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Pretranslational upregulation of microsomal CYP4A in rat liver by intake of a high-sucrose, lipid-devoid diet containing orotic acid

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Abstract

In rodents, high-fat diets promote hepatic lipid accumulation in rodents, activation of peroxisome proliferator activated receptor-α (PPARα) and upregulation of cytochrome P450 (CYP) 4A gene expression. Lipid-devoid diets containing sucrose and orotic acid (S/OA-diet) also cause lipid infiltration by stimulating intrahepatic lipid synthesis and preventing lipoprotein transport through the Golgi apparatus. This study evaluated the impact of the lipid-deficient S/OA-diet on CYP4A expression and PPARα activation in rodent liver. CYP4A protein and laurate ω-hydroxylation activity were increased in rat liver after S/OA-feeding for 21 days. CYP4A1 and CYP4A2 mRNAs were induced to 2.1- and 2.6-fold of control, but mRNAs corresponding to CYP4A3 and the peroxisomal acyl-CoA oxidase (AOX) were unchanged. Coadministration of clofibric acid and the S/OA-diet prevented hepatic lipid accumulation and upregulated CYP4A protein to levels comparable with clofibric acid alone (five-fold of control). Clofibric acid, alone and in combination with the S/OA-diet, upregulated CYP4A1-3 and AOX mRNAs. Hepatic PPARα protein was decreased by the S/OA-diet but was increased to 5.7-fold of control by clofibric acid; retinoid X-receptor-α (RXRα) protein was decreased to 26–41% of control by all treatments. In further studies, administration of the S/OA-diet to control and PPARα-null mice promoted hepatic lipid deposition; microsomal CYP4A protein was induced in wild-type but not PPARα-null mice. These findings implicate PPARα in the induction of CYP4A in rodent liver by the lipid-devoid S/OA-diet. Decreased availability of hepatic PPARα and RXRα after intake of the diet may contribute to the selective upregulation of hepatic CYP4A1 and CYP4A2 in this model.

Keywords: Cytochrome P450; Gene regulation; Orotic acid; Peroxisome proliferator-activated receptor-α; Retinoid X-receptor-α; Hepatic lipid synthesis

1. Introduction

It is well established that diet has a major influence on hepatic microsomal CYP-mediated drug and xenobiotic biotransformation [1], but detailed mechanistic information is currently unavailable for many nutritional factors. Constitutive xenobiotic and steroid hydroxylating CYPs from the 2C and 3A subfamilies are down-regulated in several species following ingestion of high-fat diets. Thus, CYP suppression was proportional to the extent of hepatic lipid deposition in rats [2] and force-fed geese [3]. Down-regulation of CYPs 2C11 and 3A has also been reported in rat liver following intake of the S/OA-diet [4]. The S/OA-diet does not contain fat [5], but the high carbohydrate content stimulates hepatic acetyl-CoA and L-α-glycerophosphate production, which enhances long chain fatty acyl-CoA formation and lipid synthesis [6]. OA then impairs the transport of lipoproteins through the Golgi apparatus [7], so that the S/OA combination results in extensive deposition of lipid in liver. These findings suggest that increased intrahepatic lipid synthesis and accumulation exerts a similar suppressive effect on CYP drug oxidation to that produced by direct intake of dietary fat.

In addition to down-regulation of drug oxidising CYPs, high fat-containing diets have been found to upregulate

Abbreviations: AOX, acyl-CoA oxidase; CYP, cytochrome P450; S/OA-diet, sucrose/orotic acid-containing diet; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TBAR, thiobarbituric acid-reactive substance

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CYP4A fatty acid ω-hydroxylases in liver of rats and mice [8,9]. CYPs 4A may contribute to ongoing injury following lipid deposition. The ω-hydroxylated metabolites of arachidonic acid are vasoactive and may contribute to local decreases in blood pressure, which has been reported in fatty liver of obesity [10]. In addition, studies in experimental animals have shown that CYP4A increases free radical production during uncoupled oxidation of NADPH and substrate; these free radicals may contribute to long term injury [8]. Lipids are established ligands of PPARα, which forms heterodimers with RXR to activate target gene transcription [11]. In the present study, the relationship between ingestion of the lipid-devoid S/OA-diet, expression of PPARα-inducible CYPs 4A and activation of PPARα was explored in rodents. In comparative experiments clofibric acid, an established chemical PP agent, was administered to animals in control and S/OA-containing diets and evaluated for its impact on CYP4A expression and PPARα activation.

2. Materials and methods

2.1. Chemicals

[α-^{32}P]-dCTP (specific activity 3000 Ci/mmol), Hyperfilm-MP Hyperfilm-ECL, ACS II, Hybond-N^{+} filters, [1-^{14}C]-lauric acid (specific activity ca. 58 mCi/mmol) and enhanced chemiluminescence reagents were from Amersham Pharmacia Biotech. Terminal deoxynucleotidyl transferase was from Promega and clofibric acid and biochemicals were from Sigma Aldrich or Roche. Reagents for electrophoresis were from Bio-Rad. HPLC solvents were from Rhone-Poulenc and analytical reagents were from Ajax.

2.2. Animal treatments

Experimental studies followed National Health and Medical Research Council guidelines as approved by Institutional Animal Ethics Committees; animals received humane care. The diet consisted of sucrose (600 g/kg), casein (200 g/kg), cellulose (110 g/kg), corn oil (40 g/kg), salt mixture (#4179 ICN Biochemicals; 40 g/kg), ICN vitamin fortification mixture (10 g/kg), α-tocopherol (20 mg/kg) and retinyl acetate (8.7 mg/kg). Inbred male Wistar rats (ca 200 g) were obtained from the institutional animal facility and received the diet with or without 1% OA for 21 days [4]. Pair-fed animals were allowed free access to water. In another experiment animals were placed on diets that also contained clofibric acid (10 mg/kg) over a 21 day period. In a further experimental protocol, rats were placed on diets that were fortified with additional vitamin E (200 mg/kg) over a 21 day period.

Female C57BL/6N and PPARα null mice (8–10 weeks of age), originally provided by Dr. F.J. Gonzalez, NCI, Bethesda, MD, USA [12], were bred in the transgenic facility of the Westmead Milennium Institute and were administered the S/OA- or control diets for 21 days.

2.3. Hepatic subcellular fractions, lipid extraction and peroxidation

Animals were killed under enflurane anaesthesia, which was found in preliminary studies not to affect CYP expression or function. Livers were removed, perfused with cold saline and then taken for histology. The remainder was snap frozen in liquid nitrogen or used in the preparation of hepatic microsomal fractions and homogenates [13].

Hepatic lipids were extracted, dried to constant weight in a vacuum dessicator and quantified [14]. Esterified triglycerides (Periodochrom GPO-PAP; Boehringer-Mannheim GmbH) and free fatty acids (Half-micro kit; Roche Diagnostics) were quantified. Lipid peroxides, were assayed in rat hepatic homogenates as TBARs [15]. Kidneys were also harvested but there was no increase in weight or lipid content after ingestion of the experimental diet. Further, there was no evidence of lipid accumulation in other organs, thus indicating the liver-specific effect of the S/OA-diet.

2.4. Microsomal laurate hydroxylation assay

Microsomal laurate hydroxylation (50 μM; 0.15 μCi) was measured as described previously [16]. Incubations (0.3 mL, 5 min, 37 °C) contained 200 μg protein in 0.1 M phosphate buffer, pH 7.4 and were initiated with NADPH (1 mM). Reactions were terminated by 10% sulphuric acid (0.1 mL) and were extracted with ethyl acetate (2 × 2.5 mL). The extract was dried (anhydrous sodium sulphate), filtered and evaporated under nitrogen. Samples were dissolved in acetonitrile (50 μL) and applied to an Ultrasphere C18 HPLC column (5 μm, 250 mm × 4.6 mm; Beckman). The column was eluted with 33% acetonitrile in acetic acid (1%) for 17 min, followed by 100% acetonitrile for 10 min before reequilibration with 33% acetonitrile in acetic acid (1%). Fractions were collected (1 min) and subjected to β-counting (ACS II, Amersham Pharmacia Biotech). 11- and 12-Hydroxylauric acids eluted at 13 and 15.5 min and laurate at 23 min.

2.5. Sodium dodecylsulphate polyacrylamide gel electrophoresis and immunoblotting

Microsomes (5 μg/lane) were incubated at 100 °C for 5 min with 2% sodium dodecyl sulphate and 5% 2-mercaptoethanol and electrophoresed on 7.5% polyacrylamide gels [17] with minor modifications [18]. Proteins were transferred to nitrocellulose [19] and incubated with anti-CYP IgGs for 120 min (3.7 μg protein/mL). The rabbit anti-CYP4A1 IgG was a gift from Prof. G.G. Gibson, University of Surrey, the rabbit anti-CYP2E1 IgG was