Biochimie 94 (2012) 1660–1667

Contents lists available at SciVerse ScienceDirect

Biochimie

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

Research paper

Early induction of a brown-like phenotype by rosiglitazone in the epicardial adipose tissue of fatty Zucker rats

Emilie Distel [1], Graziella Penot [1], Thomas Cadoudal, Irène Balguy, Sylvie Durant, Chantal Benelli*

Institut National de la Santé et de la Recherche Médicale UMR-S 747; Université Paris Descartes Centre universitaire des Saints-Pères, Pharmacologie Toxicologie et Signalisation Cellulaire, 45 rue des Saints-Pères, 75006 Paris, France

ARTICLE INFO

Article history:
Received 21 March 2012
Accepted 10 April 2012
Available online 2 May 2012

Keywords:
Rosiglitazone
Epicardial adipose tissue
Fatty rat

ABSTRACT

The epicardial adipose tissue (EAT) is "hypertrophied" in the obese. Thiazolidinediones are anti-diabetic, hypolipidemic drugs and are selective agonists for the gamma isoform of peroxisome proliferator-activated receptor (PPARγ). We evaluated the short-term effects of the prototype rosiglitazone (RSG, 5 mg kg^{-1} day^{-1} for 4 days) on the expression of the genes and proteins (by real-time PCR and Western blot) involved in fatty acid (FA) metabolism in EAT of the obese fatty Zucker rat and compared the levels of expression with those in retroperitoneal adipose tissue (RAT). The glyceroneogenic flux leading to fatty acid re-esterification was assessed by the incorporation of 14C from [1-14C]-pyruvate into neutral lipids. RSG upregulated the mRNA for phosphoenolpyruvate carboxykinase, pyruvate dehydrogenase kinase 4, glycerol kinase, adipocyte lipid binding protein, adipose tissue triglyceride lipase and lipoprotein lipase in both RAT and EAT with a resulting increase in glyceroneogenesis that, however, was more pronounced in EAT than in RAT. Under RSG, fatty acid output was decreased in both tissues but unexpectedly less so in EAT than in RAT. RSG also induced the expression of the key genes for fatty acid oxidation [carnitine-palmitoyl transferase-1, medium chain acyl dehydrogenase and very long chain acyl dehydrogenase (VLCAD)]in EAT and RAT with a resulting significant rise of the expression of VLCAD protein. In addition, the expression of the genes encoding proteins involved in mitochondrial processing and density PPARγ coactivator 1 alpha (PGC-1α), NADH dehydrogenase 1 and cytochrome oxidase (COX4) were increased by RSG treatment only in EAT, with a resulting significant up-regulation of PGC1-α and COX4 protein. This was accompanied by a rise in the expression of PR domain containing 16 and uncoupling protein 1, two brown adipose tissue-specific proteins. In conclusion, this study reveals that PPAR-γ agonist could induce a rapid browning of the EAT that probably contributes to the increase in lipid turnover.

© 2012 Elsevier Masson SAS. All rights reserved.

1. Introduction

Epicardial adipose tissue (EAT), compared to other visceral fat depots, exhibits different fatty acid (FA) composition, lower glucose oxidation rate but higher FA metabolism, as described in animal studies [1]. Recently EAT has been described as functioning as brown adipose tissue (BAT) in Human [2]. Because of its anatomic contiguity to the heart, EAT can modulate locally the myocardium. In Human, under physiological conditions, EAT is thought to act as a buffering system against toxic levels of FA between the myocardium and the local vascular bed. Increased circulating FA lead to increased storage in EAT which could play an important role in the development of cardiac pathology [3]. It is also plausible that paracrine release of bioactive cytokines and adipokines by EAT could pass through the coronary wall by diffusion from the outside to the inside and interact with cells in each of its layers. Under pathological circumstances, such as visceral obesity, increased EAT might act as

Abbreviations: TZD, thiazolidinedione; RSG, rosiglitazone; TG, triglycerides; PPARγ, peroxisome proliferator-activated receptor gamma; RXR, retinoid X receptor; CEBP, CCAAT enhancer binding protein; RAT, retroperitoneal adipose tissue; EAT, epicardial adipose tissue; BAT, brown adipose tissue; NEFA, non esterified fatty acids; ATGL, adipose tissue triglyceride lipase; LPL, lipoproteine lipase; aP2, adipocyte lipid binding protein; PEPCK-C, cytosolic phosphoenolpyruvate carboxykinase; PDK4, pyruvate dehydrogenase kinase 4; GlyK, glycerol kinase; CPT-1, carnitine palmitoyl transferase-1; MCAD, medium chain acyl dehydrogenase; VLCAD, very long chain acyl dehydrogenase; ND1, NADH dehydrogenase 1; COX4, cytochrome oxidase; PGC-1α, PPARγ coactivator 1 alpha; Tfam, transcription factor A mitochondrial; UCP-1, uncoupling protein 1; PRDM16, PR domain containing 16.

* Corresponding author. Tel.: +33 (0) 1 42 86 38 65; fax: +33 (0) 1 42 86 38 68.
E-mail address: chantal.benelli@parisdescartes.fr (C. Benelli).

[1] These authors participated equally in the work.

0300-9084/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2012.04.014

a source of cardiotoxic FA and proinflammatory cytokines [4,5]and then, possibly, contribute to myocardial dysregulation.

It is now well established that thiazolidinediones (TZD) which selectively activate PPARγ [6], improve insulin resistance in type-2 diabetic patients, as well as in models of rodent obesity and type-2 diabetes [6,7]. Insulin sensitivity improvement by TZD is associated with a decrease in lipidemia. TZD activate various metabolic pathways that lead lower FA release into the circulation: uptake (lipoprotein lipase (LPL), adipocyte lipid binding protein (aP2), re-esterification (cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C), pyruvate dehydrogenase kinase 4 (PDK4), glycerol kinase (GlyK) and β-oxidation (carnitine palmitoyl transferase-1 (CPT-1), medium chain acyl dehydrogenase (MCAD), very long chain acyl dehydrogenase (VLCAD). Most of the studies conducted so far have focused on the effects of TZD in subcutaneous and visceral AT and there are few data regarding the influence of PPARγ activators on EAT [2].

The aim of this study was to evaluate the short-term effect of rosiglitazone (RSG), a TZD prototype, on the expression of genes involved in FA metabolism in EAT of the fatty Zucker rat, a model of obesity with insulin resistance and dyslipidemia and to analyse the metabolic consequences of the observed changes.

2. Material and methods

2.1. Materials

Dulbecco modified Eagle's medium (DMEM) was from Life Technologies (UK). Maleate RSG was from Alexis Biochemicals. All other products were purchased from Sigma (L'isle d'Abeau Chesnes, France).

2.2. Animals and treatments

Male fatty Zucker rats (BW:320 +/− 7 g) were purchased from Charles River laboratories (L'arbresle, France). Rats were kept on a 12 h light/dark cycle at constant room temperature. Conventional chow diet and tap water were provided *ad libitum*. In the morning, eight week old rats were given a dose of 5 mg kg^{-1} day^{-1}maleate RSG or vehicle (0.5% methyl cellulose) via *gavage* for four days. After the last treatment, animals were fasted for 4 h, and the neuthanized. Blood was obtained via the jugular vein and plasma aliquots were stored at −20 °C for glucose (Accu-Check, Roche), insulin (ultra sensitive rat insulin Elisa, Eurobio), TG (Vitros chemistry products TRIG), NEFA (Free fatty acids half micro test Roche) and glycerol (glycerol UV-method, R-Biopharm) determinations. Retroperitoneal (RAT) and EAT were dissected and frozen at −80 °C for further analyses. The protocol for the animal studies was conducted according to the French Guidelines for the Care and use of Experimental Animals.

2.3. Histology

Epicardial and retroperitoneal adipose tissues of 2 month-old male fatty Zucker rats was removed and immediately fixed in 4% paraformaldehyde in phosphate buffered saline (PBS), pH 7.4, for 48 h at 4 °C. After fixation, tissues were dehydrated in ethanol, cleared and finally embedded in paraffin blocks. Paraffin sections (5 μm thick) were stained by hematoxylin and eosin and observed under a microscope (Zeiss, Germany). Adipocyte sizes were measured and analyzed by using Image pro plus software (Micro Optical solutions).

2.4. RNA analysis

Total RNA was extracted from rat AT by the method of Chomczynski and Sacchi [8]. Total RNA (1.25 μg) was reverse transcribed using the High Capacity cDNA Archive kit from Applied Biosystems (Courtaboeuf, France). cDNA was amplified in an ABI prism 7900 HT (Applied Biosystems) using SYBR green, and analyzed with the SDS 2.1 real-time detection system software. Quantification of mRNA was carried out by comparison of the number of cycles required to reach reference and target threshold values (δ-δΔCt method). Ribosomal protein L13a (RPL13a) mRNA was used to normalization and was unchanged between the two depots and in response to rosiglitazone treatment. Sequences of the sense and antisense oligonucleotides corresponding to the different genes tested are given in Table 1.

2.5. Protein isolation and analysis

AT proteins were isolated from adipose tissue following disruption by five strokes in a Teflon/glass homogenizer in RIPA buffer (PBS, Na-deoxycholate 0.5%, SDS 0.1%, NP40 1%)

Table 1
Rat primer sequences of RNA tested in real-time RT-PCR.

RNA	Accession number	Forward primer (5′–3′)	Reverse primer (5′–3′)
Pnpla2 (ATGL)	NM 001108509	GCTCCACCAACATCCAC	TCCCCTCCAGTCCTCTC
Lpl	NM 012598	AGGACCCCTGAAGACAC	GGCACCCAACTCTCATA
Ap2	NM 031008	AACACCGAGATTTCCTT	ACACATTCCACCACCAG
Pck1	NM 198780	TGTTGGCTGGCTCTCACTG	ACCTTTGGGGATGGGCAC
Pdk4	NM 053551	TGTGATGTGGTAGCAGTAGTC	ATGTGGTGAAGGTGTGAAG
Gyk	NM 024381	ATCCGCTGGCTAAGAGACAACC	TGCACTGGGCTCCCAATAAGG
Cpt1b	NM 013200	GCCTCAACACAGAACACTC	TAACTCCACATCATCAGCC
Acadm (MCAD)	NM 016986	TGTGCCTACTGCGTGACAGA	TTCATCACCCTTCTTCTCTGCTT
Acadvl (VLCAD)	NM 012891	TGAATGACCCTGCCAAG	CCACAATCTCTGCCAAGC
PPARγ 1	AF156665	GAGGAGGTCAAGAAGGGG	CAAAGGAATGGGAGTGGTC
PPARγ 2	AF156666	TTATGCTGTTATGGGTGAAA	CAAAGGAATGGGAGTGGTC
Ppargc1a (PGC-1α)	NM 031347	TGCCCCTGCCAGTCACAGGA	GCTCAGCCGAGGACACGAGG
mt-Nd1	X07479	AGGGTACATACAACTACGAAAAGGCC	GAGTATTTGGAGTTTGAGGCTCATCC
Tfam	NM 031326	GTTCCGGGGAATGTGGGGCG	GACAGGCGAGGGTATGCGGC
Cox4	BC 084719	GCGGAATGTTGGCTACCAGGGC	ATGTGCCCGAAGGCACACCG
UCP-1	NM 012682	TACAGAGTTATAGCCACCACA	TGGAACGTCATCATGTTTGTG
Prdm16	NM 027504	AGGAACACGCTACACGGATG	ACTTTGGATGGGAGATGCTG
RXRα	NM 012805	CCATCTTTGACAGGGTGC	GAACAGGTGCTCCAGGC
CEBPα (CEBPA)	NM 004364	GCCAAGAAGTCGGTGGATAA	CGGTCATTGTCACTGGTCAA
CEBPβ (CEBPB)	NM 005194	CAAGCTGAGCGACGAGTACA	CAGCTGCTCCACCTTCTTCT
Rpl13	NM 031101	TGGCAGGGGCTTCAG	TGGGCATCACAGGTCC

The abbreviations of the genes, their accession number and 5′ to 3′ nucleotide sequences of the forward and reverse primers are presented.

Download English Version:

https://daneshyari.com/en/article/10804046

Download Persian Version:

https://daneshyari.com/article/10804046

Daneshyari.com