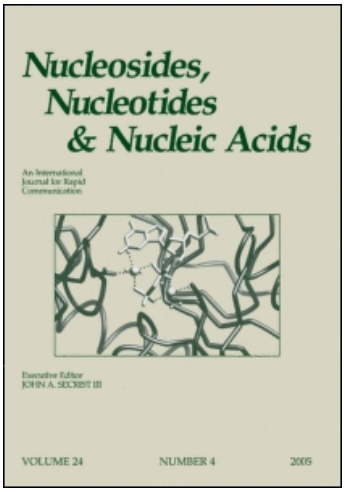

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THE EFFECT OF TESTOSTERONE UPON THE URATE REABSORPTIVE TRANSPORT SYSTEM IN MOUSE KIDNEY

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□ *It is hypothesized that hyperuricemia in males is caused by androgen-induced urate reabsorptive transport system in the kidney. The expression of urate transporter 1 (Urat1), sodium-coupled monocarboxylate transporter 1 (Smct1) and glucose transporter 9 (Glut9) were investigated in orchiectomized mice with or without testosterone replacement. Testosterone enhanced mRNA and protein levels of Smct1 while those of Glut9 were attenuated. Although the mRNA level of Urat1 was enhanced by testosterone, the corresponding levels of Urat1 protein remained unaffected. Thus, the induction of Smct1 by testosterone is a candidate mechanism underlying hyperuricemia in males.*

Keywords Urate; transporter; kidney; testosterone

INTRODUCTION

Coincidentally with Hippocratic Aphorisms concerning the sex difference of gout, serum urate levels have also been reported to increase in men following puberty.[1] Furthermore, administrated testosterone was reported to cause a definite increase in plasma uric acid levels in post-menopausal women.[2] In patients with prostate cancer, a reduction of serum urate level was observed after castration[3] or androgen deprivation therapy.[4] Consequently, sex-related differences in serum urate levels are suspected to be associated with enhanced renal urate reabsorption in response to testosterone.

Urate reabsorption in the kidney is thought to be conducted by transcellular pathway and not by paracellular pathway. Urate in glomerular filtrate flows into tubular epithelium via URAT1, which is an urate-lactate exchanger and expresses on the luminal side of tubular epithelium.[5] The driving force of urate transport via URAT1 is the concentration gradient of lactate, which is diminished in glomerular filtarate and accumulated in tubular epithelium.

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This concentration gradient of lactate was produced by Smct1 and/or Smct2, which are sodium-lactate co-transporters and express on the luminal side of tubular epithelium.[6] Intracellular urate flows out from the tubular epithelium into interstitium and blood via GLUT9, which is a facilitated urate transporter and express on the anti-luminal side of tubular epithelium.[7]

Hereditary hypouricemia was observed in the patients who lose the urate reabsorption via URAT1,[8] or GLUT9.[9] Enhancement of renal urate elimination was also demonstrated in a mouse strain which is deficit of Smct1 and Smct2.[10] Thus, there is the possibility that the hyperuricemia induced by testosterone should be caused by enhancement of the expression of URAT1, SMCT1 and/or 2, or GLUT9. The purpose of this study was to elucidate the effect of testosterone upon the expression of Urat1, Glut9, and Smct1 in the mouse kidney.

MATERIALS AND METHODS

Bilateral orchiectomies or sham operations were performed on 8-week-old male ICR mice (CLEA Japan, Inc.). Eight days after surgery, 2.5 mg/kg of testosterone enanthate (Sigma Aldrich, USA) was injected subcutaneously once a day at a dose of 5.0 mg/mL in sunflower oil into some orchiectomized mice ($n = 4$) for 8 days. Sham-operated ($n = 4$) and the remaining orchiectomized mice ($n = 4$) were injected with only sunflower oil as a vehicle, in accordance with a previous report.[11] Animals were placed into metabolic cages for 24 hours in order to obtain daily urine samples, and were then anesthetized by intra-peritoneal injections of 50 mg/kg pentobarbital to obtain samples of plasma and kidney. Urate concentrations in plasma and urine samples were determined by HPLC and ODS columns (Unison US-C18, 250 mm × 2 mm, Imtakt Corp.), as described previously.[12]

Total RNA and kidney microsomal fractions were prepared as described previously.[13] After cDNA was prepared from the total RNA using a High-Capacity cDNA Reverse Transcription Kit, real-time PCR amplifications were conducted using an Applied Biosystems 7300 Real-Time PCR System using a TaqMan Gene Expression Master Mix with TaqMan probes corresponding to Urat1 (Mm00486206_m1), Smct1 (Mm00520629_m1), Smct2 (Mm01276455_m1), or Glut9 (Mm01211147_m1), and glyceraldehyde-3-phosphate dehydrogenase as an internal standard. Microsomal proteins (5 μg) were separated by SDS-PAGE and transferred to a poly vinilidene difluoride membrane using an iBlot module (Life Technologies Japan Ltd., Japan). After blocking, the blotted membranes were treated overnight at 4°C using anti-Urat1 antibody (0.21 μg/mL),[12] anti-Smct1 antibody (0.25 μg/mL; sc-34194 Santa Cruz Biotechnology, Inc., USA) or anti-Glut9 antibody (4.58 μg/mL). The anti-Glut9 antibody was prepared as an affinity-purified polyclonal antibody by immunizing rabbits with keyhole limpet

hemocyanin conjugated synthetic peptides CSQTEPDSSSTLDSYGQNKIV in accordance with a previous report.[14] Protein levels were determined with Quantity One software ver. 4.3.0 (Bio-Rad Laboratories, USA) from the density of the band detected by an ECL or ECL plus kit (GE Healthcare UK Ltd., UK). Coomassie brilliant blue staining was performed to verify the equality of protein loading. All data were expressed as mean ± S.E. Statistical analyses were performed using the unpaired t-test. p values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Smct1 and Urat1 mRNA levels were significantly reduced by orchiectomy and were completely and incompletely recovered by testosterone replacement, respectively. On the other hand, Glut9 mRNA levels were significantly increased by orchiectomy but decreased by testosterone replacement (Figure 1). Therefore, the dose of testosterone was adequate for a replacement of androgen effect in orchectomized mice. On the contrary, the mRNA level of another sodium-coupled lactate transporter, Smct2, was increased significantly by orchiectomy. Although promoter activity of the Urat1 gene

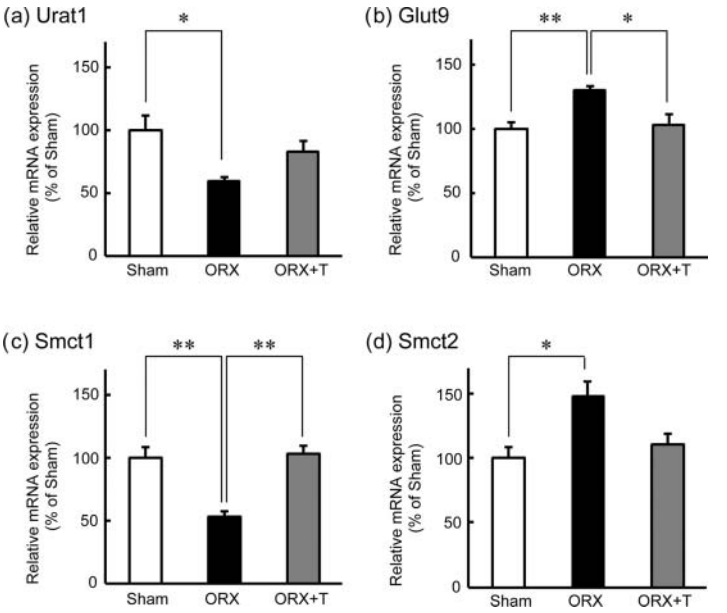

FIGURE 1 mRNA levels of urate reabsorptive transporter genes in orchiectomized mice with or without testosterone replacement. Renal mRNA levels of Urat1 (a), Glut9 (b), Smct1 (c), and Smct2 (d) in sham-operated (Sham), orchiectomized (ORX), and orchiectomized + testosterone-treated (ORX+T) male mice. mRNA levels in Sham mice were fixed at 100%. Date represent mean ± S.E., n = 4, $^{*}p < 0.05$ $^{**}p < 0.01$. mRNA levels of Urat1 and Smct2 in ORX mice were lower and higher than those in Sham mice, respectively. mRNA levels of Glut9 and Smct1 in ORX mice were higher and lower than those in Sham mice and ORX+T mice, respectively.

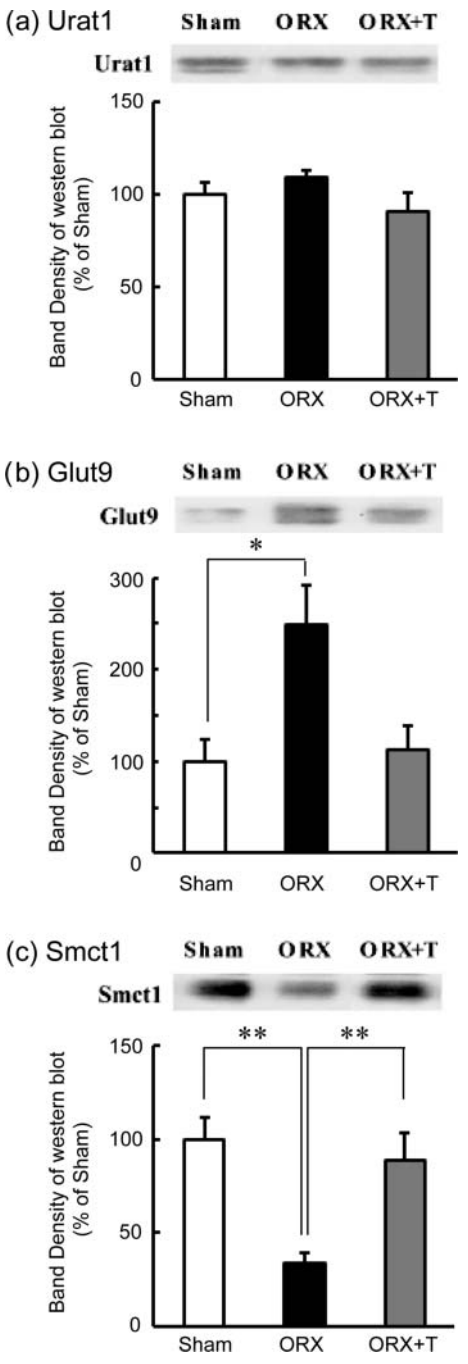

FIGURE 2 Protein levels of urate reabsorptive transporter genes in orchiectomized mice with or without testosterone replacement. Renal protein levels of Urat1 (a), Glut9 (b), and Smct1 (c) in sham-operated (Sham), orchiectomized (ORX), and orchiectomized + testosterone-treated (ORX+T) male mice. Protein levels in Sham mice were fixed at 100%. Date represent mean ± S.E., n = 4, $^{*}p < 0.05$, $^{**}p < 0.01$. Protein levels of Smct1 and Glut9 in ORX mice were lower than those in Sham or ORX+T mice, and higher than those in Sham mice, respectively. The upper photographs represent the protein bands of each gene as visualized by Western blot analyses.

has been observed in vitro in response to testosterone,[15] a change in the mRNA levels of Smct1 and Glut9 in response to testosterone has yet to be reported.

Smct1 and Glut9 protein levels were increased and decreased by orchiectomy, respectively. Testosterone replacement recovered these changes (Figure 2). Therefore, the protein levels of the genes studied herein were correlated with their transcription levels. Although the mRNA level of Urat1 was decreased by orchiectomy, protein levels remained unchanged. Urat1 was reliably detected using the anti-Urat1 antibody which had been previously verified to specifically recognize the Urat1 molecule in a study involving Urat1 knockout mice.[12] One possible explanation is that orchiectomy might alter levels of other hormones besides testosterone, and might also induce post-transcriptional changes in the expression of Urat1. Another possible explanation is that the turnover of Urat1 might be slower than those of Smct1 and Glut9.

There was no significant difference in plasma urate concentration detected among the three groups: sham-operated mice (0.36 ± 0.06 mg/dL); orchitectomized mice (0.42 ± 0.09 mg/dL); and testosterone-replaced orchitectomized mice (0.38 ± 0.14 mg/dL). The effect of urate renal excretion upon plasma urate levels in mice appears to be difficult to detect because of the higher rates of urate degradation by urate oxidase in the mouse liver. On the other hand, the rate of urinary urate excretion increased significantly in orchiectomized mice (373 ± 32 mmol/mol Cr; $p < 0.05$) and testosterone replacement (456 ± 43 mmol/mol Cr; $p < 0.05$) as compared to sham-operated mice (252 ± 18 mmol/mol Cr; $p < 0.05$). Consequently, we suggest that Smct1 is down-regulated by the loss of testosterone, which induced the decrease of urate reabsorption in the kidney and the increase of urate excretion into the urine. However, testosterone replacement in orchiectomized mice failed to reverse the enhanced rate of urate excretion (456 ± 43 mmol/mol Cr). This failure of reverse of the enhanced rate of urate excretion might be due to the increase of urate production in the mouse liver by the testosterone, which might be overwhelmed the increase of urate reabsorption in the kidney.

In conclusion, the Smct1 was up-regulated by testosterone among the urate reabsorptive transport system, and is a possible causative factor for hyperuricemia in males.

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