Interactions of Human Organic Anion Transporters with Diuretics

Habib Hasannejad, Michio Takeda, Kentarou Taki, Ho Jung Shin, Ellappan Babu, Promsuk Jutabha, Suparat Khamdang, Mahmoud Alboyeh, Maristela Lika Onozato, Akihiro Tojo, Atsushi Enomoto, Naohiko Anzai, Shinichi Narikawa, Xi-Lin Huang, Toshimitsu Niwa, and Hitoshi Endou

Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan (H.H., M.T., H.J.S., E.B., P.J., S.K., M.A., N.A., H.E.); Department of Clinical Preventive Medicine, Nagoya University School of Medicine, Nagoya, Japan (K.T., A.E., T.N.); Department of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan (M.L.O., A.T.); and Kobuchizawa Laboratories, Fuji Biomedix Co., Yamanashi, Japan (S.N., X.-L.H.)

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ABSTRACT

The tubular secretion of diuretics in the proximal tubule has been shown to be critical for the action of drugs. To elucidate the molecular mechanisms for the tubular excretion of diuretics, we have elucidated the interactions of human organic anion transporters (hOATs) with diuretics using cells stably expressing hOATs. Diuretics tested were thiazides, including chlorothiazide, cyclothiazide, hydrochlorothiazide, and trichlormethiazide; loop diuretics, including bumetanide, ethacrynic acid, and furosemide; and carbonic anhydrase inhibitors, including acetazolamide and methazolamide. These diuretics inhibited organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4 in a competitive manner. hOAT1 exhibited the highest affinity interactions for thiazides, whereas hOAT3 did those for loop diuretics. hOAT1, hOAT3, and hOAT4 but not hOAT2, mediated the uptake of bumetanide. hOAT3 and hOAT4, but not hOAT1 mediated the efflux of bumetanide. hOAT1 and hOAT3, but not hOAT2 and hOAT4 mediated the uptake of furosemide. In conclusion, it was suggested that hOAT1 may play an important role in the basolateral uptake of thiazides, whereas hOAT3 and hOAT4 in the uptake of loop diuretics. In addition, it was also suggested that bumetanide taken up by hOAT3 and/or hOAT1 is excreted into the urine by hOAT4.

Diuretics cause natriuresis and are therefore used to treat patients with volume overload, including hypertension, liver cirrhosis, nephrotic syndrome, and congestive heart failure (Ives, 2001). Thiazides and loop diuretics exhibit their diuretic effects from the luminal side by inhibiting the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter of the distal tubule and the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter of the loop of Henle, respectively (Ives, 2001). In addition, the binding of diuretics to plasma proteins is generally high (more than 90%), tubular secretion is the main route of urinary excretion of the diuretics. Thus, tubular secretion has been thought to be critical for the action of loop and thiazide diuretics. Renal tubular secretion of diuretics has been demonstrated in studies including the secretion of bumetanide and furosemide in the isolated perfused rat kidney (Bekersky and Popick, 1986; Lee et al., 1986) and renal tubular secretion of chlorothiazide and hydrochlorothiazide in the avian kidney (Odlin and Lonnerholm, 1982). As shown in Table 1, thiazide and loop diuretics, which carry as a common chemical characteristic a sulfamoyl group (sulfonamide diuretics), are weak organic acids. Consistent with this, the involvement of the organic anion transport system in the tubular secretion of diuretics has been suggested in studies including bumetanide inhibition of para-aminohippuric acid (PAH) transport in rat renal slices (Gemba et al., 1981) and PAH inhibition of furosemide excretion in the rabbit (Bidiville and Roch-Ramel, 1986).

Carbonic anhydrase plays an important role in bicarbonate reabsorption from the proximal tubule (Ives, 2001). Acetazolamide, a major carbonic anhydrase inhibitor, blocks the reabsorption of sodium bicarbonate, resulting in an alkaline diuresis with loss of sodium and bicarbonate in the urine.

ABBRIDAVATIONS: PAH, para-aminohippuric acid; hOAT, human organic anion transporter; PGF\(_2\alpha\), prostaglandin F\(_2\alpha\); rOAT, rat organic anion transporter; ES, estrone sulfate; oatp, organic anion-transporting polypeptide; HPLC, high-performance liquid chromatography; MRP, multidrug resistance-associated protein; DPBS, Dulbecco’s modified phosphate-buffered saline.
Tubular secretion of acetazolamide was demonstrated to be important for the elimination of this drug in the study using the isolated rat perfused kidney (Taft and Sweeney, 1995).

The secretion of numerous organic anions, including endogenous metabolites, drugs, and xenobiotics, is an important physiological function of the renal proximal tubule. The process of secreting organic anions through the proximal tubule cells is achieved via unidirectional transcellular transport involving the uptake of organic anions into the cells from the blood across the basolateral membrane, followed by extrusion across the brush-border membrane into the proximal tubule fluid. Recently, cDNAs encoding the human organic anion transporter (hOAT) family have been successively cloned, including hOAT1 (Reid et al., 1998; Hosoyamada et al., 1999), hOAT2 (Enomoto et al., 2002), hOAT3 (Cha et al., 2001), and hOAT4 (Cha et al., 2000). As shown in Fig. 1, hOAT1, hOAT2, and hOAT3 have been shown to be localized to the basolateral side of the proximal tubule (Hosoyamada et al., 1999; Cha et al., 2001; Enomoto et al., 2002; Motohashi et al., 2002), whereas hOAT4 is localized to the apical side of the proximal tubule (Babu et al., 2002a).

Although Uwai et al. (2000) have already demonstrated the interactions of rat OAT1 (rOAT1) with diuretics using an oocyte expression system, the molecular mechanisms underlying the renal handling of diuretics have been poorly clarified. Thus, the purpose of this study was to elucidate the interactions of hOATs with diuretics using the second segment of the proximal tubule (S₂) cells stably expressing hOAT1, hOAT2, hOAT3, and hOAT4 (S₂ hOAT1, S₂ hOAT2, S₂ hOAT3, and S₂ hOAT4). Diuretics tested in the current study are listed in Table 1.

### Materials and Methods

#### Materials

- [14C]PAH (1.86 GBq/mmol), [3H]prostaglandin F₂α (PGF₂α) (6808 GBq/mmol), and [3H]estrone sulfate (ES) (1961 GBq/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA).
- [3H]Bumetanide (185.6 GBq/mmol) was purchased from Muromachi Chemicals (Tokyo, Japan).
- Diuretics were obtained from Sigma-Aldrich (St. Louis, MO).
- Other materials used included fetal bovine serum, trypsin, and geneticin from Invitrogen (Carlsbad, CA); recombinant epidermal growth factor from Wakunaga (Hiroshima, Japan); insulin from Shimizu (Shizuoka, Japan); RITC 80-7 culture medium from Iwaki Co. (Tokyo, Japan); and TFX-50 from Promega (Madison, WI).

#### Cell Culture

S₂ cells were established by culturing the microdissected S₂ segment derived from transgenic mice harboring the temperature-sensitive simian virus 40 large T-antigen gene. The establishment and characterization of S₂ hOAT1, S₂ hOAT2, S₂ hOAT3, and S₂ hOAT4 were reported previously (Takeda et al., 2002a,b). Briefly, the full-length cDNAs of hOAT1, hOAT2, hOAT3, and hOAT4 were subcloned into pcDNA 3.1 (Invitrogen), a mammalian expression vector. S₂ hOAT1, S₂ hOAT2, S₂ hOAT3, and S₂ hOAT4 were obtained by transfecting S₂ cells with pcDNA3.1-hOAT1, pcDNA3.1-hOAT2, pcDNA3.1-hOAT3, and pcDNA3.1-hOAT4, using
Bumetanide, 50 μM/assay, was added to the cell culture medium containing 5% fetal bovine serum, 10 ng/ml recombinant epidermal growth factor, and 400 μg/ml geneticin. The cells were subcultured in a medium containing 0.05% trypsin-EDTA solution (containing 137 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 8 mM NaHCO3, 0.5 mM EDTA, and 5 mM Hepes; pH 7.2) and used for ~25 to 35 passages. Clonal cells were isolated using a cloning cylinder and screened by determining the optimal substrate for each transporter, i.e., [14C]PAH for hOAT1 (Hosoyamada et al., 1999), [3H]PGF2α for hOAT2 (Enomoto et al., 2002), and [3H]ES for hOAT3 and hOAT4 (Cha et al., 2000, 2001).

**Uptake Experiments.** Uptake experiments were performed as described previously (Takeda et al., 2002a,b). The S2 cells were seeded in 24-well tissue culture plates at a density of 1 × 10^5 cells/well. After the cells were cultured for 2 days, the cells were washed three times with Dulbecco’s modified phosphate-buffered saline solution (DPBS) (containing 137 mM NaCl, 3 mM KCl, 8 mM NaHCO3, 1 mM KH2PO4, 1 mM CaCl2, and 0.5 mM MgCl2; pH 7.4) and then preincubated in the same solution in a water bath at 37°C for 10 min. The cells were then incubated in a solution containing various substrates at 37°C for 30 min. The uptake was stopped by the addition of ice-cold DPBS, and the cells were washed three times with DPBS. The cells in each well were lysed with 0.5 ml of 0.1 N sodium hydroxide and 2.5 ml of aquasol-2, and radioactivity was determined using a β-scintillation counter (LSC-3100; Aloka, Tokyo, Japan).

**Inhibition Study.** To evaluate the inhibitory effects of diuretics on organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4, the cells were incubated in a solution containing either [14C]PAH for 2 min (hOAT1), [3H]PGF2α for 20 s (hOAT2), or [3H]ES for 2 min (hOAT3 and hOAT4) in the absence or presence of various concentrations of diuretics at 37°C. Each hOAT mediates the transport of each substrate with a high affinity, which is consistent with the original reports (Hosoyamada et al., 1999; Cha et al., 2000, 2001; Enomoto et al., 2002). The Km value of hOAT1 for PAH is 20.1 μM, that of hOAT2 for PGF2α is 425 nM, that of hOAT3 for ES is 2.2 μM, and that of hOAT4 for ES is 9.9 μM. Diuretics were dissolved in dimethyl sulfoxide and diluted with the incubation medium. The final concentration of dimethyl sulfoxide in the incubation medium was adjusted to less than 0.2%. We have chosen the incubation time for the inhibition experiment based on the following reasons. One is that the uptake of the standard substrate by each hOAT increased linearly. Another is that the uptake of the standard substrate by each hOAT was much higher than that by mock, as described in previous reports (Enomoto et al., 2002; Takeda et al., 2002a). The uptake by hOAT1, hOAT2, hOAT3, and hOAT4 were approximately 16-, 26-, 37-, and 31-fold higher than that by mock, respectively. Thus, even if diuretics exerted any inhibitory effects on organic anion uptake by mock, it would be negligible. Based on these results, we have not performed the inhibition experiments on mock.

**Kinetic Analysis of Inhibition.** After preincubation as described above, S2 hOAT1, S2 hOAT2, S2 hOAT3, and S2 hOAT4 were incubated in a solution containing [14C]PAH (hOAT1), [3H]PGF2α (hOAT2), and [3H]ES (hOAT3 and hOAT4) at various concentrations in the absence or presence of diuretics at 37°C either for 2 min (hOAT1, hOAT3, and hOAT4) or 20 s (hOAT2). The concentrations of diuretics used were as follows: 100 μM hydrochlorothiazide, 20 μM bumetanide, 50 μM furosemide, and 150 μM acetazolamide for hOAT1; 2000 μM hydrochlorothiazide, 300 μM bumetanide, and 1000 μM furosemide for hOAT2; 2000 μM hydrochlorothiazide, 5 μM bumetanide, 25 μM furosemide, and 1500 μM acetazolamide for hOAT3; and 750 μM bumetanide, 100 μM furosemide, and 1000 μM acetazolamide for hOAT4. Analyses of Lineweaver-Burk plots were performed as described previously by us (Babu et al., 2002a).

**High-Performance Liquid Chromatography (HPLC) Analysis of Furosemide.** After preincubation as described above, S2 hOAT1, S2 hOAT2, S2 hOAT3, S2 hOAT4, and mock were incubated in the same solution containing 500 μM bumetanide at 37°C for 2 min. After washing the cells with DPBS, the cells were extracted with 0.1 N NaOH, centrifuged at 14,000 rpm for 10 min, and the supernatants were neutralized with 8 N HCl. The intracellular concentrations of furosemide were determined by HPLC as follows. High-performance liquid chromatograph was equipped with a model 880-PU pump (Jasco, Tokyo, Japan), a model 875-UV variance wavelength detector (Jasco), and a model chromato-tocorder 12 (SIC Co., Tokyo, Japan) under the following conditions: a reverse phase column (Development ODS-UG-5, 250 × 4.6 mm i.d.; Nomura Chemicals Co., Aichi, Japan) with a guard column (Development ODS-UG-5, 10 × 4.0 mm i.d.; Nomura Chemicals Co.), mobile phase, acetonitrile/water/acetic acid (70/30/0.1) (v/v); flow rate, 1.0 ml/min; wavelength, 270 nm; and temperature, ambient temperature.

**Bumetanide Efflux.** The efflux study was performed as described previously (Babu et al., 2002a, 2002b; Takeda et al., 2002a). The S2 hOAT1, S2 hOAT3, S2 hOAT4, and mock were seeded in 24-well tissue culture plates at a cell density of 1 × 10^5 cells/well. After the cells were cultured for 2 days, the cells were washed three times with DPBS and then preincubated in the same solution for 10 min in a water bath at 37°C. S2 hOAT1 exhibited the highest uptake activity compared with mock for 5-min incubation (data not shown). Thus, the monolayers were incubated at 37°C in a solution containing 300 nM [3H]bumetanide for 5 min for hOAT1 and 30 min for hOAT3 and hOAT4, washed immediately with DPBS, and incubated at 37°C in 500 μl of DPBS. After incubation for the indicated periods, 50 μl of supernatant was collected. After the incubation, the medium was aspirated immediately, and the cell monolayers were washed three times with the medium and solubilized in 0.5 ml of 0.1 N sodium hydroxide. The amount of bumetanide in the supernatant and in the cell lysate was measured. The rate of efflux at each time point was calculated using the following formula: (effluxed bumetanide by S2 hOATs – effluxed bumetanide by mock)/(bumetanide accumulated by S2 hOATs at time 0 – bumetanide accumulated by mock at time 0).

**Statistical Analysis.** Data are expressed as means ± S.E. Statistical differences were determined using one-way analysis of variance with Dunnett’s post hoc test for the inhibition experiments and Student’s t test for the uptake experiments. Differences were considered significant at P < 0.05.

**Results**

Effects of Diuretics on Organic Anion Uptake Mediated by hOATs. We examined the inhibitory effects of various concentrations of diuretics on the organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4. Figure 2 shows the effects of various concentrations of bumetanide on the organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4. Bumetanide inhibited the organic anion uptake mediated by hOAT1 (A), hOAT2 (B), hOAT3 (C), and hOAT4 (D) in a dose-dependent manner (*P < 0.05, **P < 0.01, ***P < 0.001 versus control). Similarly, other diuretics tested also inhibited the organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4 in a dose-dependent manner, whereas acetazolamide and methazolamide did not inhibit that by hOAT2, and cyclohexazine, hydrochlorothiazide, and metazolamide did not inhibit that by hOAT4. The IC50 values are listed in Table 2.

To further elucidate the inhibitory effects of diuretics on the organic anion uptake mediated by hOATs, we performed the kinetic analyses of inhibitory effects. As shown in Fig. 3, bumetanide competitively inhibited the organic anion uptake mediated by hOAT1 (A), hOAT2 (B), hOAT3 (C), and hOAT4 (D) in a competitive manner, whereas acetazolamide and methazolamide did not inhibit that by hOAT1, and cyclohexazine, hydrochlorothiazide, and metazolamide did not inhibit that by hOAT4. The IC50 values are listed in Table 2.
(D). Competitive inhibition was also seen for the inhibitory effects of hydrochlorothiazide, furosemide, and acetazolamide for hOAT1; hydrochlorothiazide and furosemide for hOAT2; hydrochlorothiazide, furosemide, and acetazolamide for hOAT3; and furosemide and acetazolamide for hOAT4 (data not shown).

**TABLE 2**

IC_{50} values of various diuretics for organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4. S_0 hOAT1, S_0 hOAT2, S_0 hOAT3, and S_0 hOAT4 were incubated in a solution containing either 5 μM [14C]PAH (hOAT1), 50 nM [3H]PGF_{2α} (hOAT2), or 50 nM [3H]ES (hOAT3 and hOAT4) in the absence or presence of various concentrations of diuretics for 2 min (hOAT1, hOAT3, and hOAT4) or 20 s (hOAT2). Each value represents the mean ± S.E. of eight monolayers from two separate experiments.

<table>
<thead>
<tr>
<th>Diuretics</th>
<th>IC_{50} (μM) hOAT1</th>
<th>IC_{50} (μM) hOAT2</th>
<th>IC_{50} (μM) hOAT3</th>
<th>IC_{50} (μM) hOAT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazide</td>
<td></td>
<td></td>
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<tr>
<td>Chlorothiazide</td>
<td>3.78 ± 0.41</td>
<td>2205 ± 143</td>
<td>65.3 ± 4.83</td>
<td>2632 ± 132</td>
</tr>
<tr>
<td>Cyclothiazide</td>
<td>84.3 ± 7.32</td>
<td>39.2 ± 4.10</td>
<td>27.9 ± 2.12</td>
<td>5000&gt;</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>67.3 ± 5.32</td>
<td>1023 ± 53.2</td>
<td>942 ± 76.5</td>
<td>5000&gt;</td>
</tr>
<tr>
<td>Nitrothiazide</td>
<td>19.2 ± 1.12</td>
<td>1220 ± 101</td>
<td>71.2 ± 4.32</td>
<td>1505 ± 98.3</td>
</tr>
<tr>
<td>Loop diuretic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bumetanide</td>
<td>7.60 ± 0.58</td>
<td>77.5 ± 5.38</td>
<td>0.75 ± 0.07</td>
<td>348 ± 23.2</td>
</tr>
<tr>
<td>Ethacrynic acid</td>
<td>29.6 ± 1.63</td>
<td>121 ± 9.83</td>
<td>0.58 ± 0.08</td>
<td>8.76 ± 0.91</td>
</tr>
<tr>
<td>Furosemide</td>
<td>18.0 ± 1.10</td>
<td>603 ± 46.3</td>
<td>7.31 ± 0.81</td>
<td>44.5 ± 2.53</td>
</tr>
<tr>
<td>Carbonic anhydrase inhibitor</td>
<td>75.0 ± 6.52</td>
<td>5000&gt;</td>
<td>816 ± 67.3</td>
<td>425 ± 39.3</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>438 ± 32.1</td>
<td>5000&gt;</td>
<td>97.5 ± 9.84</td>
<td>5000&gt;</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effects of various concentrations of bumetanide on the organic anion uptake mediated by hOATs. S_0 hOAT1 (A), S_0 hOAT2 (B), S_0 hOAT3 (C), and S_0 hOAT4 (D) were incubated in solution containing 5 μM [14C]PAH (hOAT1), 50 nM [3H]PGF_{2α} (hOAT2), or 50 nM [3H]ES (hOAT3 and hOAT4) in the presence of various concentrations of bumetanide at 37°C for 2 min (hOAT1, hOAT3, and hOAT4) or 20 s (hOAT2). Each value represents the mean ± S.E. of eight monolayers from two separate experiments. *, P < 0.05; **, P < 0.01; and ***, P < 0.001 versus control.

**Bumetanide Uptake Mediated by hOATs.** To determine whether hOATs mediate the uptake of diuretics, we evaluated the uptake activities of [3H]bumetanide by hOATs. As shown in Fig. 4, A to C, the uptake rates of [3H]bumetanide by hOAT1, hOAT3, and hOAT4, but not by hOAT2, were higher than those by mock (*P < 0.05, **P < 0.01, and
To further elucidate the transport property of bumetanide mediated by hOAT3 and hOAT4, we performed kinetic analysis of bumetanide uptake in S2 hOAT3 and S2 hOAT4. As shown in Fig. 5, hOAT3 (A) and hOAT4 (C) mediated the dose-dependent uptake of bumetanide, and analysis of the Eadie-Hofstee revealed that the K_m values for hOAT3 (B) and hOAT4 (D)-mediated bumetanide uptake were 1586 ± 69.4 nM and 306 ± 63.2 nM, respectively.

**Bumetanide Efflux by hOATs.** To determine whether hOAT1, hOAT3, and hOAT4 mediate the efflux of bumetanide, we measured the efflux of bumetanide from S2 hOAT1, S2 hOAT3, S2 hOAT4, and mock preincubated in a solution containing bumetanide. As shown in Fig. 6, when the efflux from mock was subtracted, S2 hOAT3 (A) and S2 hOAT4 (B) mediated the efflux of bumetanide, whereas S2 hOAT1 did not (data not shown).

**HPLC Analysis of Furosemide.** To elucidate whether hOATs mediate the uptake of furosemide, we have measured intracellular furosemide content using HPLC. As shown in Fig. 7, the uptake rates of furosemide by hOAT1 and hOAT3, but not hOAT2 and hOAT4, were higher than those by mock (n = 6, *P < 0.05 and **P < 0.01 versus mock).

**Discussion**

HOAT1 and hOAT3 have been shown to mediate the transport of nonsteroidal anti-inflammatory drugs, antitumor drugs, histamine H1-receptor antagonist, prostaglandins, anti-virals, angiotensin-converting enzyme inhibitors, and β-lactam antibiotics (Hosoyamada et al., 1999; Cha et al., 2001; Khamdang et al., 2002; Takeda et al., 2002a,b). In addition, hOAT1 and hOAT3 exhibit transport properties as an exchanger (Bakhiya et al., 2003; Sweet et al., 2003). However, some differences in characteristics exist between hOAT1 and hOAT3, such as substrate specificity and localization: hOAT1 is localized at S2 segment of the proximal tubule (Hosoyamada et al., 1999), whereas hOAT3 is local-
HOAT2 also mediates the basolateral uptake of organic anions, including salicylate and PGF₂α (Enomoto et al., 2002). On the other hand, hOAT4 also mediates the apical transport of various anionic drugs in the proximal tubule (Babu et al., 2002a); however, this transporter exhibits relatively narrow substrate recognition compared with hOAT1 and hOAT3 (Cha et al., 2000). Using the cells stably expressing hOATs, we have elucidated the interactions of hOATs with diuretics.

Comparing the IC₅₀ values of diuretics among hOATs, hOAT1 exhibited the highest affinity for chlorothiazide, hydrochlorothiazide, trichlorothiazide, and acetazolamide; and hOAT3 exhibited that for bumetanide, ethacrynic acid, furosemide, and methazolamide. In contrast, hOAT2 exhibited the lowest affinity for ethacrynic acid, furosemide, and acetazolamide; hOAT4 exhibited that for cyclothiazide, hydrochlorothiazide, and bumetanide; and hOAT2 and hOAT4 exhibited that for chlorothiazide, trichlorothiazide and methazolamide. Thus, hOAT1 and hOAT3 generally exhibited higher affinity interactions with diuretics than hOAT2 and hOAT4. In addition, generally, hOAT1 seems to exhibit the highest affinity interactions with thiazides except cyclothiazide, and hOAT3 seems to do that with loop diuretics. On the other hand, it was suggested that hOAT4 exhibits higher affinity with loop diuretics than with thiazides. Furthermore, the interactions of hOATs with carbonic anhydrase inhibitors were the weakest among the diuretics tested.

HOAT3 mediated the uptake as well as efflux of bumetanide, and hOAT1 mediated the uptake of bumetanide. It is possible that the reason why hOAT1 exhibited no efflux activity of bumetanide is because it did not show as much bumetanide uptake activity as hOAT3 and hOAT4, as shown in Fig. 4. The magnitude of bumetanide uptake by hOAT3 was approximately 20-fold larger than that by hOAT1. The
IC₅₀ values of bumetanide for hOAT1 and hOAT3 in our system were similar to that of bumetanide for the basolateral PAH transport system in the isolated S₂ segments of rabbit kidney proximal tubules, 3 µM (Bartel et al., 1993). Based on these findings, it was suggested that hOAT3 mediates the uptake as well as efflux of bumetanide, and hOAT1 mediates the uptake of bumetanide in the basolateral side of the proximal tubule.

HOAT1 and hOAT3 exhibited high-affinity interactions with furosemide. In addition, hOAT1 and hOAT3 mediated the uptake of furosemide. It was reported that furosemide inhibited the basolateral PAH transport system in the isolated S₂ segments of rabbit kidney proximal tubules, with an IC₅₀ value of 40 µM (Bartel et al., 1993). The value was similar to those of furosemide for hOAT1 and hOAT3. Bidiville and Roch-Ramel (1986) demonstrated that probenecid inhibited the renal secretion of furosemide by 95%, whereas PAH depressed the renal secretion of furosemide by only 44 to 66% in rabbit kidneys. Based on this, Uwai et al. (2000) hypothesized the existence of a transporter besides OAT1 that mediates the renal excretion of furosemide. Considering these lines of evidence, it was suggested that hOAT1 and hOAT3 mediate the transport of furosemide in the basolateral side of the proximal tubule.

Furosemide uptake activity by hOAT1 and the IC₅₀ value of furosemide for hOAT1 were consistent with those of rOAT1 (Uwai et al., 2000). However, the IC₅₀ values of acetazolamide, bumetanide, and hydrochlorothiazide for hOAT1 were different between humans and rats (more than 3-fold difference; Zhang et al., 1998). The reason for this may be due to the interspecies difference in the interactions of OAT1 with these drugs between humans and rats or the difference concerning the expression system, i.e., mammalian expression system for hOAT1 versus Xenopus oocyte expression system for rOAT1.

In addition to bumetanide and furosemide, because hOAT1 and hOAT3 exhibited high-affinity interactions with ethacrynic acid and thiazides, it is speculated that these transporters play important roles in the basolateral uptake of these diuretics in the proximal tubule. The IC₅₀ value of hydrochlorothiazide for hOAT1 (67.3 µM) was comparable with that of hydrochlorothiazide for cellular PAH uptake across the basolateral membrane of isolated S₂ segments in rabbits, i.e., 140 µM (Bartel et al., 1993). In contrast, the interactions of hOAT1 and hOAT3 with carbonic anhydrase inhibitors were the weakest among the diuretics tested. However, because hOAT1 and hOAT3 seem to be a major transporters mediating the basolateral uptake of various anionic drugs (Hosoyamada et al., 1999; Cha et al., 2001; Khamdang et al., 2002; Takeda et al., 2002a,b), it is possible that these two transporters are involved in the transport of carbonic anhydrase inhibitors. The involvement of transporters other than hOAT1 and hOAT3 in the basolateral transport of carbonic anhydrase inhibitors in the proximal tubule should also be considered. In contrast, comparing the affinities of hOATs with diuretics, hOAT2 may not significantly contribute to the transport of all the diuretics tested except cyclothiazide.

HOAT4 mediated the uptake and the efflux of bumetanide and exhibited higher affinity interactions with loop diuretics than with other diuretics. Thus, it is suggested that hOAT4 mediates the reabsorption or the efflux of loop diuretics in the

Fig. 5. Dose-dependent uptake of bumetanide by hOAT3 and hOAT4, and its kinetic analysis. S₂ hOAT3 (A), S₂ hOAT4 (C), and mock were incubated in solution containing various concentrations of [³H]bumetanide at 37°C for 1 min. Eadie-Hofstee plot analysis of bumetanide uptake by hOAT3 (B) and hOAT4 (D) was performed. Each value represents the mean ± S.E. of four monolayers from one typical experiment of two separate experiments.
apical side of the proximal tubule. In addition, considering the above-mentioned evidence that hOAT1 and hOAT3 mediate the basolateral uptake of bumetanide, it is possible that bumetanide taken up by hOAT1 and/or hOAT3 is effluxed into the urine by hOAT4, which represents the mechanism of the unidirectional transport of bumetanide. Other than the hOAT family, the interactions of other human transporters and human homologs of rodent transporters mediating organic anion transport in the apical side of the proximal tubule with diuretics should be investigated, including OAT-K1 (Saito et al., 1996), OAT-K2 (Masuda et al., 1999), organic anion-transporting polypeptide (oatp)1 (Jacquemin et al., 1994), oatp2 (Noe et al., 1997), oatp3 (Abe et al., 1998), multidrug resistance-associated protein (MRP)2 (Leier et al., 2000), MRP4 (van Aubel et al., 2002), and human-type I sodium-dependent inorganic phosphate transporter (Uchino et al., 2000). In this regard, the interaction of OAT-K1 with furosemide, as well as that of OAT-K2 with furosemide and spironolactone, was reported (Saito et al., 1996; Masuda et al., 1999). In addition, furosemide was shown to inhibit N-ethylmaleimide glutathione uptake mediated by MRP2 (Bakos et al., 2000).

The therapeutically relevant plasma concentration of a drug is considered to be within 5-fold of the maximum steady-state plasma concentration of a drug (Zhang et al., 2000). Considering this as well as the plasma concentrations of diuretics and their unbound fractions (Hardman and Limbird, 2001), the therapeutically relevant concentrations of unbound hydrochlorothiazide, trichloromethiazide, bumetanide, furosemide, and acetazolamide are considered to be 2.62, 0.173, 0.22, 0.41, and 33.8/μM, respectively. Thus, the IC50 values of hOAT1 for hydrochlorothiazide, trichloromethiazide, and acetazolamide are approximately 25, 100, and 2 times higher than the therapeutically relevant concentrations of unbound drugs, and those of hOAT3 for bumetanide and furosemide are approximately 3 and 18 times higher than the therapeutically relevant concentrations of unbound drugs. Thus, it is predicted that hOATs could transport these diuretics in vivo, whereas the rates of transport vary from drug to drug.

In conclusion, it was suggested that hOAT1 plays an important role in the basolateral uptake of thiazides, and hOAT3 in the uptake of loop diuretics in the proximal tubule. In addition, it was also suggested that bumetanide taken up by hOAT1 and/or hOAT3 is excreted into the urine by hOAT4.
hOAT4, which may be the mechanism underlying the tubular secretion of bumetanide.

References


Address correspondence to: Dr. Hitoshi Endou, Department of Pharmacology and Toxicology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka-shi, Tokyo 181-8611, Japan. Email: endouh@kyorin-u.ac.jp