Expression of peroxisome proliferator-activated receptors in urinary tract of rabbits and humans

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Guan, YouFei, YaHua Zhang, Linda Davis, Matthew D. Breyer. Expression of peroxisome proliferator-activated receptors in urinary tract of rabbits and humans. Am. J. Physiol. 273 (Renal Physiol. 42): F1013–F1022. 1997.— Peroxisome proliferator-activated receptors (PPARs, α, β/δ, and γ) are members of the nuclear receptor superfamily of ligand-activated transcription factors. PPARs regulate the expression of genes involved in lipid metabolism. 8(S)-hydroxyeicosatetraenoic acid (8-S-HETE), leukotriene B4 (LTB4), and hypolipidemic fibrates activate PPARα, whereas PPARγ is activated by prostaglandin metabolites. The present studies examined the intrarenal and tissue distribution of rabbit and human PPARα, β/δ, and γ mRNAs. Nuclease protection showed PPARα predominated in liver, heart, and kidney, whereas PPARγ, a putative adipose-specific transcription factor, was in white adipose tissue, bladder, and ileum, followed by kidney and spleen. Lower expression levels of PPARβ/δ were observed in several tissues. In situ hybridization of kidney showed PPARα mRNA predominated in proximal tubules and medullary thick ascending limbs of both rabbit and human. PPARγ was exclusively expressed in medullary collecting duct and papillary urothelium. Immunoblot confirmed the expression of PPARγ protein in freshly isolated inner medullary collecting ducts. mRNAs for all the PPARs were expressed in the ureter and bladder in both rabbit and human, but PPARγ expression was greatest. This distinct distribution of PPAR isoforms has important implications for lipid-activated gene transcription in urinary epithelia.

PEROXISOME PROLIFERATORS comprise a group of structurally diverse compounds including hypolipidemic fibrates (e.g., clofibrate) and leukotriene analogs. When administered to rodents, these compounds induce proliferation of peroxisomes and upregulate several enzymes involved in lipid metabolism (18, 36). Peroxisome proliferators are now known to bind to a family of nuclear receptors designated peroxisome proliferator-activated receptors (PPARs). PPARs were originally identified as members of the steroid hormone receptor superfamily of nuclear transcription factors which includes the thyroid hormone receptors and retinoic acid receptors (41). PPARs form heterodimers with the 9-cis retinoic acid receptor, RXRα (25). These heterodimers bind to characteristic DNA sequences termed peroxisome proliferator response elements (PPRE) located in the 5′-flanking region of target genes (12, 30, 44, 47). After binding the PPREs, PPARs activate transcription of several genes including acyl-CoA synthase, acyl-CoA oxidase (44), cytochrome P-450 fatty acid ω-hydroxylase (27, 30) and phosphoenolpyruvate carboxykinase (40).

Since the first PPAR (PPARα) was cloned from mouse liver (18), two additional PPAR genes have been recognized (8). These genes are designated PPARβ (also referred to as PPARβ or NUC1) and PPARγ (19, 24). These PPARs are differentially activated by a variety of fatty acids (19, 24, 45). Whereas PPARα is activated by fibrates, 8(S)-hydroxyeicosatetraenoic acid (8-S-HETE), and leukotriene B4 (LTB4) (7, 45), PPARγ is activated by 15-deoxy-Δ12,14-prostaglandin J2 (15-deoxy-Δ12,14-PGJ2), a metabolite of PGD2 (12, 23). Importantly, PPARγ is also activated by the anti-diabetic thiazolidinediones. A PPARβ/δ-selective ligand has not yet been identified. A growing body of evidence demonstrates that PPARα, β/δ, and γ are not only activated by different ligands but that they are also expressed in distinct tissues. Whereas PPARα is expressed in liver, heart, brain, muscle, and kidney (1, 19, 24), PPARγ has been relatively selectively expressed in adipose tissue (42). Lower expression levels of PPARγ have also been reported in other tissues (9). In the present studies, we examined the expression of PPARα, β/δ, and γ in rabbit tissues and determined their distribution along the urinary tract in both rabbits and humans.

MATERIALS AND METHODS

Partial cloning of rabbit PPARα, β/δ, δ, and γ. Reverse transcription-polymerase chain reaction (RT-PCR) was used to amplify a portion of rabbit PPARα, β/δ, δ, and γ from mRNA isolated from female New Zealand White rabbits (1.5–2.0 kg) as described below. Primers were selected from conserved sequences in the human, rat, mouse, and Xenopus homologs. For rabbit PPARα, a cDNA comprising a portion of the DNA binding and ligand binding domains (D and E/F) (8) was obtained by RT-PCR using liver RNA as a template and primers derived from human PPARα cDNA sequence (5′ AGA ACT TCA ACA TGA AGG TCA 3′ for sense and 5′ GCC AGG ACG ATC TCC ACA GCA AAT 3′ for antisense) (28). A cDNA comprising a portion of the transactivation and DNA binding (A/B and C) domains of rabbit PPARβ was amplified from kidney cDNA using the primers derived from mouse PPARβ (upstream primer, 5′ CGG GAA GAG GAG AAA GAG GAA GTG 3′; downstream primer, 5′ CTT GTT GCG GTT CTT GGT GAT 3′) (24). For rabbit PPARγ, a pair of primers based on human homolog (sense, 5′ CCC TCA TGG CAA TTG AAT GTC GTG 3′; and antisense, 5′ TCG CAG CTC TAG AAA CTC CCT 3′) were used to amplify a cDNA sequence comprising part of the DNA binding and ligand binding (C and E/F) domains (14). Total RNA was purified from rabbit kidney and liver using Trizol-Reagent (GIBCO-BRL) and reverse transcribed to single-stranded cDNAs using Moloney murine leukemia virus reverse transcriptase and 2.5 µM of random hexamers according to manufacturer's
instructions (GeneAmp RNA PCR kit; Perkin-Elmer Cetus, Norwalk, CT). The cDNAs were then amplified using PPAR-selective primers. PCR reactions were carried out in 10 mM tris(hydroxymethyl)aminomethane (Tris) hydrochloride (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, 58°C for 0.5 min, and 72°C for 0.5 min for 35 cycles in a thermal cycler (model 9600, Perkin-Elmer Cetus). Amplified cDNAs were ligated into pCR II vector (Invitrogen) and sequenced. Nucleotide and predicted amino acid sequence were compared using the GenBank database and BLAST and CLUSTAL programs at the National Institutes of Health data bank.

Preparation of human PPARα, -β, and -γ probes. Three human PPAR cDNA fragments were generated by RT-PCR using human liver and kidney total RNA (Clontech, Palo Alto, CA) and human PPAR isoform-specific oligonucleotides, as follows: 5′ AGA ACT TCA ACA TGA ACA AGG TCA 3′ (sense) and 5′ GCC AGG ACG ATC TCC ACA GCA AAT 3′ (antisense) for human PPARα; 5′ AGC AGC CTC TTC CTC AAC GAC CAG 3′ (sense) and 5′ GGT GCT GGT GCT CTT GAT 3′ (antisense) for PPARβ/δ; and 5′ CCC TCA TGG CAA TTG AAT GTG GTG 3′ (sense) and 5′ TCG CAG CGT CTT TAG AAG CTT CCT 3′ (antisense) for PPARγ. After amplification, a 524-bp PPARα, a 471-bp PPARβ/δ, and 761-bp PPARγ cDNA were sequenced and subcloned in Blue-script SK(−) vector (Stratagene). Antisense and sense probes were transcribed using appropriate RNA polymerases (MAXIscript; Ambion, Austin, TX) and [35S]-UTP as labeled isotope for in situ hybridization.

Solution hybridization/ribonuclease protection assays. Total RNA from various rabbit tissues was isolated by using Trizol-Reagent (GIBCO-BRL). Briefly, 1 mg of tissue sample was homogenized in 10 ml Trizol reagent, and 1/10 vol of chloroform was added and vortex mixed for 15 s. The phases were separated by centrifugation (12,000 g, 10 min), and isopropanol alcohol was added to the aqueous phase to precipitate total RNA. The resulting RNA was dissolved in diethyl pyrocarbonate-treated water.

Ribonuclease (RNase) protection assay was performed as described earlier (3). Briefly, the plasmids [pBluescript SK(−), Stratagene] containing rabbit PPARα (109 bp of Xma I fragment), PPARβ (337 bp of Pvu II fragment), and PPARγ (316 bp of Eco R I fragment) inserts were linearized with appropriate restriction enzymes. Radioactive riboprobes were synthesized in vitro from 1 µg of linearized plasmids containing three different cDNA fragments of PPAR isoforms using MAXIscript kit (Ambion) for 1 h at 37°C in a total volume of 20 µl. The reaction buffer contained 10 mM dithiothreitol (DTT), 0.5 mM each of ATP, CTP, and GTP, 2.5 µM of UTP, and 5 µl of 800 Ci/mmol [α-32P]UTP at 10 mcCi/ml (DuPont, NEN, Boston, MA). Hybridization buffer included 80% deionized formamide, 100 mM sodium citrate, pH 6.4, and 1 mM EDTA (RPA II, Ambion). Twenty micrograms of total RNA were incubated at 45°C for 12 h in hybridization buffer with 5 × 10⁶ cpm labeled riboprobes. After hybridization, RNase digestion was carried out at 37°C for 30 min, and precipitated protected fragments were separated on 4% polyacrylamide gel at 200 V for 3 h. The gel was exposed to Kodak XAR-5 film overnight at −80°C with intensifying screens.

Medullary interstitial cell and cortical collecting duct cell culture. Rabbit medullary interstitial cells (RMICs) were cultured as previously described (15). Briefly, the kidney of a female New Zealand White rabbit (1.5–2.0 kg) was removed. The medulla was dissected and minced in 5 ml of sterile RPMI 1640 plus 20% (vol/vol) fetal bovine serum (FBS, Hyclone). The homogenate was injected subcutaneously in the abdominal wall. Twenty to thirty days post-surgery, the subcutaneous renal medullary nodules were minced into 1-mm fragments and explanted in culture plates. Cells were cultured in RPMI-1640 tissue culture medium supplemented with 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer, l-glutamine (2 mM), 20% (vol/vol) FBS, streptomycin, and penicillin. Cultures were incubated at 37°C in 95% O2-5% CO2. Cells in their third to fourth passage were generally used for experimentation.

Cortical collecting ducts. Primary cultures of rabbit cortical collecting ducts (CCDs) were grown on semipermeable supports (Transwell; Costar, Cambridge, MA) as previously described (6). Briefly, two rabbit kidneys were perfused with Krebs-Ringer. The renal cortex was separated from the capsule and medulla via gross dissection and passed through a tissue press. The dispersed tissue was digested with collagenase (0.1%), deoxyribonuclease (100 U/ml), and soybean trypsin inhibitor (1,000 U/ml, 37°C) in Krebs-Ringer. This suspension was then poured over plates precoated with monoclonal antibody specific for rabbit CCD (3G10) and incubated for 10 min. Nonadherent cells were removed by gentle aspiration. The adherent CCD cells were resuspended by sharp mechanical blow and plated onto collagen-coated semipermeable supports (Costar). Cells were grown to confluence in Dulbecco's modified Eagle's medium with 1 µM aldosterone, 1% penicillin-streptomycin-neomycin, supplemented with 10% FBS at 37°C in 95% O2-5% CO2.

Preparation of freshly isolated inner medullary collecting duct cells. Inner medullary collecting ducts (IMCDs) were isolated by a modification of a method described by Zeidel et al. (46). Rabbits were killed, and kidneys were perfused free of blood with 30 ml of ice-cold noncarbonate Ringer solution diluted 1:1 with j oklik minimum essential medium containing 5% FBS. Kidneys were then perfused with 5 ml of j oklik medium containing 0.2% collagenase. Inner medullar cells were excised, finely minced, and incubated with 0.2% collagenase in j oklik medium for 90 min at 37°C in a shaking water bath. The resulting mixed inner medullary cell suspension was fractionated over 16% Ficoll layer in noncarbonate Ringer solution by centrifugation for 45 min at 2,300 g. IMCDs were located at the top of the 16% Ficoll layer. The cells were collected and washed twice with j oklik medium supplemented with 10% FBS. Cell viability was measured by trypan blue exclusion and assessed by phase-contrast microscopy.

Immunoblotting. Confluent RMICs and CCDs and freshly isolated IMCDs were harvested in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (120 mM Tris-HCl, pH 6.5, 4% SDS, 5 mM DTT, and 20% glycerol). This material was then heated in boiling water for 3 min, and protein concentration was determined. Ten milligrams of protein extract were loaded onto a 10% SDS-PAGE minigel and run at 100 V. Proteins were transferred to nitrocellulose membrane at 14 V overnight at 4°C. The membrane was washed three times with phosphate-buffered saline (PBS) and incubated in blocking buffer (Tris-buffered saline which contained 150 mM NaCl, 50 mM Tris, 0.05% Tween 20 detergent, and 5% Carnation nonfat dry milk, pH 7.5) for 1 h at 24°C, followed by three washings with blocking buffer at 5-min intervals. The nitrocellulose membrane was then incubated in the anti-PPARγ antibody (rabbit anti-mouse PPARγ1,2 polyclonal antibody; Biomol, Plymouth Meeting, PA) diluted 1:2,000 in blocking buffer for 2 h at room temperature. Following three additional washings, the membrane was incubated with biotinylated anti-rabbit immunoglobulin G antibody (1:2,000; Vector, Burlingame, CA) for 1 h, followed by three 15-min washings. Antibody labeling was visualized by addition of chemiluminescence.
RESULTS

Cloning and sequencing of rabbit PPARα, -β/δ, and -γ CDNAs. Sequencing of a 524-bp rabbit PPARα fragment amplified by RT-PCR revealed a predicted amino acid sequence that was 96.0% and 97.1% identical to the human and mouse PPARα, respectively (Fig. 1).

The predicted amino acid sequences of a 337-bp rabbit PPARβ/δ fragment demonstrated 92.9% and 87.5% amino acid identity to human and mouse homologs. Finally, a 758-bp rabbit PPARγ fragment was 96.4% and 98.0% identical to human and mouse homologs at the amino acid level (Fig. 1). At the nucleotide level, there was less than 42% identity between the cloned cDNA fragments of rabbit PPARα, β/δ, and γ (although these cDNAs fragments represent different regions of the full-length PPAR).

PPARα, -β/δ, and -γ are differentially expressed in renal tissues. The distribution of rabbit PPARα, -β/δ, and -γ mRNA was determined by nuclease protection (Fig. 2). Comparable mRNA loading was confirmed by simultaneous nuclease protection with a rabbit glyceroldehyde-3-phosphate dehydrogenase (GAPDH) riboprobe. PPARα expression was highest in liver, kidney, and heart, followed by ileum, adrenal, and urinary bladder. Lower but significant PPARα message expression was observed in lung, stomach, and brain. PPARγ was the only PPAR detected by nuclease protection in white adipose tissue (data not shown). No PPARα or -β/δ were observed in mRNA harvested from white adipose tissue. PPARγ mRNA expression in white adipose tissue was greater than levels observed in any other...
Nuclease protection also demonstrated high PPARγ expression in bladder and ileum (Fig. 2). Lower levels were expressed in kidney, spleen, adrenal, heart, liver, lung, and brain. Although PPARβ/δ mRNA was widely distributed, expression levels were generally much lower than for PPARα and -γ. PPARβ/δ mRNA was not detected in significant amounts in the liver. Thus the PPAR isoforms are differentially expressed in adult rabbit tissues.

Intrarenal localization of PPARα, -β/δ, and -γ. In situ hybridization was used to examine the distribution of the three PPAR isoforms in the kidney, ureter, and
bladder. A PPARα antisense riboprobe predominantly hybridized to tubules in the renal cortex and outer medulla of both rabbit and human kidney (Fig. 3). Photomicrographs demonstrated intense labeling of proximal tubules and distal nephron segments including thick ascending limb. In contrast no labeling of glomeruli or collecting duct was noted (Fig. 3). In renal outer medulla, PPARα hybridized to thick ascending limb and the S3 segment of the proximal straight tubule. No labeling of cortical thick ascending limbs, collecting ducts, or any structures in the inner medulla was detected.

In contrast to PPARα, no significant expression of PPARβ/δ or PPARγ was detected in the renal cortex; however, significant expression of PPARγ was detected in the IMCDs of both rabbit and human (Fig. 4). PPARγ mRNA was also detected in the urothelium lining the renal papillae. PPARγ expression in the kidney was specific for the medullary collecting duct, without significant detection in other portions in the kidney. Furthermore, neither PPARα nor β/δ were detected in the medullary collecting duct. No hybridization of sense riboprobes for PPARα or either of the other two PPARs (PPARβ/δ or PPARγ) was observed (data not shown).

PPARγ protein is highly expressed in IMCDs. Immunoblots of protein extracts from RMICs, CCDs, and IMCDs using a polyclonal anti-PPARγ antibody demonstrated that IMCDs highly expressed PPARγ with lower expression in cultured CCDs and RMICs (Fig. 5). The presence of PPARγ protein in IMCDs corresponds with in situ hybridization data.
PPAR expression in bladder and ureter. PPARγ mRNA labeling was particularly intense in the transitional urothelium of rabbit and human ureter and bladder (Figs. 6 and 7). The expression of PPARγ was restricted to the epithelium, with no expression detected in surrounding smooth muscle. PPARα and PPARβ/δ were also detected in the urothelium of the ureter and bladder of both species, albeit less intensely. Uroepithelial expression of PPARα was significantly lower than PPARγ, with PPARβ/δ expression appearing to be intermediate.

**DISCUSSION**

The kidney is a major site of fatty acid, ketone body, and prostaglandin metabolism (8, 15, 26), as well as prostaglandin synthesis (9). One major mechanism regulating lipid metabolism is the transcriptional con-
control of enzymes involved in oxidation of fatty acids (10). The PPARs are ligand-activated transcription factors intimately involved in the expression of several of these enzymes. We cloned cDNA fragments of rabbit PPARα, -β/δ, and -γ and mapped their distribution in normal rabbit tissues and the urinary tracts of both rabbit and human. The rabbit PPAR fragments cloned for these studies are highly homologous to their human and murine counterparts. PPAR mRNA species were highly expressed in kidney as well as other tissues more classically identified with lipid metabolism, such as liver and adipose tissue. The mRNA for these PPAR isoforms display distinct distribution in normal tissues and within the kidney.

Recent evidence suggests PPARα, -β/δ, and -γ complex with and are activated by distinct endogenous lipid ligands (24, 45). Among the ligands demonstrated to activate PPAR-mediated gene transcription are arachidonate metabolites including LTB4, PGA2, PGJ2, PGD2, PGJ12, and 8-S-HETE (7, 12, 23, 45). PPARα is uniquely activated by LTB4 and 8-S-HETE, whereas prostaglandins PGA2, PGD2, and PGJ2 uniquely activate PPARγ (12, 21, 23, 45). Upon binding their respective ligands, PPARs form heterodimers with the retinoid X receptor (RXR). This complex binds to a specific response element [peroxisome proliferator response element (PPRE)] in target genes (33, 35, 41, 43, 48). The genes downstream of these PPREs include enzymes implicated in regulation of fatty acid metabolism, cholesterol metabolism, and adipogenesis (38). The present studies suggest unique intrarenal localization of the PPARs, implying differential control of their activation along the urinary tract.

As demonstrated in a previous report examining PPAR expression in rat (1), the present results show that PPARα is abundantly expressed in tissues with high mitochondrial and β-oxidation activity including liver, renal cortex, intestinal mucosa, and heart. This may correspond with the demonstrated role of PPARα in regulating genes encoding mitochondrial and peroxisomal activities involved in the metabolism of fatty acids. In situ hybridization demonstrates that PPARα mRNA is highly expressed in proximal tubules, with little labeling of glomeruli or collecting ducts. PPARα induces the expression of a variety of genes in the rabbit including cytochrome P-450 4A6 (CYP4A6) (27). CYP4A6 is an ω-hydroxylase for arachidonate, laurate, and other fatty acids (36). CYP4A6 has been shown...
have an upstream PPRE, and enzyme expression is induced by fibrates via PPARα (27). Although the intrarenal expression of CYP4A6 in rabbit has not yet been mapped, the cytochrome P-450 4A family is highly expressed in rat proximal tubule (2) and suggests PPARα may regulate fatty acid catabolism via induction of these enzymes in the proximal tubule. There is less data on candidate genes that may be activated by PPARα in the medullary thick ascending limb, the other major site of PPARα mRNA expression along the nephron.

Of the three PPAR isoforms, the biochemical and physiological role of PPARβ/δ is least clearly defined. Although PPARβ/δ was detected by nucleic acid protection in rabbit kidney, no region-specific labeling was observed by in situ hybridization. In contrast, significant expression of PPARβ/δ mRNA in transitional epithelium of the urinary bladder and ureter was detected, suggesting this PPAR may play a role in regulating gene expression in these tissues. To date, no specific ligand that activates PPARβ/δ has been identified. A recent reported (20) showed that PPARβ/δ can competitively inhibit the activity of other PPARs either at the level of the PPRE or by titrating out a limiting factor required for the transcription activity of PPARα (e.g., RXRα). Since PPARγ is also highly expressed in the urothelium, such a mechanism may play a role in PPARβ/δ action this tissue.

PPARγ is highly expressed in adipose tissue, but lower expression levels have been previously reported in other tissues (9). After binding a peroxisome proliferator, such as clofibrate, WY-14,643, 15-deoxy-12,14-PGJ2, or thiazolidinediones, PPARγ activates adipogenesis, transforming fibroblasts into adipocytes (12, 23, 38). Importantly, the thiazolidinediones have recently been approved for use in the treatment of diabetes mellitus (29) and have been shown to be particularly high-affinity ligands for PPARγ (12, 23). The present studies demonstrate for the first time that PPARγ is not only expressed in adipose tissue but is also highly expressed in the distal urinary tract both at the mRNA and protein level. In both rabbit and human kidney, PPARγ mRNA was predominantly detected in IMCD. It is also highly expressed in the transitional urothelium of ureter and bladder. This contrasts with previous studies in rat where expression of PPARγ in the renal medulla was not reported (1). PPARγ expression in urinary bladder was not examined in those studies. Although the localization of the PPAR mRNAs in rabbit and human kidney are consistent with each other, one must be cautious in interpreting their distribution in humans, given the limited number of patients studied.

Little is known about the biological roles of PPARγ in collecting duct, ureter, or bladder epithelium; however, its expression in these tissues could have implications for renal effects of the antidiabetic thiazolidinediones as well as in bladder carcinogenesis (32). It is relevant to note that PPARγ is not only activated by prostaglandins but that the medullary collecting is also the major site of prostaglandin synthesis in the kidney (11, 37). Although the urothelium has not been demonstrated to be a major site of prostaglandin biosynthesis, prostaglandin concentrations in the urine are in the nanomolar range, well above those in plasma (9, 31). Importantly, PGJ2 metabolites, proposed ligands for PPARγ, have been reported in human urine (16). Thus high endogenous prostaglandin concentrations in the urine could activate PPARγ in the medullary collecting duct, ureter, and bladder.

Diverse biological effects of the prostaglandin ligands for PPARγ, including PGA2, PGD2, and PGJ2, have been described. These effects range from inhibition of cell cycle progression and induction of apoptosis to suppression of viral replication (13, 17, 22, 34). Although many of the biological effects of the prostaglandins are mediated by G protein coupled, membrane-spanning receptors (4, 39), the effects of prostaglandins on cell proliferation appear to be mediated by nuclear binding proteins (13, 26). PPARs have not been directly implicated as the nuclear receptors mediating these effects of PGA2, PGD2, or PGJ2 on cell growth and death; however, these or other, similar, prostaglandin-activated nuclear transcription factors may be involved (22, 26). Roles for PPARs in processes not directly involved in lipid metabolism remain to be established. Even so, PPAR-mediated regulation of prostaglandin metabolism might modify local concentrations of prostaglandins and their effects on urinary epithelia.

In summary, we have cloned fragments of rabbit PPARα, -β/δ, and -γ and described their tissue distribution. Within the kidney and in the lower urinary tract, there is distinct distribution of these PPAR isoforms. In both human and rabbit kidney, PPARα is predominantly expressed in proximal tubules, with lower expression in medullary thick ascending limb. In contrast, PPARγ is predominantly located in IMCDs. We have also demonstrated that PPARγ protein is expressed in the IMCD. No distinct intrarenal localization of PPARβ/δ was observed. High levels of expression of PPARγ mRNA were detected in the urothelium of ureter and bladder of both rabbit and human. Lower but significant expression of PPARβ/δ was also detected in the urothelium. The physiological roles of these lipid-activated transcription factors in urinary epithelia remain to be determined.

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