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Research Paper

Involvement of Uric Acid Transporters in Alteration of Serum Uric Acid Level by Angiotensin II Receptor Blockers

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Purpose. To examine the mechanisms of the alteration of serum uric acid level by angiotensin II receptor blockers (ARBs), the effects of ARBs on renal uric acid transporters, including OAT1, OAT3, OAT4, and MRP4, were evaluated.
Materials and Methods. Uptakes of uric acid by OAT1-expressing Flp293 cells, by *Xenopus* oocytes expressing OAT3 or OAT4, and by membrane vesicles from Sf9 cells expressing MRP4 were evaluated in the presence or absence of ARBs.
Results. All ARBs inhibited uptake of uric acid or estrone-3-sulfate by OAT1, OAT3 and OAT4 in concentration dependent manners. Among them, the IC_{50} values of valsartan, olmesartan and pratosartan for OAT3 were comparable to clinically observed unbound maximum plasma concentration of ARBs. Candesartan, losartan, and telmisartan inhibited ATP-dependent uptake of uric acid by MRP4 at 10 μM. The IC_{50} value of losartan for MRP4 was comparable to the estimated kidney tissue concentration of losartan. No ARBs showed *trans*-stimulatory effects on the uptake of estrone-3-sulfate by OAT4.
Conclusion. Valsartan, olmesartan, and pratosartan could inhibit the OAT3-mediated uric acid secretion in clinical situations. Furthermore losartan could inhibit ATP-dependent uric acid secretion by MRP4. These effects may explain partially the alteration of serum uric acid level by ARBs.

KEY WORDS: angiotensin II receptor blockers; kidney; MRP4; OAT; uric acid transporter.

INTRODUCTION

Epidemiological studies have shown that elevated serum uric acid (SUA) level promotes the development of cardiovascular disease in patients with hypertension (1,2). Therefore, an increase of SUA level should be prevented in patients with hypertension. It is known that angiotensin II receptor blockers (ARBs) exhibit differential effects on SUA level. Namely, candesartan (3) and valsartan (4) increase SUA level, whereas losartan (5–7) and pratosartan decrease it (Ogihara *et al.*, "The 21st Scientific Meeting of The International Society of Hypertension", Abstract PO3-18, 2006). The possible mechanisms of the change of SUA level induced by pharmacologically active agents include changes in the generation of uric acid in liver and/or in the secretion and reabsorption of uric acid in kidney (8,9). Among these mechanisms, alteration of renal handling of uric acid by ARBs could be significant; for example, losartan, which is known to decrease the SUA level, increases the renal clearance of uric acid (5–7).

In humans, the so-called "four-component hypothesis" has been proposed to explain the renal handling of uric acid (9). Reabsorption and secretion of uric acid depend on transporters that reside in the renal tubular epithelial cell membranes (10). After glomerular filtration, uric acid is mostly reabsorbed from the tubular lumen to blood via the uric acid transporter URAT1 (11) and recently the involvement of organic anion transporter OAT4 was suggested (12). Tubular secretion of uric acid after reabsorption is thought to be mediated by plural transporters, including organic anion transporters (13,14), and multidrug resistance associated protein 4 (15). So, interaction between uric acid and ARBs on these renal transporters could be the mechanism of the altered SUA level similar to the transporter-mediated drug–drug interaction reported previously on renal transporters such as organic cation transporters (OCTs), organic cation/carnitine transporter 2 (OCTN2) and *P*-glycoprotein (*P*-gp; 16–18).

Based on this background, we have studied the mechanisms of the effect of ARBs on SUA level and demonstrated

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ABBREVIATIONS: ARB, angiotensin II receptor blocker; $E_1$3S, estrone-3-sulfate; MRP, multidrug resistance-associated protein; OAT, organic anion transporter; SUA, serum uric acid; URAT1, uric acid transporter.

that ARBs exhibited variable effects on URAT1, including *cis*-inhibitory and/or *trans*-stimulatory effects on URAT1 depending on the concentrations of ARBs (19). The ARBs such as candesartan, valsartan, olmesartan, losartan, and pratosartan, though not telmisartan, all had the potential to show a *trans*-stimulatory effect on uric acid transport by URAT1, which means that they may increase reabsorption of uric acid via URAT1. On the other hand, losartan and pratosartan exhibited a *cis*-inhibitory effect on the uptake of uric acid by URAT1, which clinically corresponds to a reduction of reabsorption of uric acid via URAT1. The Ki values of losartan and pratosartan for URAT1 were 7.7 and 6.7 nM, respectively, and these values are comparable with the clinically observed unbound maximum plasma concentrations of the drugs (19). These observations may explain partially the variable effects of ARBs on SUA level, while the involvement of other mechanisms on the secretion via basolateral membrane transporters and other reabsorption transporters cannot be excluded.

It has been demonstrated that uric acid is a substrate of the renal transporters OAT1 (13) and OAT3 (14) in the basolateral membrane, as well as OAT4 (12) and MRP4 (15) in the apical membrane. Therefore, alteration of those transporter activities by ARBs may lead to changes in SUA level. Accordingly, in the present study, we examined the possible involvement of OAT1, OAT3, OAT4 and MRP4 in the change of SUA level by ARBs in addition to the previously investigated URAT1.

MATERIALS AND METHODS

Chemicals

[^{14}C]Uric acid (1.96 TBq/mol) was purchased from Moravek Biochemicals, Inc. (Brea, CA). [^{3}H]Estrone-3-sulfate ([^{3}H]$E_1$3S, 2.12 TBq/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Losartan and telmisartan were purchased from Zhejiang Tianyu Pharmaceutical (Zhejiang, China). Valsartan was purchased from Livzon Group Changzhou Kony Pharmaceutical (Changzhou, China). Pratosartan (Patterson, 2003), candesartan and olmesartan were synthesized and kindly supplied by Kotobuki Pharmaceutical (Nagano, Japan). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan).

cDNA Cloning of OAT1, OAT3 and OAT4

The *OAT1* gene was PCR-amplified using a Marathon ready human kidney cDNA library (Clontech Laboratory Inc., Mountain View, CA) as a template, with upstream primer 5′-CAATGGCCTTTAATGACCTC-3′ and downstream primer 5′-GTCCTCAGAGTCCATTC-3′ (both synthesized by Hokkaido System Science, Sapporo, Japan), based on the reported *OAT1* gene sequence (20; GenBank accession no. NM004790). A major 1.6-kilobase-polymerase chain reaction product was ligated into pCR2.1-TOPO cloning vector (Invitrogen, Carlsbad, CA) and the OAT1–cDNA was digested with *Eco*RI and ligated into plasmid vector pcDNA3.1 (Invitrogen).

The *OAT3* gene was PCR-amplified using Cap Site cDNA dT human kidney (Nippon Gene, Tokyo, Japan) as a template, with upstream primer 5′-CACCAGCCCCATC GGATCCA-3′ and downstream primer 5′-TCAC CAAGCTCTCAGAAGGCTTCA-3′ and Ex Taq DNA polymerase (Takara Shuzo Co., Shiga, Japan), based on the reported *OAT3* gene sequence (14; GenBank accession no. AB042505). A major 1.8-kilobase polymerase chain reaction product was ligated into the TA cloning vector pGEM-T Easy (Promega, Madison, WI), and then OAT3 complementary DNA (cDNA) was digested with *Eco*RI and ligated to pGEMHE (21). The obtained cDNA sequences were analyzed and confirmed to be the same as the reported ones.

The method of cDNA cloning of OAT4 was described previously (22).

Establishment of Flp293 Cells Expressing OAT1

cDNA of OAT1 in the expression vector pcDNA3.1 was digested with *Nhe*I and *Bam*HI and ligated into the same site of pcDNA5/FRT (Invitrogen). The resulting vectors containing OAT1 or pcDNA5/FRT (termed MOCK) were co-transfected with pOG44 (Invitrogen) into the Flp293 host cell line (Invitrogen) using Lipofectamine 2000 (Invitrogen) (23). Stable integrants were selected by culture in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1×10^5 U/l penicillin G, 100 mg/l streptomycin and 200 mg/l hygromycin B (Invitrogen) for about 2 weeks.

Transport Study by Cultured Cells

For the uptake study with OAT1, Flp293-OAT1 cells were seeded in 24-well tissue culture plates at a density of 4×10^5 cells/well. The cells were cultured for 2 days, then washed three times with transport medium (125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 25 mM HEPES, pH 7.4), and preincubated in the same solution in a water bath at 37°C for 10 min. The uptake reaction was initiated by adding cell to transport medium containing [^{14}C]uric acid, and the mixture was incubated at 37°C for 5 min. In inhibition studies, ARB or 1% dimethyl sulfoxide (DMSO) was added simultaneously with [^{14}C]uric acid. The uptake was terminated by addition of ice-cold transport medium, and the cells were washed three times with transport medium. The cells in each well were lysed with 0.2 ml of 1 N NaOH and neutralized with HCl.

Transport Study Using *Xenopus* Oocytes

Complementary RNAs (cRNAs) of OAT3 and OAT4 were prepared by *in vitro* transcription with T7 RNA polymerase in the presence of ribonuclease inhibitor and an RNA cap analog using a mMESSAGE mMACHINE kit (Ambion, Austin TX). Uptake experiments were conducted as described previously (22). Briefly, for the uptake study with OAT3, defolliculated oocytes were injected with 25 ng of OAT3 cRNA or the same volume of water, and cultured in modified Barth's solution (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, and 10 mM HEPES, pH 7.4) containing 50 μg/ml gentamycin at 18°C for 3 days. The oocytes were transferred

to 24-well tissue culture plates and incubated with ND96 buffer (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.4) containing [^{14}C]uric acid with or without unlabeled uric acid at 25°C for 60 min. In inhibition studies, ARB or 1% DMSO was added simultaneously with [^{14}C]uric acid. The uptake was terminated by washing the oocytes three times with ice-cold ND96 buffer. The oocytes were solubilized with 5% sodium dodecyl sulfate solution.

Uptake study with OAT4 was done in the same manner as described for OAT3. In the *cis*-inhibition study, ARB or 1% DMSO was added simultaneously with [^{3}H]$E_1$3S. In the *trans*-stimulation study, the oocytes were microinjected with 50 nl of drug solution or water as a control containing 1% DMSO. Immediately after the microinjection (within approximately 2 min), the oocytes were transferred to ND96 buffer containing [^{3}H] $E_1$3S. The transport study was terminated and the oocytes were solubilized in the same manner as described above.

For the efflux study with OAT3 and OAT4, defolliculated oocytes were injected with 25 ng of OAT3 cRNA or 10 ng of OAT4 cRNA or the same volume of water and cultured in modified Barth's solution containing 50 μg/ml gentamycin at 18°C for 3 days as described above. The oocytes were injected with 1 nCi of [^{14}C]uric acid, which corresponds to 19 pmol of uric acid, in a injected volume of 50 nl. On the basis of the intracellular volumes of the oocytes (about 0.8 μl: 19), the intracellular concentration in the oocytes would be approximately 20 μM. The oocytes were then immediately transferred to 24-well culture plates with 700 μl of ND96 buffer and incubated for 90 min. At 30, 60, and 90 min, a 100 μl aliquot of efflux buffer was sampled. At 90 min, the oocytes were washed three times with ice-cold ND96 buffer, and then solubilized with 5% sodium dodecyl sulfate solution.

Transport Study Using Membrane Vesicles

Membrane vesicles from Sf9 cells expressing MRP4 were obtained from GenoMembrane Inc. (Yokohama, Japan). Uptake experiments were conducted as described previously (24). Briefly, membrane vesicles were thawed at 37°C for 1 min, added to prewarmed assay medium (1 M KCl, 1 M $MgCl_2$, and 100 mM MOPS–Tris, pH 7.0) containing [^{14}C]uric acid, and preincubated at 37°C for 5 min. Uptake was initiated by addition of 10 mM MgATP or 10 mM MgAMP to the reaction mixture in a final volume of 50 μl, and incubation was continued at 37°C for 10 min. Uptake was terminated by adding 1 ml of ice-cold stop buffer (1 M KCl, and 100 mM MOPS–Tris, pH 7.0), then the mixture was filtered through a nitrocellulose filter (0.45-μM pore size, Millipore, Bedford, MA). The filters were washed twice with 5 ml of ice-cold stop buffer, and dissolved in Clearsol-I (Nacalai Tesque, Kyoto, Japan) for liquid scintillation counting.

Analytical Method

For uptake study, the radioactivity was measured using a liquid scintillation counter (LSC-5100, Aloka, Tokyo). Uptake was expressed as the cell-to-medium ratio (microliters per milligram protein or per oocyte), obtained by dividing the uptake amount by the concentration of substrate in the uptake medium. The results are shown as mean±SEM. obtained from three samples for OAT1 and MRP4, and from ten oocytes for OAT3 and OAT4. Transporter-mediated initial uptake rates were obtained after subtraction of the uptake by MOCK cells from that by OAT1-expressing cells, or the uptake by water-injected oocytes from that by OAT3 or OAT4 cRNA-injected oocytes. MRP4-mediated uptake by membrane vesicles was evaluated by subtracting the uptake in presence of AMP from that in the presence of ATP. For the evaluation of the kinetic parameters, the rates were fitted to the following equation (1) by means of nonlinear least-squares regression analysis using Kaleidagraph (Synergy Software, Reading, PA):

$$v = V_{max} \times s/(K_m + s) \quad (1)$$

where v, s, K_m, and V_{max} are the initial uptake rate of substrate (picomoles per indicated time per mg protein or per oocyte), the substrate concentration in the medium (micromole/liter), the apparent Michaelis–Menten constant (micromoles/liter), and the maximal uptake rate (picomoles per indicated time per mg protein or per oocyte), respectively. The inhibitory effect was expressed as % of control, and the inhibitor concentration giving half-maximum inhibition (IC_{50}) was calculated by application of the following equation (2):

$$\%\ of\ control = 100 \times IC_{50}^{n}/\left(IC_{50}^{n} + [I]^{n}\right) \quad (2)$$

where n and $[I]$ are the Hill coefficient and the inhibitor concentration (micromolar), respectively.

For efflux study, the radioactivity was measured with a liquid scintillation counter, using Clearsol-I as a liquid scintillation fluid. Total [^{14}C]uric acid was calculated as the sum of [^{14}C]uric acid remaining in the oocytes and total efflux.

For protein assay, cellular protein content was measured according to the method of Bradford (1976) using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) with BSA as the standard.

Statistical Analysis

Statistical significance of differences was determined with Student's t test or by analysis of variance followed by Dunnett's test, and a p value of less than 0.05 was considered statistically significant.

RESULTS

Influx and Efflux Transports of Uric Acid by OAT1, OAT3, OAT4, and MRP4

First of all, uptake of uric acid by OAT1-, OAT3-, and OAT4-expressing cells or oocytes (OAT1: Flp293 cells, OAT3 and OAT4: oocytes) was measured. As shown in Fig. 1, uptake of [^{14}C]uric acid by OAT1, OAT3, or OAT4 was significantly higher than the control (MOCK cells or water-injected oocytes). In MRP4-expressing membrane vesicles, ATP-dependent uptake of [^{14}C]uric acid was significantly higher than in the control membrane vesicles (Fig. 1d). The uptake of [^{14}C]uric acid by OAT1-, OAT3-,

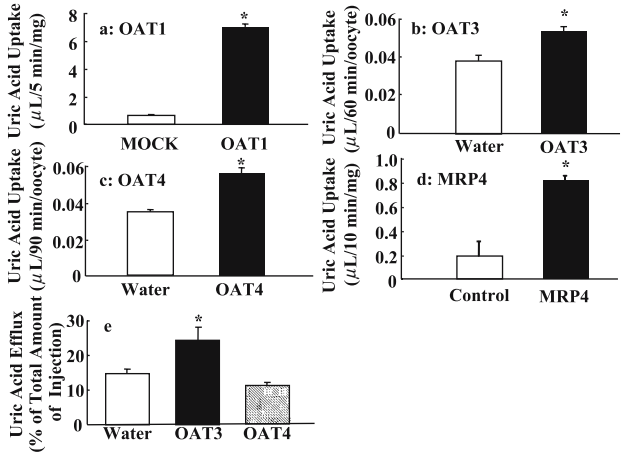

Fig. 1. Uptake and efflux of [^{14}C]uric acid by OAT1, OAT3, OAT4 and MRP4. **a** Uptake of [^{14}C]uric acid (10 μM) by Flp293–MOCK cells and Flp293–OAT1 cells was measured for 5 min. **b** Uptake of [^{14}C]uric acid (20 μM) by water-injected and OAT3–cRNA-injected oocytes was measured for 60 min. **c** Uptake of [^{14}C]uric acid (20 μM) by water-injected and OAT4–cRNA-injected oocytes was measured for 90 min. **d** Uptake of [^{14}C]uric acid (65 μM) by membrane vesicles derived from Sf9 cells expressing or not expressing MRP4 was measured in the presence of 4 mM AMP or 4 mM ATP for 10 min. ATP-dependent uptake of [^{14}C]uric acid was calculated from the difference between in the presence and absence of ATP, and the MRP4-specific uptake was calculated as the difference in ATP-dependent uptake between membrane vesicles obtained from the Sf9 cells expressing and not expressing MRP4. **e** Efflux of [^{14}C]uric acid from oocytes injected with water, OAT3–cRNA or OAT4–cRNA was measured for 60 min immediately after microinjection of [^{14}C]uric acid (19 pmol) into the oocytes. Efflux is shown as the efflux percent of total injected [^{14}C]uric acid in 60 min. Each point represents the mean+SEM (n=3 for **a** and **d**, and 10 for **b**, **c**, and **e**). An *asterisk* indicates a significant difference from the uptake by Flp293–MOCK (**a**), by water-injected oocytes (**b**) and (**c**), and control membrane vesicles (**d**), and from the efflux by water-injected oocytes, respectively (p<0.05).

and OAT4-expressing cells linearly increased for over 10, 90, and 120 min, respectively, and was significantly greater than the corresponding control uptake (data not shown). Therefore, uptake at 5, 60, and 90 min by OAT1, OAT3 and OAT4, respectively, was routinely used for evaluation of the initial uptake rate of [^{14}C]uric acid in the subsequent studies.

To estimate the direction of OAT4-mediated uric acid transport, efflux of uric acid from OAT4-expressing oocytes was examined. Figure 1e shows the efflux of [^{14}C]uric acid from OAT4-expressing oocytes, as well as that from OAT3-expressing oocytes as a positive control for efflux transport. There was no difference in the efflux of uric acid between OAT4-expressing oocytes and water-injected control oocytes, whereas the efflux from OAT3-expressing oocytes was significantly higher than that from water-injected oocytes.

The concentration dependence of uric acid uptake by OAT1, OAT3 and OAT4 was examined to compare the affinities of the transporters for uric acid. The OAT1-, OAT3-, OAT4-mediated uric acid uptakes were saturable, and the K_m values, determined by nonlinear regression analysis, were 197.6±67.6 μM, 380.3±106.2 μM, and 106.0±45.9 μM, respectively. Thus, the OATs showed comparable affinities for uric acid, and the K_m values are close to the plasma concentration of uric acid (Figs. 2a–c).

Cis–Inhibitory Effect of ARBs on Uric Acid Uptake by OAT1, OAT3, OAT4, and MRP4

The effects of ARBs on the uptake of [^{14}C]uric acid via OAT1, OAT3, and MRP4 were examined. All ARBs reduced the uptake of [^{14}C]uric acid by OAT1 and OAT3 in a concentration-dependent manner. The results are showed in Fig. 3 and the IC_{50} values are summarized in Table I. Olmesartan showed the strongest effect on both OAT1- and OAT3-mediated uptakes of uric acid, with IC_{50} values of 280 nM and 27 nM, respectively (Table I). ATP-dependent uric acid uptake by MRP4-expressing membrane vesicles was reduced by losartan, telmisartan, and candesartan with IC_{50} values of 1.5, 11, and 16 μM, while other ARBs showed less potent inhibitory effects with higher IC_{50} values.

In the case of OAT4, although it showed significant uptake of uric acid (Fig. 1c), the extent of the uptake was not large enough to allow kinetic analysis. Accordingly, we used [^{3}H]$E_1$3S as the substrate of OAT4 for the evaluation of ARB effect, since [^{3}H]$E_1$3S is easy to detect. All ARBs evaluated in this study reduced OAT4-mediated uptake of [^{3}H]$E_1$3S in a concentration-dependent manner (Fig. 3c). The IC_{50} values were significantly higher than the estimated kidney tissue concentrations (see Table I).

Trans-Stimulatory Effect of ARBs on OAT4-Mediated Uptake of [^{3}H]Estrone − 3 − Sulfate

The *trans*-stimulatory effect of ARBs on the uptake of [^{3}H]$E_1$3S by OAT4 was examined, since it had been shown that OAT4 exhibited uptake of uric acid, and it was considered that OAT4 is involved in reabsorption of uric acid. Oocytes microinjected with cRNA of OAT4 or water were preinjected with 5 mM succinate, 50 μM telmisartan, or 100 μM other ARBs and immediately incubated with [^{3}H]$E_1$3S for 60 min. The uptakes of [^{3}H]$E_1$3S at 60 min, after correction for uptake by water-injected oocytes, are shown in Fig. 4. OAT4-mediated uptake of [^{3}H]$E_1$3S was increased when oocytes were preloaded with succinate. On the other hand, no ARBs induced an increase in the uptake of [^{3}H]$E_1$3S by OAT4. Accordingly, OAT4 is not likely to exhibit *trans*-stimulation of the uptake of uric acid by ARBs.

DISCUSSION

Previously, we demonstrated that ARBs affect reabsorption of uric acid via URAT1 owing to their *cis*-inhibitory and/or *trans*-stimulatory effects (19). Although *trans*-stimulation of URAT1 apparently explains the increase of SUA level by ARBs, the possibility of interaction between ARBs and uric acid on the transporters responsible for tubular secretion cannot be excluded. Furthermore, since glomerularly filtered uric acid is almost exclusively reabsorbed, the final amount of urinary excretion is largely dependent on the tubular

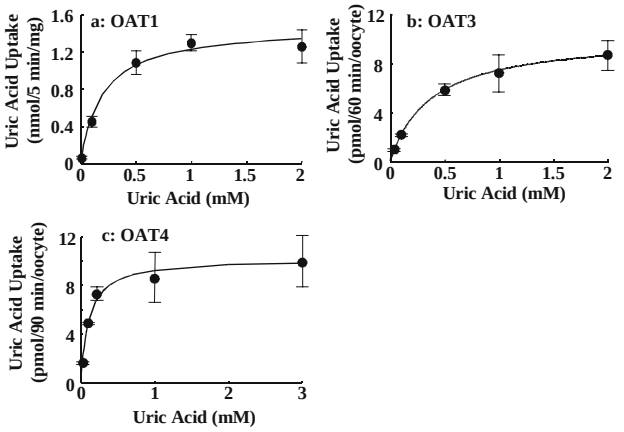

Fig. 2. Concentration dependence of OAT1-, OAT3- and OAT4-mediated uric acid uptake. **a** Uptake of [^{14}C]uric acid by Flp293–MOCK cells and Flp293–OAT1 cells at various concentrations (10–2,000 μM) was measured for 5 min and the OAT1-specific uptake, obtained as the difference between the two types of cells, is shown. Each point represents the mean±SEM from three wells with cells. **b** Uptake of [^{14}C]uric acid by water-injected and OAT3–cRNA-injected oocytes at various concentrations (20–2,000 μM) was measured for 60 min. OAT3-specific uptake, determined by subtracting the uptake in water-injected oocytes from that in OAT3–cRNA-injected oocytes, is shown. Each point represents the mean±SEM from ten oocytes. **c** Uptake of [^{14}C]uric acid by water-injected and OAT4–cRNA-injected oocytes at various concentrations (30–3,000 μM) was measured for 90 min. OAT4-specific uptake, determined by subtracting the uptake in water-injected oocytes from that in OAT4–cRNA-injected oocytes, is shown. Each point represents the mean±SEM from 9–11 oocytes.

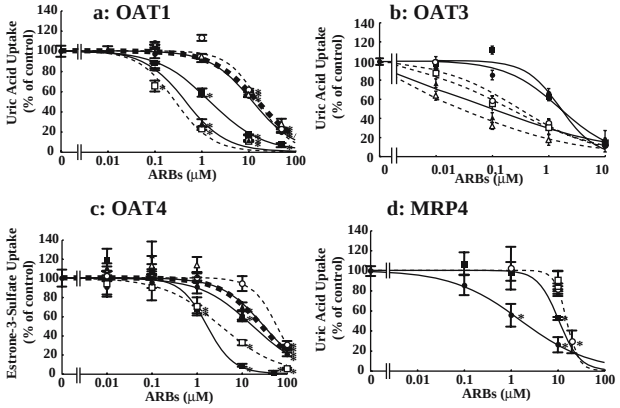

Fig. 3. *Cis*-Inhibitory effects of ARBs on OAT1, OAT3, OAT4 and MRP4. **a** [^{14}C]Uric acid (10 μM) uptake by Flp293–MOCK cells and Flp293–OAT1 cells was measured for 5 min in the presence and in the absence (control) of ARBs (0.1–50 μM for candesartan, valsartan, losartan, and pratosartan, 0.1–10 μM for olmesartan, and 0.01–10 μM for telmisartan). Results are shown as follows (presence and absence of ARB, respectively): candesartan (*open circles* and *dotted line*), valsartan (*open triangles* and *dotted line*), olmesartan (*open squares* and *dotted line*), losartan (*closed circles* and *solid line*), pratosartan (*closed triangles* and *solid line*), and telmisartan (*closed squares* and *solid line*). OAT1-mediated uptake was determined by subtracting the uptake in Flp293–MOCK cells from that in Flp293–OAT1 cells, and the values obtained were divided by the control value in each assay. Each point represents the mean±SEM from three wells with cells. **b** Uptake of [^{14}C]uric acid (20 μM) by water-injected and OAT3–cRNA-injected oocytes was measured in the presence and in the absence (control) of ARBs (0.01–10 μM) as described above for 60 min. OAT3-mediated uptake was determined by subtracting the uptake in water-injected oocytes from that in OAT3 cRNA-injected oocytes, and the values obtained were divided by the control value in each assay. Each point represents the mean±SEM from ten oocytes. **c** Uptake of [^{3}H] $E_1$3S (10 nM) by water-injected and OAT4–cRNA-injected oocytes was measured in the presence and in the absence (control) of ARBs (0.01–100 μM, except for telmisartan 0.01–50 μM) as described above for 60 min. OAT4-mediated uptake was determined by subtracting the uptake in water-injected oocytes from that in OAT4–cRNA-injected oocytes, and the values obtained were divided by the control value in each assay. Each point represents the mean±SEM from ten oocytes. **d** Uptake of [^{14}C]uric acid (65 μM) by membrane vesicles expressing MRP4 was measured for 10 min in the presence and in the absence (control) of ARBs, including candesartan (1–20 μM), valsartan (10 μM), olmesartan (10 μM), losartan (0.1–10 μM), pratosartan (10 μM) and telmisartan (0.1–10 μM). ATP-dependent uptake by MRP4 was determined by subtracting the uptake in the presence of 4 mM AMP from that in the presence of 4 mM ATP, and the values obtained were divided by the control value in each assay. Each symbol is the same as in the case of OAT1 described above. Each point represents the mean±SEM from three tubes with suspended membrane vesicles. An *asterisk* indicates a significant difference from the control by Dunnett's test ($p<0.05$).

secretion. Accordingly, the inhibition of the secretion of uric acid could also be due to the alteration of the SUA level by ARBs. It is also known that uric acid is transported by renal transporters OAT1, OAT3, OAT4, and MRP4 (12–15). Accordingly, in the present study, we examined the possible involvement of these uric acid transporters in the changes of SUA level induced by ARBs. All of the present observations and previously reported values are summarized in Table I, including K_i or IC_{50} values and the pharmacokinetic parameters of ARBs.

First of all, when OAT4 was expressed in *Xenopus* oocytes, a significant increase in the uptake of [^{14}C]uric acid was observed, while there was no increase in the efflux of uric acid (Fig. 1). Accordingly, it was suggested that OAT4 is involved in reabsorption, but not secretion, of uric acid under the present conditions. These observations corresponded well to those by Hagos *et al.* (12). To estimate the effect of ARBs on OAT4-mediated uric acid transport, we examined the *cis*-inhibitory and *trans*-stimulatory effects of ARBs on the uptake of $E_1$3S, a better OAT4 substrate than uric acid. Although all ARBs inhibited OAT4-mediated transport of [^{3}H]$E_1$3S in a concentration-dependent manner, their IC_{50} values were much higher than clinically observed unbound maximum plasma concentrations (Table I). Similar effects of ARBs on OAT4 were reported recently (25). We further examined the *trans*-stimulatory effect of ARBs on OAT4-mediated uptake of [^{3}H]$E_1$3S, to see whether ARBs might stimulate the reabsorption of uric acid via OAT4. However, no ARBs exhibited a *trans*-stimulatory effect on OAT4-mediated $E_1$3S uptake at the estimated intracellular concentration of ARBs 4.3 μM in the oocyte based on the intracellular volume of oocytes 0.8 μl, recovery ratio after microinjection 70%, the microinjected volume (50 nl), and the injected concentration of ARBs (100 μM, except for telmisartan, 50 μM) as reported previously (19). Since these concentrations are much higher than the estimated maximum tissue concentrations of ARBs in kidney (Table I), ARBs are unlikely to have a *trans*-stimulatory effect in the clinical context. Accordingly, these results indicated that OAT4

Table I. The Effects of ARBs on Uric Acid Transporters

ARB (Dose)		Candesartan (4 mg)	Valsartan (80 mg)	Olmesartan (40 mg)	Losartan (50 mg)	Pratosartan (80 mg)	Telmisartan (80 mg)
Total Cmax (nM)		125	6,500	2,330	600	2,340	710
Protein binding (%)		>99	95	99	98.7	98	>99
Unbound Cmax (nM)		<1.25	325	23.3	7.8	46.8	<7.1
K_p value (kidney)		0.6	NA	NA	1.44	1.27	NA
Total Cmax in kidney (nM)		75	(6,500)	(2,330)	864	2,972	(710)
Reabsorption							
URAT1[a]	K_i (nM)	NE	NE	NE	7.7	6.7	19
	Trans (%)[b]	178	136	123	131	132	NE
OAT4	IC_{50} (nM)	60,000	26,000	4,400	18,000	31,000	1,200
	Trans	NE	NE	NE	NE	NE	NE
Secretion							
OAT1	IC_{50} (nM)	17,000	16,000	280	12,000	1,500	460
OAT3	IC_{50} (nM)	300	200	27	1,600	95	1,600
MRP4	IC_{50} (nM)	16,000	NE at 10,000	NE at 10,000	1,500	NE at 10,000	11,000

Total Cmax: bound and unbound maximum plasma concentration; Total Cmax in kidney: Total Cmax; *K_p value in kidney (the concentrations of valsartan, olmesartan and telmisartan were estimated by assuming K_p=1)
[a] Data from Iwanaga *et al.* (19)
[b] Percent of control (microinjected concentration: 10 μM)
NA = not available; NE = no effect

functions in the reabsorption of uric acid, but is not involved in the alteration of the SUA level by ARBs.

Candesartan, which increased the SUA level, inhibited uptake of uric acid by OAT1, OAT3 and MRP4. However, the observed IC_{50} values for the uptake of uric acid by OAT1 and OAT3, 17 μM and 300 nM, respectively, were higher than the unbound maximum plasma concentration of candesartan (<1.25 nM), and that for MRP4-mediated uric acid uptake (16 μM) is also higher than the estimated maximal kidney tissue concentration of candesartan (75 nM). Therefore, it is considered that candesartan does not inhibit OAT1, OAT3 or MRP4 in the clinical situation. On the other hand, we have previously demonstrated that candesartan exhibited a strong *trans*-stimulatory effect on URAT1-mediated uptake of uric acid (19). Accordingly, the increase of SUA level induced by candesartan could be due to enhanced reabsorption of uric acid via a *trans*-stimulatory effect on URAT1, but not due to inhibition of urinary secretion of uric acid via OAT1, OAT3 or MRP4.

Valsartan reduced the uptake of uric acid by OAT1 and OAT3 in a concentration-dependent manner, with IC_{50} values of 16 and 0.2 μM, respectively. Since the unbound maximum plasma concentration of valsartan (0.325 μM) is higher than the IC_{50} value for OAT3, valsartan may clinically inhibit secretion of uric acid via OAT3. Furthermore, valsartan also could enhance the reabsorption of uric acid via URAT1 through its *trans*-stimulatory effect (19), though it is unlikely to inhibit MRP4 (Table I). Accordingly, the increment of SUA level by valsartan may be due to both the stimulation of reabsorption via URAT1 and the inhibition of secretion via OAT3, although the clinically observed increment of SUA level by valsartan is minimal (from 5.9 to 6.0 mg/dl) (4). Nevertheless, since valsartan has the potential to increase the SUA level via plural mechanisms, care may be needed in the clinical use of valsartan.

Olmesartan exhibited similar effects to valsartan on OAT1-, OAT3-, and MRP4-mediated uptake of uric acid. The unbound maximum plasma concentration of olmesartan (0.023 μM) is comparable to the IC_{50} value for OAT3 (0.027 μM), while other transporters are unlikely to be affected at clinically achievable concentrations. Moreover, olmesartan exhibited a *trans*-stimulatory effect on URAT1-mediated uptake of uric acid (19). These results suggested that olmesartan could have similar effect on renal uric acid transporters such as URAT1 and OAT3 as seen with valsartan. Therefore, olmesartan might clinically increase SUA level, while no clinical report is yet available about alteration of SUA level by olmesartan.

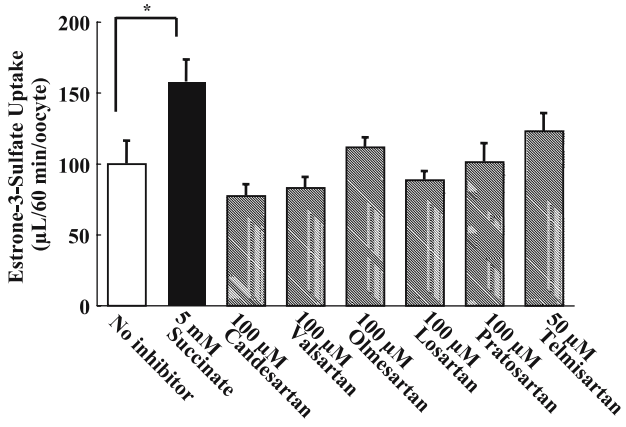

Fig. 4. *Trans*-Stimulatory effect of ARBs on OAT4-mediated [^{3}H] $E_1$3S uptake. Uptake of [^{3}H] $E_1$3S (10 nM) by water-injected or OAT4–cRNA-injected oocytes was measured in the absence (control) or presence of ARBs (100 μM, except for telmisartan 50 μM) for 60 min. OAT4-mediated uptake was determined by subtracting the uptake in water-injected oocytes from that in OAT4 cRNA-injected oocytes, and the values obtained were divided by the control value in each assay. Each point represents the mean+SEM from 9–10 oocytes. An *asterisk* indicates a significant difference from the control by Dunnett's test (p<0.05). *CAS* Candesartan, *VAS* valsartan, *OLS* olmesartan, *LOS* losartan, *PRS* pratosartan, *TES* telmisartan.

Losartan inhibited uptake of uric acid by OAT1 and OAT3 at much higher IC_{50} values than its unbound maximum plasma concentration (Table I). However, the observed IC_{50} value of losartan for MRP4 (1.5 μM) is close to the estimated maximum concentration of losartan in kidney (864 nM). Pratosartan inhibited uptake of uric acid by OAT1 and OAT3 in a concentration-dependent manner, with IC_{50} values of 1.5 and 0.095 μM, respectively. The unbound maximum plasma concentration of pratosartan (0.047 μM; unpublished observation) was close to the IC_{50} values for OAT3 (Table I). Therefore, losartan and pratosartan may potentially inhibit secretion of uric acid via MRP4 and OAT3, respectively, though clinically, these ARBs decrease the SUA level. This apparent discrepancy can be explained by the reduction of reabsorption via URAT1 by these two ARBs, since in our previous study they exhibited strong *cis*-inhibitory effects on URAT1-mediated uptake of uric acid (Table I).

Telmisartan inhibited the uptake of uric acid by OAT1 and OAT3 at concentrations higher than the unbound maximum plasma concentration (Table I). Furthermore, the IC_{50} value of telmisartan for MRP4 was estimated to be higher than the kidney tissue concentration (Table I). Therefore, telmisartan may not affect the secretion of uric acid via OAT1, OAT3, or MRP4. In addition, although telmisartan exhibits a potent *cis*-inhibitory effect on URAT1-mediated uric acid transport (K_i: 19 nM), telmisartan may not affect the SUA level in view of its pharmacokinetic properties, as discussed previously (19).

In this study, it was suggested that OAT3 could be also involved in renal uric acid reabsorption based on the bi-directional transport property of OAT3. Since it was shown that ARBs have affinity for OAT3 in this study, ARBs could have ability to alter OAT3-mediated uric acid reabsorption from proximal tubular cells to blood. To clarify the effect of ARBs on OAT3-mediated uric acid transport, it is needed to understand the role of OAT3 in renal uric acid handling, and to evaluate the effect of ARBs on OAT3-mediated efflux of uric acid in addition to the effect on OAT3-mediated influx of uric acid. Furthermore, it should be also examined whether OAT1 transports uric acid bi-directionally as does OAT3.

In conclusion, the results of this study suggest that interaction of some ARBs, including losartan, olmesartan, pratosartan, and valsartan, with uric acid transport by OAT3 and MRP4 could lead to an elevation of the SUA level. However, losartan and pratosartan have been clinically observed to decrease the SUA level. This apparent discrepancy is considered to be due to the predominance of their inhibitory effects on URAT1-mediated reabsorption. In the case of valsartan, inhibition of OAT3 as well as *trans*-stimulation of URAT1 could be involved in the elevation of the SUA level. Although there is no clinical report on the effect of olmesartan on the SUA level, olmesartan may have similar effects to valsartan. Candesartan is unlikely to affect SUA level via renal secretion transporters OAT1, OAT3 or MRP4 and the clinically observed increase of SUA level by candesartan may be explained by the *trans*-stimulation effect via URAT1. Furthermore, we found that OAT4 can transport uric acid in the direction of reabsorption, though this is unlikely to be involved in the ARB-mediated changes of the SUA level. It remains necessary to examine the possible effects of these ARBs on uric acid-related enzymes and other transporters such as NPT1 (26), but the present and previous two observations (19) suggest that the clinically observed effects of ARBs on the SUA level can be largely explained by the interaction between ARBs and uric acid on the renal uric acid transporters OAT3, MRP4 and URAT1. This information should be useful in estimating the effects of other drugs, as well as ARBs, on the SUA level.

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