Interactions of Human Organic Anion Transporters and Human Organic Cation Transporters with Nonsteroidal Anti-Inflammatory Drugs

SUPARAT KHAMDANG, MICHIO TAKEDA, RIE NOSHIRO, SHINICHI NARIKAWA, ATSUSHI ENOMOTO, NAOHIKO ANZAI, PAWINEE PIYACHATURAWAT, and HITOSHI ENDOU

Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan (S.K., M.T., R.N., S.N., A.E., N.A., H.E.); and Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand (S.K., P.P.)

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ABSTRACT

The purpose of this study was to elucidate the interactions of human organic anion transporters (hOATs) and human organic cation transporters (hOCTs) with nonsteroidal anti-inflammatory drugs (NSAIDs) using cells stably expressing hOATs and hOCTs. NSAIDs tested were acetaminophen, acetylsalicylate, salicylate, diclofenac, ibuprofen, indomethacin, ketoprofen, mefenamic acid, naproxen, piroxicam, phenacetin, and sulindac. These NSAIDs inhibited organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4. By comparing the IC50 values of NSAIDs for hOATs, it was found that hOAT1 and hOAT3 exhibited higher affinity interactions with NSAIDs than did hOAT2 and hOAT4. HOAT1, hOAT2, hOAT3, and hOAT4 mediated the uptake of either ibuprofen, indomethacin, ketoprofen, or salicylate, but not acetylsalicylate. Although organic cation uptake mediated by hOCT1 and hOCT2 was also inhibited by some NSAIDs, hOCT1 and hOCT2 did not mediate the uptake of NSAIDs. In conclusion, hOATs and hOCTs interacted with various NSAIDs, whereas hOATs but not hOCTs mediated the transport of some of these NSAIDs. Considering the localization of hOATs, it was suggested that the interactions of hOATs with NSAIDs are associated with the pharmacokinetics and the induction of adverse reactions of NSAIDs.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for their anti-inflammatory and analgesic properties. The indications of NSAIDs are broadening from rheumatic diseases and various pain states, such as cancer pain, and biliary and colic pain, to include possibly Alzheimer’s disease and colon cancer prevention (Day et al., 2000). Table 1 shows the chemical structures of NSAIDs tested in the current study. Although all of these NSAIDs are weak organic acids, they are grouped in several classes based on their chemical structures. Although the chemical diversity yields a broad range of pharmacokinetic characteristics (Furst and Munster, 2000), they have some general properties in common. NSAIDs have been shown to induce various forms of adverse drug reactions including adverse gastrointestinal effects (Day et al., 2000), renal dysfunction and nephrotoxicity (Day et al., 2000), and rhabdomyolysis (Ross and Hoppel, 1987; Leventhal et al., 1989; Delrio et al., 1996).

The secretion of numerous organic anions and cations, including endogenous metabolites, drugs, and xenobiotics, is an important physiological function of the renal proximal tubule. The process of secreting organic anions and cations through the proximal tubule cells is achieved via unidirectional transcellular transport involving the uptake of organic anions and cations into the cells from the blood across the basolateral membrane, followed by extrusion across the brush-border membrane into the proximal tubule fluid (Pritchard and Miller, 1993). 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 (Y. Kobayashi, unpublished observation), hOAT3 (Cha et al., 2001), and hOAT4 (Cha et al., 2000). The human organic cation transporters (hOCTs) isolated thus far are hOCT1 (Gorboulev et al., 1981; Wood et al., 1985; Purcell et al., 1991; Day et al., 2000), adverse neurological effects (Hoppman et al., 1991; Day et al., 2000), and rhabdomyolysis (Ross and Hoppel, 1987; Leventhal et al., 1989; Delrio et al., 1996).

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that anionic drugs such as PGE2 and PGF2α have been poorly clarified. In addition, we recently found mechanisms underlying the pharmacokinetics of NSAIDs.

Thus, the purpose of this study was to elucidate the interactions of hOATs and hOCTs with NSAIDs using the proximal tubule cells stably expressing hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, and hOCT2.

### Materials and Methods

**Materials.** [14C]parao-Aminomhippuric acid (PAH) (1.86 GBq/mmol), [3H]PGF2α (6808 GBq/mmol), [3H]estrone sulfate (1961 GBq/mmol), and [14C]TEA (2.035 GBq/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). [3H]Acetylsalicylate (2.04 GBq/mmol), [14C]salicylate (2.05 GBq/mmol), [3H]ibuprofen (0.5 GBq/mmol), [3H]indomethacin (0.74 GBq/mmol), and [3H]ketoprofen (1.11 GBq/mmol) were purchased from Muromachi Chemicals (Tokyo, Japan). NSAIDs 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 TIX-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, S2 hOAT4, S2 hOCT1, and S2 hOCT2 were reported previously (Enomoto et al., 2002; Kimura et al., 2002; Takeda et al., 2002). Briefly, the full-length cDNAs of hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, and hOCT2 were subcloned into pcDNA 3.1 (Invitrogen), a mammalian expression vector. S2 hOAT1, S2 hOAT2, S2 hOAT3, S2 hOAT4, S2 hOCT1, and S2 hOCT2 were obtained by transfecting S2 cells with pcDNA 3.1-hOAT1, pcDNA 3.1-hOAT2, pcDNA 3.1-hOAT3, pcDNA 3.1-hOAT4, pcDNA 3.1-hOCT1, and pcDNA 3.1-hOCT2 coupled with pSV2neo, a neomycin resistance gene, using TIX-50 according to the manufacturer's instructions. S2 cells transfected with pcDNA 3.1 lacking an insert and pSV2neo were designated as S2 pcDNA 3.1 (mock) and used as control. These cells were grown in a humidified incubator at 33°C and under 5% CO2 using RITC 80-7 medium containing 5% fetal bovine serum, 10 mg/ml transferrin, 0.08 U/ml insulin, 10 ng/ml recombinant epidermal growth factor, and 400 mg/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, 4 mM NaHCO3, 0.5 mM EDTA, and 5 mM Hepes; pH 7.2) and used for 25–35 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), [3H]estrone sulfate for hOAT3 and hOAT4 (Cha et al., 2000, 2001), and [3H]TEA for hOCT1 and hOCT2 (Gorboulev et al., 1997; Zhang et al., 1998).

**Uptake Experiments.** Uptake experiments were performed as previously described (Enomoto et al., 2002; Kimura et al., 2002; Takeda et al., 2002). The S2 cells were seeded in 24-well tissue culture plates at a density of 1 × 10⁵ cells/well. After the cells were cultured for 2 days, the cells were washed three times with Dulbecco's modified phosphate-buffered saline solution (containing 137 mM NaCl, 3 mM KCl, 8 mM NaHPO4, 1 mM KH2PO4, 1 mM CaCl2, and 0.5 mM MgCl2, pH 7.4) supplemented with 5.5 mM glucose 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 either 30 µM [3H]acetylsalicylate, 30 µM [14C]salicylate, 500 nM [3H]ibuprofen, 5 µM [3H]indomethacin, or 50 nM [3H]ketoprofen at 37°C for up to 30 min. The uptake was stopped by the addition of ice-cold Dulbecco's modified phosphate-buffered saline solution, and the cells were washed three times with the same solution. The cells in each well were lysed with 0.5 ml of 0.1 N sodium hydroxide and 2.5 ml of 0.5 M NaOH, and radioactivity was determined using a β-scintillation counter (LSC-3100; Aloka, Tokyo, Japan).

**Inhibition Study.** To evaluate the inhibitory effects of NSAIDs on organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4, and organic cation uptake mediated by hOCT1 and hOCT2, the cells were incubated in a solution containing either [14C]PAH for 2 min (hOAT1), [3H]PGF2α for 20 s (hOAT2), [3H]estrone sulfate for 2 min (hOAT3 and hOAT4), or [3H]TEA for 5 min (hOCT1 and hOCT2).

### Table 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical Structure</th>
<th>Urinary Excretion Rate of Unchanged Drug (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>Hydrophilic NSAIDs</strong></td>
<td></td>
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<td></td>
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<td>Acetylsalicylate</td>
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</tr>
<tr>
<td>Salicylate</td>
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<td>2–30</td>
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<tr>
<td><strong>Hydrophobic NSAIDs</strong></td>
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<td></td>
</tr>
<tr>
<td>Didofenac</td>
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<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
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<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>16</td>
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<tr>
<td>Ketoprofen</td>
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<td></td>
</tr>
<tr>
<td>Mefenamic acid</td>
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<td></td>
</tr>
<tr>
<td>Naproxen</td>
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</tr>
<tr>
<td>Piroxicam</td>
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<td>4–10</td>
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<tr>
<td>Phenacetin</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td></td>
</tr>
<tr>
<td>Sulindac</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>7</td>
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</tbody>
</table>

1997; Zhang et al., 1998), hOCT2 (Gorboulev et al., 1997; Busch et al., 1998), and hOCT3 (Wu et al., 2000).

Although we have previously demonstrated the interaction of rat OAT1 (rOAT1) with NSAIDs using an oocyte expression system (Apisattanakul et al., 1999), the molecular mechanisms underlying the pharmacokinetics of NSAIDs have been poorly clarified. In addition, we recently found that anionic drugs such as PGE2 and PGF2α are transported by not only hOATs but also hOCTs (Kimura et al., 2002). Thus, the purpose of this study was to elucidate the interactions of hOATs and hOCTs with NSAIDs using the proximal tubule cells stably expressing hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, and hOCT2.
hOCT2) in the absence or presence of various concentrations of NSAIDs at 37°C. Acetaminophen, acetylsalicylate, salicylate, and phenacetin were dissolved in H2O. Diclofenac, ibuprofen, indomethacin, ketoprofen, mofemenic acid, naproxen, piroxicam, and sulindac 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%.

**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. Differences were considered significant at \( P < 0.05 \).

**Results**

**Effects of NSAIDs on Organic Anion Uptake Mediated by hOATs.** We examined the inhibitory effects of various concentrations of NSAIDs on the organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4. Figure 1 shows the effects of diclofenac on the organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4. Diclofenac inhibited the organic anion uptake mediated by hOAT1 (Fig. 1A), hOAT2 (Fig. 1B), hOAT3 (Fig. 1C), and hOAT4 (Fig. 1D) in a dose-dependent manner (\( *P < 0.001 \), \( **P < 0.01 \), and \( ***P < 0.05 \) versus control). Similarly, all of the other NSAIDs tested dose-dependently inhibited the organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4 (data not shown). The IC50 values are listed in Table 2.

**Effects of NSAIDs on Organic Cation Uptake Mediated by hOCTs.** Since anionic drugs such as PGE2 and PGF2α were recently shown to be transported by not only hOATs but also hOCTs (Kimura et al., 2002), we examined the effects of 0.5 mM (Fig. 2A) and 2 mM (Fig. 2B) concentrations of NSAIDs on the organic cation uptake mediated by hOCT1 and hOCT2. When the concentration of NSAIDs was set at 0.5 mM (100-fold higher than the substrate concentration), among the various NSAIDs tested, diclofenac, ibuprofen, indomethacin, ketoprofen, mofemenic acid, and sulindac significantly inhibited hOCT1-mediated TEA uptake (Fig. 2A; \( *P < 0.001 \) and \( ***P < 0.05 \) versus control), and indomethacin, naproxen, piroxicam, and sulindac significantly inhibited hOCT2-mediated TEA uptake (Fig. 2A; \( *P < 0.001 \), \( **P < 0.01 \), and \( ***P < 0.05 \) versus control). In contrast, when the concentration of inhibitor was set at 2 mM (400-fold higher than the substrate concentration), as shown in Fig. 2B, all NSAIDs except salicylate significantly inhibited TEA uptake mediated by hOCT1 (\( *P < 0.001 \) and \( **P < 0.01 \) versus control), and all NSAIDs except acetaminophen significantly inhibited TEA uptake mediated by hOCT2 (\( *P < 0.001 \), \( **P < 0.01 \) and \( ***P < 0.05 \) versus control).

**NSAID Uptake Mediated by hOATs and hOCTs.** To determine whether hOATs and hOCTs mediate the uptake of NSAIDs, we evaluated the uptake activities of either [14C]acetylsalicylate, [14C]salicylate, [3H]ibuprofen, [3H]indomethacin, or [3H]ketoprofen by hOATs and hOCTs. The uptake rates of [14C]acetylsalicylate by hOAT1, hOAT2, hOAT3, and hOAT4 were not higher than those by mock (Fig. 3A; N.S.); those of [14C]salicylate by hOAT1, hOAT2, hOAT3, and hOAT4 were 1.98-, 1.75-, 2.04-, and 1.49-fold higher than those by mock (Fig. 3B; \( *P < 0.001 \) versus mock); those of [3H]ibuprofen by hOAT1 and hOAT3 but not hOAT2 and hOAT4 were 1.38- and 1.74-fold higher than those by mock (Fig. 3C; \( *P < 0.001 \) and \( **P < 0.01 \) versus mock); those of [3H]indomethacin by hOAT1 and hOAT3 but not hOAT2 and hOAT4 were 1.47- and 1.18-fold higher than those by mock (Fig. 3D; \( *P < 0.001 \) and \( **P < 0.01 \) versus mock); and those of [3H]ketoprofen by hOAT1, hOAT3, and hOAT4 but not hOAT2 were 1.75-, 1.39-, and 1.23-fold higher than those by mock (Fig. 3E; \( *P < 0.001 \) and \( **P < 0.01 \) versus mock). For reference, hOAT1-mediated PAH uptake, hOAT2-mediated PGF2α uptake, and hOAT3- and hOAT4-mediated estrone sulfate uptake were 16-, 16.7-, 37-, and 31-fold higher, respectively, than those by control (Enomoto et al., 2002; Takeda et al., 2002). In contrast to hOATs, hOCT1 and hOCT2 did not mediate the transport of various NSAIDs tested in the current study (data not shown).

**Discussion**

hOAT1 and hOAT3 have been shown to mediate the transport of NSAIDs, antitumor drugs, histamine H2-receptor antagonist, prostaglandins, diuretics, angiotensin-converting enzyme inhibitors, and β-lactam antibiotics (Hosoyamada et al., 1999; Cha et al., 2001). Some differences in characteristics exist between hOAT1 and hOAT3, such as substrate specificity and localization: hOAT1 is localized at the basolateral side of the S2 segment of the proximal tubule (Hosoyamada et al., 1999), whereas hOAT3 is localized at the first, second, and third segments (S1, S2, and S3) of the proximal tubule (Cha et al., 2001). In addition, hOAT1, but not hOAT3, exhibits transport properties as an exchanger (Hosoyamada et al., 1999; Cha et al., 2001). HOAT2, also shown to be localized at the basolateral side of the proximal tubule, mediates the transport of organic anions including salicylate and PGF2α (Enomoto et al., 2002). HOAT4
also mediates the apical transport of various anionic drugs in the proximal tubule (Babu et al., 2002); however, this transporter exhibits relatively narrow substrate recognition compared with hOAT1 and hOAT3 (Cha et al., 2000). HOCT1 is mainly localized in the liver and mediates polyspecific pH-independent transport of organic cations. In contrast, hOCT2 is mainly localized in the kidney and mediates pH-independent, electrogenic, and polyspecific transport of organic cations (Gor-

**TABLE 2**  
The IC₅₀ values of various NSAIDs for organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4

<table>
<thead>
<tr>
<th>Drugs</th>
<th>hOAT1</th>
<th>hOAT2</th>
<th>hOAT3</th>
<th>hOAT4</th>
</tr>
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<tbody>
<tr>
<td>Acetylsalicylate</td>
<td>769</td>
<td>2000&gt;</td>
<td>717</td>
<td>2000&gt;</td>
</tr>
<tr>
<td>Salicylate</td>
<td>325</td>
<td>2000&gt;</td>
<td>50.0</td>
<td>2000&gt;</td>
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<tr>
<td>Diclofenac</td>
<td>4.46</td>
<td>14.3</td>
<td>7.78</td>
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<td>Ibuprofen</td>
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<td>64.1</td>
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<td>10.1</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>4.34</td>
<td>400</td>
<td>5.98</td>
<td>70.3</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>0.83</td>
<td>21.7</td>
<td>0.78</td>
<td>61.7</td>
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<tr>
<td>Naproxen</td>
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<td>486</td>
<td>4.67</td>
<td>55.4</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>275</td>
<td>1878</td>
<td>19.4</td>
<td>2000&gt;</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>62.8</td>
<td>70.3</td>
<td>2.52</td>
<td>84.9</td>
</tr>
<tr>
<td>Sulindac</td>
<td>36.2</td>
<td>440</td>
<td>3.62</td>
<td>617</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effects of NSAIDs on organic cation uptake mediated by hOCT1 and hOCT2. S₂ hOCT1 and S₂ hOCT2 were incubated in solution containing 5 μM [³¹C]TEA in the presence of 0.5 mM (A) or 2 mM (B) NSAIDs at 37°C for 5 min. Each value represents the mean ± S.E. of eight monolayers from two separate experiments. *, P < 0.001; **, P < 0.01, and ***, P < 0.05 versus control.

**Fig. 3.** NSAID uptake by hOATs. S₂ hOAT1, S₂ hOAT2, S₂ hOAT3, S₂ hOAT4, and mock were incubated in solution containing either 30 μM [³¹C]acetylsalicylate (A), 30 μM [³¹C]salicylate (B), 500 nM [³¹H]ibuprofen (C), 5 μM [³¹H]indomethacin (D), or 50 nM [³¹H]ketoprofen (E) at 37°C for 2 min (acetylsalicylate, salicylate, indomethacin, and ketoprofen) or 5 min (ibuprofen). Each value represents the mean ± S.E. of eight monolayers from two separate experiments. *, P < 0.001 and ***, P < 0.01 versus mock.
bolou et al., 1997). Using stable cell lines, we have elucidated the interactions of hOATs and hOCTs with various NSAIDs. 

By comparing the IC_{50} values of NSAIDs among hOATs, it was found that hOAT1 and hOAT3 generally exhibited high affinity for NSAIDs. In contrast, hOAT2 exhibited the lowest affinity for ibuprofen, indomethacin, ketoprofen, and naproxen; hOAT4 exhibited the lowest affinity for diclofenac, mefenamic acid, and phenacetin; hOAT2 and hOAT3 exhibited the lowest affinity for acetylsalicylate, salicylate, and sulindac. Thus, hOAT2 and hOAT4 generally appear to exhibit the lowest affinity for NSAIDs among the hOATs.

By comparing the IC_{50} values of various NSAIDs for hOAT1-mediated PAH uptake with the K_{i} values for rOAT1-mediated PAH uptake (Apiwattanakul et al., 1999), it was found that the IC_{50} values of acetylsalicylate, salicylate, indomethacin, naproxen, phenacetin, and piroxicam for hOAT1 were similar to the K_{i} values for rOAT1 (within 3-fold difference; Zhang et al., 1998), whereas those of acetaminophen and ibuprofen were different (more than 3-fold). It is reported that when the substrate concentration is low compared with the K_{m} value, K_{i} values will be identical with the IC_{50} values despite the mechanism of inhibition (Cheng and Prusoff, 1973). In this regard, all of the experiments in the current study were performed using substrate concentrations less than the K_{m} values. In addition, in contrast to the fact that rOAT1 mediated the uptake of acetylsalicylate (Apiwattanakul et al., 1999), hOAT1 did not exhibit acetylsalicylate uptake activity. Although there was a difference in the expression system between the results of hOAT1 and rOAT1, i.e., cultured cells versus oocyte expression system, some interspecies differences between human and rat appear to exist for the interactions of OAT1 with some of the NSAIDs. Similarly, Morita et al. (2001) have demonstrated that the K_{i} values of ketoprofen, diclofenac, and ibuprofen for salicylate uptake in LLC-PK1 cells stably expressing rat OAT2 were 1.84, 49.3, and 155 μM, respectively, whereas the IC_{50} values for hOAT2 in the current study were 400, 14.3, and 692 μM, respectively. Thus, there appears to exist a significant difference between human and rat in the interactions of OAT2 with some of NSAIDs.

In a previous study using oocytes expressing rOAT1 (Apiwattanakul et al., 1999), we also found that all hydrophobic NSAIDs potently inhibited PAH uptake, whereas hydrophilic NSAIDs inhibited PAH uptake to a lesser degree. As shown in Table 2, this tendency was true not only for PAH uptake by hOAT1, but also for PGF_{2α} uptake by hOAT2 and estrone sulfate uptake by hOAT3 and hOAT4.

Unexpectedly, some of the NSAIDs inhibited organic cation uptake mediated by hOCT1 and hOCT2, although these two transporters did not mediate the uptake of NSAIDs. The transport of substrates by carrier proteins consists of three processes: substrate binding, translocation, and dissociation. Thus, it was suggested that some of the NSAIDs inhibited TEA binding with hOCT1 and hOCT2 molecules; however, they were not translocated by hOCT1 and hOCT2. The results showing the inhibitory effect of NSAIDs on TEA uptake mediated by hOCT1 and hOCT2 do not contradict the fact that NSAIDs possess anionic moieties. This is due to the assumption that the structures of the binding sites of OATs and OCTs are quite similar, except for the charge recognition sites (Sekine et al., 1999). Similarly, we have previously demonstrated that hOATs and hOCTs interacted with PGF_{2α} and PGF_{2α}, which possess anionic moieties (Kimura et al., 2002).

As demonstrated in the urinary excretion rate of unchanged drugs in Table 1, NSAIDs are highly metabolized in the liver, some by phase I and phase II mechanisms, and others by direct glucuronidation (phase II alone) (Frustr and Munster, 2000). In addition, NSAIDs including acetylsalicylate, acetaminophen, sulindac, and diclofenac have been shown to induce liver injury (Zimmerman 1981; Wood et al., 1985; Purcell et al., 1991). In this regard, hOAT2 was shown to be localized at the basolateral side of the liver (Y. Kobayashi, unpublished observation). In the current study, hOAT2 interacted with all of the NSAIDs tested and mediated the transport of some of these NSAIDs. Thus, it was suggested that hOAT2 mediates the uptake of NSAIDs in the basolateral side of the hepatocyte, leading to the metabolism of NSAIDs or the induction of liver injury.

The significant aspects of the interactions of hOATs with NSAIDs in the kidney are suggested to be as follows. The first is to mediate the urinary excretion of NSAIDs. So far, renal handling of salicylate has been studied extensively in animal experiments (Ferrier et al., 1983; Schild and Roch-Ramel, 1988). In humans, as shown in Table 1, 2–30% of administered salicylate is eliminated by urinary excretion in the unchanged form. In the current study, we found that hOAT1 and hOAT3 interacted with salicylate and mediated its transport. Thus, it was suggested that hOAT1 and hOAT3 mediate the uptake of salicylate in the basolateral membrane of the proximal tubule in humans. The second is associated with the induction of renal papillary necrosis. In humans, NSAIDs, including ibuprofen and mefenamic acid, were reported to induce renal papillary necrosis (Robertson et al., 1980; Shah et al., 1981). The mechanism of the induction of renal papillary necrosis has been postulated to be as follows: the accumulation of NSAIDs and the subsequent secretion of these drugs into the lumen lead to high concentrations of these drugs in papillary tips, thereby causing renal papillary necrosis. In the current study, we found that hOAT1 and hOAT3 mediated the transport of ibuprofen and hOAT1, hOAT2, hOAT3, and hOAT4 interacted with ibuprofen and mefenamic acid. Thus, NSAIDs transport mediated by hOAT1, hOAT2, hOAT3, and hOAT4 may be associated with the induction of renal papillary necrosis.

NSAIDs have been shown to induce various forms of adverse neurological effects including cognitive dysfunction, confusion, somnolence, behavioral disturbances, seizures and dizziness (Hoppman et al., 1991; Day et al., 2000). In this regard, we found that hOAT3 mRNA was expressed in the skeletal muscle using Northern blot analysis (Cha et al., 2001). In the current study, we found that hOAT3 interacted with all of the NSAIDs tested and mediated the transport of some of these NSAIDs. Based on these observations, it is possible that hOAT3-mediated uptake of NSAIDs in the brain leads to the induction of adverse neurological effects.

Acetaminophen, salicylate, diclofenac, and ibuprofen were reported to induce rhabdomyolysis in humans (Ross and Hopple, 1987; Leventhal et al., 1989; Delrio et al., 1996). In this regard, we found that hOAT3-mediated transport of salicylate and ibuprofen, and interacted with acetaminophen and diclofenac. Although precise immunohistochemical analysis should be performed, it is possible that hOAT3 me-
diates the accumulation of NSAIDs, which leads to the induction of rhabdomyolysis.

Acetylsalicylate, ibuprofen, indomethacin, ketoprofen, and naproxen were reported to potentially induce adverse effects on the fetus including increased cutaneous and intracranial bleeding, premature closure of the ductus arteriosus, pulmonary hypertension, and impaired renal function (Janssen and Genta, 2000). In addition, acetylsalicylate and diclofenac were shown to possess teratogenic potential in animals (Kimmel et al., 1971; Chan et al., 2001). In this regard, hOAT4 was shown to be localized in the placenta using Northern blot analysis (Cha et al., 2000). In the current study, hOAT4 interacted with all of the NSAIDs tested and mediated the uptake of ketoprofen. Thus, although precise immunohistochemical analysis of hOAT4 in the human placenta should be performed, it is possible that hOAT4 mediates the delivery of NSAIDs into the fetus, which may be associated with the induction of adverse drug reactions and teratogenesis.

In addition to the hOAT family, the interactions of other human transporters and human homologs of rodent transporters mediating organic anion transport with NSAIDs should be investigated, including OAT-K1 (Saito et al., 1996), OAT-K2 (Masuda et al., 1999), organic anion-transporting peptide 1 (oatp1) (Jacquemin et al., 1994), oatp2 (Noe et al., 1997), multidrug resistance protein 2 (Leier et al., 2000), and human type I sodium-dependent inorganic phosphate transporter (NPT1) (Uchino et al., 2000). In this regard, it was already reported that OAT-K1-mediated methotrexate transport was inhibited by NSAIDs including ibuprofen, indomethacin, ketoprofen, and phenylbutazone (Uwai et al., 2000); OAT-K2-mediated taurocholate transport was inhibited by indomethacin (Masuda et al., 1999); and NPT1-mediated PAH transport was inhibited by indomethacin and salicylate (Uchino et al., 2000).

In conclusion, hOATs and hOCTs interacted with various NSAIDs, whereas hOATs but not hOCTs mediated the transport of NSAIDs. It was suggested that hOATs are associated with the pharmacokinetics and the induction of adverse actions of NSAIDs.

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. E-mail: endou@kyorin-u.ac.jp


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. E-mail: endou@kyorin-u.ac.jp