Biochemical and Biophysical Research Communications 430 (2013) 573–578

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Induction of mitochondrial biogenesis and respiration is associated with mTOR regulation in hepatocytes of rats treated with the pan-PPAR activator tetradecylthioacetic acid (TTA)

Hanne R. Hagland [a], Linn I.H. Nilsson [a], Lena Burri [b], Julie Nikolaisen [a], Rolf K. Berge [b,c], Karl J. Tronstad [a,*]

[a] Department of Biomedicine, University of Bergen, Norway
[b] Institute of Medicine, University of Bergen, Haukeland University Hospital, Norway
[c] Department of Heart Disease, Haukeland University Hospital, Norway

ARTICLE INFO

Article history:
Received 23 November 2012
Available online 7 December 2012

Keywords:
Rat hepatocytes
Mitochondrial biogenesis
Mitochondrial respiration
PPAR
mTOR
Hypertrophy

ABSTRACT

The hypolipidemic effect of peroxisome proliferator-activated receptor (PPAR) activators has been explained by increasing mitochondrial fatty acid oxidation, as observed in livers of rats treated with the pan-PPAR activator tetradecylthioacetic acid (TTA). PPAR-activation does, however, not fully explain the metabolic adaptations observed in hepatocytes after treatment with TTA. We therefore characterized the mitochondrial effects, and linked this to signalling by the metabolic sensor, the mammalian target of rapamycin (mTOR). In hepatocytes isolated from TTA-treated rats, the changes in cellular content and morphology were consistent with hypertrophy. This was associated with induction of multiple mitochondrial biomarkers, including mitochondrial DNA, citrate synthase and mRNAs of mitochondrial proteins. Transcription analysis further confirmed activation of PPARα-associated genes, in addition to genes related to mitochondrial biogenesis and function. Analysis of mitochondrial respiration revealed that the capacity of both electron transport and oxidative phosphorylation were increased. These effects coincided with activation of the stress related factor, ERK1/2, and mTOR. The protein level and phosphorylation of the downstream mTOR actors eIF4G and 4E-BP1 were induced. In summary, TTA increases mitochondrial respiration by inducing hypertrophy and mitochondrial biogenesis in rat hepatocytes, via adaptive regulation of PPARs as well as mTOR.

1. Introduction

Metabolic adaptation is linked to life span regulation [1] and important diseases, such as cancer [2], neurodegeneration [3] and obesity-related disorders [4]. Accordingly, mechanisms of metabolic adaptation represent potential targets for prevention and treatment of disease.

The peroxisome proliferator-activated receptors (PPARs) [5,6] and the mammalian target of rapamycin (mTOR) [7,8] are major regulators of metabolism. They converge on the cell survival-associated protein kinase AKT [9], which is involved in insulin signalling and metabolic balancing. The PPARs crosstalk with central metabolic sensors, such as AMP-dependent protein kinase (AMPK) activated upon low energy stress, and PPARγ co-activator 1α (PGC1α) involved in mitochondrial biogenesis, to control adaptive responses [5,6]. The mTOR protein exists in two complexes, named mTOR complex 1/2 (mTORC1/2), where mTORC1 is the primary nutrient responder [8]. Upon activation, mTORC1 promotes protein translation and cell growth via regulation of the downstream eukaryotic translation initiation factor 4E (eIF4E) and the eIF4E-binding protein 1 (4E-BP1) [10]. Upstream regulators of mTORC1 include AKT [11], AMPK [12] and MAP-kinase-extracellular regulated kinase (ERK) [13].

Metabolic signalling is closely associated with regulation of mitochondrial function and biogenesis. Mitochondria are essential organelles in cellular energy metabolism, and house several catabolic pathways, including fatty acid oxidation, TCA-cycle and mitochondrial respiration. In mitochondrial respiration, electron transport by the respiratory protein complexes is coupled to oxidative phosphorylation yielding ATP from ADP. Regulation of mitochondrial metabolism and respiration is a crucial mechanism of cellular adaptation, e.g. in response to nutritional alterations or energy depletion. For instance, induction of mitochondrial biogenesis can be observed in muscle as a reaction to physical exercise [14] or mitochondrial mutations [15], and is linked to the actions of AMPK, PPARs and PGC1α. [16]. Multiple important functions of mitochondrial regulators have previously been characterized in rat liver after nutritional and pharmacological interventions [17].

* Corresponding author. Address: Department of Biomedicine, University of Bergen, Jonas Lies vei 91, N-5009 Bergen, Norway. Fax: +47 55586360.
E-mail address: karl.tronstad@biomed.uib.no (K.J. Tronstad).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.11.111

Tetradecylthioacetic acid (TTA) is a modified fatty acid that has hypolipidemic effects in rats [18,19]. This agent activates all the PPAR members, in the ranking order PPARα > PPARδ > PPARγ [19]. Treatment with TTA dramatically increases the mitochondrial oxidative capacity in rat hepatocytes, which seems only partly to be explained by PPAR-activation [17,20]. Here, we used TTA-treatment to investigate the impact and mechanisms of mitochondrial adaptation in relation to key signalling pathways involved in metabolic regulation in rat hepatocytes.

2. Materials and methods

2.1. Materials

TTA was synthesized as described previously [21]. The mtHSP70 and β-actin antibodies were from Abcam (Cambridge, UK), whereas the antibodies for Akt, p-Akt (S473), mTOR, 4E-BP1, p-4E-BP1, eIF4G, p-eIF4G, ERK and p-ERK were all from Cell Signaling Technology Inc (Danvers, MA, USA).

2.2. Animals and treatments

The animal study was conducted according to the guidelines for the care and use of experimental animals, and the protocol was approved by the Norwegian State Board of Biological Experiments with Living Animals. Eight to ten week old (150–200 g) male Wistar rats were obtained from Taconic Europe (Ry, Denmark) and acclimatized for one week prior to experiment. Control groups were given a normal chow diet, while the TTA-treated group was given chow pellets sprayed with 0.3% (*w*/*w*) TTA; both groups were fed ad libitum. The daily intake of TTA was estimated to 60 mg based on a consumption of 20 g of feed each day. For each experiment, two animals in each group were sacrificed, one for collection of biopsies and one for liver perfusion. Hepatocytes were isolated using in vitro collagenase perfusion as previously described [22,23].

2.3. Transmission electron microscopy (TEM)

Cells and tissue biopsies were fixed in cold 0.1 M Na-Cacodylate buffer, pH 7.4, containing 2.5% glutaraldehyde for 15 min. Samples were rinsed with 0.1 M Na-Cacodylate buffer for 10 min and post-fixed in 1% osmium tetraoxide (OsO4) for 60 min. Following ethanol dehydration, the samples were embedded in epoxy resin. Ultra-thin sections were prepared and double-stained with uranyl acetate and lead citrate. Microscopy was performed on a Jeol JEM-1230 instrument (Jeol Ltd., Tokyo, Japan) at the Molecular Imaging Centre (University of Bergen). Pictures were acquired using the GATAN multiscan camera (Gatan Inc, Pleasanton, CA, USA).

2.4. Mitochondrial respiration

Oxygen consumption rates were analyzed using Oxygraph O2K and DatLab software (Oroboros Instruments, Austria). The oxygen consumption rates were normalized to the number of cells in each chamber. Measurements in intact hepatocytes were performed in RPMI 1640 cell culture medium (without FBS) after sequential additions of oligomycin (1.8 μg/ml), carbonylcyanide- 4-(trifluoromethoxy)-phenylhydrazone (FCCP) (titrated to maximal activity, titration range 0.1–0.27 mM), rotenone (0.5 μM) and antimycin A (2.5 μM). The respiration medium for permeabilized cells was 0.5 mM EGTA, 3 mM $MgCl_2 \cdot 6H_2O$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 1 g/l bovine serum albumin. The additional supplements were malate (1.8 mM), glutamate (9.1 mM), succinate (9.1 mM), digitonin (3.7 μM) and ADP (2.3 mM).

2.5. Citrate synthase measurements

Citrate synthase activity was measured in 2×10^5 cells/ml in incubation medium as previously described [24].

2.6. Flow cytometry

Viable cells were analyzed only for their FSC-H and SSC-H properties in a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). For protein analysis, hepatocytes were fixed (3.7% para-formaldehyde in PBS), permeabilized (methanol) and immunostained according to the protocol provided by Cell Signaling Technology Inc. Following labelling with Alexa Fluor 488-conjugated secondary antibody (Invitrogen, Carlsbad, CA, USA), the analysis was performed using an Accuri C6 flow cytometer (Accuri Cytometers Inc., Ann Arbor, MI, USA).

2.7. RNA isolation and quantitative real-time PCR

RNA was extracted from tissue samples using RNAeasy Mini kit (Qiagen, Hilden, Germany). Reverse transcription was performed using High-Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative PCR was performed in the LightCycler® 480 Real-Time PCR system using the LightCycler® 480 Probes master kit (Roche, Basel, Switzerland). The following genes were analyzed using specific FAM probes and primers from Applied Biosystems: Cytochrome *c* oxidase (COX), *Cox4i1*, Rn00665001_g1; Cytochrome *c* somatic (Cyt *c*), Rn00820639_g1; Transcriptional Factor A mitochondrial (TFAM), Rn_00580051_m1; Carnitine palmitoyl transporter 1 (CPT-1), Rn0050702_m1; CPT-2, Rn00563995_m1; Carnitine/acylcarnitine translocase (CACT) (Slc25a20), Rn00588652; Acyl-CoA oxidase-1 (ACOX-1) , Rn00569216; 3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial) (HMGCS-2), Rn00597339: Nuclear respiratory factor 1 (NRF-1), Rn01455958_m1; Uncoupling protein 3 (UCP3), Rn00565874_m1. For reference we used the 18s rRNA control kit FAM-TAMRA (RT-CKFT-18s) from Eurogentec (Liége, Belgium).

2.8. Mitochondrial DNA quantification

DNA was isolated from primary hepatocytes using DNA Blood and Tissue Kit (Qiagen). Specific primer for mitochondrial DNA (mtDNA), mitochondrially encoded NADH dehydrogenase 1 (MT-ND1) (Rn03296764_s1), was purchased from Applied Biosystems and nuclear DNA (nDNA) primer 18S (RT-CKFT-18s) from Eurogentec. Real-time PCR was carried out in the Lightcycler® 480 detection system (Roche) and the mtDNA/nDNA ratio was calculated [25].

2.9. Western blots

Protein concentrations were measured using the Thermo Scientific Pierce BCA Protein Assay Kit (Thermo Scientific Pierce Protein Biology, Rockford, IL, USA). SDS–PAGE and Western blotting were performed using the Invitrogen system, with PVDF membranes. Detection was done using secondary antibody conjugated to HRP from BioRad Laboratories Inc (Hercules, CA, USA) and ECL Western Blotting Substrate (Thermo Scientific Pierce Protein Biology) in a LAS-3000 imaging system (Fujifilm, Tokyo, Japan). Pixel analysis was performed using Multi Gauge V2.2. software (Fujifilm).