-3β, Sirt 1, FAS, pAKT, pAMPK, ferritin, eNOS, peNOS, adiponectin and phosphorylation of insulin receptor (IR)

Frozen tissues were used for determination of HO-1, Bach 1, GSK-3β (serine 9), Sirt 1, FAS, AMPK-alpha, pAMPK (Thr172), pAKT (serine 473), adiponectin, eNOS and peNOS (serine 1177), ferritin and insulin receptor phosphorylation. The nuclear fractions were obtained using Nuclear Extraction Reagents (Thermo Scientific, Rockford, IL, USA) and were done according to the manufacturer's protocol. Immunoblotting was performed in aorta and adipose tissue as previously described [8,15].

2.6. O_2^- production

Aorta and adipose tissues were placed in scintillation vials respectively (2 per vial) containing 1 mL of Krebs-HEPES buffer, pH 7.4, and lucigenin (5 μmol/L) for 30 min at 37 °C. Lucigenin chemiluminescence was measured in a liquid scintillation counter (LS6000TA, Beckman Instruments) and superoxide production quantified as previously described [22].

2.7. Cytokines/chemokines and plasma insulin measurements

The levels of IL-6, TNF alpha and the high molecular weight (HMW) form of adiponectin were determined using an ELISA assay as previously described [8,15]. Insulin concentration in plasma was determined by ELISA (Linco Research, St. Charles, MO, USA) according to the manufactures' protocol.

2.8. Statistical analysis

Data are expressed as means ± S.E.M. Significance of difference in mean values was determined using one-way analysis of variance followed by the Newman-Keul's post hoc test. $p < 0.05$ was considered to be significant.

3. Results

3.1. Effect of a HF diet on body weight and fat content

As seen in Fig. 1A, a HF diet for 16 weeks significantly increased body weight of SD rats as compared to age-matched controls (516 ± 9.4 vs. 407 ± 9.4 g, $p < 0.01$). This increase in body weight was attenuated in rats, on HF diet, treated with NUDSA for 2 weeks ($p < 0.01$). The decrease in body weight was reversed by the concurrent administration of SnMP and NUDSA to rats being fed a HF diet. These findings paralleled the change in subcutaneous fat content, Fig. 1B. Rats fed a HF diet showed marked increase in subcutaneous fat content as compared to control animals (14.7 ± 0.7 and