Liver X receptor agonist TO-901317 upregulates SCD1 expression in renal proximal straight tubule

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THE LIVER X RECEPTORS (LXRs) are members of the superfamily of nuclear hormone receptor of ligand-activated transcription factors that were originally identified in the liver. Two isoforms of LXRs exist, designated LXRα (NR1H3) and LXRβ (NR1H2). They share high sequence homology and similar protein structure (9, 17). Both LXRα and LXRβ bind as heterodimers with retinoic acid receptor RXR-α to a specific DNA sequence named LXR response element in the promoter regions of their target genes, thereby modulating the target gene transcription. Target genes of LXRs include cytochrome P-450 7A1 (CYP7A1), ATP-binding cassette transporter (ABC) A1, ABCG5, ABCG8, apolipoprotein E, cholesterol ester transport protein, lipoprotein lipase, fatty acid synthase, and sterol response element-binding protein 1c (SREBP1c), consistent with a key role for LXRs in regulation of cholesterol and fatty acid metabolism (34). The LXR ligand TO-901317 significantly improves insulin resistance and lowers plasma glucose level by inhibition of hepatic gluconeogenesis in type II diabetic rats (6). Recent studies demonstrate that an LXR-specific activator increases high-density lipoprotein (HDL) cholesterol in mice, whereas gene disruption of LXRα and LXRβ increases low-density lipoprotein cholesterol and decreases HDL cholesterol in plasma. LXR gene knockout is also associated with massive lipid accumulation in macrophages in the liver, spleen, and lung (26, 32). These findings suggest that LXR plays an important role not only in cholesterol and fatty acid metabolism but also in insulin sensitivity and glucose homeostasis.

Although LXRα and LXRβ display distinct tissue distribution patterns, both are expressed in the kidney (3, 17, 37). A recent report shows that LXRα mRNA is ubiquitously expressed along the nephron, and its activation mediates cholesterol efflux via ABCA1 in cultured glomerular mesangial cells (37). LXRβ is also widely expressed at high levels in the late stage of embryonic kidney (3). The role of these receptors in the kidney with respect to lipid metabolism is largely unknown. To provide insight into the role of LXRs in renal physiology, the present study utilized Affymetrix GeneChip to perform a systematic study of the genomewide expression profiles of the kidney from mice treated with an LXR-specific agonist, TO-901317. We provide evidence suggesting that treatment of the LXR agonist TO-901317 markedly increases the gene expression of stearoyl-CoA desaturase-1 (SCD1), possibly via increased SREBP1 expression in the proximal straight tubules. The results suggest that LXR may play an important role in regulating lipid metabolism and maintaining proximal tubule function.

MATERIALS AND METHODS

Chemical reagents. TO-901317 was obtained from Cayman Chemical (Ann Arbor, MI). Goat anti-mouse SCD1 antibody, rabbit anti-human SREBP1c antibody, and goat anti-human Tamm-Horsfall protein antibody were purchased from Santa Cruz (Santa Cruz, CA) and Organon-Technika.

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Animal treatment. Male C57 Bl/6 mice (8 wk old) were purchased from the Jackson Laboratories (Bar Harbor, ME) and fed with mouse chow ad libitum. At 10 wk of age, animals were treated with LXR agonist TO-901317 (3 mg-kg^{-1} day^{-1}) or vehicle alone by gavage for 3 days. At the end of study, mice were killed, and tissue samples were snap-frozen in liquid nitrogen and then stored at −80°C until further processes. Kidney samples were also fixed in 4% paraformaldehyde solution for immunostaining and in situ hybridization.

RNA extraction and purification. Total RNA from murine kidneys and cultured renal cells was isolated by using Trizol-Reagent (GIBCO-BRL). The tissue and cell samples were homogenized in 10 ml Trizol reagent. Phase separation of RNA was performed by adding one-tenth volume of chloroform and vortex mixing for 15 s followed by centrifugation at 12,000 g for 10 min. Isopropanol alcohol (0.5 ml/ml Trizol) was added to the aqueous phase to precipitate total RNA, followed by two washes with 75% ethanol. For Affymetrix analysis, the RNA sample was dried and then redissolved. RNA quality was determined by the ratio of absorbance at 260 nm to that at 280 nm (A260/A280). Total RNA extracted was further purified using the RNeasy Clean Up kit (Qiagen) to increase the A260/A280 readings.

Affymetrix GeneChip analysis. Total RNA isolated from three controls and three treated kidneys using Trizol reagent described above was treated with RNeasy to obtain a ratio of optical density at 260 to 280 nm between 1.9 and 2.1 for each sample. Gene expression level of each sample was studied using the Affymetrix mouse chip at the Vanderbilt Microarray Shared Resource (VMSR, http://array.uc.vanderbilt.edu/products/affymetrix.htm). The chip measured the expression of 12,488 probe sets. The Signal Log Ratio (SLR) estimates the magnitude and direction of change of a transcript when two arrays are compared (experiment vs. control). A transcript is significantly increased when the SLR is >1.0, indicating a twofold increase, and significantly decreased when the SLR is less than −1.0, indicating a twofold decrease. The gene expression data were analyzed using Affymetrix Microarray Suite version 5.0 and Gene Traffic at VMSR. The data were further analyzed independently using Gene Spring.

Lipid analysis. Fatty acid profiles of lipid esters in the kidneys were analyzed by gas-liquid chromatography at Vanderbilt Lipids/Lipid Peroxidation Core. Briefly, total lipids were extracted from mouse kidneys and were separated by silica gel TLC using hexane-diethyl ether-acetic acid (80:30:1 vol/vol/vol) as a developing solvent. The lipids were visualized by cupric sulfate in 8% phosphoric acid. The lipids were then scraped, methylated, and analyzed by gas-liquid chromatography (20).

RT-PCR analysis. To determine whether the LXRs are expressed in mouse kidney and cultured murine proximal tubule cells (MCT cells; see Ref. 14), RT-PCR analysis was utilized. Total RNA isolated from three normal male C57Bl6 mice (10 wk old) was reverse transcribed to single-stranded cDNA using Moloney murine leukemia virus RT and 2.5 μM of random hexamers according to the manufacturer’s protocol (GeneAmp RNA PCR kit; Perkin-Elmer Cetus, Norwalk, CT). The cDNAs were then amplified using LXRα, LXRβ, and glyceroldehyde-3-phosphate dehydrogenase (GAPDH) selective primers. Mouse LXRα primer sequences are 5′-TCC ATC ACA CAC CCC CAC GAC-3′ for sense and 5′-CAG CCA GAA ACCCAA CCT-3′ for antisense. Mouse LXRβ primer sequences are 5′-TGCACTTCAAACAACACCAACC CACGAC-3′ for sense and 5′-CAG CCA GAA ACCCAA CCT-3′ for antisense. Mouse GAPDH primer sequences are 5′-TGCACTTCAAACAACACCAACC CACGAC-3′ for sense and 5′-GAGTGGGTTGGTGTGATG-3′ for antisense. These primers were used to amplify a 328-bp product from LXRα, a 514-bp product from LXRβ, and a 178-bp GAPDH cDNA fragment. PCR reactions were carried out in 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM dNTPs, and 1 μM primers at 94°C for 0.5 min, 60°C for 0.5 min, and 72°C for 1.0 min for 35 cycles in a Perkin-Elmer Cetus 2400 thermal cycler. PCR products were separated by 1% agarose gel and further confirmed by sequencing.

RT-PCR was also used to amplify a 352-bp product of SCD-1 cDNA fragment from mouse fat tissues for use in situ hybridization. A pair of primers with the sequences 5′-CTTTCTTCGGGATAACACCTTCGACTTCTGCTGTG-3′ for sense and 5′-AGGAACTTGAACCCTAAAGCCTTCTGCTGTG-3′ for antisense were used. The resulting 352-bp PCR fragment was cloned into pcRII2.0 vector (Invitrogen) and sequenced.

Northern blot analysis. Total RNA was extracted from the kidneys of mice treated with or without the LXR agonist TO-901317. Total RNA (20 μg) was size-separated in 1% agarose gel and blotted to a nylon membrane; the blot was prehybridized in 6× saline-sodium citrate (SSC), 5× Denhardt’s solution, 1% SDS, and 150 μg/ml of freshly denatured salmon sperm DNA for 4 h and then hybridized in same buffer with 1 × 10⁶ counts·min⁻¹·ml⁻¹ of 32P-labeled mouse SCD1 or SCD2 cDNA probe (a gift from Dr. N. Wang at the Institute of Cardiovascular Science, Peking University Health Science Center) at 62°C overnight. After hybridization, the blot was washed one time in 2× SSC, 0.1% SDS for 30 min at room temperature followed by two washes in 0.1× SSC, 0.1% SDS at 62°C for 30 min and exposed to X-ray film at −80°C for 1 day.

In situ hybridization. In situ hybridization was performed as previously reported (10). Riboprobes were made for in situ hybridization using the 352-bp product from mouse SCD1 cDNA. The plasmid containing mouse SCD1 cDNA was linearized, and 35S-UTP-labeled antisense riboprobe was synthesized and hybridized to mouse kidney sections and then washed as previously described (10). Slides were dehydrated with graded ethanol containing 300 mM ammonium acetate, dipped in emulsion (Ilford K5; Knutsford, Cheshire, UK), and exposed for 4–5 days at 4°C. After being developed in Kodak D-19, some slides were counterstained with hematoxylin, and photomicrographs were taken using a Zeiss Axioskop microscope and either darkfield or brightfield optics.

Immunohistochemistry. Immunohistological examination and additional staining of in situ hybridization slides was performed using a goat anti-mouse SCD1 antibody (1:500; Santa Cruz), rabbit anti-aquaporin-2 (AQP2) antibody (1:200; a gift from Dr. M. A. Knepper), and goat anti-human Tamm-Horsfall antibody (1:1,000; Organon Technika), which specifically recognize mouse SCD1, AQP2, and Tamm-Horsfall protein. Briefly, the slides were incubated first with 3% H₂O₂ to eliminate endogenous peroxidase activity and thereafter with antibodies for 60 min. The sections were rinsed with Tris-buffered saline containing 0.1% Tween 20 and a biotinylated secondary antibody against rabbit or goat immunoglobulin for 30 min. Then sections were incubated with horseradish peroxidase (HRP)-conjugated streptavidin for 20 min. HRP labeling was detected by peroxidase substrate solution and counterstained with hematoxylin before being examined under a light microscope.

Construction of a mouse SCD1 promoter-driven luciferase reporter plasmid. A 716-bp fragment of mouse SCD1 promoter was cloned from mouse embryonic stem cell (ES cell) genomic DNA using a set of primers (upstream primer: 5′-GTCGCGCTGAGTGGGCT-3′ and downstream primer: 5′-TCTCGGGATGGGTGT-TCAGC-3′). The fragment was then cloned into the pGL3-luciferase plasmid (Promega), and the resultant construct designated mSCD1 716-Luc was sequenced to confirm the orientation and sequence. The promoter activity was assayed in transient transfections of mSCD1 716-Luc in NIH3T3 cells, which specifically recognize mouse SCD1, AQP2, and Tamm-Horsfall protein. Briefly, the cells were incubated first with 3% H₂O₂ to eliminate endogenous peroxidase activity and thereafter with antibodies for 60 min. The sections were rinsed with Tris-buffered saline containing 0.1% Tween 20 and a biotinylated secondary antibody against rabbit or goat immunoglobulin for 30 min. Then sections were incubated with horseradish peroxidase (HRP)-conjugated streptavidin for 20 min. HRP labeling was detected by peroxidase substrate solution and counterstained with hematoxylin before being examined under a light microscope.

Transient transfections and assays for mSCD1 716-luciferase and SREBP1c (2.6 kb)-luciferase reporter activity. MCT cells, a murine proximal cell line, were kindly provided by Dr. Eric Neilson at Vanderbilt University and were transfected with mSCD1 716-Luc, a reporter construct containing 1 putative SRE [nuclear transcript (nt)] −412 to 422; see Ref. 4] with or without a constitutive active form of SREBP1c expression vector (SREBP1cN; a gift from Dr. Y. Zhu at the University of California at Riverside), or a rabbit LXRα expression vector (37) using the Effectene Transfection Reagent as recommended by the supplier (Qiagen, Valencia, CA). After incubation for 18 h, the cells were then harvested in 1× luciferase lysis buffer (Dual
Increased genes

- Stearoyl-CoA desaturase 1 (M21285) - Fatty acid Δ9-desaturation +8.0
- Stearoyl-CoA desaturase 1 (M26270) - Fatty acid Δ9-desaturation +2.5
- PRP39 pre-mRNA processing factor 39 homolog (AV369904) - Unknown +3.5
- Major urinary protein group 1 (M17818) - Unknown +3.5
- SREBP-1 (A1843895) - Fatty acid synthase +2.3
- Calpain 5 (Y10656) - ABCA1 degradation +2.3

Decreased genes

- P53 binding protein (P53BP1) (AW048394) - Cell growth, apoptosis −9.8
- Interleukin-15 (U14332) - Renal epithelial cells survival −4.9
- Lipocortin 1 (AV003419) - Ca2+-dependent phospholipid-binding protein, apoptosis −4.3
- Aryl-hydrocarbon receptor (M94623) - Environmental sensor and cell cycle checkpoint −2.8
- Prostaglandin E2 receptor 3 (D10204) - Sodium and water homeostasis −2.8
- Carboxypeptidase (X61233) - Neuropeptide conversion −2.8
- Cyclin G (L49507) - Cell cycle −2.0
- TNF-α receptor-associated factor (U59864) - Apoptosis −2.3
- 3-Hydroxyisobutyryl-CoA hydrolase (AW121399) - Amino acid catabolism −2.1
- Lipoprotein lipase (M63355) - Triglyceride metabolism −2.0
- Cytochrome P-450 7B1 (U36993) - Bile acid synthesis −2.0
- FBJ osteosarcoma oncogene (V00727) - Oncogene −2.0

**LXRs are expressed in mouse kidney.** As shown in Fig. 1, LXRα mRNA was detected by RT-PCR yielding the expected 328-bp fragments in three individual kidney samples. RT-PCR for LXRβ mRNA also indicated the presence of a single 514-bp band corresponding to the predicted size of the LXRβ product in all three mouse kidney samples. In addition, both LXRα and LXRβ mRNA were detected in cultured MCT cells, a murine proximal tubule cell line (data not shown).

**Effect of LXR agonist TO-901317 on gene expression profile in the kidney.** The renal gene expression profile regulated by the LXR agonist TO-901317 was examined using the Affymetrix GeneChip Microarray. Of the 51 genes differentially expressed in the kidneys from the mice treated with or without TO-901317, 11 known genes were upregulated by TO-901317 and 40 genes were downregulated. One of the most striking changes revealed by GeneChip was the induction of a group of genes that are involved in catalyzing the synthesis of fatty acids, including SCD1, SCD2, and SREBP1 (Table 1). SCD1 and SCD2 mRNA were expressed at much higher levels in the

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**Table 1. A partial list of genes regulated by LXR agonist TO-901317 treatment in mouse kidney**

<table>
<thead>
<tr>
<th>Name of Gene</th>
<th>GenBank Accession No.</th>
<th>Gene Function</th>
<th>Degree of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waxman-CoA desaturase 1</td>
<td>M21285</td>
<td>Fatty acid Δ9-desaturation</td>
<td>+8.0</td>
</tr>
<tr>
<td>Waxman-CoA desaturase 1</td>
<td>M26270</td>
<td>Fatty acid Δ9-desaturation</td>
<td>+2.5</td>
</tr>
<tr>
<td>PRP39 pre-mRNA processing factor 39 homolog</td>
<td>AV369904</td>
<td>Unknown</td>
<td>+3.5</td>
</tr>
<tr>
<td>Major urinary protein group 1</td>
<td>M17818</td>
<td>Unknown</td>
<td>+3.5</td>
</tr>
<tr>
<td>SREBP-1</td>
<td>A1843895</td>
<td>Fatty acid synthase</td>
<td>+2.3</td>
</tr>
<tr>
<td>Calpain 5</td>
<td>Y10656</td>
<td>ABCA1 degradation</td>
<td>+2.3</td>
</tr>
<tr>
<td>P53 binding protein (P53BP1)</td>
<td>AW048394</td>
<td>Cell growth, apoptosis</td>
<td>−9.8</td>
</tr>
<tr>
<td>Interleukin-15</td>
<td>U14332</td>
<td>Renal epithelial cells survival</td>
<td>−4.9</td>
</tr>
<tr>
<td>Lipocortin 1</td>
<td>AV003419</td>
<td>Ca2+-dependent phospholipid-binding protein, apoptosis</td>
<td>−4.3</td>
</tr>
<tr>
<td>Aryl-hydrocarbon receptor</td>
<td>M94623</td>
<td>Environmental sensor and cell cycle checkpoint</td>
<td>−2.8</td>
</tr>
<tr>
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<td>−2.0</td>
</tr>
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LXR, liver X receptor; SREBP, sterol regulatory element-binding protein; ABC, ATP-binding cassette.
kidneys of mice treated with LXR agonist TO-901317 compared with vehicle-treated animals. Based on Affymetrix analysis, SCD1 and SCD2 were upregulated by ~8-fold and 2.5-fold in TO-901317-treated mice, respectively (Table 1).

Induction of SCD1 and SCD2 gene expression in mouse kidney by TO-901317. As shown in Fig. 2A, Northern blot analysis confirmed a marked increase in both SCD1 and SCD2 gene expression. Consistent with the data from the Affymetrix study, quantitative analysis further revealed 5.4- and 2.9-fold upregulation of SCD1 and SCD2 genes in mouse kidneys by TO-901317 treatment, respectively (Fig. 2B).

Increased fatty acid desaturation index in the kidneys by TO-901317 treatment. To assess the effect of TO-901317 on SCD activity in the kidney, the long-chain fatty acid profile was analyzed. As shown in Fig. 3, SCD1 desaturation indexes as reflected by oleate + palmitoleate/stearate + palmitate (C16:0 + C18:01/C16:0 + C18:0) in the triglyceride fraction of the kidneys of mice treated by TO-901317 were significantly greater than the control mice (P < 0.01).

Intrarenal localization of SCD1. In situ hybridization was used to define the intrarenal expression of SCD1 mRNA. In
both vehicle- and TO-901317-treated mice, SCD1 mRNA was predominantly expressed in the outer stripe of the outer medulla. TO-901317 treatment for 3 days dramatically increased SCD1 mRNA expression in this region (Fig. 4).

In agreement with the result from in situ hybridization analysis, immunohistochemical studies confirmed this pattern of intrarenal localization of SCD1. SCD1 immunoreactivity was observed in a subset of tubular structures in the region of the outer medulla in mouse kidney (Fig. 5). The localization, size, and cuboidal shape of epithelial cells expressing SCD1 protein suggest SCD1 may be expressed in the proximal straight tubule cells, medullary collecting ducts, or medullary thick ascending limb cells rather than the cells in the thin limb of the loop of Henle and the vasa recta (Fig. 5).

Segmental expression of SCD1 mRNA. To further define the tubule segments expressing SCD1, double-labeling studies using both in situ hybridization and immunostaining with segmental specific markers were performed. Anti-AQP2 antibody was used to specifically stain medullary collecting ducts (Fig. 6). SCD1 mRNA expression as assessed by in situ hybridization was not expressed in AQP2-positive renal tubules. Double-labeling studies using in situ hybridization for SCD1 mRNA and immunohistochemistry for Tamm-Horsfall protein also showed that SCD1 gene expression was not colocalized with Tamm-Horsfall-positive tubules (Fig. 7). These findings suggest that SCD1 was expressed in proximal straight tubule.

LXR activation induced SREBP1c expression in MCT cells. Sequence analysis failed to reveal whether a putative LXRE consensus site exists in the mouse SCD1 promoter region (TESS software http://agave.upenn.edu/tess/index.html), suggesting SCD1 may not be a direct transcriptional target of LXRs. To determine the mechanisms by which LXR agonist...
TO-901317 upregulated SCD1 expression in renal proximal tubule cells, MCT cells were cultured and treated with TO-901317 (10 μM for 24 h) to examine the effect of LXR activation on SREBP1c expression. Consistent with the findings revealed by the Affymetrix gene chip analysis (Table 1), LXR activation markedly increased the levels of the 68-kDa NH2-terminal fragment that is responsible for activating SREBP1c target gene transcription (Fig. 8A). In support of this, activation of LXRs by TO-901317 treatment significantly enhanced mouse SREBP1c promoter activity (Fig. 8B) and resulted in a 3.5-fold increase in SREBP1c mRNA expression, as assessed by quantitative real-time PCR ($P < 0.01$, n = 3; data not shown). In addition, SCD1 mRNA levels were increased by 140% after TO-901317 treatment for 24 h in MCT cells ($P < 0.05$, n = 3; data not shown).

SREBP1c increased mouse SCD1 promoter activity. Consistent with a previous report (4), sequence analysis of our cloned 716 bp of mouse SCD1 promoter region by the TESS software revealed a putative SREBP binding site (SRE; nt = 412 to 422; Fig. 9A). Cotransfection of MCT cells with a constitutively active SREBP1 expression vector (SREBP1cN) significantly increased mouse SCD1 promoter activity (Fig. 9B). However, cotransfection of a LXRα expression vector had little effect on SCD1 transcription (data not shown), further supporting the idea that enhanced SREBP1c expression may be responsible for TO-901317-mediated SCD1 induction.

DISCUSSION

LXRs are nuclear receptor transcription factors and play an important role in cholesterol metabolism and lipid biosynthesis (15). Although LXRs are predominantly expressed in adipose tissue and liver, moderate levels of LXRα and LXRβ were also found in the kidney (17). To date, little is known about the biological function of LXR receptors in the kidney. The present studies reveal for the first time that an LXR agonist significantly increases expression of SCD1 in renal proximal straight tubules of mouse (Fig. 10). SCD1 is the rate-limiting enzyme catalyzing the conversion of saturated long-chain fatty acids into monounsaturated fatty acids (MUFAs; see Refs. 22 and 23); thus, LXRs may be involved in regulating lipid metabolism, especially in biosynthesis of triglycerides, cholesterol esters, and phospholipids in this nephron segment.

Our results demonstrated that both LXRα and LXRβ are expressed in mouse kidney, suggesting these receptors may play an important role in regulating renal physiology. We and others (30, 37) have previously reported that LXRα was highly expressed in rabbit kidney, with wide expression in every segment along the nephron including the glomeruli, proximal tubules, and cultured glomerular mesangial cells. Activation of LXR in mesangial cells resulted in marked induction of gene transcription of ABCA1 (30), a membrane-associated transporter mediating cholesterol efflux (18). These findings suggest LXRα may be an important modulator in regulating lipid metabolism in the kidney, especially in maintaining intraglomerular lipid homeostasis (37). Consistent with previous reports in which LXRβ was found to be widely expressed in late stages of embryonic kidney development (3, 17), the present study also indicated the LXRβ receptor is expressed in adult kidney. Although, in general, no clear role for this receptor has yet been identified, expression of LXRβ at high levels in
developing and adult organs, including brain, thyroid gland, adrenal, testis, ovary, and kidney, suggests LXRβ may be involved in the morphogenesis or physiology of these tissues (3, 17, 37).

To gain further insight into the role of LXRo and LXRβ in renal function, gene expression profiling using microarrays (mouse-specific array; Affymetrix) was employed for the investigation of the effect of LXR agonist TO-901317 on renal gene expression. Among 51 genes differentially regulated by LXRs in the kidney, a group of genes involved in catalyzing the synthesis of fatty acids and converting the saturated fatty acids into MUFAs were found to be most markedly increased. These genes include SREBP1, SCD1, and SCD2. SREBP1 is a membrane-bound transcription factor and has been postulated to be a master regulator in the synthesis of fatty acids, cholesterol, and triglycerides (1, 11) and a direct target gene of LXRo and LXRβ (29). The mammalian genome contains only one SREBP1 gene, but as a result of alternative transcription start sites, two SREBP1 isoforms exist, designated SREBP1a and SREBP1c (5). SREBP1a is a potent activator of all SREBP response genes and enhances both fatty acid and cholesterol synthesis, whereas the role of SREBP1c is more restricted and it preferentially participates in fatty acid biosynthesis (11). Although SREBP1 is highly expressed in the liver, steroidogenic organs, and adipose tissue, significant levels of SREBP1a and -1c are also found to be present in the kidney (33). The induction of SREBP1c gene expression after LXR activation in the kidney raises the possibility that, in addition to liver and adipose tissue, LXRs may also regulate renal lipid metabolism through activating a SREBP1 transcriptional pathway. At present, it remains unclear which nephron segments are significantly affected by LXR (30, 31). Such information will be fundamental to understand the LXR-modulated lipid homeostasis in the kidney.

The present studies also demonstrated a marked induction of a group of genes that mediate the conversion of saturated fatty acids into MUFAs, including SCD1 and SCD2 in the kidney after LXR agonist treatment. SCDs are the rate-limiting enzymes catalyzing the synthesis of oleate (18:1) and palmitoleate (C16:1) from stearate (C18:0) and palmitate (C16:0), respectively (24). Oleate and palmitoleate, the main product of SCDs, represent the major MUFAs of membrane phospholipids, triglycerides, and cholesterol esters, and the ratio of saturated fatty acid to MUFAs affects phospholipids composition and contributes to the pathogenesis of many diseases, including atherosclerosis, obesity, and diabetes (23). Studies in genetically engineered mice clearly indicate that SCD1 activity is inversely associated with insulin sensitivity and adiposity and correlates with triglyceride and cholesterol ester biosynthesis in vivo (7, 8, 25, 27). Thus the induction of SCD1 and SCD2 gene expression and enzymatic activity by LXR activation may alter the composition of membrane phospholipids and triglyceride and cholesterol ester content in the kidney, thereby affecting renal function.

Reduced SCD1 expression, which is associated with decreased fatty acid synthesis and increased fatty acid oxidation in primary hepatocytes (12), was previously reported in the...
kidney of diabetic NOD mice (36), suggesting SCD1 may play an important role in pathogenesis of diabetic nephropathy. The present studies provide the first in vivo evidence that renal SCD1 gene expression is differentially increased in the proximal straight tubules by an LXR agonist. Because SCD1 catalyzes the Δ9-cis desaturation of saturated fatty acyl-CoAs, including palmitoyl- and stearoyl-CoA, its activation by LXR agonist may increase the ratio of oleate to palmitoleate to stearate to palmitate in epithelial cells of the proximal straight tubules, thereby improving cell membrane fluidity and enhancing cell function and integrity (22). In addition, increased expression of SCD1 in proximal straight tubules by LXR agonists may indirectly protect these cells from harmful effects of free cholesterol and free saturated fatty acids by converting them to cholesterol ester and triglyceride (13, 16, 21). It has been previously shown that excessive intracellular free cholesterol induces a number of cellular events, including cholesterol crystallization and cell death (1, 13), and increased SCD1 activity promotes desaturation of saturated fatty acids and enhances the synthesis of less toxic cholesterol ester (21). With respect to fatty acids, excess unsaturated oleic acid leads to triglyceride accumulation and is well tolerated, whereas over-load-saturated palmitic acid caused marked apoptosis and is poorly incorporated into triglyceride (16). In addition, enhanced SCD1 expression has been reported to be associated with an increase in cholesterol efflux to HDL2 (35), possibly due to more unsaturated lipid composition (19). Considered together, the present studies suggest the LXRs could play an important role in maintaining the lipid homeostasis in renal proximal tubules by promoting fatty acid desaturation. Increased SCD1 expression may lead to enhanced intracellular levels of unsaturated fatty acids (35), thereby serving a protective function against lipotoxicity through promotion of tri-glyceride and cholesterol ester biosynthesis and passive efflux of free cholesterol.

At present, little is known about the mechanisms by which TO-901317 upregulates SCD1 gene expression in the proximal straight tubules. Sequence analysis of 716 bp of mouse SCD1 promoter sequence revealed no LXRE exists in this region, suggesting SCD1 gene transcription is not under direct control of LXR receptors. In support of this, overexpression of an LXRα expression vector did not induce SCD1-luciferase activity. Among multiple genes induced by TO-901317 in the kidney, SREBP1 is a direct target gene of LXRs (38) and a key regulator of many genes involved in lipid metabolism, including SCD1 (4, 28). SCD1 is reported to be directly regulated by SREBP1c via binding to the SREBP binding element (SRE) in its promoter region (4). In the present studies, we demonstrated that TO-901317 enhanced SREBP1c transcription and increased active SREBP1c (SREBP1cN) expression in cultured renal proximal tubule cells, which may contribute to increased SCD1 expression in these cells.

In conclusion, pharmacological activation of LXRs by TO-901317 results in marked upregulation of SCD1 gene expression via the SREBP1c pathway in renal proximal straight tubule cells (Fig. 9), where enhanced SCD1 activity may mediate fatty acid desaturation and therefore modulate cellular function and lipid homeostasis.

GRANTS

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REFERENCES