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Decreased activity of basolateral organic ion transports in hyperuricemic rat kidney: roles of organic ion transporters, rOAT1, rOAT3 and rOCT2

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Abstract

We investigated organic anion and cation transport activity and the expression of several organic ion transporters in hyperuricemic rat kidney. Feeding oxonic acid, an inhibitor of uric acid metabolism, and uric acid for 10 days significantly increased plasma uric acid level. Plasma creatinine and blood urea nitrogen concentrations also increased in hyperuricemic rats, indicating impaired renal function. The accumulation of organic anions, *p*-aminohippurate (PAH) and methotrexate, and cations, tetraethylammonium (TEA) and cimetidine, into renal slices was markedly decreased, suggesting decreased transport activity for organic anions and cations at the basolateral membrane in the kidney. The expression levels of basolateral organic anion transporters rOAT1 and rOAT3, and organic cation transporter, rOCT2, significantly decreased in hyperuricemic rat kidney as assessed by mRNA and protein levels. In contrast, the expression of rOCT1 was unaltered by hyperuricemia at both mRNA and protein levels. Moreover, the mRNA expression of kidney-specific organic anion transporters, OAT-K1 and OAT-K2, and organic anion transporting polypeptide (oatp) 1, which localize at the brush-border membrane in the kidney, was unchanged in hyperuricemic rats. In conclusion, we showed decreased basolateral organic anion and cation transport activity, accompanied by a specific decrease in rOAT1, rOAT3 and rOCT2 expression in hyperuricemic rat kidney. These phenomena partly contribute to the changed renal disposition of organic anions and cations in hyperuricemia.

Keywords: Kidney; Hyperuricemia; Renal secretion; Organic anion transporter; Organic cation transporter

1. Introduction

The organic ion transport system in the proximal tubules of the kidney plays an important role in the elimination of a wide variety of ionic compounds including endogenous metabolites, drugs and xenobiotics [1–3]. Organic ions are taken up in the proximal tubules from the blood and secreted into the luminal fluid. Studies with intact kidneys, renal cortical slices, isolated renal tubules, cultured cells and renal membrane vesicles have provided a great deal of information about the organic ion transport systems. Renal tubular secretion of organic anions including PAH is mediated by an organic anion/α-ketoglutarate exchanger at the basolateral membrane and by an anion exchanger and/or potential-sensitive transport system at the brush-border membrane [1,2,4,5]. On the other hand, the transport of organic cations including TEA is facilitated by the transmembrane potential difference at the basolateral membrane and mediated by the H^+/organic cation antiporter at the brush-border membrane in the kidney [1,2,6].

Several cDNA clones encoding organic anion and cation transporters have been identified from rat kidney to date. In 1994, rat organic cation transporter (rOCT) 1 was identified in the kidney [7]. We identified the second member of the OCT family, rOCT2 [8]. Both rOCT1 and rOCT2 are potential-sensitive organic cation transporters [8,9] mediating transport of organic cations such as TEA and the H_2-receptor antagonist cimetidine [10], and are present at the basolateral membrane in the kidney [11]. Rat organic

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Abbreviations: PAH, *p*-aminohippurate; TEA, tetraethylammonium; SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction.

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anion transporter (rOAT) 1 mediates organic anion/α-ketoglutarate exchange at the basolateral membrane of the proximal tubules, and transports various organic anions such as PAH and an anticancer drug methotrexate [12–14]. Another member of the OAT family, OAT3 is also present at the basolateral membrane, and mediates the transport of some organic anions including PAH and methotrexate [15].

Hyperuricemia is often the first clinical manifestation of gout and is associated with renal disease. Recently, serum uric acid was found to be an independent risk factor for development of renal insufficiency in a study of 6403 subjects [16]. Hyperuricemic rats, induced with oxonic acid, an inhibitor of uric acid metabolism, and uric acid, also showed impaired renal functions such as concentrating ability, sodium, calcium and phosphate reabsorption, and glomerular filtration [17]. However, urinary acidifying ability and the capacity to form titratable acid remained intact [17]. These phenomena suggested that renal functions were not affected in a uniform manner in hyperuricemic rats. On the other hand, substrate-induced stimulation of organic anion transport at the basolateral membrane was suggested [18,19], and uric acid is an endogenous anionic substrate for rOAT1 [12]. Therefore, we investigated the basolateral transport activity of organic anions in hyperuricemic rats, in comparison with organic cations, and also evaluated the expression of several organic ion transporters in the kidney of hyperuricemic rats.

2. Materials and methods

2.1. Materials

D-[1-^{3}H(*N*)]Mannitol (973 GBq/mmol) and *p*-[glycyl-1-^{14}C]aminohippuric acid (1.9 GBq/mmol) were obtained from NEN™ Life Science Products. [3′,5′,7-^{3}H(*N*)]-Methotrexate disodium salt (992 GBq/mmol) and [1-^{14}C]-D-mannitol (2.07 GBq/mmol) were purchased from Moravek Biochemicals. [1-^{14}C]Tetraethylammonium bromide (2.04 GBq/mmol) was obtained from American Radiolabeled Chemicals. [*N*-methyl-^{3}H]Cimetidine (814 GBq/mmol) was from Amersham Biosciences. Oxonic acid and uric acid were purchased from Aldrich Chemical and Wako Pure Chemical Industries, respectively. All other chemicals used were of the highest purity available.

2.2. Animals

All animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University. The Animal Research Committee, Graduate School of Medicine, Kyoto University, approved the experimental protocol (Med Kyo 01200). Male Wistar rats weighing 170–240 g were fed ground standard rat chow and water freely for 10 days. Hyperuricemia was induced with ground standard rat chow containing 5% oxonic acid and 2.5% uric acid, following the previous reports [17,20]. The concentrations of blood urea nitrogen and plasma creatinine were measured with an i-STAT™ portable clinical analyzer (i-STAT) and the Jaffé method using a kit obtained from Wako Pure Chemical Industries, respectively.

2.3. Histological analyses

Kidneys of control or hyperuricemic rats were removed and immediately fixed for 1 day at room temperature in carnoy fixative (ethanol:chloroform:acetic acid: 6:3:1) and preserved in 70% ethanol. Conventional histological sections were stained with periodic acid-Schiff (PAS) reagent.

2.4. Uptake by rat renal slices

The kidneys were decapsulated, and a thin renal slice was prepared with a Stadie–Riggs microtome as described previously [21]. Renal slices from control and hyperuricemic rats were stored in ice-cold oxygenated incubation buffer composed 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. Renal slices were randomly selected and placed for incubation in flasks containing 3 mL of the incubation buffer with [^{14}C]PAH (5 μM, 0.93 kBq/mL), [^{3}H]methotrexate (1 μM, 1.85 kBq/mL), [^{14}C]TEA (5 μM, 1.03 kBq/mL) or [^{3}H]cimetidine (5 μM, 1.85 kBq/mL). The uptake of these compounds was carried out at 25° under an atmosphere of 100% oxygen. [^{3}H]Mannitol (5 μM, 22.8 kBq/mL) was used to calculate the extracellular trapping and non-specific uptake of [^{14}C]PAH or [^{14}C]TEA as well as to evaluate the viability of slices. [^{14}C]Mannitol (1 or 5 μM, 0.37 kBq/mL) was used for [^{3}H]methotrexate or [^{3}H]cimetidine. After incubation for a specified period, the incubation buffer containing radiolabeled compounds was rapidly removed from the flask, washed twice with 3 mL of ice-cold incubation medium, blotted on filter paper, weighed and solubilized in 0.5 mL of NCSII (Amersham Biosciences). Then, the radioactivity was determined in 10 mL of ACSII (Amersham Biosciences) by liquid scintillation counting.

2.5. Western blot analysis

Crude membrane fractions were prepared from the kidney of control and hyperuricemic rats and Western blot analyses with specific antibodies for rOAT1, rOAT3, rOCT1, rOCT2 and Na^{+}–K^{+}–ATPase α-1 subunit (Upstate Biotechnology) were performed as previously reported [11,22]. After the detection of rat OAT1, OAT3, OCT1 or OCT2, the detection of Na^{+}–K^{+}–ATPase α-1 subunit was performed with the same polyvinylidene difluoride membranes (Immobilon-P, Millipore). The density of bands was determined using NIH Image 1.61 (National Institutes of Health).

2.6. Northern blot analysis

Total RNA was extracted from kidney using TRIZOL™ reagent (Invitrogen). Then, Northern blot analysis was performed as previously described [23]. In brief, 5 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 with cDNA encoding rOAT1, rOCT1, rOCT2 or rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) labeled with [α-^{32}P]dCTP. The cDNA probes corresponded to the nucleotide positions at 1–2217 (rOAT1, accession number AF008221), 399–1882 (rOCT1, X78855), 362–2114 (rOCT2, D83044) and 10–1047 (GAPDH, M17701) of the published sequences in the GenBank/EBI Data Bank. The blots were washed finally with 0.2× SSC/0.5% SDS at 65°. Dried membranes were exposed to the imaging plates of a FUJIX BIO-Imaging Analyzer BAS 2000 II (Fuji Photo Film).

2.7. RNase protection assay

Total RNA was extracted from rat kidney using the RNeasy Mini Kit (QIAGEN KK). The RNase protection assay for OAT-K1 and OAT-K2 was performed as described previously [24]. Antisense cRNA probe for oatp1 was synthesized as follows. Rat liver total RNA was extracted using RNeasy Mini Kit (QIAGEN KK), reverse transcribed with random primers using a Superscript® II reverse transcriptase (Life Technologies) and subjected to RNase H (Life Technologies) digestion. By PCR amplification, a fragment corresponding to nucleotides 1–499 of oatp1 was isolated and subcloned into the pGEM®-T Easy Vector (Promega), and the resulting plasmid was named 5′oatp1/pGEM®-T Easy. The primers used were 5′-CATGAGTGTACTTCTCTCTTGG-3′ as the sense strand and 5′-TGTGTTCGGTTCTCCATACAC-3′ as the antisense strand (accession number L19031). The DNA sequence of 5′oatp1/pGEM®-T Easy was verified and found to be identical to published sequences in the GenBank/EBI Data Bank. After linearization of 5′oatp1/pGEM®-T Easy by digestion with *Apa*I, antisense oatp1 cRNA labeled with [α-^{32}P]CTP (29.6 MBq/mmol, Amersham Biosciences) was generated by *in vitro* transcription using SP6 RNA polymerase (Promega). The antisense cRNA probes were purified using mini Quick Spin™ RNA Columns (Boehringer, Mannheim).

2.8. In vivo clearance method

Control or hyperuricemic rats were anesthetized with sodium pentobarbital and the femoral artery and vein were cannulated with polyethylene tubing (SP31, Natsume Seisakusho). The bladder was also cannulated with PE-50 tubing (Becton Dickinson) for urine collection. Blank urine was collected for 10 min and blank blood was sampled. Thereafter, in the experiment on methotrexate clearance, bolus doses of 86 μg/kg of methotrexate, 146 mg/kg of mannitol and 73.4 mg/kg of inulin were administered, followed by a constant infusion of methotrexate (61 μg/mL), mannitol (40 mg/mL) and inulin (20 mg/mL) at a rate of 2.2 mL/hr using an automatic infusion pump (Natsume Seisakusho). In the experiment on cimetidine clearance, bolus doses of 8 mg/kg of cimetidine and a constant infusion of cimetidine (0.5 mg/mL) at a rate of 2.2 mL/hr were administered with mannitol and inulin. Inulin clearance was used as the glomerular filtration rate. After 60 min equilibration of a constant infusion, urine samples were collected three times at 10 min intervals and blood samples were obtained at the mid point of urine collection. The plasma unbound fractions of methotrexate and cimetidine were determined by ultrafiltration using a Micropartition System (MPS-1, Amicon).

2.9. Measurement of the concentration of urate, cimetidine and methotrexate

The concentration of urate and cimetidine in plasma and urine was determined according to previous reports with slight modifications [25,26]. A high-performance liquid chromatograph LC-10AS (Shimadzu) was equipped with an UV spectrophotometric detector (SPD-10AV; Shimadzu) adjusted to 254 nm for urate or 235 nm for cimetidine and an integrator (Chromatopac C-R6A; Shimadzu). The stationary phase was a reversed phase Chemcosorb 5-ODS-H column (4.6 mm inside diameter × 150 mm, Chemco Scientific). The flow rate was 1.0 mL/min and the column temperature was maintained at 40°. The mobile phase consisted of 98% phosphate buffer (40 mM, pH 2.2) and 2% methanol for urate, and 95% phosphate buffer (50 mM, pH 5.5) and 5% acetonitrile for cimetidine. The concentration of methotrexate was measured by a fluorescence polarizing immunoassay on a TDx instrument (Dainabot Laboratories).

2.10. Statistical analysis

The statistical significance of differences between mean values was calculated using the non-paired *t*-test. *P* values of less than 0.05 were considered significant.

3. Results

Table 1 shows the effect of diet containing 5% oxonic acid and 2.5% uric acid on body weight and biochemical parameters in rats. Rats treated with oxonic acid and uric acid showed a 5.7-fold higher plasma uric acid concentration than control rats. The increase in body weight of hyperuricemic rats was much less than that of control rats, and the values of plasma creatinine and blood urea nitrogen

Table 1
Body weight and biochemical parameters in control and hyperuricemic rats

	Weight change (%)	Plasma uric acid (mg/dL)	Plasma creatinine (mg/dL)	BUN (mg/dL)
Control	51.5 ± 1.6 (24)	0.93 ± 0.18 (12)	0.51 ± 0.06 (15)	13.2 ± 0.8 (19)
Hyperuricemia	3.0 ± 2.4* (25)	5.29 ± 0.74* (16)	0.74 ± 0.06* (17)	41.2 ± 3.0* (20)

Note: Values are the mean ± SE from the number of rats indicated in parentheses. Weight change ((body weight after the experiments − body weight before the experiments)/body weight before the experiments) × 100; BUN, blood urea nitrogen.
* $P < 0.05$, significantly different from control.

concentrations were significantly increased. Results of histological examinations are shown in Fig. 1. The tubular lumen was dilated in a diffuse area filled with detached tubular epithelial cells and infiltrated mono- and polynuclear cells in the hyperuricemic rats. Inflammatory cells were also infiltrated into the interstitium and in tubules (tubulitis). In the papillary area, tubular dilatation was also noticed in hyperuricemic rats, but urate crystals were not visible.

To evaluate the activity for renal organic ion transport at the basolateral membranes, we measured the accumulation of organic anions, PAH and methotrexate, and organic cations, TEA and cimetidine, into renal slices of hyperuricemic rats (Fig. 2). The accumulation of all organic ions examined was significantly decreased in hyperuricemic rats at each time point.

Crude plasma membranes were isolated from kidneys of control and hyperuricemic rats and immunoblot analyses were performed for organic ion transporters, rOAT1, rOAT3, rOCT1 and rOCT2 (Fig. 3). Immunoblot analysis for the Na^+–K^+–ATPase α-1 subunit was also performed, since it was present at the basolateral membrane in the proximal tubules as well as organic ion transporters examined [27]. The expression of the Na^+–K^+–ATPase α-1 subunit was not changed in hyperuricemic rats, compared with control rats. We evaluated the alteration in the expression of organic ion transporters as the ratio to that of the

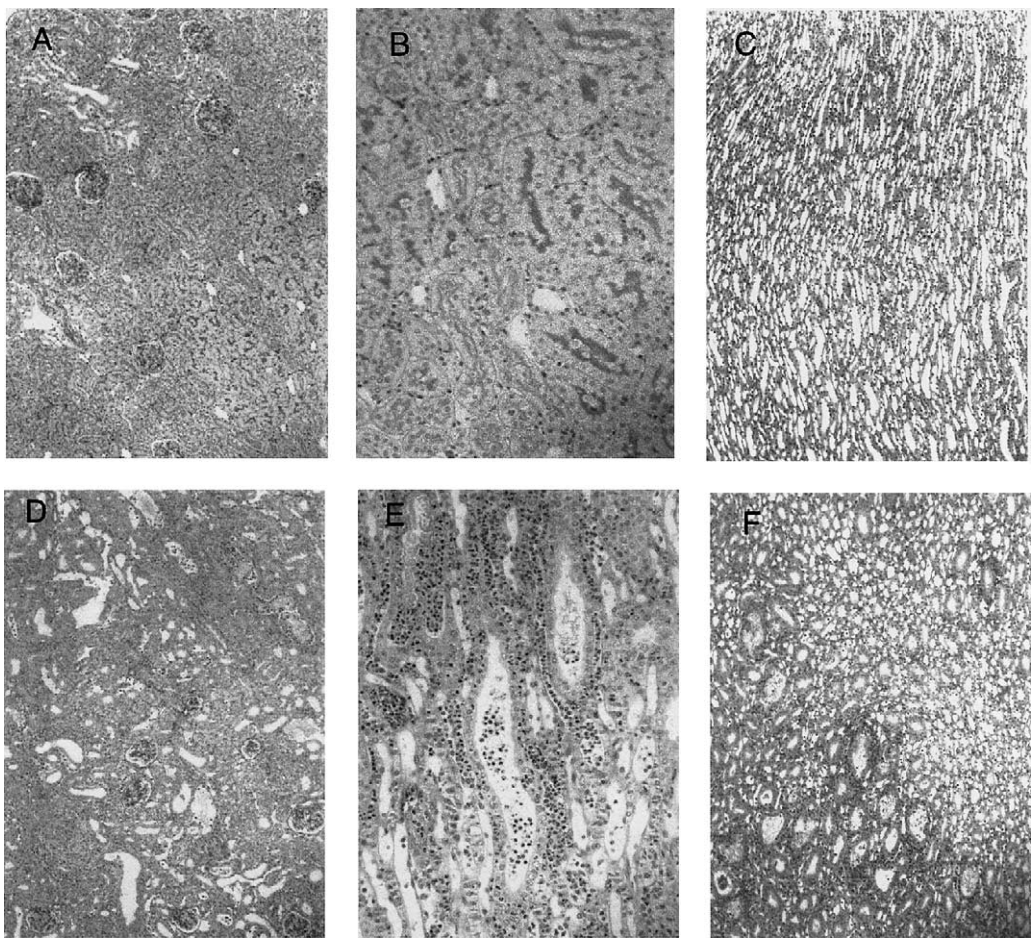

Fig. 1. Histological studies of the kidney in control (A–C) and hyperuricemic rats (D–F). Control rats showed normal histology at lower (A, C) and higher (B) magnifications. Tubular dilatation with cellular casts composed of mostly detached cells and mono and polynuclear infiltrating cells and tubulitis were visible (D, F). Tubular dilatation was noted in the papillary area, although no crystal formation was noticed (F). PAS staining. (A, D) Cortex; (B, E) medulla; (C, F) papilla; (A, C, D, F) 100×; (B, E) 400×.

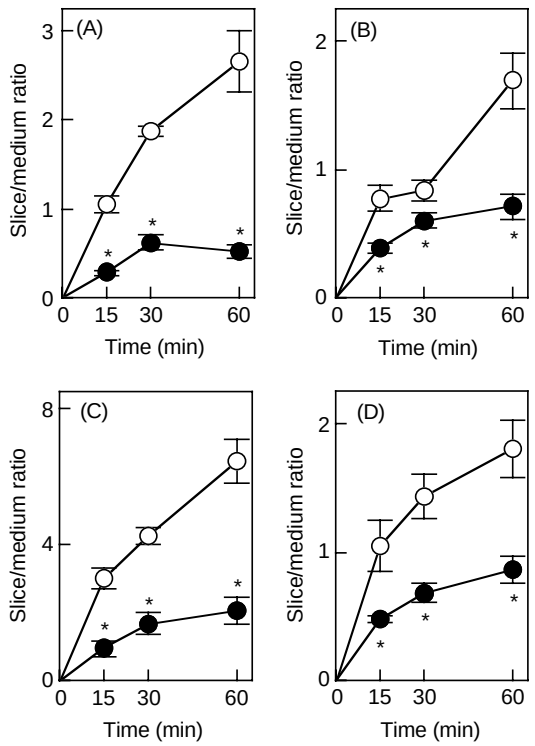

Fig. 2. PAH (A), methotrexate (B), TEA (C) and cimetidine (D) accumulation by renal slices of control (○) and hyperuricemic (●) rats. Renal slices were incubated at 25° in incubation buffer containing 5 μM [^{14}C]PAH, 1 μM [^{3}H]methotrexate, 5 μM [^{14}C]TEA or 5 μM [^{3}H]cimetidine, for the periods indicated. D-[^{3}H]Mannitol or [^{14}C]mannitol was used to estimate the extracellular trapping and non-specific uptake of [^{14}C]PAH and [^{14}C]TEA or [^{3}H]methotrexate and [^{3}H]cimetidine, respectively. Each point represents the mean ± SE for 4–6 slices from different rats. (*)$P < 0.05$, significantly different from control.

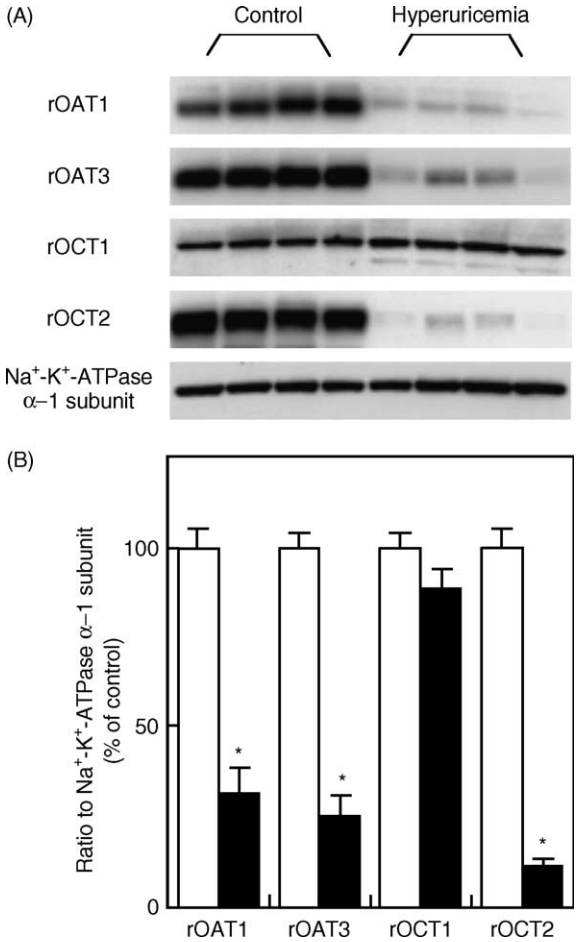

Fig. 3. Western blotting of renal crude plasma membranes with antibodies for rOAT1, rOAT3, rOCT1 and rOCT2 in control and hyperuricemic rat kidneys. (A) Crude membrane (50 μg) from the kidney of control and hyperuricemic rats was separated by SDS–PAGE. rOAT1, rOAT3, rOCT1, rOCT2 and Na^+–K^+–ATPase α-1 subunit were identified with antibodies. The results in four control and hyperuricemic rats from a typical experiment are shown. (B) The ratio of rOAT1, rOAT3, rOCT1 and rOCT2 density to Na^+–K^+–ATPase α-1 subunit density. Each column represents the mean ± SE for seven control (open columns) and hyperuricemic (closed columns) rats from two experiments. (*)$P < 0.05$, significantly different from control.

Na^+–K^+–ATPase α-1 subunit. The expression level of rOCT1 was not changed in hyperuricemic rats. However, the expression levels of rOAT1, rOAT3 and rOCT2 proteins were significantly decreased in hyperuricemic rats.

Total RNA from kidneys of control and hyperuricemic rats was analyzed by Northern blot hybridization for the mRNA expression of rOAT1, rOCT1, rOCT2 and GAPDH (Fig. 4). The mRNA expression of GAPDH in hyperuricemic rats was not significantly different from that in control rats. Therefore, the density of the bands for various transporters was corrected with that for GAPDH. The expression of rOAT1 and rOCT2 mRNA was significantly lower in hyperuricemic rats than in control rats. On the other hand, the expression of rOCT1 mRNA did not differ between control and hyperuricemic rats. Similar results were observed in other rats, in which total RNA was extracted with RNeasy Mini Kit (N = 6–7, data not shown).

We further investigated the mRNA expression of OAT-K1, OAT-K2 and oatp1, which are present at the brush-border membrane in the proximal tubules [3]. The expression of OAT-K1, OAT-K2 and oatp1 in hyperuricemic rats was similar to that in control rats (Fig. 5).

We investigated urinary excretion of methotrexate and cimetidine in control and hyperuricemic rats. As shown in Table 2, the plasma concentration of methotrexate and cimetidine was 2-fold higher in hyperuricemic rats than control rats, accompanied by a decrease in the renal clearance of methotrexate and cimetidine. The unbound fraction of methotrexate was significantly increased in hyperuricemic rats. The value of unbound renal clearance for methotrexate was markedly decreased, and the ratio of unbound renal clearance of methotrexate to the glomerular filtration rate was significantly decreased in hyperuricemic rats. On the other hand, the ratio of unbound renal clearance of cimetidine to the glomerular filtration rate was not altered.

4. Discussion

Experimental hyperuricemia is difficult to induce because of the extremely rapid metabolism of uric acid

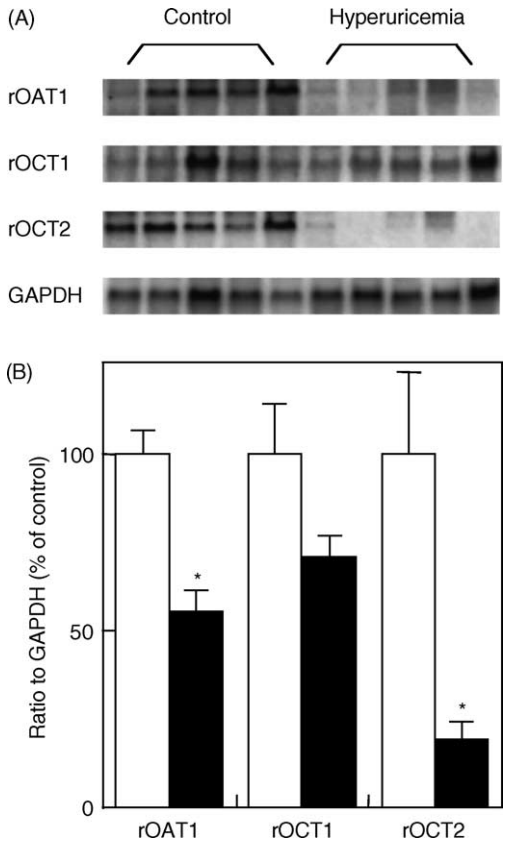

Fig. 4. Northern blotting of rOAT1, rOCT1 and rOCT2 in control and hyperuricemic rats. (A) Total RNA (5 μg) from control and hyperuricemic rat kidneys hybridized with rOAT1, rOCT1, rOCT2 and GAPDH cDNA probe under high stringency. (B) Densitometric quantitation of rOAT1, rOCT1 and rOCT2, corrected for loading with GAPDH. The density in hyperuricemic rats was compared with that in control rats. Each column represents the mean ± SE for five control (open columns) and hyperuricemic (closed columns) rats. (*)$P < 0.05$, significantly different from control.

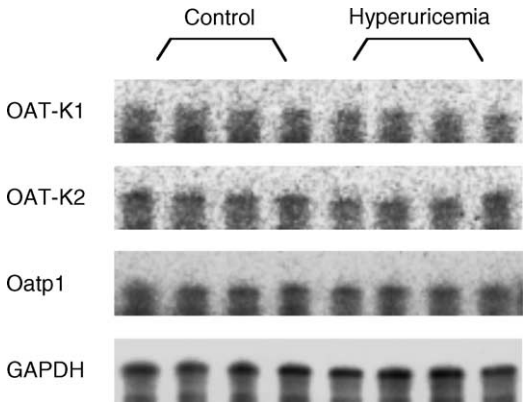

Fig. 5. mRNA detection of OAT-K1, OAT-K2, oatp1 and GAPDH in the kidney of control and hyperuricemic rats by RNase protection assay. Aliquots of 2 μg of total RNA were hybridized with OAT-K1, OAT-K2, oatp1 and GAPDH probes, and an RNase protection assay was carried out. The results for four control and hyperuricemic rats in an experiment are shown.

by uricase in many animal species except humans and higher apes. However, the combined oral administration of oxonic acid, an inhibitor of uric acid metabolism, and uric acid in rats induced a sustained hyperuricemia, hyperuricosuria, an increase in the concentration of uric acid in whole kidney tissue and morphological changes [20,28,29]. We observed a great increase in plasma uric acid concentration and morphological changes in rats simultaneously treated with oxonic acid and uric acid. Administration of oxonic acid alone was reported not to induce morphological change in the kidney [28,30]. Therefore, the morphological changes in the renal tubules of hyperuricemic rats were mostly caused by the direct or indirect effect of hyper uric acid. Since urate crystals were not detected in the kidney, these changes were not due to the formation of casts. Recently, Mazzali *et al.* [30] reported that a crystal-independent mechanism contributed to renal injury with collagen deposition, macrophage infiltration and increased osteopontin expression in hyperuricemia.

Organic anion transport activity at the basolateral membrane was reduced in hyperuricemic rat kidney, since the accumulation of PAH and methotrexate into renal slices was significantly decreased. Moreover, we found that the expression of rOAT1 and rOAT3 significantly decreased in hyperuricemic rat kidney. rOAT1 mediates transport of PAH and several pharmacological agents, such as methotrexate, β-lactam antibiotics and non-steroidal anti-inflammatory drugs [3]. On one hand, rOAT3 mediates estrone sulfate transport effectively and transports of several anionic compounds including PAH, methotrexate, prostaglandin E_2 or cAMP [3]. Therefore, we considered that the reduced expression of both rOAT1 and rOAT3 should be responsible for the decreases in PAH and methotrexate transport at the basolateral membrane in hyperuricemic rat

Table 2
Pharmacokinetic parameters of methotrexate and cimetidine in control and hyperuricemic rats

	Cpss (μM)	CLr (mL/min)	fu	CLr,u (mL/min)	GFR (mL/min)	CLr,u/GFR
Methotrexate						
Control	0.74 ± 0.06	1.86 ± 0.08	0.39 ± 0.02	4.82 ± 0.39	2.32 ± 0.25	2.10 ± 0.16
Hyperuricemia	1.68 ± 0.18*	0.41 ± 0.08*	0.57 ± 0.03*	0.74 ± 0.15*	0.44 ± 0.09*	1.71 ± 0.05*
Cimetidine						
Control	11.6 ± 1.0	5.44 ± 0.28	0.80[a]	6.84 ± 0.42	1.67 ± 0.12	4.13 ± 0.14
Hyperuricemia	23.3 ± 2.3*	1.83 ± 0.19*	0.72 ± 0.04	2.58 ± 0.43*	0.49 ± 0.03*	5.40 ± 1.14

Note: Values are the mean ± SE from 3 to 5 rats. Cpss, plasma concentration at steady-state; CLr, renal clearance; fu, unbound fraction; CLr,u, unbound renal clearance; GFR, glomerular filtration rate. (a) The value is the mean from two rats and was used to estimate CLr,u of the other rat.

* $P < 0.05$, significantly different from control.

kidney. We further investigated the *in vivo* renal excretion of methotrexate in hyperuricemic rats. The renal clearance of methotrexate was markedly decreased in hyperuricemic rats. Notably, the ratio of unbound renal methotrexate clearance to the glomerular filtration rate was significantly decreased in hyperuricemic rats, suggesting that renal secretion was impaired more than the glomerular filtration. Uric acid is a substrate for rOAT1, but its affinity is considered to be low since 2 mM uric acid did not completely inhibit PAH uptake via rOAT1 [12]. The plasma uric acid concentration in hyperuricemic rats was 5.3 mg/dL, which corresponds to 0.32 mM. Therefore, we considered that renal methotrexate secretion *in vivo* was not competitively inhibited by plasma uric acid in hyperuricemic rats.

Both TEA and cimetidine are substrates for rOCT1 and rOCT2 [10]. Basolateral organic cation transport activity and rOCT2 expression was decreased in hyperuricemic rats, although rOCT1 expression was not changed. Therefore, we assumed that the reduced expression of rOCT2 would contribute to the decrease in basolateral organic cation transport activity. We previously reported that organic cation transport activity at the renal basolateral membrane was higher in male than female rats, which is attributable to higher expression of rOCT2 in male than female, although renal expression of rOCT1 and rOCT3 was not different between male and female [23]. Moreover, the rOCT2 expression level correlated with unbound renal clearance of cimetidine in 5/6 nephrectomized rats [22]. Therefore, we considered that rOCT2 would be a major transporter in the renal excretion of cationic compounds. Since the slice to medium ratio of TEA was 2.1 at 60 min after incubation in hyperuricemic rats, constitutively expressed rOCT1 in the kidney might also contribute to the concentrative accumulation of TEA in renal slices of hyperuricemic rats.

In vivo unbound renal clearances of methotrexate and cimetidine significantly decreased in hyperuricemic rats. Renal clearance of methotrexate and cimetidine may be affected by organic anion and cation transport activity not only at the basolateral membrane but also at the brush-border membrane, since renal secretion is consisted of two transport steps in both membranes. It was reported that organic anion transporters, OAT4, OAT-K1, OAT-K2, multidrug resistance-related protein (MRP)2 and MRP4, localized at the brush-border membrane in kidney and mediated methotrexate transport [3,31]. In 5/6 nephrectomized rats, renal clearance of methotrexate was reduced with decreased expression of OAT-K1 and OAT-K2, although the MRP2 expression and basolateral methotrexate transport activity were not changed [24]. Since OAT-K1 and OAT-K2 mRNA expression was not changed in hyperuricemic rats, the decrease in basolateral organic anion transport activity via rOAT1 and rOAT3 would be responsible for the decrease in renal clearance of methotrexate in hyperuricemic rats. On the other hand, cimetidine transport at the brush-border membrane in the kidney was mediated by the H^+/organic cation antiporter [32], although the molecular identity of organic cation transporter at the brush-border membrane is not yet clarified. Taking into consideration that unbound renal clearance of cimetidine correlated with the expression level of rOCT2 in 5/6 nephrectomized rats [22], the decreased basolateral organic cation transport via rOCT2 should contribute at least in part to the decreased renal clearance of cimetidine in hyperuricemic rats.

All transporters examined in this study were shown to be present in proximal tubules of the rat kidney. Although the expression levels of several transporters were unchanged, those of rOAT1, rOAT3 and rOCT2 were significantly decreased in hyperuricemic rats. Therefore, we considered that the decreased expression of rOAT1, rOAT3 and rOCT2 could not be simply explained by cell death in renal injury and was specifically regulated in hyperuricemia. The decreased expression of rOAT3 and rOCT2 was also suggested in cisplatin-induced nephropathy [33]. On the other hand, the renal expression of MRP1, MRP2 and P-glycoprotein was increased in cisplatin-induced nephropathy in rats [33,34]. The induction of MRP2 was also reported in chronic renal failure caused by subtotal nephrectomy [35]. On the other hand, the expression of rOCT2 was reported to be up-regulated by testosterone and down-regulated by estradiol in rats [36], and we found that plasma concentration of testosterone was significantly decreased in hyperuricemic rats (control, 3.2 ± 0.6, hyperuricemia, 1.1 ± 0.1 μg/mL; mean ± SE, $N = 6$). Ji *et al.* [22] reported that the expression of rOCT2 was reduced in 5/6 nephrectomized rats, and suggested that the lowered plasma level of testosterone was responsible for the decreased rOCT2 expression. Further study on the regulatory factors for the expression of renal transporters is necessary to understand the change in the renal excretion of various compounds in disease states.

In conclusion, we showed decreased activity of organic anion and cation transport at the basolateral membrane and specific down-regulation of rOAT1, rOAT3 and rOCT2 in hyperuricemic rats. These phenomena partly contribute to the changed renal disposition of organic anions and cations in hyperuricemia.

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References

[1] Pritchard JB, Miller DS. Mechanisms mediating renal secretion of organic anions and cations. Physiol Rev 1993;73:765–96.

[2] Inui K, Okuda M. Cellular and molecular mechanisms of renal tubular secretion of organic anions and cations. Clin Exp Nephrol 1998;2:100–8.

[3] Inui K, Masuda S, Saito H. Cellular and molecular aspects of drug transport in the kidney. Kidney Int 2000;58:944–58.
[4] Shimada H, Moewes B, Burckhardt G. Indirect coupling to Na^+ of *p*-aminohippuric acid uptake into renal basolateral membrane vesicles. Am J Physiol 1987;253:F795–801.
[5] Ohoka K, Takano M, Okano T, Maeda S, Inui K, Hori R. *p*-Aminohippurate transport in rat renal brush-border membranes: a potential-sensitive transport system and an anion exchanger. Biol Pharm Bull 1993;16:395–401.
[6] Takano M, Inui K, Okano T, Saito H, Hori R. Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. Biochim Biophys Acta 1984;773: 113–24.
[7] Gründemann D, Gorboulev V, Gambaryan S, Veyhl M, Koepsell H. Drug excretion mediated by a new prototype of polyspecific transporter. Nature 1994;372:549–52.
[8] Okuda M, Saito H, Urakami Y, Takano M, Inui K. cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. Biochem Biophys Res Commun 1996;224:500–7.
[9] Busch AE, Quester S, Ulzheimer JC, Waldegger S, Gorboulev V, Arndt P, Lang F, Koepsell H. Electrogenic properties and substrate specificity of the polyspecific rat cation transporter rOCT1. J Biol Chem 1996;271:32559–604.
[10] Urakami Y, Okuda M, Masuda S, Akazawa M, Saito H, Inui K. Distinct characteristics of organic cation transporters, OCT1 and OCT2, in the basolateral membrane of renal tubules. Pharm Res 2001;18:1528–34.
[11] Sugawara-Yokoo M, Urakami Y, Koyama H, Fujikura K, Masuda S, Saito H, Naruse T, Inui K, Takata K. Differential localization of organic cation transporters rOCT1 and rOCT2 in the basolateral membrane of rat kidney proximal tubules. Histochem Cell Biol 2000;114:175–80.
[12] Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H. Expression cloning and characterization of a novel multispecific organic anion transporter. J Biol Chem 1997;272:18526–9.
[13] Sweet DH, Wolff NA, Pritchard JB. Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. J Biol Chem 1997;272:30088–95.
[14] Tojo A, Sekine T, Nakajima N, Hosoyamada M, Kanai Y, Kimura K, Endou H. Immunohistochemical localization of multispecific renal organic anion transporter 1 in rat kidney. J Am Soc Nephrol 1999; 10:464–71.
[15] Cha SH, Sekine T, Fukushima J, Kanai Y, Kobayashi Y, Goya T, Endou H. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. Mol Pharmacol 2001;59:1277–86.
[16] Iseki K, Oshiro S, Tozawa M, Iseki C, Ikemiya Y, Takishita S. Significance of hyperuricemia on the early detection of renal failure in a cohort of screened subjects. Hypertens Res 2001;24:691–7.
[17] Brown EA, Kliger AS, Hayslett JP, Finkelstein FO. Renal function in rats with acute medullary injury. Nephron 1980;26:64–8.
[18] Stopp M, Bräunlich H. *In vitro* analysis of drug-induced stimulation of renal tubular *p*-aminohippurate (PAH) transport in rats. Biochem Pharmacol 1980;29:983–6.
[19] Stopp M, Hartwich R, Bräunlich H. Enhancement of *p*-aminohippurate accumulation in renal cortical slices after repeated administrations of various organic anionic drugs to rats of different ages. Experientia 1978;34:1493–4.
[20] Spencer HW, Yarger WE, Robinson RR. Alterations of renal function during dietary-induced hyperuricemia in the rat. Kidney Int 1976; 9:489–500.
[21] Ito T, Yano I, Masuda S, Hashimoto Y, Inui K. Distribution characteristics of levofloxacin and grepafloxacin in rat kidney. Pharm Res 1999;16:534–9.
[22] Ji L, Masuda S, Saito H, Inui K. Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy. Kidney Int 2002;62:514–24.
[23] Urakami Y, Nakamura N, Takahashi K, Okuda M, Saito H, Hashimoto Y, Inui K. Gender differences in expression of organic cation transporter OCT2 in rat kidney. FEBS Lett 1999;461:339–42.
[24] Takeuchi A, Masuda S, Saito H, Doi T, Inui K. Role of kidney-specific organic anion transporters in the urinary excretion of methotrexate. Kidney Int 2001;60:1058–68.
[25] Kaneniwa N, Funaki T, Furuta S, Watari N. High-performance liquid chromatographic determination of cimetidine in rat plasma, urine and bile. J Chromatogr 1986;374:430–4.
[26] Kojima T, Nishina T, Kitamura M, Kamatani N, Nishioka K. Reversed-phase liquid-chromatographic determination of compounds in serum applied to studies of hypouricemia. Clin Chem 1986;32:287–90.
[27] Wetzel RK, Sweadner KJ. Immunocytochemical localization of Na–K–ATPase α- and γ-subunits in rat kidney. Am J Physiol Renal Physiol 2001;281:531–45.
[28] Stavric B, Johnson WJ, Grice HC. Uric acid nephropathy: an experimental model. Proc Soc Exp Biol Med 1969;130:512–6.
[29] Waisman J, Bluestone R, Klinenberg JR. A preliminary report of nephropathy in hyperuricemic rats. Lab Invest 1974;30:716–22.
[30] Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY, Kivlighn S, Johnson RJ. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension 2001;38:1101–6.
[31] van Aubel RA, Smeets PH, Peters JG, Bindels RJ, Russel FG. The MRP4/ABCC4 gene encodes a novel apical organic anion transporter in human kidney proximal tubules: putative efflux pump for urinary cAMP and cGMP. J Am Soc Nephrol 2002;13:595–603.
[32] Takano M, Inui K, Okano T, Hori R. Cimetidine transport in rat renal brush border and basolateral membrane vesicles. Life Sci 1985;37: 1579–85.
[33] Huang Q, Dunn II RT, Jayadev S, DiSorbo O, Pack FD, Farr SB, Stoll RE, Blanchard KT. Assessment of cisplatin-induced nephrotoxicity by microarray technology. Toxicol Sci 2001;63:196–207.
[34] Demeule M, Brossard M, Béliveau R. Cisplatin induces renal expression of P-glycoprotein and canalicular multispecific organic anion transporter. Am J Physiol Renal Physiol 1999;277:F832–40.
[35] Laouari D, Yang R, Veau C, Blanke I, Friedlander G. Two apical multidrug transporters, P-gp and MRP2, are differently altered in chronic renal failure. Am J Physiol Renal Physiol 2001;280:F636–45.
[36] Urakami Y, Okuda M, Saito H, Inui K. Hormonal regulation of organic cation transporter OCT2 expression in rat kidney. FEBS Lett 2000; 473:173–6.