The liver is a central organ for the detoxification and elimination of a wide variety of organic compounds. Several distinct liver-predominant multispecific organic anion transporters such as multidrug resistance associated proteins (MRP1–5[ABCC1–5]) and organic anion-transporting polypeptides (OATP1A2[SLCO1A2], 1B1[SLCO1B1], 1B3[SLCO1B3], 2B1[SLCO2B1]), and Oat5[SLC22A10]) have been isolated and well characterized (Cole and Deeley, 1993; Simonson et al., 1994; Allikmets et al., 1996; Ito et al., 1998; Sun et al., 2001; Hagenbuch and Meier, 2004). To date, five different human Oat isoforms (hOat1–5) have been isolated (Sun et al., 2001). Although the role of hOat5 has not been characterized yet, hOat2 is considered to be one of the key molecules in hepatic handling of organic anions because this isoform is highly expressed in the human liver (Sun et al., 2001).

Theophylline, 1,3-dimethylxanthine, has been widely used as a bronchodilatory drug for the treatment of neonatal apnea in premature newborns and patients with chronic obstructive airway disease such as asthma and bronchiitis. Theophylline is metabolized by N-demethylation to 1-methylxanthine and 3-methylxanthine and by 8-hydroxylation to 1,3-dimethyluric acid in the liver (Fuhr et al., 1992). It is well known that many drugs increase or decrease the clearance of theophylline because this isoform is highly expressed in the human liver (Sun et al., 2001).

Macrolide antibiotics such as erythromycin have been used for treatment of a variety of infections and are often combined with other drug therapies. For example, patients with chronic asthma receiving continuous therapy with theophylline may require short courses of erythromycin for unrelated pyogenic infections. It has been reported that serum theophylline concentrations increased in patients with continuous administration of erythromycin by inhibiting hepatic P450s (Cummins et al., 1976; Kozak et al., 1977; Pfeifer et al., 1979). However, it is not clear at this time whether such a drug interaction could be mediated by drug transporters. In the present study, therefore, we investigated the possible involvement of hOat2 in the interaction of theophylline with erythromycin.

**Materials and Methods**

**Chemicals.** [14C]Theophylline (52 mCi/mmol) and [14C]erythromycin (55 mCi/mmol) were purchased from American Radiolabeled Chemicals (St. Louis, MO). All other chemicals were of the highest grade commercially available.

**Xenopus laevis Oocyte Preparation and cRNA Synthesis.** Isolation and preparation of Xenopus oocytes was performed as described elsewhere (Kobayashi et al., 2002b). The hOat2 cDNA was linearized with BamHI, and the capped cRNA was transcribed in vitro by T7 RNA polymerase. Defolliculated oocytes were microinjected with 50 ng of in vitro transcribed cRNA under a stereomicroscope using a microdispenser (Drummond Scientific, Broomall, PA) and incubated for 2 days in a modified Barth’s solution containing gentamicin (50 μg/ml) at 18°C.

**Transport Assays.** Uptake experiments of radiolabeled substrates were performed in an ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5 mM HEPES, pH 7.4) at room temperature. Oocytes were incubated in an ND96 solution containing radiolabeled substrates for 1 h at room temperature. For the kinetic study, concentration-dependent uptake experiments of [14C]theophylline and [14C]erythromycin via hOat2 were performed with each compound at a final concentration ranging from 1, 2, 5, 10, 15, and 20 μM, and 1, 5, 10, 20, 40, and 60 μM, respectively. The compounds were incubated with oocytes expressing hOat2 for 1 h at room temperature. The uptake was terminated by the addition of 2 ml of an ice-cold ND96
resulting in theophylline clearance may result in lack of a sufficient bronchodilation of theophylline (Adebayo et al., 1986; Periti et al., 1992). The exact mechanism of interference with theophylline disposition by erythromycin would clearly be of clinical importance because of its toxicity. Several reports suggest that macrolide antibiotics may alter theophylline clearance. The interaction between theophylline and erythromycin would clearly be of clinical importance because of its toxicity. The exact mechanism of interference with theophylline disposition by erythromycin is still unclear; it might be explained by inhibition of the 1-demethylation pathway by erythromycin by inhibiting hepatic CYP1A2 and 3A4 and resulting in an increase of the blood concentration of theophylline (Adebayo et al., 1986; Periti et al., 1992). Because the therapeutic range of theophylline is very narrow, changes in theophylline clearance may result in lack of a sufficient bronchodilating effect or in the appearance of a toxic effect.

Oat2 was initially called the novel liver-specific transport protein (NLT) (Simonson et al., 1994). Oat2, as well as OATP/Oatp, is a multispecific organic anion transporter and is highly expressed at the sinusoidal membrane of the liver (Simonson et al., 1994; Sekine et al., 1998). However, as far as we know, it is not clear at this time whether the theophylline-erythromycin interaction can occur via hepatic drug transporters. Therefore, we focused on the role of hOat2 and assumed that hOat2 could transport theophylline and erythromycin.

Using Xenopus oocytes injected with hOat2 cRNA, we first examined the transport of theophylline and erythromycin mediated by hOat2. Since the uptake of [14C]theophylline by uninjected oocytes was equal to that of the oocytes injected with 50 nl of water (data not shown), uninjected oocytes were used as the control instead of water-injected oocytes throughout this study. As shown in Fig. 1, both [14C]theophylline and [14C]erythromycin were significantly transported via hOat2 to about 5.9-fold and 33.3-fold that of the controls, respectively. These compounds are identified for the first time as hOat2 substrates. We have revealed that mouse Oat2 (mOat2) is a multispecific organic anion transporter (Kobayashi et al., 2002b). Therefore, we assumed that mOat2, as well as hOat2, also mediates the transport of theophylline and erythromycin. As expected, both compounds were significantly transported via mOat2 (data not shown). Taken together, previous data indicate that theophylline and erythromycin are conserved substrates of hOat2 and mOat2.

To determine the affinity of theophylline and erythromycin with hOat2, based on these findings, concentration dependence of the uptake of [14C]theophylline and [14C]erythromycin via hOat2 was subsequently examined. As shown in Fig. 2, hOat2 mediated the transport of [14C]theophylline, and [14C]erythromycin showed saturable kinetics and followed the Michaelis-Menten equation. Eadie-Hofstee plots of the concentration dependence of theophylline and erythromycin uptake after subtraction of the uptake by noninjected oocytes revealed that the estimated \( K_m \) values are 12.6 \( \mu M \) and 18.5 \( \mu M \), respectively. Thus, the affinities of both compounds with hOat2 are very similar. These results indicate that theophylline and erythromycin are transported via hOat2.

The therapeutic range of plasma concentration and unbound fraction of theophylline were reported to be 55 to 110 \( \mu M \) and 40 to 60\%, respectively (Tenenbein, 1989). Therefore, the steady-state concentration of unbound theophylline in the plasma is estimated to be approximately 41.3 \( \mu M \). In addition, the maximum plasma concentration and unbound fraction of erythromycin were reported to be 4.8 \( \mu M \) and 84\%, respectively (Periti et al., 1989); the steady-state concentration of unbound erythromycin in the plasma is estimated to be approximately 4.0 \( \mu M \). Comparing the plasma concentration and our kinetic data, hOat2 may be a responsible molecule for the transport of theophylline and the interaction of theophylline with erythromycin in the human liver.

To confirm hOat2 involvement in the theophylline-erythromycin drug interaction, \textit{cis}-inhibitions of hOat2-mediated drug uptake experiments were performed. The results are shown in Fig. 3. The hOat2-mediated transport of [14C]theophylline was strongly inhibited by erythromycin and slightly inhibited by clarithromycin. No inhibitory effect was observed by valproate. Similarly, transport of [14C]erythromycin via hOat2 was inhibited by unlabeled theophylline, but not by clarithromycin and valproate. Although some reports were
unable to confirm any significant effects of theophylline-erythromycin drug interaction (Kelly et al., 1981; Melethil et al., 1982), our present findings, together with the membrane localization, suggest that theophylline-erythromycin drug interaction may occur at the sinusoidal membrane of the liver by inhibiting hOat2-mediated transport.

Regarding the interaction between theophylline and clarithromycin, von Rosensteli and Adam (1995) have reported that there is a slight increase in serum concentration of theophylline AUC and a 16.4% decrease in theophylline clearance with concurrent administration of both drugs. Supportive evidence has been reported by Gillum et al. (1996). Taking their reports (von Rosensteli and Adam, 1995; Gillum et al., 1996) and our inhibition experiment into consideration, theophylline-clarithromycin interaction would be rare, or if it occurred, such interaction may not be mediated by hOat2. Further study is required.

The expression of Oat isoforms (Oat1–Oat3) exhibits a sex difference in rats and mice (Buist et al., 2002; Kobayashi et al., 2002a,b; Buist and Klaassen, 2004). Rat Oat2 in the liver is expressed at a higher level in male rats than in females (Buist et al., 2002; Kobayashi et al., 2002a), whereas mOat2 mRNA of the liver is detected predominantly in females rather than in males (Kobayashi et al., 2002b).

Recently, Buist and Klaassen (2004) have extensively investigated the theory that there is no sex-related differential gene expression of mOat2 in the liver using branched DNA analysis. Although there are no reports concerning the gender-related differential expression of hOat2 mRNA in the liver of men and women, Nafziger and Bertino (1989) have reported that theophylline is cleared significantly higher in women than in men, suggesting that such sex-dependent theophylline clearance might be caused by the differential gene expression of hOat2 between men and women. In this respect, additional study is needed.

In conclusion, our paper is the first report concerning the possible involvement of hOat2 on theophylline-erythromycin interaction. Our present findings, therefore, indicate that hOat2 may, at least partly, be involved in the interaction of theophylline with erythromycin at the sinusoidal membrane of the human liver. It would be interesting to elucidate whether other transporters such as OATP1B1, 1B3, and 2B1 (Kool et al., 1999; Hagenbuch and Meier, 2004) are also involved in the theophylline-erythromycin interaction.

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