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The gene encoding the human ileal bile acid-binding protein (I-BABP) is regulated by peroxisome proliferator-activated receptors

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Abstract

Peroxisome proliferator-activator receptors (PPAR) are involved in cholesterol homeostasis through the regulation of bile acids synthesis, composition, and reclamation. As ileal bile acid-binding protein (I-BABP) is thought to play a crucial role in the enterohepatic circulation of bile acids, we investigated whether I-BABP gene expression could also be affected by PPAR. Indeed, treatment with the PPARα-PPARβ/δ agonist bezafibrate led to the up-regulation of I-BABP mRNA levels in the human intestine-derived Caco-2 cells. Cotransfections of the reporter-linked human I-BABP promoter (hI-BABP$^{-2769/+44}$) together with PPAR and RXR expression vectors demonstrated that the fibrate-mediated induction of the I-BABP gene is dependent on PPARα or PPARβ/δ. Using progressive 5′ deletions of the hI-BABP promoter and sequence analysis, we identified a putative PPAR-binding site located at the position −198 and −186 upstream of the transcription initiation site. Electrophoretic mobility shift assays showed that the PPAR/RXR heterodimer can specifically bind to this PPRE-like motif. The deletion of the PPRE within the hI-BABP promoter abolished the PPAR-mediated transactivation in transient transfection assays. The regulation of the I-BABP promoter by PPAR appears species-specific, as the mouse I-BABP promoter, which lacks a conserved PPRE, was not responsive to exogenous PPAR expression in the presence of bezafibrate. Our findings show that the I-BABP gene may be a novel target for PPAR in humans and further emphasize the role for PPAR in the control of bile acid homeostasis.
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Keywords: I-BABP; Bile acid; PPAR; Fibrate

Fibrates are widely used drugs to treat hypertriglyceridemia in humans. Fibrates function via binding to, and activating, the peroxisome proliferator-activated receptor alpha (PPARα, NR1C1), a nuclear receptor endogenously activated by fatty acids and fatty acid-derived compounds [1,2]. PPARα expression is especially high in the liver and, to a lesser extent, in the small intestine and both cardiac and skeletal muscles. In these tissues, PPARα promotes β-oxidative degradation of fatty acids. An increased incidence of cholesterol gallstone formation is also found in patients chronically treated with fibrates. This adverse side effect suggests that PPARα, in addition of its triglyceride-lowering action, can also alter bile composition, leading to increased cholesterol saturation. Given that the lithogenic index (i.e., cholesterol/bile acids and cholic acid/chenodeoxycholic acid ratios) depends on the composition and levels of bile acids suggests that PPARα might also have a role in controlling bile acid metabolism.

Primary bile acids are synthesized from cholesterol in the liver via the classic pathway, initiated by the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1), and via the

Abbreviations: ASBT, apical sodium-dependent bile acid transporter; I-BABP, ileal bile acid-binding protein; CYP7A1, cholesterol 7α-hydroxylase; CYP27A1, sterol 27-hydroxylase; CYP8B1, sterol 12α-hydroxylase; PPAR, peroxisome proliferator-activated receptors; RXR, retinoid-X-receptor; FXR, farnesoid-X-receptor; LXR, liver-X-receptor; SREBP, sterol regulatory element-binding proteins; PPRE, PPAR responsive element; BARE, bile acid responsive element; DR1, direct repeat 1; CAT, chloramphenicol acetyl transferase; CDCA, chenodeoxycholic acid; EMSA, electrophoretic mobility shift assay

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alternative pathway, which depends on the sterol 27-hydroxylase (CYP27A1) [3–5]. Qualitative control of bile acid synthesis is achieved by the sterol 12α-hydroxylase (CYP8B1), which regulates the ratio of cholic acid to chenodeoxycholic acid. This ratio determines the biliary capacity to solubilize hydrophobic molecules, such as cholesterol [6]. In the small intestine, bile acids are actively reclaimed in the ileum by a 38-kDa integral brush border protein, apical sodium-dependent bile acid transporter (ASBT) [7,8]. Both inherited ASBT mutations in humans and targeted deletion of ASBT in mice lead to dramatic bile acid malabsorption, demonstrating a crucial role for ASBT in ileal bile acid uptake [9,10]. Once inside ileocytes, bile acids are reversibly bound to the ileal bile acid-binding protein (I-BABP) [11], an abundant 14-kDa member of the fatty acid-binding protein family. Although its physiological role is not yet fully established, its binding specificity, abundance, localization, physical interactions with ASBT, and regulation imply that I-BABP may exert a pivotal role in intestinal bile acid uptake and trafficking [12]. In healthy humans, more than 95% of bile acids is reabsorbed along the small intestine and returned to the liver to be secreted again into bile. This enterohepatic circulation is essential for the maintenance of bile acid and cholesterol pool sizes. To ensure the efficiency of enterohepatic circulation, hepatic synthesis and intestinal reclamation of bile acids are subjected to coordinate regulation [13]. The homeostatic role of bile acid-activated farnesoid-X-receptor (FXR, NR1H4) in this pathway has been recently and abundantly illustrated [14,15]. Experimental evidence also supports a role for PPARα in the control of bile acid homeostasis. PPARα has been shown to down-regulate the hepatic expression of CYP7A1 and CYP27A1 [16] and to up-regulate the expression of CYP8B1 and ASBT in the liver and the ileum, respectively [17,18]. These data correlate with the low bile acid excretion levels and the high cholic acid/chenodeoxycholic acid ratio [17] found in mice and humans treated with fibrates [16,19]. This also provides a possible molecular explanation for lithogenicity resulting from chronic treatment of patients with fibrates, such as increased saturation of cholesterol in bile promoting gallstone formation [19].

Here, we show that PPAR can positively regulate the expression of the I-BABP gene, providing further support for an important role for this nuclear receptor in controlling bile acid homeostasis.

1. Materials and methods

1.1. Cell culture

Caco-2 cells (passages 55–60) were cultured in humidified atmosphere (37 °C, 5% CO_2) in Dulbecco's modified eagle medium (Invitrogen), supplemented with 4 mM glutamine, 1% non-essential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin supplemented, and 20% fetal calf serum. The medium was changed every 2 days. For RNA preparation, after reaching confluence, the cells were incubated for 24 h in the medium containing 10% delipidated serum (v/v) and 50 μM chenodeoxycholic acid (Sigma) alone (Control), or 50 μM chenodeoxycholic acid together with various concentrations of (0 to 150 μM) bezafibrate (Sigma).

1.2. Northern blotting

Total RNAs were extracted with Trizol reagent (Invitrogen). 30 μg of RNAs was electrophoresed on a 1% agarose gel and then transferred to GeneScreen membranes (NEN Life Science Products) using previously published procedures [20]. cDNAs derived from the human I-BABP and rat L-FABP genes were used as probes, which were radiolabelled with [α-^{32}P]dCTP (3000 Ci/mmol; ICN) using a Prime-It RmT Random Primer Labeling Kit (Stratagene). A 24-residue oligonucleotide specific for the rat 18S rRNA was used as probe to ensure that equivalent amounts of RNAs were loaded and transferred. This oligonucleotide was 5′ end-labeled using T4 polynucleotide kinase and [γ-^{32}P]-ATP (3000 Ci/mmol, ICN).

1.3. Plasmid construction

Wild type −2769/+44 (hI-BABP 2769^{wt}), −1204/+44 (hI-BABP 1204^{wt}), −800/+44 (hI-BABP 800^{wt}), −342/+44 (hI-BABP 342^{wt}), and −183/+44 (hI-BABP 183^{wt}) bp fragments of the human I-BABP promoter were cloned upstream the chloramphenicol acetyltransferase gene in the pCAT3-basic vector (Promega). The deletion of the PPRE (hI-BABP 2769^{Del}) was generated by site-directed mutagenesis (Quickchange™ site-directed mutagenesis kit, Stratagene) using the following oligonucleotides: 5′-CTCCTCAAACCCGTTGCCATGGGGTGAACAG-CACTTCCCC-3′ and the reverse one. All constructs were confirmed by restriction digestions. The −913/+52 (mI-BABP 913^{wt}) fragment of the mouse I-BABP promoter [21] was subcloned into the pCAT3-basic vector.

1.4. Transfection assays

Caco-2 cells were seeded in 6-well plates in Dulbecco's modified eagle medium supplemented with 10% fetal calf serum at 40–50% confluence. Cotransfection mixes contained 4 μg of I-BABP-CAT reporter plasmid, 500 ng of β-galactosidase expression vector, 250 ng of the mouse PPARα, PPARβ/δ expression vectors, and 250 ng of human RXRα expression vector. Cells were transfected overnight by the calcium phosphate precipitation method. After this, the medium was changed to DMEM supplemented with 10% delipidated serum and 50 μM bezafibrate in dimethyl sulfoxide, after which the cells were incubated for a further

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