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Research Paper

Androgen Receptor is Responsible for Rat Organic Cation Transporter 2 Gene Regulation but not for rOCT1 and rOCT3

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Purpose. Organic cation transporters 1–3 (OCT1–3; Slc22a1–3) mediate the membrane transport of organic cations in the kidney. We previously reported that rat (r)OCT2 expression in the kidney was regulated by testosterone. In this study, we examined the transcriptional mechanisms underlying the testosterone-dependent regulation of rOCT2 expression.
Methods. Approximately 3000-bp fragments of the rOCT1–3 promoter region were isolated, and promoter activities were measured in the renal epithelial cell line LLC-PK_1 with the coexpression of rat androgen receptor.
Results. Among reporter constructs tested, only rOCT2 promoter activity was stimulated by testosterone. This stimulation was suppressed by nilutamide, an antiandrogen drug. Reporter assays using deletion constructs and mutational constructs of putative androgen response elements (ARE) in the rOCT2 promoter region suggested that two AREs, located at approximately −3000 and −1300, respectively, play an important role in the induction by testosterone.
Conclusions. Testosterone induces the expression of rOCT2, but not of rOCT1 and rOCT3, via the AR-mediated transcriptional pathway. This is the first study to address the transcriptional mechanisms of testosterone-dependent gene regulation of the Slc22 family.

KEY WORDS: gender difference; kidney; promoter; rOCT2; testosterone.

INTRODUCTION

Proximal tubules play important roles in the renal elimination of drugs. Cationic drugs are secreted from blood to urine by combined efforts of two distinct classes of organic cation transporters: one driven by the transmembrane electrical potential difference in the basolateral membranes, and the other driven by the transmembrane H^+ gradient in the brush-border membranes (1). Molecular cloning studies identified three kinds of organic cation transporters (OCT1–3), and their physiological and pharmacokinetic roles have been evaluated (2,3). Rat (r)OCT1 (Slc22a1) is expressed abundantly in the liver and kidney (4), whereas rOCT2 (Slc22a2) is expressed in the kidney, but not in the liver (5). These transporters are localized to the basolateral membranes of renal proximal tubules (6,7). rOCT3 (Slc22a3) is expressed predominantly in the placenta, and also in the intestine, heart, brain, lung, and very weakly in the kidney (8). Functional studies using heterologous expression systems revealed that all OCTs recognized a variety of organic cations with different molecular structures including tetraethylammonium, 1-methyl-4-phenylpyridinium, N^1-methylnicotinamide, choline, and dopamine (8,9).

It was reported that the uptake of tetraethylammonium was greater in renal cortical slices of male rats than female rats (10), suggesting gender differences in the basolateral membrane transport activity for organic cations. We found that expression level of rOCT2, but neither rOCT1 nor rOCT3, in the kidney was much higher in males than females and suggested that rOCT2 is responsible for gender differences in renal basolateral membrane organic cation transport activity (11). Furthermore, we demonstrated that treatment of male and female rats with testosterone significantly increased rOCT2 expression in the kidney (12). These results suggested that testosterone plays a pivotal role in the transcriptional regulation of the rOCT2 gene. However, no information has been available to demonstrate this process.

Androgens, such as testosterone, are main hormones responsible for the male phenotype (13). As with other steroid hormones, many effects of androgen are mediated by a specific intracellular androgen receptor (AR; NR3C4). AR activated by testosterone binds to the androgen response element (ARE) in the 5′-flanking region of target genes and is responsible for the expression of various genes such as the C3 subunit of prostatin (14) and prostate-specific antigen gene (15). Based on our previous studies, we hypothesized that AR could be involved in the regulation of the rOCT2 gene.

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ABBREVIATIONS: AR, androgen receptor; ARE, androgen response element; bp, base pair; CYP, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MMTV, mouse mammary tumor virus; OCT, organic cation transporter; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends.

In the present study, therefore, we examined the effects of testosterone on the promoter activities of rOCTs to understand the role of this hormone in the gender differences of rOCT2 expression.

MATERIALS AND METHODS

Materials

Restriction enzymes were obtained from New England BioLabs (Beverly, MA, USA). T4 kinase and T4 DNA ligase were purchased from TaKaRa (Ootu, Japan). [α-^{32}P] CTP was obtained from Amersham Biosciences, Inc. (Buckinghamshire, UK). Testosterone was purchased from Nacalai Tesque (Kyoto, Japan). Nilutamide was obtained from Sigma (St. Louis, MO, USA).

Determination of Putative Transcriptional Start Sites

The putative transcriptional start sites for rOCT1-3 were determined by 5′-rapid amplification of cDNA ends (5′-RACE) using the rat Marathon-Ready cDNA kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. The rOCT2 gene-specific primers for the 5′-RACE were designed and synthesized based on the genomic sequence. The 5′-RACE was performed with adapter primer 1 that came with the kit and a gene-specific primer of rOCT1 (accession number NM_012697), 5′-CACAACCAGGGAGCCCAGGAAGAAG CCC-3′ (538 to 511). Nested polymerase chain reaction (PCR) was performed with adapter primer 2 and a nested gene-specific primer of rOCT1, 5′-CAGGAAGGCTTGTTTCTGGAAC CAGCCA-3′ (106 to 79). The rOCT2 gene-specific primers are as follows: a gene-specific primer of rOCT2 (accession number NM_031584), 5′-CCTTCATAAGAGGTTGTTAA GCCTGCCACTGGA-3′ (605 to 577); a nested gene-specific primer of rOCT2, 5′-GGAGGCACCAGACAGCAGGCT AAGAGG-3′ (187 to 160). The rOCT3 gene-specific primers are as follows: a gene-specific primer of rOCT3 (accession number NM_019230), 5′-GCCCAGGAAGACCACACCAA CGAAGAG-3′ (520 to 493); a nested gene-specific primer of rOCT3, 5′-GTCAGGCACAGCAGCAGGAACACGCG CC-3′ (474 to 450).

Genomic Cloning of rOCT1, rOCT2, and rOCT3 Promoters

rOCT1 and rOCT3 promoters were isolated from the rat genome (Clontech) by a PCR-based method using the following primers designed based on the rat genomic DNA (accession number AC114389): rOCT1 sense 5′-GGACGCGTCCA TGCTCTGCGAACTGAGGT-3′ and antisense 5′-GGCTC GAGGACTGCCACCAGGGGTTCAT-3′; rOCT3 sense 5′-GGACGCGTCCCTTCGAAGCAGAGGGAAAA-3′ and antisense 5′-GGAGATCTTGCAGGAATAGCCTCCAGT GC-3′. On the other hand, the rOCT2 promoter was isolated from the rat genomic library (Clontech) with a conventional plaque hybridization method. The probe was prepared by PCR using rat genomic DNA (Clontech) as a template. The primers, designed based on the rat genomic DNA (accession number AC114389), were as follows: 5′-GGCTTGGGAGAT GGCTAAGTA-3′ and 5′-TCACAGCCATGTGGGACA TGT-3′. Phage DNA with a long rOCT2 promoter was prepared with a QIAGEN lambda midi kit (Qiagen, Hilden, Germany) and partially sequenced. The transcription factor-binding sites were predicted with TRANSFAC 5.0 software (http://www.gene-regulation.com/cgi-bin/pub/programs/match/bin/match.cgi?), with a core similarity of 0.95 and a matrix similarity of 0.90.

Construction of Reporter Gene and Rat Androgen Receptor Expression Plasmid

Approximately 3-kb fragments corresponding to the 5′-flanking regions of the rOCT genes were subcloned into a pGL3-Basic luciferase gene vector (Promega, Madison, WI, USA) to yield rOCT1 (−3025/+23), rOCT2 (−3036/+242), and rOCT3 (−3001/+31). The deletion constructs rOCT2 (−1895/+242), rOCT2 (−819/+242), rOCT3 (−1095/+31), and rOCT3 (−515/+31) were prepared with the restriction enzymes. The mouse mammary tumor virus (MMTV) gene excised from pMSG (Amersham Biosciences) was subcloned into pGL3 to yield MMTV-pGL3.

ARE mutants were constructed using QuikChange® II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. Primer sequences are listed in Table I.

Table I. Sequences of Mutation Primers

Primer	Sequence
ARE-1 S	5′-GGCCTCTGTGGTAGAGGA**GCC**ACTTAATCTTGCTGC-3′
ARE-1 AS	5′-GCAGCAAGATTAAGTGGCTCCTCTACCACAGAGGCC-3′
ARE-2 S	5′-CCTATGAGGACCAAGC**GCC**ACTCTCATGTCCTTCCTG-3′
ARE-2 AS	5′-CAGGAAGGACATGAGAGTGGCGCTTGGTCCTCATAGG-3′
ARE-3 S	5′-CCTTGGCACAGGA**GCC**TCTCCTTGACTCTCACCTG-3′
ARE-3 AS	5′-CAGGTGAGAGTCAAGGAGAGGCTCCTGTGCCAAGG-3′
ARE-4 S	5′-GCGTCCTGATACAGAC**GCC**ACCCATGAGTCAGTCAC-3′
ARE-4 AS	5′-GTGACTGACTCATGGGTGGCGTCTGTATCAGGACGC-3′
ARE-5 S	5′-CAGCAGGAAAGAGAGACTACC**GCC**TTCCCTGGCATTTGG-3′
ARE-5 AS	5′-CCAAATGCCAGGGAAGGCGGTAGTCTCTCTTTCCTGCTG-3′

Underlined sequences are putative ARE sequences, and bold characters indicate positions of the ARE mutation.
ARE = androgen response element.

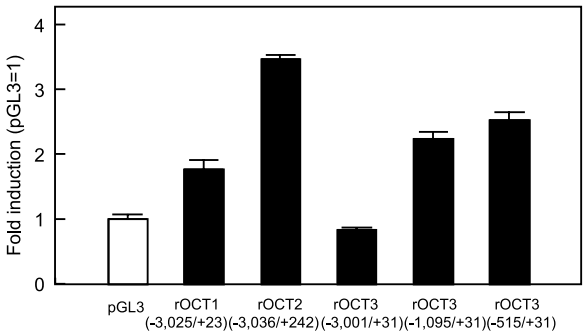

Fig. 1. Promoter activities of rat organic cation transporter (rOCT) genes. rOCT1–3 promoter constructs were transfected into LLC-PK_1 cells for luciferase assays. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Each column represents the mean ± SE of three independent experiments.

cDNA for rat AR (rAR: accession number, NM_012502) was isolated from rat kidney cDNA by a PCR-based method using the following primers: sense 5′-GGGATCCAGGATG GAGGTGCAGTTAGGG-3′ (991 to 1011) and antisense 5′-GGCTCGAGTTTCCAAATCTTCACTGTGTG-3′ (3713 to 3693). PCR was performed using Pfu polymerase (Stratagene) as follows: 95°C for 3 min; 35 cycles of 95°C for 1 min, 60°C for 1 min, 72°C for 8 min; and a final extension at 72°C for 10 min. The PCR product was subcloned into the expression vector pBK-CMV (Stratagene).

Cell Culture and Luciferase Assay

The porcine kidney epithelial cell line LLC-PK_1 was obtained from American Type Culture Collection (ATCC CRL-1392; Rockville, MD) and cultured as described previously (16). For the luciferase assay, the cells were seeded at 1.5×10^5 cells into 24-well plates in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% charcoal-stripped fetal bovine serum (FBS). Cells were transfected by 5-h exposure to LipofectAMINEplus (Invitrogen Japan KK, Tokyo, Japan), with each well containing 0.6 μg of the rOCT2 (−3036/+242) or equimolar amount of other reporter constructs, 0.1 μg of the rAR expression vector, and 30 ng of an internal control vector for transfection efficiency, namely, the *Renilla* luciferase (pRL-TK) reporter plasmid (Promega) in serum-free DMEM. The medium was changed to DMEM supplemented with 10% charcoal-stripped FBS, containing testosterone, nilutamide, or the vehicle control, dimethyl sulfoxide. After 43-h incubation, the cells were harvested and lysed, and luciferase activity was determined using a dual luciferase assay kit (Promega) and a LB940 luminometer (Berthold, Bad Wildbad, Germany). Each reporter construct was assayed in triplicate wells, and each experiment was repeated three times.

Statistical Analysis

The data were expressed as the mean ± SE. The significance of differences between the vehicle-treated and testosterone-treated groups was analyzed using Dunnet's *post hoc* analysis. Other analyses were conducted with Student's *t* test. Significance was set at $p < 0.05$.

RESULTS

Determination of the Transcriptional Start Site(s) for rOCTs in Rat Kidney Using 5′-RACE

The transcriptional start site(s) for rOCTs in the rat kidney were identified using 5′-RACE. The putative transcriptional start sites were determined using the longest RACE product. Sequencing of the amplified bands revealed that the terminal position of rOCT1 cDNA with the longest 5′-untranslated region was located 63 nucleotides above the start codon, which is 26 bp upstream of the 5′-end of rOCT1 cDNA reported previously (4). The terminal position of rOCT2 cDNA was located 306 nucleotides above the start codon, which is 266 bp upstream of the 5′-end of rOCT2 cDNA (5). The terminal position of rOCT3 cDNA is 35 nucleotides above the start codon, which is 359 bp downstream of the 5′-end of rOCT3 cDNA (8). Therefore, the terminal position of

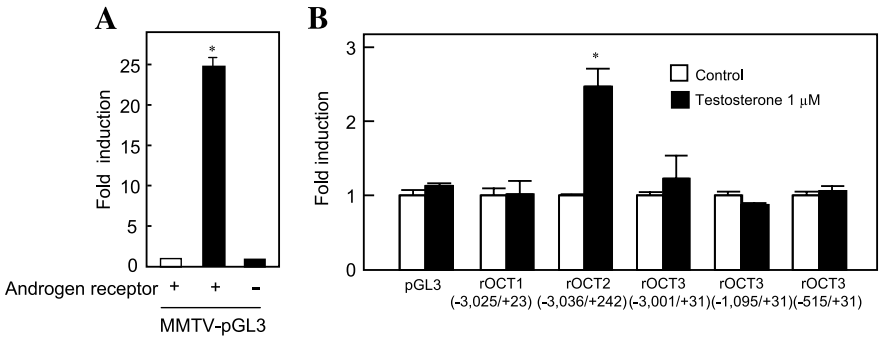

Fig. 2. (A) *Trans*-activation of the mouse mammary tumor virus (MMTV) promoter by rat androgen receptor (rAR) in the presence of testosterone. (B) *Trans*-activation of rOCT1–3 promoters by rAR in the presence of testosterone. Constructs were transiently transfected into LLC-PK_1 cells with rAR and pRL-TK. The cells were cultured for 43 h with vehicle or 1 μM testosterone, and luciferase activity was measured. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Each column represents the mean ± SE of three independent experiments. *$p < 0.05$, significantly different from control.

rOCT cDNA with the longest 5′-untranslated region was numbered with +1 as the transcription start site in this study.

Isolation and Analysis of 5′-Flanking Region of rOCT Genes

Based on the transcriptional start site, we then isolated the promoter region (about 3 kb) of each transporter and prepared reporter constructs. For luciferase assay, we used LLC-PK_1 cells because LLC-PK_1 cells possessed organic cation transport activities (16,17) and pig organic cation transporter OCT2p (18). Figure 1 shows the basal promoter activities of each transporter in LLC-PK_1 cells. Reporter constructs for rOCT1 and rOCT2 showed significant promoter activity. A reporter construct for rOCT3 (−3001/+31) did not have promoter activity, but those for rOCT3 (−1095/+31) and rOCT3 (−505/+31) did, suggesting that a repressive region is located in the rOCT3 promoter region −3001 to −1095. These findings suggest that all promoter constructs function appropriately.

Using these constructs, the effects of testosterone on the promoter activities were assessed. The functional activity of rAR was confirmed by a reporter assay using MMTV reporter construct in the presence of 1 μM testosterone (Fig. 2A). AR has been shown to *trans*-activate MMTV promoter using testosterone (19). In the absence of rAR, MMTV promoter activity was not enhanced by testosterone; native AR was not expressed in LLC-PK_1 cells. As shown in Fig. 2B, the activity of the rOCT2 promoter was significantly enhanced by testosterone, but that of the rOCT1 and rOCT3 promoters was not. These results were consistent with our previous results of Northern blotting (11). We therefore further characterized the transcriptional mechanisms by which the rOCT2 promoter is stimulated by testosterone.

Figure 3 shows the nucleotide sequence for 1000 bp upstream of the translation start site of the rOCT2 gene. Putative binding sites for many transcription factors were identified by TRANSFAC with a core similarity of 0.95 and a matrix similarity of 0.90, including activating protein (AP)-1, octamer-binding factor (Oct)-1, HNF-3/Fkh Homolog (HFH)-3, and a CCAAT box.

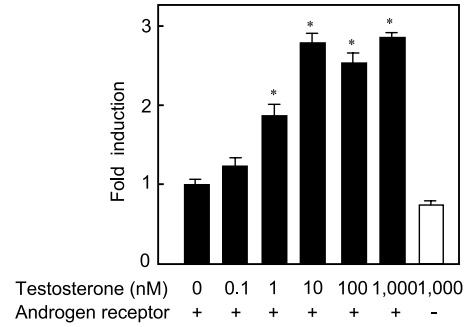

Fig. 4. *Trans*-activation of the rOCT2 promoter (−3036/+242) by rAR in the presence of testosterone. Constructs were transiently transfected into LLC-PK_1 cells with rAR and pRL-TK. The cells were cultured in the presence or absence of testosterone for 43 h. The vector pBK-CMV was used instead of rAR, and luciferase activity was measured. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Each column represents the mean ± SE of three independent experiments. *$p < 0.05$, significantly different from 0 nM testosterone.

Region of 5′-Flanking Sequence Required for Response to Testosterone

As shown in Fig. 4, a reporter construct for rOCT2 (−3036/+242) was significantly activated by testosterone in a concentration-dependent manner, and about a 3-fold increase was observed with 10 nM testosterone. Testosterone did not activate the rOCT2 promoter construct in the absence of the rAR expression vector. Nilutamide, an antiandrogen drug, acts as a competitive inhibitor of the androgen receptor (20). Nilutamide blocked the activation of the rOCT2 promoter by testosterone in a dose-dependent manner (Fig. 5), but nilutamide is a partial agonist of androgen receptor; rOCT2 activity is not completely suppressed by nilutamide. These findings suggest that rOCT2 promoter activity is stimulated by AR. Therefore, we tried to identify ARE(s) that work to stimulate rOCT2 promoter activity.

Table II shows sequences, positions, and homology to the consensus sequence of ARE within the 3000 bp of rOCT2

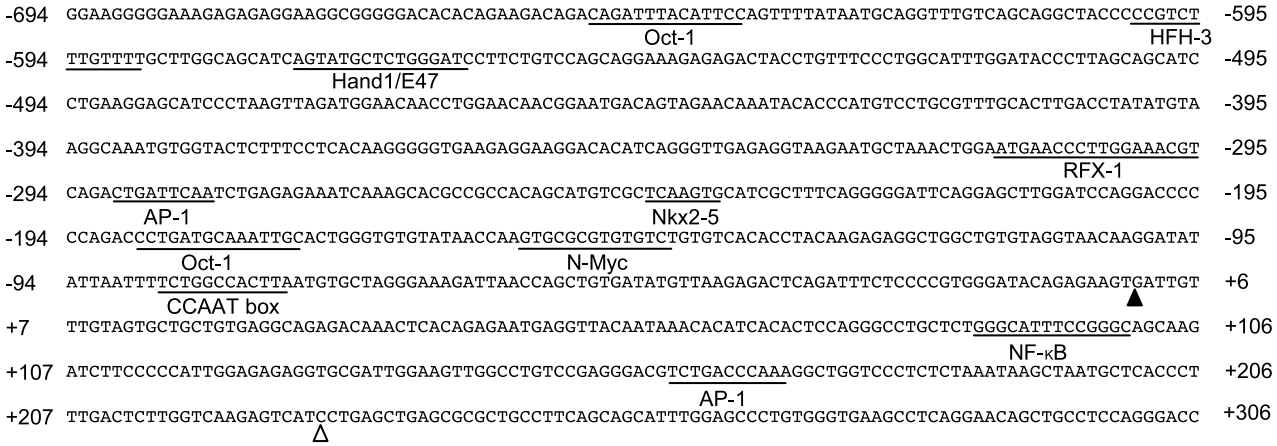

Fig. 3. Transcriptional elements of the rOCT2 promoter. A 1000-base genomic DNA sequence immediately upstream of the start codon site is listed. An open triangle indicates the putative transcriptional start position, and a closed triangle indicates the 5′-end of rOCT2 cDNA published so far (NM_031584).

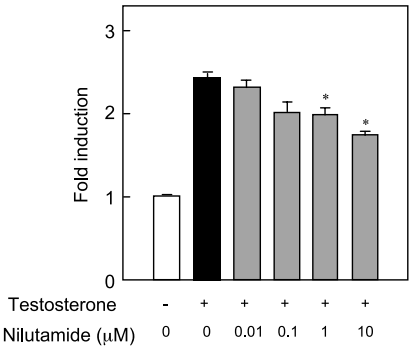

Fig. 5. The effect of nilutamide on *trans*-activation of the rOCT2 promoter (−3036/+242) by rAR in the presence of testosterone. Constructs were transiently transfected into LLC-PK_1 cells with rAR and pRL-TK. The cells were cultured for 43 h with vehicle and 10 nM testosterone in the absence or presence of various concentrations of nilutamide, and luciferase activity was measured. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Each column represents the mean ± SE of three independent experiments. $*p < 0.05$, significantly different from testosterone (+) in the absence of nilutamide.

promoter region. The ARE located furthest from the transcriptional start site was designated ARE-1 and that located closest was designated ARE-5. To determine which region(s) in the 5′-flanking region of rOCT2 gene is involved in expressional regulation by testosterone, constructs with deletions of the 5′-flanking region of rOCT2 gene were prepared and their luciferase activities were measured (Fig. 6). On deletion upstream to position −819, there was no induction by testosterone, suggesting that ARE-5 is not involved in the testosterone induction. But, on deletion up to position −1895, testosterone produced a 2-fold increase in activity, indicating that ARE-3 and/or -4 may work as response element(s). Furthermore, as the full-length promoter showed a 3-fold increase in activity, ARE-1 and/or -2 may also function as response elements.

To identify the functional sites for AR's activation, each ARE was mutated. As AR binds to specific response elements organized as an imperfect palindrome sequence (GGTACA nnnTGTTCT), we decided that the 3 bp at position 10–12 were changed to GCC in this sequence because left half-site of ARE is important for androgen receptor and ARE interaction (21). The promoter activity of rOCT2 with a mutation in each ARE revealed that mutated constructs of ARE-1 and ARE-3 were not affected by testosterone (Fig. 7). The other mutated constructs exhibited promoter activity to various extents in response to testosterone. These findings suggested that ARE-1 and ARE-3 play important roles in the activation of the rOCT2 promoter by testosterone.

DISCUSSION

Previously, we and others reported that the expression of rOCT2 mRNA in the kidney differed with gender, but neither rOCT1 nor rOCT3 (11,22), and that exogenous testosterone significantly stimulated only rOCT2 expression in the kidney of both male and female rats (12,23). Serum levels of testosterone were increased to about 1 μM in testosterone-administered rats. In the present study, we demonstrated that rOCT2 promoter activity was stimulated by 1 μM testosterone, whereas rOCT1 and rOCT3 promoters were not (Fig. 2B). These results are consistent with previous *in vivo* findings. 5′-Flanking region about 3000 bp of rOCT2 gene contained five putative AREs. The reporter assay using a series of deletion constructs and mutant constructs for each ARE revealed that ARE-1 (−2975 to −2960) and ARE-3 (−1340 to −1325), which have more similarity to the ARE consensus sequence than any other region, were responsible for the stimulation of rOCT2 promoter activity by testosterone (Figs. 6–7). As the promoter regions of rOCT1 and rOCT3 used in the present study do not have sequences highly homologous to ARE, the absence of an effect by testosterone on these two promoters is reasonable.

It was reported that there was no gender difference in rOCT2 mRNA expression in organs such as the liver and cerebellum (22). This may be a result of the low basal levels of rOCT2 mRNA or of the weak expression of AR in these organs compared to the kidney (24). We previously demonstrated that rOCT2 is predominantly expressed in the kidney, suggesting that some unidentified kidney-specific transcription factors cooperate to stimulate the rOCT2 promoter activity in the presence of testosterone. HEK293 cells (a human embryo kidney cell line) did not show any organic transporter activity (25). When HEK293 cells were used to measure rOCT2 promoter activity in the presence of AR and testosterone, there was no stimulative effect by testosterone (data not shown). It is probable that LLC-PK_1 cells express some transcription factor(s) necessary to express organic cation transporters. Further studies are needed to identify the kidney-specific transcription factors required for rOCT2 expression.

Other transporters have gender differences. For example, the level of rat organic anion transporter 2 (rOAT2) mRNA in the kidney and liver is higher in female than male rats (26,27), and the level of rOAT3 mRNA in male kidney is higher than

Table II. Comparison of the AREs in the Rat OCT2 Promoter Region

Position	Sequence	Homology (%)
ARE consensus	5′-**GGTACAnnnTGTTCT**-3′	–
ARE-1 (−2975 to −2960)	5′-**GGTAGAggaAGCTCT**-3′	80
ARE-3 (−1340 to −1325)	5′-**GGCACAggaTGCTCT**-3′	87
ARE-4 (−1268 to −1253)	5′-**GATACAgacTGTACC**-3′	80
ARE-5 (−548 to −533)	5′-**GAGACTaccTGTTTC**-3′	73

OCT = organic cation transporter.

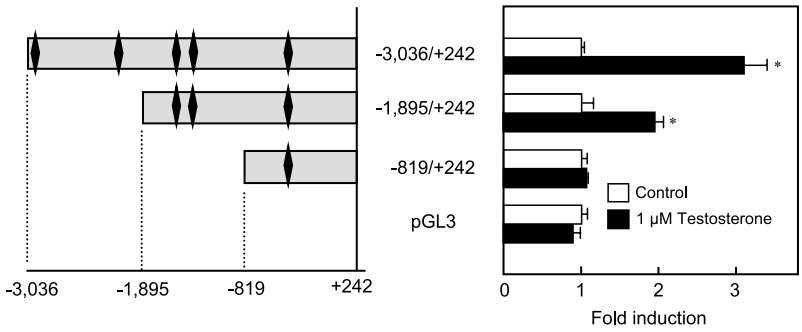

Fig. 6. *Trans*-activation of serial deletions of the rOCT2 promoter by rAR in the presence of testosterone. Various deletion constructs [equimolar amounts of the −3036/+242 construct (0.6 μg)] were transiently transfected into LLC-PK_1 cells with rAR and pRL-TK. The cells were cultured for 43 h with vehicle or 1 μM testosterone, and luciferase activity was measured. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Black diamonds indicate AREs. Each column represents the mean ± SE of three independent experiments. *$p < 0.05$, significantly different from control.

in females (27). Also, gender differences in rat renal cortical OAT1 and OAT3 levels (male > female) are caused by a stimulatory effect of androgens (28). Recently, Ohtsuki *et al.* (29) demonstrated that the expression of OAT3 in rat brain capillary endothelial cells was regulated by testosterone. Although Ohtsuki *et al.* (29) did not carry out reporter assay, it is conceivable that the expressions of the rOAT1 and rOAT3 genes are mediated by the interaction of AR and ARE on these promoters. The present study will be helpful to clarify transcriptional mechanisms of the induction of promoter activities of rOAT1 and rOAT3 by testosterone.

In humans, an evidence is the accumulation of gender differences in the efficacy and toxicity of drugs, and it is thought that physiological factors including body weight, plasma volume, and gastric emptying time are responsible for the variation in drug sensitivity between men and women (30). Recent studies have revealed that other differences, such as cytochrome P450 (CYP), cause gender-related variations in the pharmacokinetics of drugs. It is known that erythromycin (31), nifedipine (32), and verapamil (33), which are metabolized by CYP3A, have greater clearance in women than men. A sex-based difference in the expression of CYP3A4 was detected in the liver (34), but not in the intestine (35). In the renal clearance, amantadine and pramipexole, which are transported by OCT2 (36,37), also exhibit a gender difference (38,39). The renal clearance of amantadine was greater in men than women and was significantly reduced by quinine and quinidine only in men (40). In contrast, the pharmacokinetic parameters of cimetidine and procainamide do not differ between the sexes (41). It is noted that because the luminal efflux may be a rate-limiting step for renal secretion of organic cations (42), large differences in expression of a basolateral human (h)OCT2 may not result in similar large differences in renal clearance. There are some putative AREs in the promoter region of hOCT2, but the positions and sequences are different from those of rOCT2. Further studies are needed to clarify whether the expression of hOCT2 differs with gender, and whether the hOCT2 promoter interacts with the human androgen receptor.

In conclusion, a physiological concentration of testosterone (~10 nM) specifically enhanced transcription of rOCT2 gene, but not of rOCT1 or rOCT3 genes. ARE-1 (−2975 to

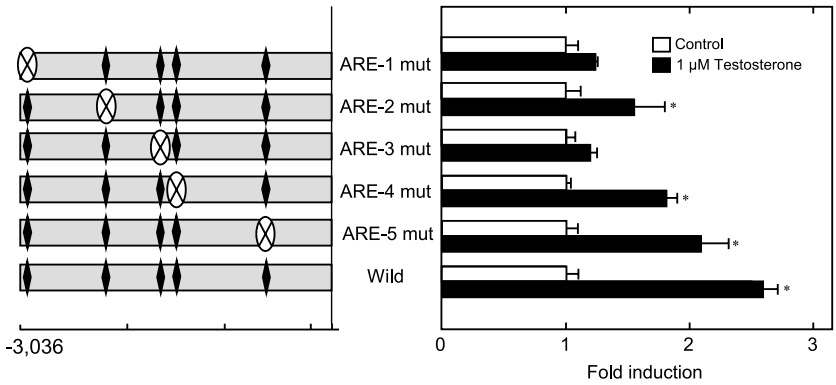

Fig. 7. *Trans*-activation of ARE-mutated rOCT2 promoters by rAR in the presence of testosterone. Constructs were transiently transfected into LLC-PK_1 cells with rAR and pRL-TK. The cells were cultured for 43 h with vehicle or 1 μM testosterone, and luciferase activity was measured. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Black diamonds indicated AREs. Each column represents the mean ± SE of three independent experiments. *$p < 0.05$, significantly different from control.

−2960) and ARE-3 (−1340 to −1325) in the rOCT2 promoter region would play important roles for the enhanced transcription of rOCT2 gene. These findings would account for the transcriptional mechanisms underlying the gender difference in the renal expression of rOCT2 and provide useful information to understand the renal handling of organic cations.

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