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Coactivator PGC-1α regulates the fasting inducible xenobiotic-metabolizing enzyme CYP2A5 in mouse primary hepatocytes

Satu Arpiainen [a], Sanna-Mari Järvenpää [a], Aki Manninen [b], Pirkko Viitala [a], Matti A. Lang [c], Olavi Pelkonen [a], Jukka Hakkola [a,*]

[a] Department of Pharmacology and Toxicology, University of Oulu, Oulu, Finland
[b] Biocenter Oulu, Department of Medical Biochemistry and Molecular Biology, University of Oulu, Oulu, Finland
[c] Division of Pharmaceutical Biochemistry, Uppsala Biomedical Center, Uppsala University, Uppsala, Sweden

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ABSTRACT

The nutritional state of organisms and energy balance related diseases such as diabetes regulate the metabolism of xenobiotics such as drugs, toxins and carcinogens. However, the mechanisms behind this regulation are mostly unknown. The xenobiotic-metabolizing cytochrome P450 (CYP) 2A5 enzyme has been shown to be induced by fasting and by glucagon and cyclic AMP (cAMP), which mediate numerous fasting responses. Peroxisome proliferator-activated receptor γ coactivator (PGC)-1α triggers many of the important hepatic fasting effects in response to elevated cAMP levels. In the present study, we were able to show that cAMP causes a coordinated induction of PGC-1α and CYP2A5 mRNAs in murine primary hepatocytes. Furthermore, the elevation of the PGC-1α expression level by adenovirus mediated gene transfer increased CYP2A5 transcription. Co-transfection of *Cyp2a5* 5′ promoter constructs with the PGC-1α expression vector demonstrated that PGC-1α is able to activate *Cyp2a5* transcription through the hepatocyte nuclear factor (HNF)-4α response element in the proximal promoter of the *Cyp2a5* gene. Chromatin immunoprecipitation assays showed that PGC-1α binds, together with HNF-4α, to the same region at the *Cyp2a5* proximal promoter. In conclusion, PGC-1α mediates the expression of CYP2A5 induced by cAMP in mouse hepatocytes through coactivation of transcription factor HNF-4α. This strongly suggests that PGC-1α is the major factor mediating the fasting response of CYP2A5.

Introduction

The cytochrome P450 (CYP) enzymes, particularly in the subfamilies CYP1–3, play key roles in the phase I metabolism of xenobiotics, such as drugs, environmental toxins and dietary compounds (Nebert and Dalton, 2006). Similar to many other liver processes, the metabolism of xenobiotics is affected by nutritional homeostasis. Fasting and energy balance related diseases, including diabetes and obesity, alter the expression of many CYP enzymes, and this, consequently, changes the metabolism of drugs and other xenobiotics (Kim and Novak, 2007).

Although it is well established that alterations in the nutrition balance change the expression pattern of drug metabolizing enzymes, the mechanisms behind this effect are still largely unknown. Nutrition related hormonal changes, predominantly in circulating insulin and glucagon levels, are known to affect the regulation of several CYP enzymes (Kim and Novak, 2007). Insulin has been shown to down-regulate CYP2E1 expression both by transcriptional and post-transcriptional mechanisms and through several signal transduction pathways. Studies with chemical kinase inhibitors suggest that PI3, Akt, mTOR and p70S6 kinases may be involved in CYP2E1 suppression by insulin (Kim and Novak, 2007, Woodcroft et al., 2002). Glucagon regulates various drug metabolizing CYP enzymes differently. It down-regulates CYP2C11, CYP2B1 and CYP2B2 while it up-regulates CYP2A5 and CYP2E1 (Iber et al., 2001, Sidhu and Omiecinski 1995, Viitala et al., 2001, Woodcroft and Novak 1999). The effects of glucagon are mediated by a specific membrane bound G protein-coupled receptor that activates adenylate cyclase and increases the intracellular cAMP level (Mayo et al., 2003). All the known effects of glucagon on CYP enzyme expression appear to be mediated by cAMP and predominantly through transcriptional regulation. However, the molecular constituents of the signal transduction pathways and the transcription factors involved remain unknown.

Abbreviations: CYP, cytochrome P450; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; cAMP, cyclic adenosine monophosphate; HNF-4α, hepatocyte nuclear factor 4α; PPAR, peroxisome proliferator-activated receptor; COH, coumarin 7-hydroxylase; Q-PCR, quantitative polymerase chain reaction; CREB, cAMP-response element binding protein; CAR, constitutive androstane receptor; PXR, pregnane X receptor; db-cAMP, dibutyryl cAMP; PEPCK, phosphoenolpyruvate carboxykinase.

* Corresponding author. Department of Pharmacology and Toxicology, University of Oulu, POB 5000 (Aapistie 5B), 90014 University of Oulu, Oulu, Finland. Fax: +358 8 537 5247.
E-mail address: jukka.hakkola@oulu.fi (J. Hakkola).

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Peroxisome proliferator-activated receptor γ coactivator (PGC)-1α is involved in numerous biological responses related to energy metabolism, including thermal regulation and glucose homeostasis. The PGC-1α level is elevated by a number of external stimuli and different signaling pathways are involved in PGC-1α regulation [see review by Handschin and Spiegelman, 2006]. cAMP signaling is a key activator of PGC-1α transcription in many tissues. PGC-1α activates nearly all known hepatic fasting responses, including gluconeogenesis, fatty acid β-oxidation, ketogenesis and bile acid homeostasis (Finck and Kelly 2006). PGC-1α induces the expression of the key gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase through interactions with the transcription factors hepatocyte nuclear factor (HNF)-4 and forkhead box O1 (FOXO-1) (Boustead et al., 2003, Puigserver et al., 2003, Rhee et al., 2003, Yoon et al., 2001). Both of these transcription factors are constitutively active and do not require an exogenous ligand for activation. HNF-4α maintains the transcription of several genes encoding for important hepatic xenobiotic-metabolizing enzymes, including CYP enzymes (Jover et al., 2001, Tirona and Kim 2005, Ulvila et al., 2004). Furthermore, a lack of PGC-1α down-regulates the expression of several HNF-4α transactivated CYP genes (Martinez-Jimenez et al., 2006).

Bauer et al. (2004) have studied the starvation response in mouse liver by microarray analysis. The expression of several CYP genes was shown to be changed after 24 or 48 h of fasting. CYP2A5 was one of the CYPs most strongly induced by starvation. We have previously shown that glucagon is an efficient inducer of CYP2A5 expression and that this induction is mediated by cAMP (Salonpaa et al., 1994; Viitala et al., 2001). We hypothesized that PGC-1α is involved in the nutritional regulation of xenobiotic metabolism and mediates the induction of CYP2A5 by cAMP. In the current study, we present evidence indicating that elevated cellular levels of PGC-1α activate *Cyp2a5* transcription through interaction with transcription factor HNF-4α. These results establish transcriptional coactivation by PGC-1α as a novel mechanism that mediates nutrition balance induced changes in the metabolism of xenobiotics.

Methods

Fasting experiments and coumarin 7-hydroxylase assay. Male DBA2/J mice, 10 weeks old, were caged in groups of five. The mice were kept at the Uppsala University animal house facilities in a 12 h light–dark cycle. The control groups had access to standard animal chow and water whereas the experimental groups had access only to water. The mice were sacrificed after 24, 48 or 72 h of fasting, and the livers were removed. The catalytic activity of CYP2A5 was determined by measuring coumarin 7-hydroxylase (COH) activity from the livers as described previously (Aitio, 1978).

Preparation of primary cultures of hepatocytes. Hepatocytes were isolated from male DBA/2 (OlaHsd) mice (Center for Experimental Animals, University of Oulu, Finland) aged 8 to 10 weeks. Livers were perfused with collagenase solution (Worthington Biochemical Co., Lakewood, NJ, USA) as described previously (Salonpaa et al., 1994). After filtration and centrifugation, the isolated hepatocytes were dispersed in William's medium E (Sigma Chemical Co., St. Louis, MO, USA) containing dexamethasone (Sigma) 20 ng/ml, ITS (insulin 5 mg/l, transferrin 5 mg/l, sodium selenate 5 μg/l) (Sigma), gentamicin (Invitrogen, Paisley, Scotland) 50 μg/ml, and 10% fetal bovine serum (Invitrogen) at a density of 2×10^7 cells/175 cm^2 flask, 1×10^7 cells/75 cm^2 flask, 1×10^6 cells/one well in six-well plates, and 3×10^5 cells/one well in twelve-well plates (FALCON Polystyrene Cell Culture Dish, BD Biosciences, San Jose, CA, USA). The cultures were maintained at 37 °C in a humidified incubator for 1 to 2 h, after which non-attached cells were discarded by aspiration, and the medium was replaced by serum-free William's E medium. The cultures were maintained for an additional 24 h before dibutyryl cAMP (db-cAMP) treatment, transient transfection or adenovirus infection.

All animal experiments were approved by the local committees for laboratory animal welfare.

Preparation of the Ad-PGC-1α virus and infection of hepatocytes. A recombinant adenovirus expressing mouse PGC-1α (Ad-PGC-1α) was prepared as follows: PGC-1α cDNA was amplified from the pcDNA3-PGC-1α expression vector (a kind gift from Dr. J. K. Kemper) with the following primers: forward 5′ CCG *CTC GAG* CCA TGG CGT GGG ACA TGT GC 3′, where italicized nucleotides code for the XhoI restriction site and bases in bold indicate the Kozak sequence, and reverse 5′ GGC *CTC GAG* TTACCT GCG AAG CTT C (XhoI and STOP codon) [based on Bhalla et al. (2004)]. The cDNA was subcloned into the XhoI sites of the adenoviral shuttle vector, pShuttle-CMV (Qbiogene Inc., Illkirch, Cedex-France). An adenoviral vector was prepared according to the manufacturer's instructions with some modifications. Briefly, the shuttle vector was linearized with PmeI and transformed into BJ5183-AD-1 competent cells using Gene Pulser Transfection Apparatus (Bio-Rad, Hercules, CA, USA). The transformants were selected for kanamycin resistance, and the homologous recombination into the pAdEasy-1 vector was confirmed by PacI digestion. The resulting pAdEasy-1-PGC-1α vector was then linearized with PacI and transfected into QBI-293A cells (Qbiogene Inc.) using Lipofectamine 2000 (Invitrogen). Adenoviruses carrying the PGC-1α gene (Ad-PGC-1α) were expanded into high-concentration stock by extracting the produced virus using freeze–thaw cycles and infecting fresh QBI-293A cells with the extracted virus. After four amplification cycles the viruses were concentrated and purified using 15%:30%:40% iodixanol (OPTIPREP, Axis-Shield PoC AS, Oslo, Norway) density gradient centrifugation (100 000 *g*, at 4 °C overnight). The purified viruses were diluted in glycerol buffer (15 mM Tris pH 8.0, 150 mM NaCl, 0.15% BSA and 50% glycerol) and in 90% glycerol (1:1:1, virus gradient: buffer: glycerol) and stored at −70 °C. The multiplicity of the infection for Ad-PGC-1α virus was titrated using the AdEasy™ Viral Titer Kit according to the manufacturer's instructions.

Mouse primary hepatocytes were infected with Ad-PGC-1α or a control adenovirus expressing a membrane-targeted GFP (Ad-GFP-GL-GPI) in Opti-MEM I medium (Invitrogen). The infected cultures were maintained at +37 °C in a humidified incubator for 1 h, after which the medium was replaced by serum-free William's E medium. The cultures were maintained for additional 12–72 h before RNA isolation and 48 h before protein extraction. Transduction efficiency of the hepatocytes with the used amounts of adenovirus is near 100% based on the observation of the cells infected with the GFP virus.

RNA preparation and quantitative PCR. Murine hepatocytes (six-well plates) were treated with 25 μM dibutyryl cAMP (Sigma) or vehicle (dimethyl sulfoxide) only, for 0.5 – 12 h, after which the total RNA was isolated using the Tri-Reagent (Sigma) according to the manufacturer's protocol for monolayer cells. 1 μg of each RNA sample was reverse transcribed to produce cDNA using a First-Strand cDNA Synthesis Kit (Amersham Biosciences, Little Chalfont, UK) as suggested in the manufacturer's instructions. The quantitative PCR reactions for PGC-1α mRNA were done with an ABI 7700 Sequence Detection System using TaqMan chemistry. The sequences for the primers and probes used were as follows: mPGC-1α-FW 5′-CAGTCTCCCCGTGGATGAA-3′, -RV 5′-GTGGTCACGGCTCCATCTG-3′, and -Tamra 5′-ACGGATTGCCCTCATTTGATGCACTG-3′, 18S-FW 5′-TGGTTGCAAAGCTGAAACTTAAAG-3′, -RV 5′-AGTCAAATTAAGCCGC-AGGC-3′ and -Tamra 5′-CCTGGTGGTGCCCTTCCGTCA-3′.

For the measurement of PGC-1α mRNA levels from Ad-PGC-1α infected cells (6-well plates) and for all CYP2A5 and PEPCK mRNA measurements, 1 μg of each RNA sample was reverse transcribed to produce cDNA using p(dN)6 random primers (Roche) and M-MLV reverse transcriptase (Promega). The AmpliQ Universal Real Time PCR