FXR regulates organic solute transporters α and β in the adrenal gland, kidney, and intestine

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Abstract Expression of the farnesoid X receptor (FXR; NR1H4) is limited to the liver, intestine, kidney, and adrenal gland. However, the role of FXR in the latter two organs is unknown. In the current study, we performed microarray analysis using RNA from H295R cells infected with constitutively active FXR. Several putative FXR target genes were identified, including the organic solute transporters α and β (OSTα and OSTβ). Electromobility shift assays and promoter-reporter studies identified functional farnesoid X receptor response elements (FXREs) in the promoters of both human genes. These FXREs are conserved in both mouse genes. Treatment of wild-type mice with 3-(2,6-dichlorophenyl)-4-(3′-carboxy-2-chloro-stilben-4-yl)-oxymethyl-5-isopropyl-isoxazole (GW4064), a synthetic FXR agonist, induced OSTα and OSTβ mRNAs in the intestine and kidney. Both mRNAs were also induced when wild-type, but not FXR-deficient ($FXR^{-/-}$), adrenals were cultured in the presence of GW4064. OSTα and OSTβ mRNA levels were also induced in the adrenals and kidneys of wild-type, but not $FXR^{-/-}$, mice after the increase of plasma bile acids in response to the hepatotoxin α-naphthylisothiocyanate. Finally, overexpression of human OSTα and OSTβ facilitated the uptake of conjugated chenodeoxycholate and the activation of FXR target genes. These results demonstrate that OSTα and OSTβ are novel FXR target genes that are expressed in the adrenal gland, kidney, and intestine.—Lee, H., Y. Zhang, F. Y. Lee, S. F. Nelson, F. J. Gonzalez, and P. A. Edwards. **FXR regulates organic solute transporters α and β in the adrenal gland, kidney, and intestine. *J. Lipid Res.* 2006.** 47: **201–214.**

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Farnesoid X receptor (FXR; NR1H4) is a ligand-activated transcription factor that contains a number of domains, including the ligand-independent activation function (activation function 1) at the N terminus, a DNA binding domain, a hinge region, a dimerization interface, a ligand binding domain, and a ligand-dependent activation function (activation function 2) at the C terminus (1, 2). FXR binds to farnesoid X receptor response elements (FXREs) after heterodimerization with its obligate partner 9-*cis* retinoic acid receptor α (RXRα) (3, 4). The optimal DNA binding sequence for the FXR/RXR heterodimer is an inverted repeat, composed of minor variants of two AGGTCA half-sites spaced by one nucleotide (IR-1) (5). FXR/RXR has also been shown to bind direct and everted repeats spaced by one and eight nucleotides (DR-1 and ER-8) and to activate target gene transcription (6, 7).

In 1999, three laboratories made the important observation that bile acids function as physiological ligands that bind to and activate both rodent and human FXR (8–10). Chenodeoxycholic acid (CDCA), a primary bile acid synthesized in the liver as a result of the oxidation and catabolism of cholesterol, is the most potent natural agonist identified to date (9, 10).

Forman et al. (11) and Seol, Choi, and Moore (12) originally identified FXR transcripts in the liver, kidney, intestine, and adrenal gland. Subsequent studies identified four murine and human FXR isoforms (FXRα1, FXRα2, FXRα3, and FXRα4) that are derived from a single gene as a result of alternative promoter use and alternative splicing of the mRNA (13, 14). The FXRα3 and FXRα4 isoforms have an extended N terminus with respect to the FXRα1 and FXRα2 isoforms. In addition, FXRα1 and FXRα3 contain a four amino acid insert (MYTG) in the hinge region immediately adjacent to the putative DNA binding domain that is absent from both FXRα2 and FXRα4 (13, 14).

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Abbreviations: ANIT, α-naphthylisothiocyanate; ASBT, apical sodium-dependent bile acid transporter; BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; FGF-19, fibroblast growth factor 19; FXR, farnesoid X receptor; FXRE, farnesoid X receptor response element; GFP, green fluorescent protein; GW4064, 3-(2,6-dichlorophenyl)-4-(3′-carboxy-2-chloro-stilben-4-yl)-oxymethyl-5-isopropyl-isoxazole; I-BABP, ileal bile acid binding protein; MRP2, multidrug resistance-related protein 2; OSTα and OSTβ, organic solute transporters α and β; PLTP, phospholipid transfer protein; RXRα, 9-*cis* retinoic acid receptor α; SHP, small heterodimer partner.

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The four FXR isoforms are expressed in a tissue-specific manner (13, 14). Many FXR target genes studied to date [small heterodimer partner (SHP), bile salt export pump (BSEP), and phospholipid transfer protein (PLTP)] are activated to similar degrees by all four FXR isoforms (14). However, certain target genes, namely ileal bile acid binding protein (I-BABP), syndecan-1, and α-crystallin, are more responsive to FXRα2 and FXRα4, the isoforms that lack the MYTG motif (6, 14, 15).

Identification of FXR target genes in the liver and intestine, together with studies of FXR-deficient ($FXR^{-/-}$) mice, has led to the proposal that FXR regulates genes involved in bile acid synthesis and lipoprotein metabolism (16–21). For example, activation of FXR results in the induction of SHP and human fibroblast growth factor 19 (FGF-19), which independently repress CYP7A1, the regulatory enzyme involved in the catabolism of cholesterol to bile acids (20, 22, 23). Currently, it is not known whether FGF-15, the proposed murine ortholog of human FGF-19 (51% amino acid identity) (24), also functions in a similar manner. Other hepatic FXR target genes encode the secreted proteins PLTP (5, 17), apolipoprotein E (25), and apolipoprotein C-II (18), all known to be involved in the metabolism of plasma lipids and lipoproteins.

FXR activation also induces the expression of several ABC transporters, including BSEP (19, 26, 27) and multidrug resistance-related protein 2 (MRP2) (7), which function to transport/export bile acids and xenobiotics out of the liver into bile. Together, these data suggest that activation of hepatic FXR results in pleiotropic effects in the metabolism of plasma lipoproteins, bile acids, and xenobiotics.

I-BABP, the first FXR target gene to be identified, is thought to play a role in enterohepatic circulation, in which bile acids that are secreted into the intestinal lumen are resorbed in the small intestine and returned to the liver (28). It has been proposed that the toxic effects of high intracellular bile acid levels in enterocytes are prevented by the sequestration by I-BABP (28). Resorption of bile acids across the ileal enterocyte brush border membrane is dependent upon the apical sodium-dependent bile acid transporter (ASBT) (29, 30). Recently, Dawson et al. (31) demonstrated that organic solute transporters α and β (OSTα and OSTβ), two genes that were originally identified in little skate, *Raja erinacea* (32, 33), may be responsible for transporting bile acids across the enterocyte basolateral membrane into the portal circulation. OSTα and OSTβ were shown to colocalize on the basolateral membrane of the same enterocytes that express ASBT (31). Moreover, because coexpression of OSTα and OSTβ supported apical-to-basolateral transport of taurocholate, as well as other major taurine- and glycine-conjugated bile acids, it was proposed that OSTα and OSTβ play a role in bile acid resorption (31).

In situ hybridization studies indicated that FXR was expressed in the adrenal cortex and the renal tubules of the kidney (11). However, the function of FXR in the adrenal gland and kidney remains an enigma. The adrenal gland, in contrast to the kidney, liver, and intestine (34), is not known to be involved in any aspect of bile acid metabolism. In this report, we infected human steroidogenic H295R cells with adenovirus expressing constitutively active FXRα1 and FXRα2 and used Affymetrix microarrays to identify putative FXR target genes. This approach identified OSTα, OSTβ, FGF-19, I-BABP, and CYP3A4. Detailed studies with cultured cells, together with in vivo studies involving loss of function of FXR and adrenal organ culture, demonstrate that OSTα and OSTβ are expressed in the adrenal gland, kidney, and intestine and are induced in an FXR-dependent manner.

EXPERIMENTAL PROCEDURES

Materials

The full-length coding regions of human OSTα and OSTβ were amplified by PCR using gene-specific primers and cloned into pcDNA3.1 Directional TOPO Expression Vectors to produce expression constructs pcDNA-hOSTα and pcDNA-hOSTβ. Expression constructs for the human FXRα1 and FXRα2 (pcDNA-hFXRα1 and pcDNA-hFXRα2) and human RXRα (pCMX-hRXRα) have been described previously (15). Adenovirus expressing constitutively active murine FXRα1 (Ad-mFXRα1VP16) and FXRα2 (Ad-mFXRα2VP16) were generated (unpublished data). The full-length coding regions of human FXRα1 and FXRα2 isoforms were cloned into the adenoviral pShuttle-IRES-hrGFP-1 vector (Stratagene). Subsequently, adenovirus expressing the human FXRα1 (Ad-hFXRα1), FXRα2 (Ad-hFXRα2), and green fluorescent protein (GFP) alone (Ad-GFP) were generated using the AdEasy™ Adenoviral Vector System according to the manufacturer's instructions (Stratagene). 3-(2,6-Dichlorophenyl)-4-(3′-carboxy-2-chloro-stilben-4-yl)-oxymethyl-5-isopropyl-isoxazole (GW4064), a synthetic ligand for FXR, was a gift from Tim Willson and Patrick Malloney (GlaxoSmithKline). CDCA, tauro-CDCA, and glyco-CDCA were purchased from Sigma.

Cell culture and adenovirus-infected cells

The maintenance of HepG2 cells has been described (35). H295R cells were maintained in DMEM/Ham's F-12 (pH 7.2) supplemented with 2.5% Nu-serum, 1% ITS (BD Biosciences), 30 mM sodium bicarbonate, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 50 U/ml penicillin G, and 50 μg/ml streptomycin sulfate in a 5% CO_2/37°C incubator. For adenovirus infection, the H295R cells were incubated for 48 h with adenovirus carrying cDNA encoding GFP alone (Ad-GFP), hFXRα1 (Ad-hFXRα1), or hFXRα2 (Ad-hFXRα2) at a multiplicity of infection of 5, followed by the indicated treatments.

RNA isolation, real-time quantitative PCR, and microarray analysis

RNA from tissues and cells was isolated using TRIzol reagent (Invitrogen). Real-time PCR was performed essentially as described (14). Briefly, 5 μg of DNase I-treated total RNA from tissues or cultured cells was reverse-transcribed with random hexamers using SuperScript II Reverse Transcriptase (Invitrogen). Each amplification mixture (20 μl) contained 50 ng of cDNA, 375 nM forward primer, 375 nM reverse primer, and 10 μl of SYBR Green Supermix (Bio-Rad). PCR thermocycling parameters were 95°C for 2.5 min and 40 cycles of 95°C for 10 s and 60°C for 45 s. Real-time PCR was carried out using the Bio-Rad MyIQ Single Color Real-Time PCR Detection System. Each sample was assayed in duplicate and normalized to either GAPDH or

cyclophilin expression. Relative changes in mRNA expression were similar whether GAPDH or cyclophilin was used for normalization. Quantitative expression values were extrapolated from separate standard curves. The sequences for primers are listed in **Table 1**.

To generate RNA for microarray analysis, H295R cells in duplicate 100 mm dishes were incubated for 48 h with adenovirus carrying cDNA encoding control VP16 alone (Ad-VP16), mFXRα1VP16 (Ad-mFXRα1VP16), or mFXRα2VP16 (Ad-mFXRα2VP16) at a multiplicity of infection of 5. The infected cells were subsequently incubated with either vehicle (Me_2SO) or GW4064 (1 μM) for 24 h. RNA was isolated using TRIzol reagent (Invitrogen) and prepared for hybridization using the RNeasy MinElute Cleanup Kit (Qiagen). Reverse transcription, hybridization to Affymetrix chips, and analysis were performed at the University of California, Los Angeles/National Heart, Lung, and Blood Institute shared Microarray Facility.

Identification of putative FXREs and electrophoretic mobility shift assay

Putative FXREs in the promoters of hOSTα and hOSTβ were identified using the NUBIScan computer algorithm. This approach is based on a weighted nucleotide distribution matrix compiled from published functional hexamer half sites (www.nubiscan.unibas.ch/).

Electrophoretic mobility shift assays were performed essentially as described (5). Human FXR isoforms or human RXRα was synthesized in vitro using the TNT T7-coupled reticulocyte system (Promega). To compare the transcription/translation efficiency of the expression constructs expressing different human FXR isoforms, equal volumes of ^{35}S-labeled lysates were loaded and separated on an 8% SDS-polyacrylamide gel. The gel was dried and autoradiographed. The bands were quantitated using a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA). Binding reactions were carried out in a buffer containing 10 mM HEPES, pH 7.8, 100 mM KCl, 0.2% Nonidet P-40, 6% glycerol, 0.3 mg/ml BSA, 1 mM dithiothreitol, 2 μg of poly(dI-dC), 1–3 μl each of in vitro-translated nuclear receptors, and ^{32}P end-labeled oligonucleotide probe. Some reactions also contained unlabeled oligonucleotide competitors at 50, 100, or 250 M excess compared with the probe. DNA-protein complexes were resolved on a 5% polyacrylamide gel in 0.5× TBE (45 mM Tris borate, 1 mM EDTA) at 4°C. Gels were dried and autoradiographed. The sense strand sequences for wild-type and mutant hOSTα and hOSTβ were 5′-tgggctgggggtgaatgacctggcgaacg-3′ (IR-1A), 5′-tgggctggggAAgaatgTTctggcgaacg-3′ (mutIR-1A), 5′-gtgggctgaggccagtgaccctctgggggg-3′ (IR-1B), 5′-gtgggctgagAAcagtgTTcctctgggggg-3′ (mutIR-1B), 5′-ccctctgggggtcagggcccttgcaggcc-3′ (IR-1C), 5′-ccctctggggAAcagggTTcttgcaggcc-3′ (mutIR-1C), 5′-agggctttgggtaattaaacccaaggcct-3′ (IR-1D), 5′-agggctttggAAaattaTTcccaaggcct-3′ (mutIR-1D), 5′-cattcaagaggtcagtcaccctggagtga-3′ (IR-1E), 5′-cattcaagagAAcagtcTTcctggagtga-3′ (mutIR-1E), 5′-ctgacctcaggtgatacacctgccttggc-3′ (IR-1F), and 5′-ctgacctcagAAgatacTTctgccttggc-3′ (mutIR-1F). The sense strand sequence for PLTP probe was 5′-gaaactgagggtcagtgacccaagtgaag-3′ (17). All mutations are shown in uppercase letters.

Reporter gene and transient transfection assays

The human OSTα (−1,489 to +12) and OSTβ (−529 to +17) proximal promoters were amplified from human genomic DNA and cloned into *Nhe*I/*Xho*I sites of the pGL3 basic vector

TABLE 1. Primer sequences used for real-time quantitative PCR

Gene	Primers
hFXRα1+α2	5′-GCTGGGATCTGGAGAGGAAGA-3′ (forward primer, F)
	5′-TTGGGTCAGAGATGGACT-3′ (reverse primer, R)
hFXRα3+α4	5′-CAAATTAGTCCTCACTGCAGCTGT-3′ (F)
	5′-TTCGCGGGCTTCATACTC-3′ (R)
mFXRα1+α2	5′-TGGGCTCCGAATCCTCTTAGA-3′ (F)
	5′-TGGTCCTCAAATAAGATCCTTGG-3′ (R)
mFXRα3+α4	5′-GGGCTTAGAAAATCCAATTCAGATTA-3′ (F)
	5′-CGTCCGGCACAAATCCTG-3′ (R)
hOSTα	5′-CTACACCTGGGTGAGCAGAA-3′ (F)
	5′-AGAGGAATAGGGAGGCGAAC-3′ (R)
hOSTβ	5′-GCAGCTGTGGTGGTCATTAT-3′ (F)
	5′-TAGGCTGTTGTGATCCTTGG-3′ (R)
mOSTα	5′-ATGCATCTGGGTGAACAGAA-3′ (F)
	5′-GAGTAGGGAGGTGAGCAAGC-3′ (R)
mOSTβ	5′-GACCACAGTGCAGAGAAAGC-3′ (F)
	5′-CTTGTCATGACCACCAGGAC-3′ (R)
hFGF-19	5′-CAGTTTGCTGGAGATCAAGG-3′ (F)
	5′-AGCACAGTCTTCCTCCGAGT-3′ (R)
mFGF-15	5′-CAGGGAGGAAATGGACTGTT-3′ (F)
	5′-GGTGAAACACGGGGATAAAG-3′ (R)
hI-BABP	5′-CTCCAGCGATGTAATCGAAA-3′ (F)
	5′-CCCCCATTGTCTGTATGTTG-3′ (R)
mI-BABP	5′-GGTCTTCCAGGAGACGTGAT-3′ (F)
	5′-ACATTCTTTGCCAATGGTGA-3′ (R)
hSHP	5′-CTTCTGGAGCCTGGAGCTTA-3′ (F)
	5′-ACTTCACACAGCACCCAGTG-3′ (R)
mSHP	5′-GTCCCAAGGAGTATGCGTACCT-3′ (F)
	5′-GCGATGTGGCAGGAGGC-3′ (R)
mBSEP	5′-ACAGAAGCAAAGGGTAGCCATC-3′ (F)
	5′-GGTAGCCATGTCCAGAAGCAG-3′ (R)
mHSD3β2	5′-GCCTTCATCTTCTCCAGCTC-3′ (F)
	5′-TCTCAGCCATCTTTTTGCTG-3′ (R)
mCYP11A1	5′-GAGACATGGCCAAGATGGTACAGT-3′ (F)
	5′-CCTGTACCAAAGTCTTGGCTGGAA-3′ (R)
mGAPDH	5′-TGTGTCCGTCGTGGATCTGA-3′ (F)
	5′-CCTGCTTCACCACCTTCTTGAT-3′ (R)
hCyclophilin	5′-TCCTAAAGCATACGGGTCCT-3′ (F)
	5′-CACTTTGCCAAACACCACAT-3′ (R)

BSEP, bile salt export pump; FGF, fibroblast growth factor; FXR, farnesoid X receptor; h, human; I-BABP, ileal bile acid binding protein; m, mouse; OSTα and OSTβ, organic solute transporters α and β; SHP, small heterodimer partner.

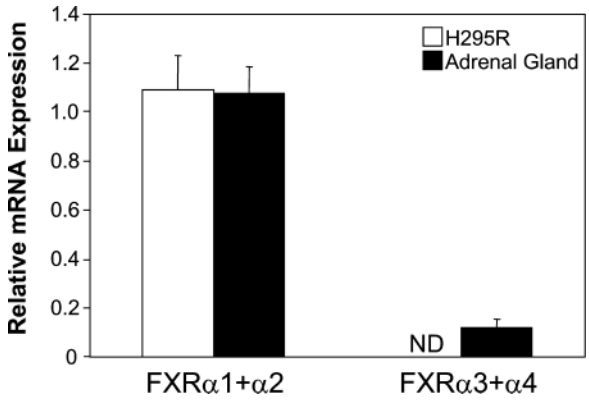

Fig. 1. Farnesoid X receptor (FXR) isoform expression in H295R cells and murine adrenal glands. RNA was isolated from H295R cells (n = 4) or wild-type murine adrenal glands (n = 4), reverse-transcribed, and subjected to real-time quantitative PCR in duplicate using specific primers for human and murine FXRα1+α2 and FXRα3+α4. The values were normalized to cyclophilin. Cycle time for FXRα1+α2 for H295R and adrenal glands was 24. Cycle times for FXRα3+α4 for H295R and adrenal glands were >40 and 27, respectively. ND, not detectable. Values shown are means ± SEM.

(Promega) to generate pGL3-hOSTα and pGL3-hOSTβ, respectively. The OSTα −1,489/+12 construct was amplified using the 5′ primer 5′-cccgctagcccccactgtgtcctgaagtt-3′ and the 3′ primer 5′-cccctcgagccggaggaatttgatcctct-3′. The OSTβ −529/+17 construct was amplified using the 5′ primer 5′-cccgctagcctctctgggtcactggtgct-3′ and the 3′ primer 5′-cccctcgaggtctttgcacttgagctctcc-3′. Four base pair mutations of IR-1A at −1376 and IR-1B at −1296 in pGL3-hOSTα were introduced using sense primers 5′-agtgggctggggAAgaatgTTctggcgaacgtg-3′ and 5′-gggtgggctgagAAcagtgTTcctctgggggtc-3′, respectively. Mutations of IR-1E at −134 in pGL3-hOSTβ were introduced using sense primer 5′-gcactcattcaagagAAcagtcTTcctggagtgatacag-3′. Mutations are shown in uppercase letters. All mutations were generated using the QuickChange Site-Directed Mutagenesis Kit (Stratagene). Sequences of all cloned fragments were confirmed. pGL3-mBSEP-luciferase was described previously (14).

Transient transfections were performed on 48-well plates. Briefly, 5 ng of pCMX-hRXRα, 100 ng of reporter plasmid, and 50 ng of pCMX-β-galactosidase, together with 50 ng of pcDNA-hFXRα1 or pcDNA-hFXRα2, were cotransfected in H295R or HepG2 cells using the MBS Mammalian Transfection Kit (Stratagene). In some experiments, the cells were also transfected with pcDNA-hOSTα and/or pcDNA-hOSTβ. The cells were treated with vehicle (Me_2SO) or the indicated ligands in 10% super stripped fetal bovine serum (HyClone) for 24 h. Luciferase activity was assayed and normalized to β-galactosidase activity. Each transfection was performed in triplicate, and experiments were repeated at least three times.

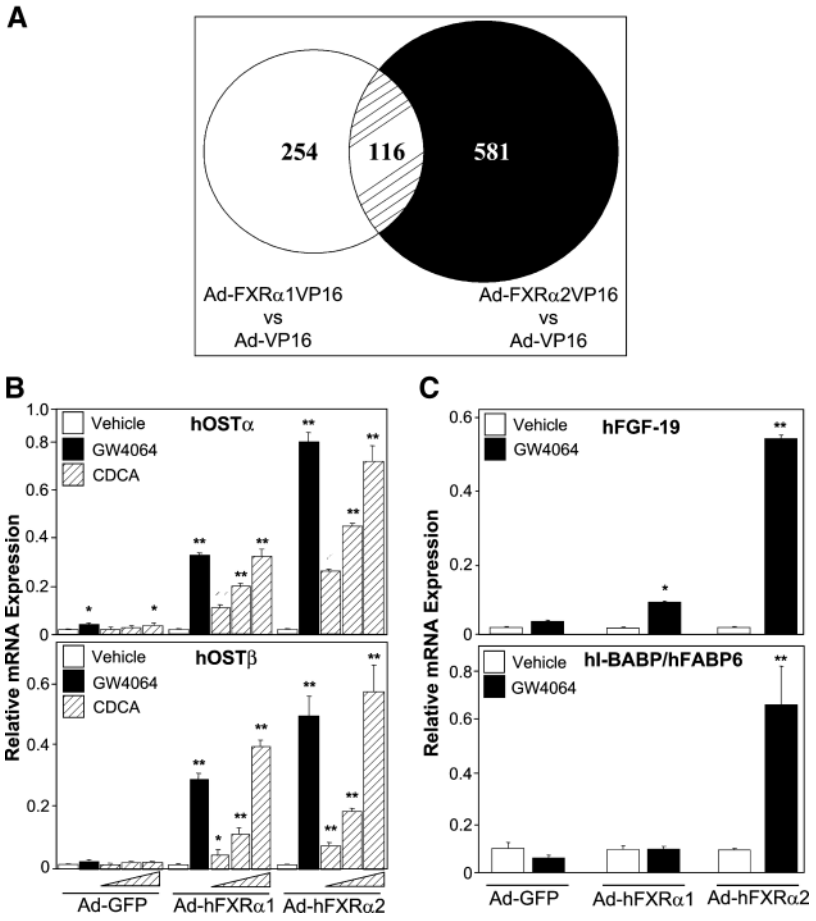

Fig. 2. Induction of human organic solute transporter α and β (OSTα and OSTβ) mRNA by activated FXR. A: Venn diagram indicating the number of genes identified from Affymetrix microarray analysis that are induced in H295R cells infected with either Ad-mFXRα1VP16 (white) or Ad-FXRα2VP16 (black) treated with 3-(2,6-dichlorophenyl)-4-(3′-carboxy-2-chloro-stilben-4-yl)-oxymethyl-5-isopropyl-isoxazole (GW4064) compared with cells infected with control Ad-VP16 treated with vehicle. Only genes with a fold change of ⩾1.8 and $P < 0.05$ were selected. The striped region indicates genes that were induced by both isoforms of FXR. B, C: H295R cells were infected in duplicate 100 mm dishes with adenovirus expressing human FXRα (Ad-hFXRα1), FXRα2 (Ad-hFXRα2), or green fluorescent protein (GFP) alone (Ad-GFP) for 48 h. The cells were then incubated for 24 h with vehicle (white bars), GW4064 (black bars; 1 μM), or chenodeoxycholic acid (CDCA; striped bars; 10, 50, or 100 μM left to right). Total RNA was isolated, reverse-transcribed, and subjected to real-time quantitative PCR in triplicate using specific primers for human OSTα or OSTβ (B) and fibroblast growth factor 19 (FGF-19) or ileal bile acid binding protein (I-BABP)/FABP6 (C); the values were normalized to cyclophilin. Quantitative expression values were extrapolated from separate standard curves. Cycle times of the control group (vehicle treatment) for each gene were as follows: OSTα, 27; OSTβ, 29; FGF-19, 29; and I-BABP, 22. Values are given as means ± SD. * $P < 0.05$, ** $P < 0.01$ versus vehicle-treated control. The data are representative of two separate experiments.

Animal experiments

Eight to 10 week old male and female FXR$^{-/-}$ mice (21) and their wild-type C57BL/6J littermates were housed in a pathogen-free barrier facility with a 12 h light/12 h dark cycle and fed a standard chow diet. For GW4064 feeding experiments, wild-type mice were gavaged vehicle (2-hydroxypropyl-β-cyclodextrin) or GW4064 at 30 mg/kg twice a day for 4 days. Animals were euthanized, and their intestine, kidneys, and adrenal glands were excised and homogenized in TRIzol reagent (Invitrogen) for RNA extraction. For α-naphthylisothiocyanate (ANIT) experiments, FXR$^{-/-}$ mice and their wild-type C57BL/6J littermates were gavaged a single dose of vehicle (corn oil) or ANIT at 150 mg/kg. Animals were euthanized at 48 h after the ANIT dose. For adrenal gland organ culture experiments, FXR$^{-/-}$ mice and their wild-type C57BL/6J littermates fed a standard chow diet were euthanized, their adrenal glands were excised, and all extraneous fat was removed. The cortex was exposed by slicing the organ in quarters, and the quartered adrenals were cultured at 37°C for 8 h in DMEM supplemented with 10% super stripped fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 50 U/ml penicillin G, 50 μg/ml streptomycin sulfate, and, where indicated, 1 μM GW4064.

Statistical analysis

Mean values and SEM were determined by the analysis of multiple independent samples, each assayed in duplicate or triplicate, as indicated in the figure legends. A two-tailed Student's *t*-test was used to calculate *P* values.

RESULTS

Human OSTα and OSTβ expression is regulated by FXR

In situ hybridization studies by Forman et al. (11) originally demonstrated that FXR mRNA expression in the rat adrenal gland was restricted to the cortex. H295R cells were chosen for the current study because they were isolated from a human adrenocortical carcinoma and have retained the ability to synthesize and secrete the major corticosteroids (cortisol, mineralocorticoids, and androgens) (36). As shown in **Fig. 1**, H295R cells express FXRα1 and FXRα2, whereas FXRα3 and FXRα4 are undetectable. Previous studies have demonstrated that murine and human adrenal glands, unlike liver, kidney, and intestine, express FXR isoforms α1 + α2 >> α3 + α4 (13, 14). Thus, H295R cells have many of the properties associated with steroidogenic cells of the adrenal cortex.

We have previously shown that overexpression of FXR isoforms fused to VP16 in cell culture upregulates FXR target genes while retaining the isoform specificity in the absence of FXR agonist (6). Moreover, treatment of the FXR-VP16-overexpressing cells with GW4064 further enhanced FXR target gene expression (6). We have observed that FXRα1 and FXRα3, the two isoforms that contain the MYTG motif, behave similarly with regard to target gene activation (unpublished data). Likewise, FXRα2 and FXRα4 also activate target genes to similar levels (6, 14, 15). Consequently, we generated adenovirus expressing either murine FXRα1 or FXRα2 fused to VP16 (Ad-mFXRα1VP16 and Ad-mFXRα2VP16). H295R adrenocarcinoma cells were then infected with Ad-mFXRα1VP16, Ad-mFXRα2VP16, or adenovirus expressing the VP16 transactivation domain alone (Ad-VP16). After 48 h of infection, the cells were treated for 24 h with either GW4064 (1 μM) or vehicle (Me_2SO). RNA was then isolated and subsequently hybridized to Affymetrix microarrays using standard procedures.

A total of 951 genes were identified that were induced by ⩾1.8-fold ($P < 0.05$) in the Ad-mFXRα1VP16- and/or Ad-mFXRα2VP16-expressing cells treated with GW4064. Interestingly, a large subset of these genes was predicted to be regulated in an FXR isoform-specific manner, whereas ∼12% (116 genes) were induced by both FXR isoforms (**Fig. 2A**). The experimental approach was validated because I-BABP (also referred to as FABP6) and FGF-19, two genes that are known to be activated in response to FXR agonists (23, 37), were induced (**Table 2**). I-BABP has been shown to be expressed in rat adrenal glands (38, 39). However, to our knowledge, the expression and regulation of FGF-19 or the regulation of I-BABP by FXR in adrenal cells has not been reported previously. Human organic transporters α and β (hOSTα and hOSTβ) were chosen for further studies because the microarray data suggested that both genes were highly induced by activated FXR.

Real-time quantitative PCR was then used to confirm the prediction from the microarray data; H295R cells were infected with adenovirus expressing human FXRα1 (Ad-hFXRα1), FXRα2 (Ad-hFXRα2), or control adenovirus that express GFP only (Ad-GFP), and the cells were then treated for 24 h with CDCA (10, 50, and 100 μM) or GW4064 (1 μM). RNA quantitation showed that both endogenous and synthetic FXR agonists resulted in a dose-dependent increase in hOSTα and hOSTβ mRNAs (Fig. 2B). Only minimal activation was seen in Ad-GFP-infected cells, presumably as a result of the endogenous FXR (Fig. 2B). The mRNA levels of FGF-19 and I-BABP were also highly induced by the FXR agonist, GW4064, after infection of the cells with Ad-FXR (Fig. 2C). It is notable that I-BABP induction requires FXRα2 (Fig. 2C), the isoform lacking the MYTG motif. Similar isoform specificity for I-BABP induction has been observed previously in studies with stably transfected cells that overexpress individual FXR isoforms or with an I-BABP promoter-reporter gene (14). Consistent with the microarray data

TABLE 2. Microarray analysis identifies FXR target genes in H295R cells

	Adenovirus and FXR Isoform		
	Ad-VP16	Ad-mFXR-VP16	
Target Gene	None	α1	α2
FGF-19	1	22.9	273.0
I-BABP	1	3.2	44.5
CYP3A4	1	18.9	1.4
OSTα	1	177.4	186.9
OSTβ	1	10.9	10.6

H295R cells were infected with the indicated adenovirus. RNA was harvested and analyzed using Affymetrix microarrays. Relative expression on the arrays of selected genes is shown.

(Table 2), both I-BABP and FGF-19 were more responsive to ligand-activated FXRα2 compared with FXRα1 (Fig. 2C). The results from Fig. 2 demonstrate that hOSTα, hOSTβ, I-BABP, and FGF-19 are induced in human adrenal steroidogenic cells by a mechanism that is dependent upon the activation of FXR.

Identification of FXREs within the proximal promoters of the human OSTα and OSTβ genes

Because both hOSTα and hOSTβ mRNAs were highly regulated after the activation of FXR, the proximal promoters of both hOSTα and hOSTβ was scanned for potential FXREs using the NUBIScan computer algorithm

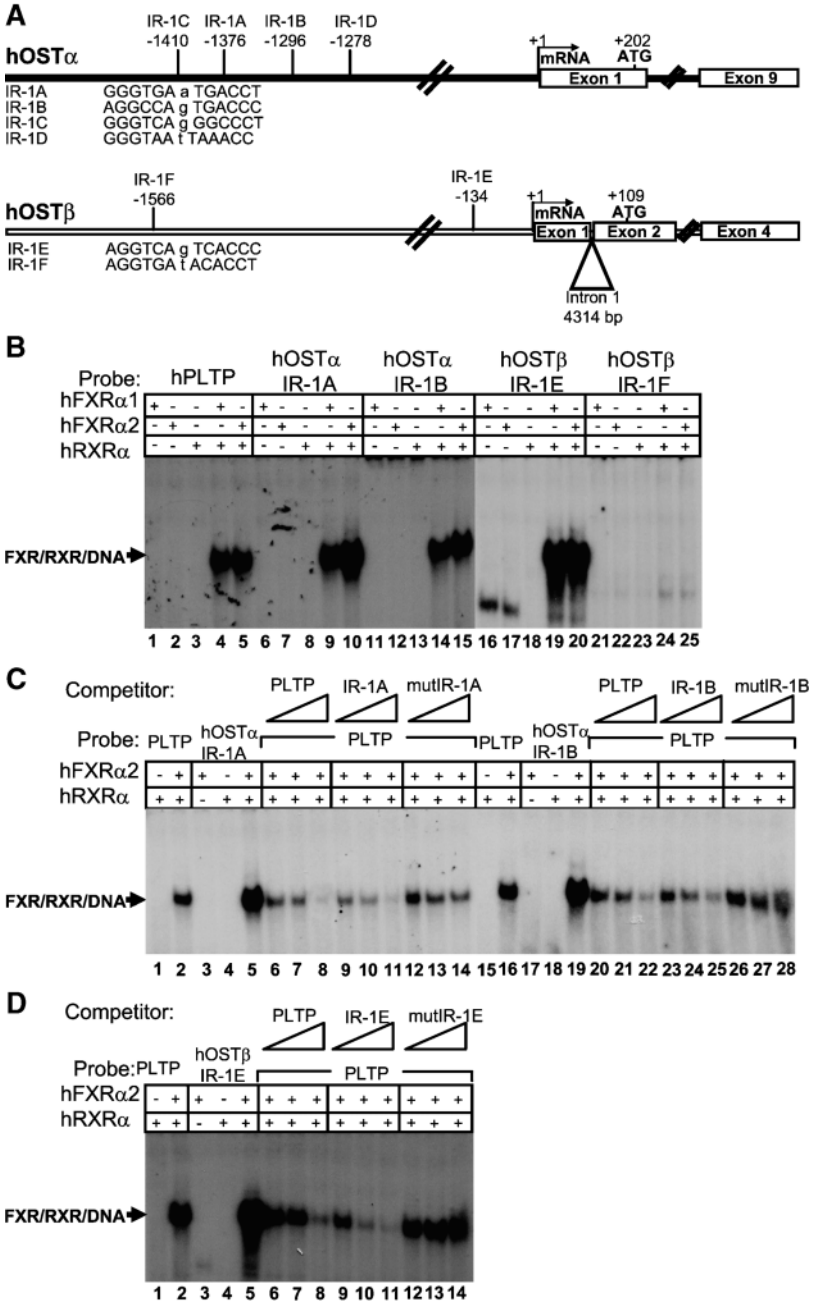

Fig. 3. FXR/9-cis retinoic acid receptor (RXR) heterodimers bind to IR-1 elements in the proximal promoters of the human OSTα and OSTβ genes. A: Scheme of the human OSTα and OSTβ proximal promoters with the putative farnesoid X receptor response elements (FXREs; IR-1A, -IB, -IC, -1D, -1E, and -1F) indicated. Nucleotides are numbered relative to the transcription start site. B: FXR/RXR binds to hOSTα IR-1A and IR-1B and hOSTβ IR-1E. Electrophoretic mobility shift assays were performed using the indicated radiolabeled probes as described in Experimental Procedures. The shifted DNA-protein complexes are indicated. C, D: FXR/RXR heterodimers bind to the hOSTα and hOSTβ IR-1 elements with high affinity. hFXRα2 was incubated with hRXRα and the indicated radiolabeled probe in the presence of unlabeled wild-type or mutant IR-1A, wild-type or mutant IR-1B (C), or wild-type or mutant IR-1E (D), as indicated. PLTP, phospholipid transfer protein.

(see Experimental Procedures). This approach identified four putative IR-1/FXREs (IR-1A, IR-1B, IR-1C, and IR-1D) and two putative IR-1/FXREs (IR-1E and IR-1F) in the proximal promoters of the hOSTα and hOSTβ genes, respectively (**Fig. 3A**). We then used in vitro-transcribed and -translated hRXRα and either hFXRα1 or hFXRα2 proteins in electrophoretic mobility shift assays to determine whether the FXR/RXR heterodimer can bind to these putative FXREs. The data in Fig. 3B show that both hFXRα1/hRXRα and hFXRα2/RXRα bind to radiolabeled probes containing hOSTα IR-1A and IR-1B (Fig. 3B, lanes 9 and 10 and lanes 14 and 15) but not to probes containing sequences corresponding to IR-1C or IR-1D (data not shown). Similarly, hFXRα1/hRXRα or hFXRα2/hRXRα bound to a radiolabeled probe containing hOSTβ IR-1E but not to a probe containing sequences corresponding to IR-1F (Fig. 3B, lanes 19 and 20 and lanes 24 and 25). Probes containing a well-characterized IR-1/FXRE from the PLTP proximal promoter (17) served as a positive control (Fig. 3). Competition assays were also performed to compare the relative affinity of FXR/RXR for the hOSTα IR-1A and IR-1B and the hOSTβ IR-1E. A ^{32}P-radiolabeled probe containing the hPLTP IR-1 sequence was incubated in the presence of increasing concentrations of unlabeled DNA

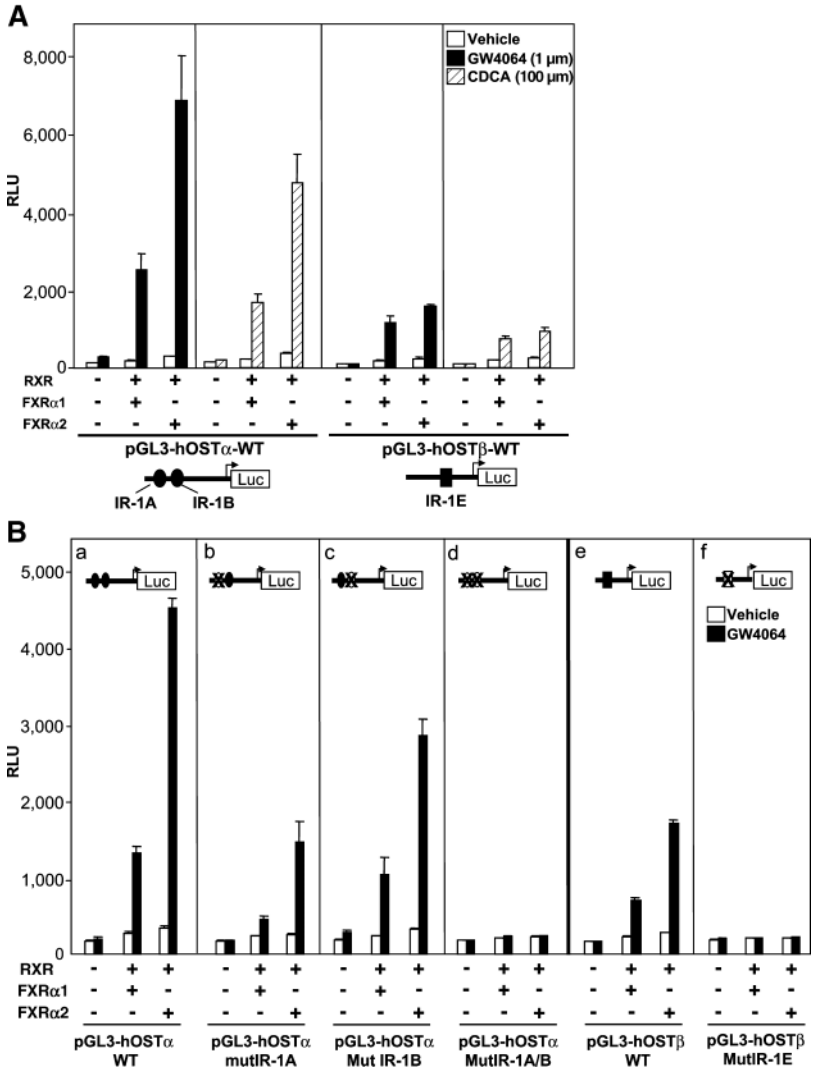

Fig. 4. Transactivation of the human OSTα and OSTβ proximal promoters by FXR. A: H295R cells were cotransfected with hFXRα1 or hFXRα2, hRXRα, and the pGL3 luciferase (Luc) reporter gene under the control of the hOSTα (−1,489 to +12; pGL3-hOSTα) or hOSTβ (−529 to +17; pGL3-hOSTβ) proximal promoter. Cells were treated with vehicle (Me_2SO; white bars), GW4064 (black bars; 1 μM), or CDCA (striped bars; 100 μM) for 24 h after the transfection. Relative light units (RLU) for the reporter genes are shown after normalization with β-galactosidase for minor changes in transfection efficiencies. WT, wild type. B: H295R cells were transfected with the indicated reporter gene and, where indicated, plasmids encoding hRXRα and hFXRα1 or hFXRα2. After the transfection, cells were incubated for 24 h with vehicle (Me_2SO; white bars) or GW4064 (black bars; 1 μM). Relative light units were determined after normalization with β-galactosidase for changes in transfection efficiencies. All transfections were performed in triplicate, and the data are given as means ± SEM. The data shown are representative of at least two experiments.

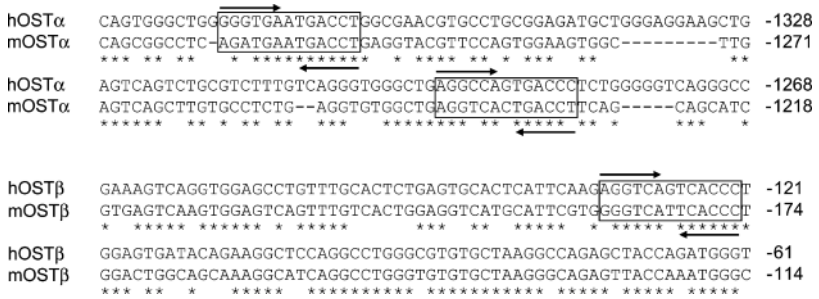

Fig. 5. Conservation of FXREs in the promoters of human and murine OSTα and OSTβ genes. The nucleotides in the proximal promoters of human and murine OSTα and OSTβ have been aligned. The human FXREs and the putative murine FXREs are boxed.

containing the indicated IR-1 or mutated IR-1 (Fig. 3C, D). The results demonstrate that the unlabeled wild-type hOSTα IR-1A and IR-1B and hOSTβ IR-1E effectively competed for the binding of hFXRα2/hRXRα heterodimers (Fig. 3C, lanes 9–11 and 23–25, and 3D, lanes 9–11), whereas the formation of the shifted complex was relatively unaffected by the presence of the unlabeled DNA containing the mutated IR-1 sequences (mutIR-1A, mutIR-1B, or mutIR-E) (Fig. 3C, lanes 12–14 and 26–28, and 3D, lanes 12–14). All competition assays shown were performed with in vitro-transcribed and -translated hFXRα2/hRXRα. Essentially identical results were seen using hFXRα1/hRXRα (data not shown).

Functional identification of the FXREs within the promoters of the human OSTα and OSTβ genes

The data in Fig. 3 suggest that hOSTα IR-1A and IR-1B and hOSTβ IR-1E are functional FXREs. To confirm this hypothesis, we constructed luciferase reporter genes under the control of the hOSTα or hOSTβ proximal promoters containing either the wild-type or mutant IR-1 elements. Each reporter construct was transiently transfected into H295R cells in the presence or absence of plasmids encoding hRXRα and hFXRα1 or hFXRα2, and the cells were then incubated for 24 h with the indicated FXR agonists (**Fig. 4**). Transfection of pGL3-hOSTα with plasmids encoding hFXR isoforms and hRXRα led to 14-fold (hFXRα1) and 23-fold (hFXRα2) increases in luciferase activity after the addition of GW4064 (1 μM). Tenfold and 16-fold increases in luciferase activity were observed after the addition of 100 μM CDCA to cells transfected with FXRα1 and FXRα2, respectively (Fig. 4A). Similarly, transfection of pGL3-hOSTβ led to 8- and 9-fold increases in luciferase activity after the addition of GW4064 and to 4- and 5-fold increases in luciferase activity after the addition of CDCA to cells expressing hFXRα1 and hFXRα2, respectively (Fig. 4A).

To definitively determine that the putative IR-1 elements in the proximal promoters are functional FXREs, we generated reporter gene constructs containing four point mutations within IR-1A (pGL3-hOSTα-mutIR-1A), IR-1B (pGL3-hOSTα-mutIR-1B), or both IR-1A and IR-1B (pGL3-hOSTα-mutIR-1E). IR-1E was also mutated within the hOSTβ promoter (pGL3-hOSTβ-mutIR-1E). Figure 4B shows that induction of the hOSTα promoter-luciferase reporter in response to ligand-activated FXR was attenuated by 50% when either IR-1A or IR-1B was mutated (Fig. 4B, b, c vs. a). Importantly, no induction was observed when the hOSTα promoter contained mutations in both IR-1A and IR-1B elements (Fig. 4B, d). Similarly, mutation of

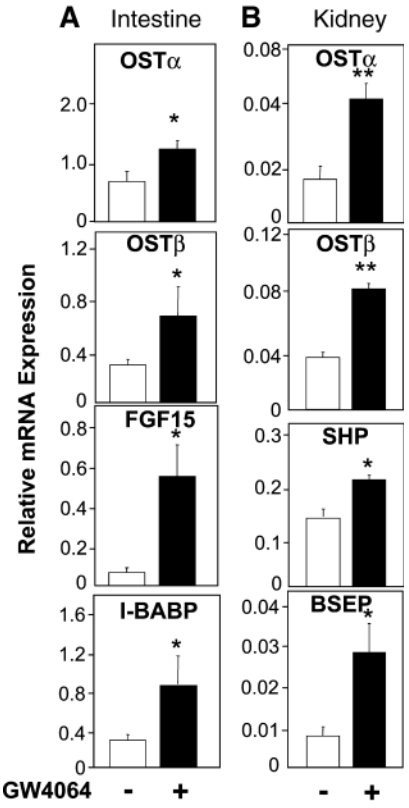

Fig. 6. Induction of murine OSTα and OSTβ mRNA in the intestine and kidney from wild-type mice in response to GW4064 treatment. Wild-type mice (five mice per group) were orally gavaged twice daily for 4 days with vehicle (2-hydroxypropyl-β-cyclodextrin) or GW4064 (30 mg/kg). Total RNAs were isolated from intestine (A) and kidney (B) and reverse-transcribed for real-time quantitative PCR analysis using the indicated gene-specific primers. Samples from each mouse were assayed in duplicate and normalized to murine GAPDH. Cycle times for the control group (vehicle treatment) for A were as follows: OSTα, 19; OSTβ, 17; FGF15, 22; and I-BABP, 15. Cycle times for the control group (vehicle treatment) for B were as follows: OSTα, 20; OSTβ, 19; small heterodimer partner (SHP), 27; and bile salt export pump (BSEP), 31. Values are shown as means ± SEM. * $P < 0.05$, ** $P < 0.01$.

the IR-1E element in the hOSTβ promoter (pGL3-hOSTβ-mutIR-1E) completely abolished gene activation in response to the FXR agonist (Fig. 4B, e vs. f). These data demonstrate that the single FXRE (IR-1E) in the proximal promoter of hOSTβ and the two FXREs (IR-1A and IR-1B) in the proximal promoter of hOSTα are critical for the transcriptional activation in response to activated FXR.

Induction of murine OSTα and OSTβ mRNAs in vivo

The results described above demonstrate that FXR agonists stimulate the transcription of human OSTα and OSTβ mRNAs in the steroidogenic cell line H295R. Comparison of the nucleotide sequences of the proximal promoters of the murine and human OSTα and OSTβ genes indicated that the three functional human FXREs are conserved in the murine genes (**Fig. 5**). Consequently, we next used wild-type and FXR$^{-/-}$ mice to determine whether these same two genes could be induced in vivo in response to FXR agonists.

The data in **Fig. 6A** demonstrate that both murine OSTα and OSTβ mRNAs are induced in the small intestine of wild-type mice in response to oral administration of GW4064. As expected, the FXR target genes, FGF-15 (24) and I-BABP (16, 28), are also induced 3- to 7-fold in the small intestine in response to GW4064 (Fig. 6A).

Figure 6B shows that OSTα and OSTβ are also highly induced in the kidneys of the wild-type mice in response to GW4064. mRNAs encoding SHP and BSEP, two well-characterized FXR target genes, were also induced in the kidneys of GW4064-treated mice (Fig. 6B). To our knowledge, BSEP expression in the kidney has not been reported previously. Nonetheless, although the BSEP mRNA level was low, expression was significantly induced ($P < 0.05$) in response to GW4064. We interpret these data to indicate that oral administration of GW4064 in mice activates FXR in the intestine and kidney. In contrast, OSTα, OSTβ, and FGF-15 mRNA levels were undetectable by quantitative PCR in the livers of the same mice, whereas a significant induction of hepatic SHP and BSEP mRNA levels was noted in the same livers (data not shown). These latter data suggest that OSTα, OSTβ, and FGF-15 are not expressed in murine liver in vivo.

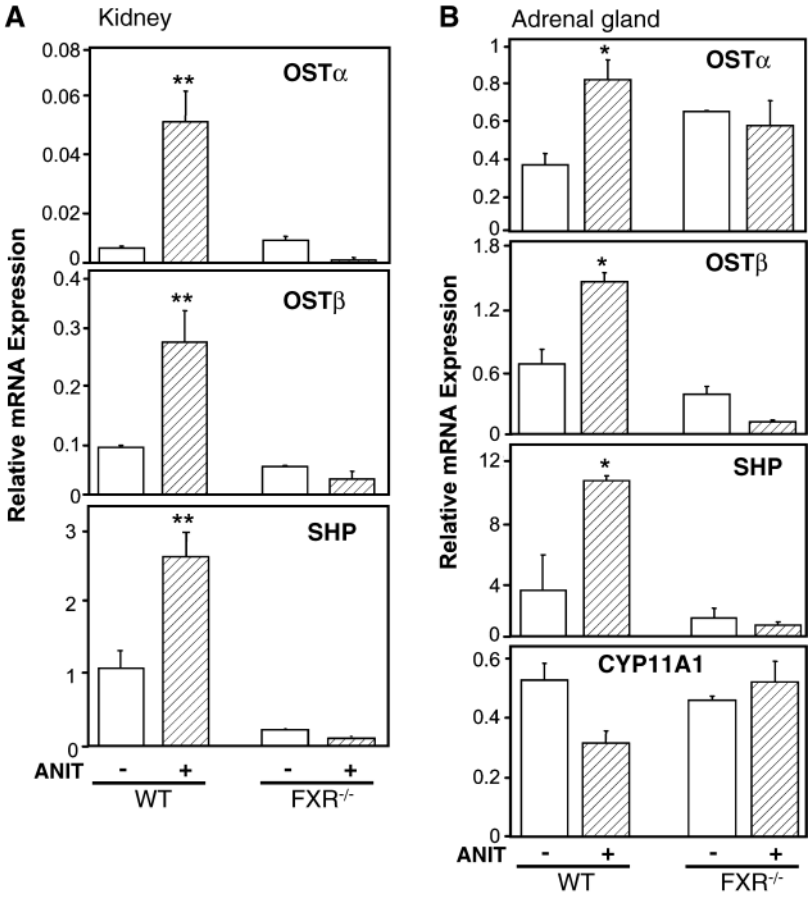

Fig. 7. Induction of OSTα and OSTβ mRNA in the kidney and adrenal gland from wild-type (WT) and FXR-deficient (FXR$^{-/-}$) mice in response to α-naphthylisothiocyanate (ANIT). Wild-type and FXR$^{-/-}$ mice were orally gavaged a single dose of vehicle (corn oil) or ANIT (150 mg/kg) as indicated. The mice were euthanized after 48 h, and total RNAs were isolated from kidney (A) and adrenal gland (B) and reverse-transcribed for real-time quantitative PCR analysis using the indicated gene-specific primers. Cycle times for the control group (wild-type vehicle) for A were as follows: OSTα, 25; OSTβ, 18; and SHP, 25. Cycle times for the control group (wild-type vehicle) for B were as follows: OSTα, 27; OSTβ, 26; SHP, 23; and CYP11A1, 15. Values are shown as means ± SEM (n = 3 mice/group). * $P < 0.05$, ** $P < 0.01$.

Surprisingly, we were unable to demonstrate any change in the mRNA levels of genes encoding OSTα, OSTβ, or SHP in the adrenal glands of mice in response to oral administration of GW4064 (data not shown). It is possible that GW4064 does not accumulate in adrenal glands in vivo in amounts sufficient to activate FXR. Consequently, we used an alternative approach to increase the concentration of endogenous FXR agonists.

Administration of ANIT to rodents induces intrahepatic cholestasis as a result of hepatocellular and biliary epithelial cell necrosis, bile duct obstruction, and biliary epithelial cell hyperplasia (40). The net effect is a decrease in bile flow and an increase in bile acid levels in the plasma (40). We reasoned that with controlled doses of ANIT, plasma bile acids would be increased to levels sufficient to activate FXR in the adrenal gland and other peripheral tissues. The data in **Fig. 7A** show that administration of ANIT resulted in a robust increase in mRNA levels of both OSTα and OSTβ in the kidneys of wild-type mice. In contrast, kidney OSTα and OSTβ mRNA levels were unaffected after the administration of ANIT to $FXR^{-/-}$ mice (Fig. 7A). ANIT treatment also increased SHP mRNA levels ~2-fold in the kidneys of wild-type, but not $FXR^{-/-}$, mice (Fig. 7A), thus mimicking the effect of GW4064 on the expression of SHP in the kidney (Fig. 6B). We conclude that administration of ANIT results in increased plasma bile acids that activate FXR in the kidney and stimulate the transcription of FXR target genes.

Importantly, OSTα, OSTβ, and SHP mRNA levels were all induced ~2-fold in the adrenal glands of ANIT-treated wild-type, but not $FXR^{-/-}$, mice (Fig. 7B). In contrast, the expression of two well-characterized steroidogenic genes (CYP11A1 and HSD3β2) that are expressed in the adrenals was not increased in response to ANIT treatment of either wild-type or $FXR^{-/-}$ mice (Fig. 7B and data not shown). Together, these data suggest that ANIT increases plasma bile acids in mice to levels that lead to the activation of FXR target genes in both the kidney and adrenal glands.

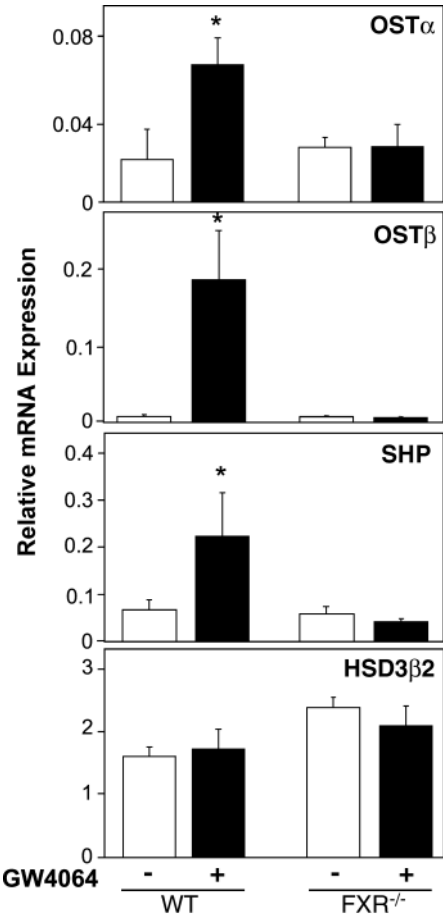

Fig. 8. Induction of OSTα and OSTβ mRNA in adrenal glands from wild-type (WT) and $FXR^{-/-}$ mice. Adrenal glands from wild-type and $FXR^{-/-}$ mice were removed, quartered, and incubated in medium containing either vehicle or GW4064 (1 μM) for 8 h. RNA isolation and quantitation using real-time quantitative PCR was as described for Fig. 5. Cycle times for the control group (wild-type vehicle) were as follows: OSTα, 28; OSTβ, 27; SHP, 24; and HSD3β2, 21. Values are shown as means ± SEM (n = 4 mice/group). * $P < 0.05$ versus vehicle-treated organs.

OSTα and OSTβ mRNAs are induced by GW4064 in adrenal gland organ culture by a process that requires FXR

To overcome problems associated with the delivery and/or retention of the synthetic FXR agonist GW4064 to the adrenal gland in vivo, we used an organ culture model. Adrenal glands were removed from wild-type and $FXR^{-/-}$ mice and incubated for 8 h in medium containing either vehicle or GW4064. Even though the wild-type adrenal glands were exposed to GW4064 for only a short time, the FXR agonist treatment resulted in a significant induction of OSTα, OSTβ, and SHP mRNA (**Fig. 8**). In contrast, these genes were not activated when the adrenal glands were derived from $FXR^{-/-}$ mice (Fig. 8). The steroidogenic enzyme, HSD3β2, was unaffected by FXR ligand exposure (Fig. 8) and served as a negative control. Together, these data provide additional evidence that OSTα and OSTβ are expressed in the adrenal gland, kidney, and intestine and that these genes are all induced after the activation of FXR.

OSTα and OSTβ facilitate the activation of FXR and the induction of FXR target genes in response to conjugated bile acids

Adrenal glands, unlike the liver, intestine, and kidney, are not known to be involved in bile acid metabolism and/or transport. Consequently, although FXR is expressed in the adrenal cortex, the role of FXR in this tissue is an enigma. Recently, Dawson et al. (31) demonstrated that OSTα and OSTβ supported apical-to-basolateral transport of taurocholate as well as other major taurine- and glycine-conjugated bile acids. In addition, previous studies demonstrated that injection of oocytes with mRNAs for OSTα and OSTβ led to increased uptake of a number of compounds, including taurocholate, estrone 3-sulfate, digoxin, and prostaglandin E_2 (32, 33). These studies are consistent with OSTα and OSTβ forming a facilitative transporter that can transport substrates in either direction, depending on the substrate concentration. Consequently, we reasoned that when plasma bile acid levels are

increased, as in cholestasis (40, 41), OSTα and OSTβ might function to recruit bile acids into the adrenal gland and, thus, activate FXR in a feed-forward process.

Conjugated (taurine and glycine) forms of bile acids are unable to efficiently enter cells in the absence of a functional bile acid transporter (9). To determine whether human OSTα and OSTβ can facilitate the uptake of conjugated bile acids into cells, activate FXR, and induce target genes, we cotransfected H295R cells with expression vectors that encode hOSTα, hOSTβ, hRXRα, and either hFXRα1 or hFXRα2, together with a luciferase reporter gene, under the control of the BSEP promoter. The latter promoter contains a functional FXRE (19). The cells were then incubated in the presence or absence of various conjugated bile acids.

The data in **Fig. 9A** demonstrate clearly that the 50- to 100-fold induction of the BSEP promoter-luciferase reporter gene by GW4064 (Fig. 9A, a) or CDCA (Fig. 9A, b) is unaffected by the cotransfection of OSTα and OSTβ. These latter results are expected, because GW4064 and CDCA are both thought to diffuse into cells independent of specific transporters. In contrast, the luciferase reporter was only induced 3- to 4-fold by conjugated (taurine and glycine) CDCA in the absence of OSTα and OSTβ (Fig. 9B, a, b). However, induction of the reporter gene was increased significantly in cells cotransfected with OSTα and OSTβ before the addition of conjugated CDCA (Fig. 9B, a, b). Luciferase activity was not enhanced when the cells were transfected with plasmids encoding either OSTα or OSTβ (Fig. 9B).

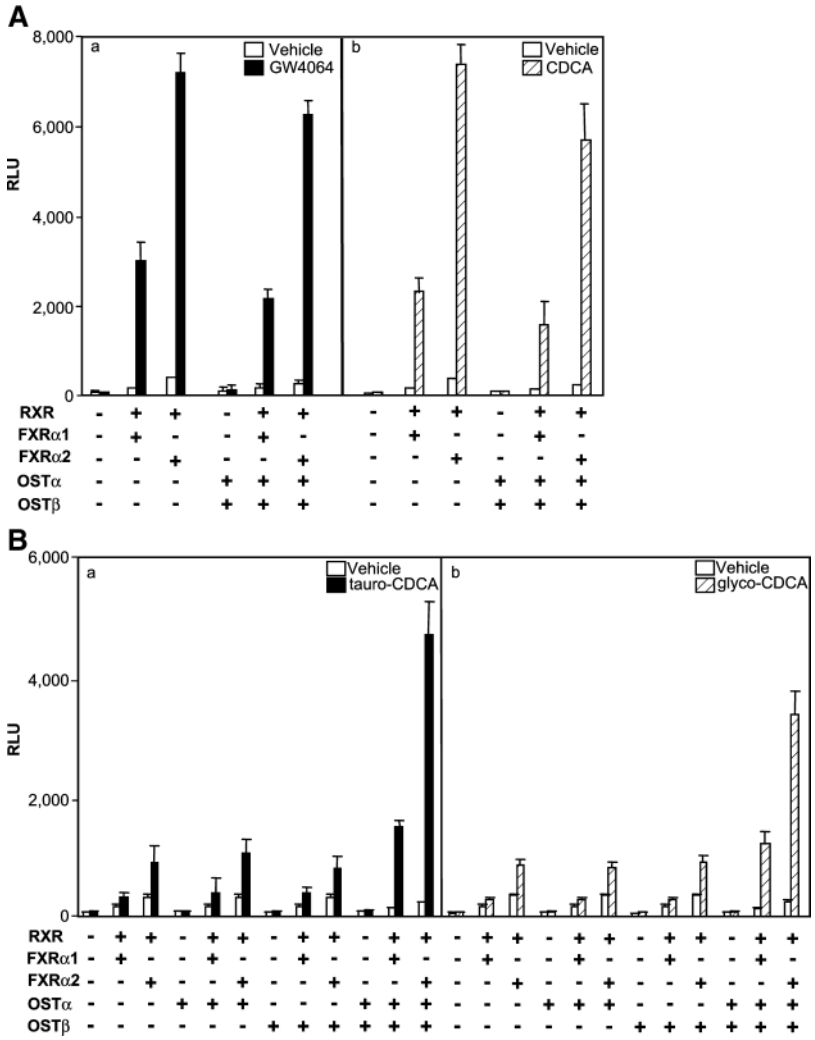

Fig. 9. Overexpression of human OSTα and OSTβ increases transactivation of the BSEP luciferase reporter by conjugated bile acids. H295R cells were cotransfected with hRXRα and hFXRα1 or hFXRα2 and the BSEP promoter-reporter gene. Where indicated, cells were also transfected with hOSTα and/or hOSTβ. Cells were then incubated for 24 h with vehicle (Me_2SO; white bars), GW4064 (black bars; 1 μM), or CDCA (striped bars; 100 μM) (A) or with vehicle (Me_2SO; white bars), tauro-CDCA (black bars; 100 μM), or glyco-CDCA (striped bars; 100 μM) (B). Relative light units (RLU) for the reporter gene (means ± SEM) are shown after normalization using β-galactosidase. All transfections were performed in triplicate. The data shown are representative of at least two experiments.

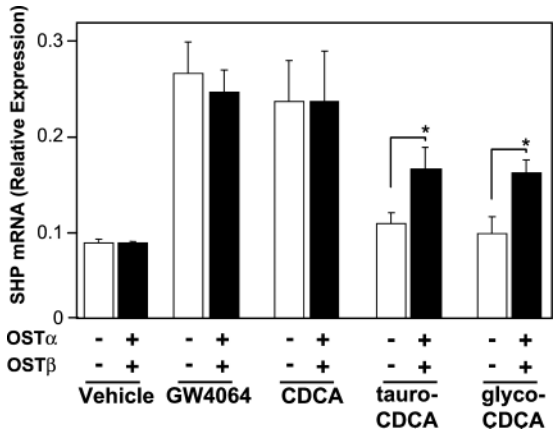

Fig. 10. OSTα and OSTβ overexpression activates FXR target genes in response to conjugated bile acids. HepG2 cells were transiently transfected with an empty vector (white bars) or plasmids encoding OSTα and OSTβ (black bars). Cells were then incubated for 24 h with vehicle (Me_2SO), GW4064 (1 μM), CDCA (100 μM), tauro-CDCA (100 μM), or glyco-CDCA (100 μM). SHP mRNA levels were determined by real-time quantitative PCR. The cycle time for the control group (vehicle treatment) was 20. Each sample was assayed in triplicate and normalized to cyclophilin. Values are means ± SEM. * $P < 0.05$.

To validate the results obtained from the reporter assay, we transiently transfected human hepatoma-derived HepG2 cells with plasmids encoding human OSTα and OSTβ and analyzed endogenous SHP mRNA levels after incubation of the cells with various conjugated bile acids. These cells were used because they express FXR and thus regulate endogenous FXR target genes in response to intracellular agonists. As shown in **Fig. 10**, overexpression of both OSTα and OSTβ resulted in an increase in the mRNA levels of SHP in response to conjugated bile acids. In contrast, induction of SHP mRNA levels by GW4064 or CDCA was unaffected by the overexpression of OSTα and OSTβ (Fig. 10). These results demonstrate that OSTα and OSTβ, when coexpressed, can facilitate the uptake of conjugated bile acids and, thus, allow the activation of endogenous FXR target genes.

DISCUSSION

In the original studies that identified the FXR cDNA (11, 12), rodent FXR was shown to be expressed in the liver, intestine, adrenal cortex, and renal tubules of the kidney. Subsequent studies have shown that the four FXR isoforms are expressed in a tissue-specific manner (13, 14). In addition, numerous studies established that FXR activates target genes involved in bile acid and lipid metabolism (6, 17–20, 25, 26, 42). In contrast, no functional role for FXR in the adrenal gland and kidney has been identified. Indeed, the adrenal gland is not known to be involved in any aspect of bile acid metabolism, whereas the kidney has been implicated in both resorption and secretion of bile acids (34).

To elucidate the spectrum of genes regulated by activated FXR, and in hopes of identifying novel target genes that function in the adrenal gland, we used adenovirus to overexpress constitutively active FXR isoforms in H295R, a human steroidogenic cell line. This approach led to the identification of OSTα and OSTβ as the first known FXR target genes in both the adrenal gland and the kidney. The findings that OSTα and OSTβ mRNA levels increase in an FXR-dependent and FXR ligand-dependent manner in cultured cells, adrenal organ culture, and in vivo, and that the promoters of both human genes contain functional FXREs, demonstrate that OSTα and OSTβ are direct FXR target genes.

Although OSTα and OSTβ cDNAs were originally identified in the livers of little skate (32, 33), their function in this marine vertebrate is unknown. In a recent study, murine OSTα and OSTβ proteins were shown to be located in the basolateral membrane of enterocytes, where, it was proposed, they function to export bile acids into the blood (31). Here, we report that OSTα and OSTβ are expressed in the murine kidney and adrenal gland, in addition to the intestine, and we demonstrate that both genes are induced in all three tissues after activation of the nuclear receptor FXR. Functional studies reported here also demonstrate that OSTα and OSTβ facilitate the uptake of conjugated bile acids into cultured cells and activate FXR target genes. Based on the current studies, we propose that when the levels of bile acids in the blood are increased, as in cholestasis or after ANIT treatment, OSTα and OSTβ facilitate the uptake of conjugated bile acids into the adrenals and that this results in a feed-forward activation of OSTα and OSTβ by FXR. However, because OSTα and OSTβ form a facilitative transporter, this heterodimer may also export specific substrates from the adrenal gland. Indeed, expression of OSTα and OSTβ in frog oocytes was shown recently to enhance the efflux of dehydroepiandrosterone 3-sulfate from preloaded cells (43). Because the human adrenal cortex synthesizes and secretes relatively large amounts of dehydroepiandrosterone 3-sulfate, this finding suggests that OSTα and OSTβ also function to export conjugated steroid intermediates from the adrenals into the blood.

At present, it is not known whether adrenal FXR is activated, not only after the uptake of bile acids but also as a result of endogenous synthesis of a yet unidentified, novel agonist. Indeed, androsterone, a metabolite derived from adrenal steroids, was reported to activate FXR (44). However, activation of a BSEP promoter-reporter gene by androsterone (100 μM) was relatively weak (<20% of CDCA) (unpublished data). Thus, it remains unclear whether androsterone or other steroid intermediates are physiological activators of FXR. The identification of other FXR target genes in the adrenal gland should provide additional insights into the role of this nuclear receptor in this organ.

The kidney is a highly specialized tissue that maintains the internal environment of the body by selectively excreting or retaining various substances according to specific bodily needs. Original studies by Forman et al. (11) showed that FXR is expressed in the renal tubules of the

rat kidney, a region responsible for the resorption and secretion of solutes. Although the precise mechanisms of renal handling of bile acids are still unclear, the expression of OSTα, OSTβ, ASBT, MRP2, and FXR in the kidney suggests that FXR may function in bile acid resorption. Definitive insights into the physiological function of OSTα and OSTβ in the adrenal gland, kidney, and intestine will likely be dependent upon the generation of OSTα and/or OSTβ null mice.

In summary, this study identifies OSTα and OSTβ as novel FXR target genes that are expressed in the adrenal gland, kidney, and intestine. We propose that OSTα and OSTβ are multifunctional facilitative transporters that mediate the uptake of bile acids from the circulation into the adrenal cortex under conditions in which blood bile acid levels are highly increased and facilitate the efflux of conjugated steroids from the cortex under normal conditions.

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