Hormonal regulation of organic cation transporter OCT2 expression in rat kidney

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Abstract Rat (r) OCT2 was identified as the second member of the organic cation transporter (OCT) family, and is predominantly expressed in the kidney. We reported previously that rOCT2 was responsible for the gender differences in renal basolateral membrane organic cation transport activity. As renal rOCT2 expression in males is much higher than that in females, we hypothesized that rOCT2 expression may be under the control of sex hormones. Treatment of male and female rats with testosterone significantly increased the expression levels of rOCT2 mRNA and protein in the kidney, whereas estradiol treatment moderately decreased the expression levels of rOCT2. There was no regulation of renal rOCT1 mRNA expression by testosterone or estradiol. Treatment of male and female rats with testosterone significantly stimulated the tetraethylammonium (TEA) accumulation by renal slices, whereas estradiol treatment caused a decrease in the TEA accumulation by slices from male but not female rats. The present findings suggested that testosterone up-regulates renal rOCT2 expression and estradiol moderately down-regulates rOCT2.

Key words: Organic cation transporter; Gender difference; Sex hormone; Basolateral membrane; Rat kidney

1. Introduction

Secretion of drugs and xenobiotics is an important physiological function of the renal proximal tubules. In the kidney, organic cation secretion is a two-step process with carrier-mediated steps at both basolateral and brush-border membranes [1–3]. These processes have been functionally characterized by uptake measurements using isolated renal membrane vesicles [4–8] and cultured renal epithelial cells [9,10]. Renal tubular secretion of cationic substances is performed by two distinct classes of organic cation transporters (OCT): one facilitated by the transmembrane potential difference in the basolateral membranes and the other driven by the transmembrane H^+ gradient in the brush-border membranes. In recent years, several members of a common gene family of organic cation transporters have been cloned and characterized [11]. The first organic cation transporter, rOCT1, was cloned from rat kidney [12]. We identified a rat organic cation transporter, rOCT2, the second member of the OCT family [13]. rOCT2 shows 67% amino acid identity with rOCT1 and is expressed predominantly in the kidney. Functional studies using *Xenopus* oocytes [13–15] and transfectants of mammalian kidney cells [16–18] suggested that both rOCT1 and rOCT2 are multispecific facilitative transporters that recognize a variety of cations with different molecular structures, tetraethylammonium (TEA), 1-methyl-4-phenylpiridinium, N^1-methylnicotinamide, choline and dopamine, and are potential-sensitive organic cation transporters.

Bowman and Hook [19] reported that the uptake of *p*-aminohippurate and TEA by renal cortical slices from male rats was greater than that by those of female rats, suggesting gender differences in the basolateral membrane transport capacities for both organic anions and cations. We examined whether rOCT1 and/or rOCT2 contribute to the gender differences in organic cation transport activity in the rat kidney [20]. We observed that the expression levels of rOCT2 mRNA and protein in the kidney of males were much higher than those of females, and there were no gender differences in mRNA expression of rOCT1. These observations suggested that rOCT2 is responsible for the gender differences in renal basolateral membrane organic cation transport activity. On the other hand, renal organic anion transporting polypeptide (oatp) mRNA levels are higher in males than in females. Furthermore, renal oatp expression is under the control of androgen, and to a lesser extent estrogen [21]. We hypothesized that rOCT2 expression may be under the control of sex hormones.

This study was performed to examine whether gender-dependent differences in rOCT2 expression are due to sex hormones. We report here that rOCT2 expression is regulated by testosterone, and moderately by estradiol.

2. Materials and methods

2.1. Materials

[1-^{14}C]TEA bromide (185.0 MBq/mmol) was obtained from Du Pont-New England Nuclear Research Products (Boston, MA, USA). HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) was purchased from Nacalai Tesque (Kyoto, Japan). Testosterone and 17β-estradiol were obtained from Nacalai Tesque (Kyoto, Japan). All other chemicals were of the highest purity available.

2.2. Treatment of rats with testosterone and estradiol

Testosterone (10 mg/0.1 ml of olive oil) or 17β-estradiol (1 mg/0.1 ml of olive oil) was administered subcutaneously in the nape of the neck to male and female rats once daily for 7 days. The control rats were given the same volume of olive oil. After administration, rats were killed and kidneys were excised for isolation of RNA and crude plasma membranes. When rats were killed, blood was taken for

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Abbreviations: OCT, organic cation transporter; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; oatp, organic anion transporting polypeptide

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measurement of serum levels of testosterone and 17β-estradiol. Serum testosterone and 17β-estradiol were assayed with the Enzyme Immunoassay Kit (Cayman Chemical Co., MI, USA). The animal experiments were performed in accordance with The Guideline for Animal Experiments of Kyoto University.

2.3. Northern blot hybridization

Total RNA was extracted from the whole kidney using the guanidine isothiocyanate method [22]. Briefly, 10 μg of total RNA was electrophoresed in 1% denaturing agarose gel containing formaldehyde and transferred onto nylon membranes. The quality of RNA was assessed by ethidium bromide staining. After transfer, blots were hybridized at high stringency (50% formamide, 5×SSPE [1×SSPE = 0.15 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA], 5×Denhardt's solution, 0.2% SDS, 10 μg/ml salmon sperm DNA at 42°C) with cDNA encoding rOCT1, rOCT2 or rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) labeled with [α-^{32}P]dCTP. The cDNA probes corresponded to nucleotide positions 399–1882 (rOCT1), 362–2114 (rOCT2) and 10–1047 (GAPDH) of the published sequences in the GenBank/EBI Data Bank. After hybridization, the blots were washed twice in 2×SSC (1×SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.0)/0.5% SDS at room temperature for 10 min, and then twice in 0.2×SSC/0.5% SDS at 65°C for 30 min. Dried membranes were exposed to the imaging plates of Fujix BIO-Imaging Analyzer BAS 2000 II (Fuji Photo Film).

2.4. Immunoblotting

The crude plasma membrane fraction of rat kidney was prepared as described [23]. The membrane fraction (50 μg) was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and analyzed by immunoblotting with anti-rOCT2 antiserum as described [20]. The blots were incubated with antiserum preabsorbed with the synthetic antigen peptide (1 μg/ml) or antiserum (1:1000) overnight at 4°C. The blots were washed, and the bound antibody was detected on X-ray film by enhanced chemiluminescence (ECL) with a horseradish peroxidase-conjugated anti-rabbit IgG antibody and cyclic diacylhydrazides (Amersham).

2.5. Uptake study by kidney slices

Slices of kidneys including renal cortex and outer medulla from male and female Wistar rats (9 weeks old, 180–260 g body weight) were put in ice-cold oxygenated incubation buffer comprised of 120 mM NaCl, 16.2 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$ and 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.5. Uptake study by kidney slices was performed as described previously [24]. Slices weighing 40–90 mg were randomly selected and placed for incubation in a flask containing 3 ml of incubation buffer with [^{14}C]TEA (50 μM, 0.89 kBq/ml). The uptake of [^{14}C]TEA was carried out at 25°C under an atmosphere of 100% oxygen. D-[^{3}H]Mannitol (50 or 5 μM, 3.7 kBq/ml) was used to estimate the extracellular trapping and non-specific uptake of [^{14}C]TEA. After incubation for an appropriate time, each slice was rapidly removed from the flask, washed twice in 3 ml of ice-cold incubation medium, blotted on filter paper, weighed and solubilized in 0.5 ml of NCS II (Amersham International, Buckinghamshire, UK). The radioactivity was determined in 5 ml of ACS II (Amersham) by liquid scintillation counting.

2.6. Statistical analysis

Statistical analysis was performed by Student's *t*-test, or by one-way analysis of variance followed by Fisher's *t*-test, when multiple comparisons against the control were needed.

3. Results

As shown in Table 1, the serum level of testosterone in intact male rats was six-fold higher than that in females. In contrast, the serum level of estradiol in intact female rats was four-fold higher than that in males. To determine whether the gender differences in rOCT2 expression were produced by these hormones, male and female rats were subcutaneously administered either testosterone or estradiol for 7 days. After treatment with either hormone, serum levels of testosterone and estradiol increased markedly in both male and female rats (Table 1). Increases in serum testosterone level agreed with those reported previously [21].

Fig. 1 shows Northern blots of renal rOCT1 and rOCT2 mRNA expression in male and female kidneys after treatment with the hormones. Administration of testosterone to rats of both sexes caused significant increases in rOCT2 mRNA expression (Fig. 1B). In contrast, administration of estradiol produced a slight, but not statistically significant, decrease in rOCT2 expression in males, but not in females. These hormone treatments had little effect on rOCT1 or GAPDH mRNA expression (Fig. 1).

To determine whether the up- or down-regulation of rOCT2 mRNA expression was accompanied by a modulation of rOCT2 protein expression, immunoblot analysis was performed. Previously, a primary band for rOCT2 with a size of 74 kDa (calculated molecular weight of 66 kDa) was detected exclusively in the membranes of the kidney [20]. Treatment of male (Fig. 2A) and female (Fig. 2B) rats with testosterone produced marked increases in rOCT2 expression. On the other hand, treatment with estradiol resulted in a slight decrease in the rOCT2 expression in males, but not in females. Thus, the modulation of rOCT2 protein expression by hormone treatment appeared to be due to regulation of its mRNA expression.

Next, we examined whether the regulation of rOCT2 expression by administration of testosterone or estradiol produced alterations in organic cation transport activity. As shown in Fig. 3, TEA accumulation was higher in slices from testosterone-treated male (Fig. 3A) and female (Fig. 3B) rat kidneys than in those from control rats given only the vehicle. In contrast, the accumulation was lower in slices from estradiol-treated male rats than in those from controls. Treatment with estradiol had no significant effect on the TEA accumulation in slices from female rats.

4. Discussion

This study demonstrated that the expression of rOCT2 in the kidney is regulated by testosterone, and in part by estra-

Table 1
Serum levels of testosterone and estradiol in male and female rats

	Testosterone (ng/ml)		Estradiol (ng/ml)	
	Male	Female	Male	Female
Intact	1.5 ± 0.026	0.25 ± 0.021	0.022 ± 0.0047	0.092 ± 0.013
Control	0.55 ± 0.082	0.74 ± 0.13	0.083 ± 0.028	0.18 ± 0.054
Testosterone	182 ± 47*	138 ± 9.1*	0.54 ± 0.022	0.50 ± 0.13
Estradiol	0.59 ± 0.079	1.6 ± 0.31	5.2 ± 0.94*	8.1 ± 1.3*

Serum concentrations of testosterone and 17β-estradiol in untreated (intact) male and female rats, and rats treated with either testosterone (10 mg/day) or 17β-estradiol (1 mg/day) for 7 days. Control rats were given vehicle. Each point represents the mean ± S.E.M. of three or four rats. *$P < 0.05$, significantly different from control.

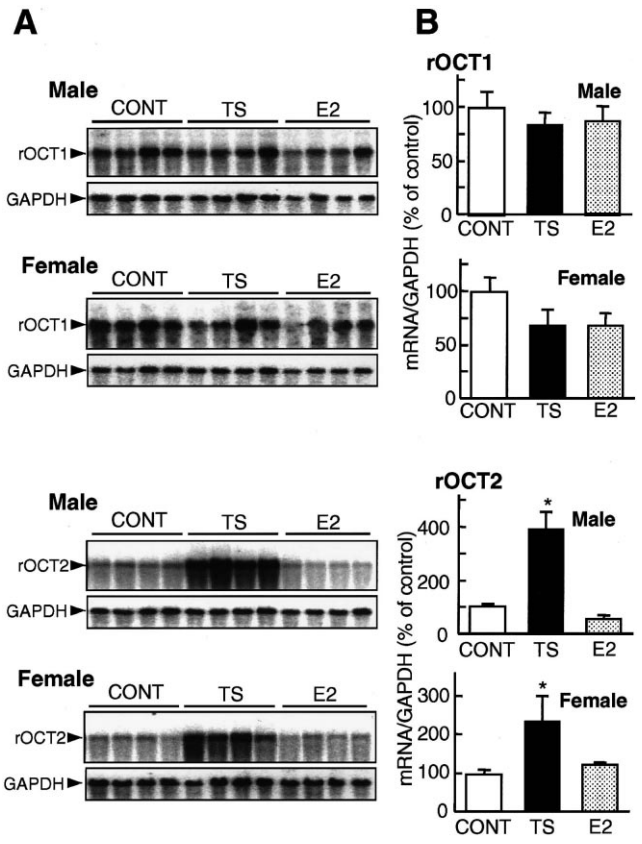

Fig. 1. Northern blot analysis of total RNA of the kidney from male and female rats treated with testosterone or estradiol. A: Total RNA (10 μg) of the kidney from rats treated with vehicle (CONT), testosterone (TS) or 17β-estradiol (E2) was hybridized with rOCT1, rOCT2 or GAPDH cDNA probe under high stringency. B: Densitometric quantitation of rOCT1 and rOCT2 mRNA, corrected for loading using GAPDH. Control levels were set at 100%. No differences were detected in GAPDH among rats. Each column represents the mean ± S.E.M. of four rats. $*P < 0.05$, significantly different from control.

diol, modulating the gender differences in organic cation transport activity of the basolateral membrane of renal tubular cells. Gender differences in organic cation transport activities of rat kidney have been described by Bowman and Hook [19], who demonstrated that accumulation of TEA was greater in renal cortical slices from male than female rats. Since uptake by renal slices is considered to predominantly reflect transport across the basolateral membranes, the gender difference in TEA transport could result from differences in the expression of basolateral membrane transporter(s). In addition, we recently demonstrated gender-related basolateral membrane organic cation transport activity using basolateral membrane vesicles and renal slices from male and female rats [20].

In our previous study, Northern blot and immunoblot analyses revealed that the levels of both rOCT2 transcript and protein expression were greater in the kidney of male than of female rats. In contrast, there were no appreciable gender differences in the expression level of rOCT1, rOCT3 or rOAT1 mRNA [20]. Thus, we postulated that rOCT2 could be an organic cation transporter whose expression is regulated by gender-related factors, whereas rOCT1 may be a constitutive type of transporter. In this study, this assumption was

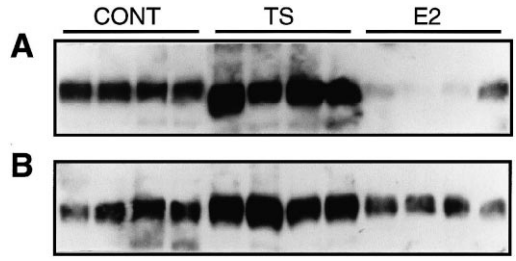

Fig. 2. Immunoblot analysis of membrane proteins isolated from male and female rat kidneys treated with testosterone or estradiol. 50 μg of crude membranes from male (A) and female (B) rat kidneys were separated by SDS–PAGE. rOCT2 was identified using polyclonal antibodies as described in Section 2. CONT, rats treated with vehicle; TS, rats treated with testosterone; E2, rats treated with 17β-estradiol.

tested by treating rats with testosterone and estradiol, since we detected gender differences in serum levels of these hormones (Table 1). Treatment with testosterone produced a marked increase in the steady-state level of rOCT2 mRNA in the male and female rat kidney. We speculated that rOCT2 mRNA expression was under strong stimulatory regulation by testosterone. In contrast to the case of rOCT2, testosterone treatment showed no effect on the expression of rOCT1 mRNA (Fig. 1). This indicated that rOCT1 could be an organic cation transporter expressed constitutively in the kidney with no gender differences. Treatment with estradiol caused a small decrease in rOCT2 expression in male rats, suggesting that the expression may be weakly down-regulated by estradiol. In the female rat kidney, administration of estradiol had no effect on rOCT2 expression (Figs. 1 and 2B). Although the precise mechanisms underlying this difference in effect are unknown, the gender-dependent differential regulation of rOCT2 expression by estradiol via other endogenous factor(s) might be involved.

The present findings support the linkage between hormonal regulation of rOCT2 expression and organic cation transport activity in the kidney. The TEA accumulation by slices from the male rat kidney was significantly enhanced by treatment with testosterone, but was depressed by estradiol treatment (Fig. 3A). On the other hand, the TEA accumulation by slices

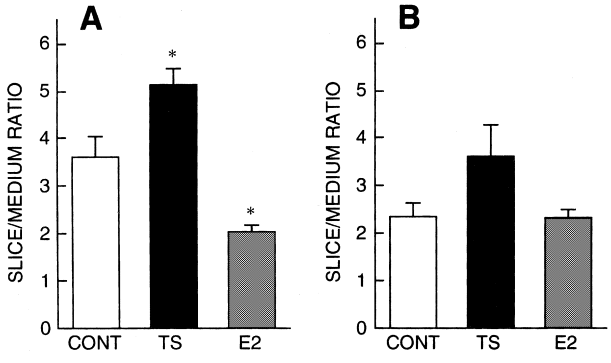

Fig. 3. TEA accumulation by kidney slices from male and female rats treated with testosterone and estradiol. Kidney slices from males (A) and females (B) were incubated at 25°C in buffer containing 50 μM [^{14}C]TEA for 60 min. CONT, rats treated with vehicle; TS, rats treated with testosterone; E2, rats treated with 17β-estradiol. Each column represents the mean ± S.E.M. of three separate experiments in three slices. $*P < 0.05$, significantly different from control.

from the female kidney was also stimulated by testosterone, but was mostly unaffected by estradiol (Fig. 3B). Thus, the up- or down-regulation of rOCT2 expression appeared to be related to the TEA transport activity in the kidneys. In other words, rOCT2 should be a primary organic cation transporter in the basolateral membranes of the renal tubular cells, whose expression would be regulated by androgens.

Gender-dependent expression of the transporter has been reported for the sodium taurocholate transporting polypeptide [25], and oatp [21]. Lu et al. [21] reported that renal oatp mRNA expression was under strong control of testosterone and weaker regulation by estrogen. They speculated that this regulation of renal oatp expression may be important in modulating the renal tubular secretion of 17β-D-glucuronide, which is a major substrate of oatp. Although it is still unclear why rOCT2, but not rOCT1, is sexually regulated by testosterone and partly by estradiol in the kidney, we speculate that this transporter might play a physiological role in the metabolic clearance of endogenous substances, which may be generated differentially in males and females, for effective removal from the circulation to maintain hormone balance.

Recently, the human organic cation transporters hOCT1 and hOCT2 were cloned [26]. Northern blot analysis demonstrated that hOCT1 is expressed primarily in the human liver, while hOCT2 is expressed mainly in the human kidney. In situ hybridization and immunohistochemical analyses indicated expression of hOCT2 mainly in the distal tubule where the transporter is localized at the luminal membrane [26]. There is no information concerning gender differences in expression of hOCT2. Further studies are required to determine whether there are gender differences in hOCT2 expression and in cation transport mediated by hOCT2 in the human kidney.

In conclusion, we demonstrated that rOCT2 is responsible for the gender differences in renal organic cation transport activity, possibly in the basolateral membranes of tubular epithelial cells, and that the differences in rOCT2 expression are regulated by the overall balance of testosterone and estradiol. These findings suggest that rOCT2 may play physiological and pharmacological roles in renal clearance of endogenous and exogenous organic cations from the blood.

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