CARRIER-MEDIATED UPTAKE OF H2-RECEPTOR ANTAGONISTS BY THE RAT CHOROID PLEXUS: INVOLVEMENT OF RAT ORGANIC ANION TRANSPORTER 3

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ABSTRACT:

The choroid plexus (CP) acts as a site for the elimination of xenobiotic organic compounds from the cerebrospinal fluid (CSF). The purpose of the present study is to investigate the role of rat organic anion transporter 3 (rOat3; *Slc22a8*) in the uptake of H2-receptor antagonists (cimetidine, ranitidine, and famotidine) by the isolated rat CP. Saturable uptake of cimetidine and ranitidine was observed in rOat3-LLC with K_m values of 80 and 120 μM, respectively, whereas famotidine was found to be a poor substrate. The steady-state concentration of the H2-receptor antagonists in the CSF was significantly increased by simultaneously administered probenecid, although it did not affect their brain and plasma concentrations. Saturable uptake of cimetidine and ranitidine was observed in the isolated rat CP with K_m values of 93 and 170 μM, respectively, whereas 50% of the uptake of famotidine remained at the highest concentration examined (1 mM). The K_i value of ranitidine for the uptake of cimetidine by the isolated CP (50 μM) was similar to its own K_m value, suggesting that they share the same transporter for their uptake. The inhibition potency of organic anions such as benzylpenicillin, estradiol 17β-glucuronide, *p*-aminohippurate, and estrone sulfate for the uptake of cimetidine by the isolated rat CP was similar to that for benzylpenicillin, the uptake of which has been hypothesized to be mediated by rOat3, whereas a minimal effect by tetraethylammonium excludes involvement of organic cation transporter(s). These results suggest that rOat3 is the most likely candidate transporter involved in regulating the CSF concentration of H2-receptor antagonists at the CP.

The choroid plexus (CP), located in the lateral, third, and fourth ventricles, is the site of production of cerebrospinal fluid (CSF) (Segal, 2000; Haselbach et al., 2001). It is well established that CP acts as a barrier between the CSF and the circulating blood, and it is referred to as the blood-CSF barrier (Suzuki et al., 1997; Ghersi-Egea and Strazielle, 2001; Haselbach et al., 2001; Kusuhara and Sugiyama, 2001). The barrier function is achieved partly by the tight monolayer of choroid plexus epithelial cells and partly by detoxification systems consisting of metabolic enzymes and multispecific transporters (Suzuki et al., 1997; Ghersi-Egea and Strazielle, 2001; Haselbach et al., 2001; Kusuhara and Sugiyama, 2001).

Histamine H2-receptor antagonists have been used clinically to cure duodenal ulcers and gastric acid hypersecretion, and adverse effects by H2-receptor antagonists on the central nervous system, ranging from mild dizziness, restlessness, and mental confusion to advanced symptoms such as myoclonic twitching and seizure, have been reported (Grimson, 1977; Schentag et al., 1979; McGuigan, 1981). Schentag et al. (1979) reported that the concentration of cimetidine in the CSF is related to the mental status. We demonstrated previously that a saturable mechanism is involved in the elimination of cimetidine from the CSF after intracerebroventricular administration (Su-

zuki et al., 1985, 1988). Transport studies revealed that the uptake of cimetidine by the isolated rat choroid plexus is saturable, and the efflux transport across the CP has been considered to account for the saturable elimination mechanism of cimetidine from the CSF (Suzuki et al., 1986). Organic anions such as benzylpenicillin and *p*-aminohippurate (PAH) inhibit the uptake of cimetidine by the isolated rat choroid plexus, whereas organic cations such as tetraethylammonium (TEA) and *N*-methylnicotinamide have no such effect (Suzuki et al., 1986). It is likely that organic anion transporter(s) play a major role in regulating the CSF concentration of cimetidine at the CP.

We have isolated rat organic anion transporter 3 (rOat3; *Slc22a8*), the third isoform of the Oat/OAT family, from the rat brain cDNA library by homology cloning (Kusuhara et al., 1999). Functional expression of rOat3 in *Xenopus laevis* oocytes and mammalian cells has revealed its broad substrate specificity for organic anions, including PAH and benzylpenicillin (Kusuhara et al., 1999; Burckhardt and Burckhardt, 2003; Dantzler and Wright, 2003). In the CP, although mRNA expression of all the Oat isoforms (rOat1~rOat3) has been detected (Sweet et al., 2002; Choudhuri et al., 2003), rOat3 is the most abundant isoform (Choudhuri et al., 2003). rOat3 has been shown to be expressed on the brush border membrane of the CP (Nagata et al., 2002). Localization of rOat3 in the CP suggests its involvement in the uptake process at the CP. Since the spectrum of inhibitors and kinetic

ABBREVIATIONS: CP, choroid plexus; CSF, cerebrospinal fluid; PAH, *p*-aminohippurate; TEA, tetraethylammonium; Oat, organic anion transporter; MEP, molecular electrostatic potential(s); LC-MS, liquid chromatography-mass spectometry; Oct, organic cation transporter.

parameters for the uptake of PAH and benzylpenicillin by the isolated rat CP were similar to those for rOat3, it has been hypothesized that rOat3 accounts for their uptake by the isolated rat CP (Nagata et al., 2002). Furthermore, the finding that the isolated choroid plexus from mOat3 knockout mice was unable to cellularly accumulate fluorescein supports the role of Oat3 in the CP (Sweet et al., 2002). H$_2$-receptor antagonists have been referred to as bisubstrates recognized by both renal organic anion and cation transporters (Ullrich et al., 1993). Indeed, cimetidine is a substrate of rOat3 as well as organic cation transporter(s) (Grundemann et al., 1999; Kusuhara et al., 1999). Therefore, it is likely that rOat3 plays a major role in the uptake of cimetidine and other H$_2$-receptor antagonists by the isolated rat CP.

The primary purpose of the present study was to investigate the importance of rOat3 in regulating the concentration of H$_2$-receptor antagonists (cimetidine, ranitidine, and famotidine). We examined whether ranitidine and famotidine are substrates of rOat3 in LLC-PK1 cells expressing rOat3 (rOat3-LLC). rOat3-LLC exhibits specific uptake of ranitidine and famotidine, but the transport activity of famotidine was quite low compared with that of cimetidine and ranitidine. Steady-state concentrations of H$_2$-receptor antagonists in the CSF and plasma were determined in rats treated with or without probenecid, a potent inhibitor of rOat3. The uptake of H$_2$-receptor antagonists was investigated using the isolated rat CP, and the kinetic parameters and spectrum of inhibitors were compared with those for benzylpenicillin.

Materials and Methods

Materials. [^{3}H]Cimetidine (16.5 Ci/mmol) and [^{14}C]urea (52 mCi/mmol) were purchased from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). All cell culture media and reagents were obtained from Invitrogen (Carlsbad, CA), except fetal bovine serum (Cansera International Inc., Ontario, Canada). All other chemicals and reagents were of analytical grade and readily available from commercial sources.

Calculation of Molecular Electrostatic Potential (MEP) of H$_2$-Receptor Antagonists. The starting structures of H$_2$-receptor antagonists were built up on the basis of standard bond lengths and angles, and the structures of H$_2$-receptor antagonists were optimized using the AM1 Hamiltonian and conductor-like screening model. Their molecular electrostatic potentials at pH 7.4 were calculated using the modified neglect of diatomic overlap Hamiltonian. All calculations were carried out by the MOPAC97 (CS Chem3D Pro; CambridgeSoft Corporation, Cambridge, MA).

Uptake Studies by rOat3-LLC. rOat3-LLC was established previously, and all the procedures have been described in detail (Sugiyama et al., 2001). Cells were seeded on a 12-well dish (BD Biosciences, Franklin Lakes, NJ) at a density of 1.2×10^5 cells/well and cultured for 3 days. Sodium butyrate (5 mM) was added to the culture medium to induce expression of the transporter 24 h before starting the experiments (Sugiyama et al., 2001). Uptake was initiated by adding medium containing ligands, with or without inhibitors, after the cells had been washed twice and preincubated with Krebs-Henseleit buffer at 37°C for 15 min. This buffer consists of 142 mM NaCl, 23.8 mM Na$_2$CO$_3$, 4.83 mM KCl, 0.96 mM KH$_2$PO$_4$, 1.20 mM MgSO$_4$, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM CaCl$_2$ adjusted to pH 7.4. The uptake was terminated at a designated time by adding ice-cold Krebs-Henseleit buffer. For [^{3}H]cimetidine uptake, cells were dissolved in 500 μl of 1 N NaOH, kept overnight, neutralized with 250 μl of 2 N HCl, and then aliquots (500 μl) were transferred to scintillation vials. The radioactivity associated with the cells and medium was determined in a liquid scintillation spectrophotometer (LS6000SE; Beckman Coulter, Fullerton, CA) after adding scintillation fluid (Hionic-Fluor; PerkinElmer Life and Analytical Sciences, Boston, MA) to the vials. For the determination of the uptake of ranitidine and famotidine, cells were dissolved in 200 μl of 0.2 N NaOH, and aliquots (50 μl) were used for LC-MS quantification as described below. The remaining portions of cell lysate were used to determine the protein concentration by the method of Lowry, with bovine serum albumin as a standard. Ligand uptake was given as the cell-to-medium concentration ratio determined as the amount of ligand associated with cells divided by the medium concentration.

Uptake Studies by Isolated Rat CP. Male Sprague-Dawley rats weighing 250 to 300g were purchased from SLC (Shizuoka, Japan). The CP was isolated from the lateral ventricles and incubated at 37°C for 1 min in 500 μl of artificial CSF, which consists of 122 mM NaCl, 25 mM NaHCO$_3$, 10 mM glucose, 3 mM KCl, 1.4 mM CaCl$_2$, 1.2 mM MgSO$_4$, 0.4 mM K$_2$HPO$_4$, and 10 mM HEPES (pH 7.3), equilibrated with 95% O$_2$/5% CO$_2$. Radiolabeled ligands, with or without inhibitors, were added simultaneously to initiate uptake. The uptake of [^{3}H]cimetidine and [^{14}C]TEA by isolated rat CP was examined by centrifugal filtration as described previously (Nagata et al., 2002). The tissue-to-medium concentration ratio of [^{3}H]cimetidine and [^{14}C]TEA was calculated with [^{14}C]urea or [^{3}H]water as a cell water space marker and corrected for the adherent water space. The ^{3}H and ^{14}C activity in the specimens was determined in a liquid scintillation spectrophotometer. To determine the uptake of nonradiolabeled H$_2$-receptor antagonists, the rapid filtration method was used since the silicon oil, used in the centrifugal filtration method, disturbed the quantification by LC-MS. The uptake of cimetidine, ranitidine, and famotidine by isolated rat CP was terminated by rapid filtration using a vacuum manifold; then the CP was rinsed three times with 300 μl of artificial CSF, the CP was dissolved in 100 μl of 0.2 N NaOH, and aliquots (50 μl) were subjected to LC-MS quantification as described below. The remaining portions of lysate were used to determine the protein concentration by the method of Lowry, with bovine serum albumin as a standard.

Constant Infusion Study in Rats. Rats were lightly anesthetized with ether, and the left femoral vein was cannulated with polyethylene tubing (PE-50; BD Biosciences). The priming dose of the H$_2$ antagonist and probenecid was 2 and 15 mg/kg, respectively. The H$_2$ antagonist and probenecid were given to rats through the femoral vein cannula at 2 and 30 mg/h/kg, respectively, for 3 h. The dose regimen was designed to obtain a CSF concentration of probenecid sufficient to inhibit rOat3. Blood samples (300 μl) were collected from the tail vein at 1 and 2 h during the infusion. At the end of the experiment, blood samples were collected from the abdominal vein, and animals were sacrificed by bleeding the abdominal aorta under ether anesthesia. Following sacrifice, 50- to 100-μl aliquots of CSF were obtained by cisternal puncture using insulin syringes (0.5-ml syringe with a 29-gauge $\times$ 1/2-inch attached needle; TERUMO Corporation, Tokyo, Japan), and whole brains were removed and weighed. To obtain plasma, blood was centrifuged at 10,000g for 5 min. For determination of the unbound plasma concentration, aliquots (0.5 ml) of plasma specimens were subjected to filtration (2500 rpm, 10 min) (MPS-1; Millipore Corporation, Bedford, MA). The brain samples were homogenized in three volumes of water using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). The concentrations of H$_2$-receptor antagonists and probenecid in plasma, plasma ultrafiltrate, and brain homogenate were quantified by LC-MS as described below.

Quantification of H$_2$-Receptor Antagonists and Probenecid by LC-MS. The quantification of cimetidine, ranitidine, famotidine, and probenecid was performed by a high-performance liquid chromatograph (Alliance 2690; Waters, Milford, MA) connected to a mass spectrometer (ZMD; Micromass UK Ltd., Manchester, UK). Aliquots (50 μl) of samples containing H$_2$-receptor antagonists were precipitated by adding 100 μl of methanol containing an internal standard (famotidine for cimetidine and ranitidine and cimetidine for famotidine), mixed, and centrifuged, and 20 μl of the supernatants was subjected to LC-MS. Aliquots (50 μl) of samples containing probenecid were precipitated by adding 100 μl methanol, mixed, and centrifuged, and aliquots (10 μl) of the supernatants were diluted with 990 μl of methanol, 20 μl of which was subjected to LC-MS. High-performance liquid chromatography analysis was performed on an Waters Xterra MS C18 column (2.5 μm, 3 mm i.d., 30 mm) at room temperature. Elution was performed with a 0 to 100% linear gradient of 10 mM ammonium acetate/methanol over 4 min at 0.8 ml/min. A portion of the eluent (split ratio = 1:3) was introduced into the MS via an electrospray interface. Detection was performed by selective ion monitoring in positive ion mode (m/z: 253, 315, 338, and 286 for cimetidine, ranitidine, famotidine, and probenecid, respectively).

Kinetic Analyses. Kinetic parameters were obtained using the Michaelis-Menten equation $v = V_{max} \times S/(K_m + S) + P_{dif} \times S$, where v is the uptake rate of the substrate (pmol/min/mg protein or pmol/min/μl tissue), S is the substrate concentration in the medium (μM), K_m is the Michaelis-Menten constant (μM), and V_{max} is the maximum uptake rate (pmol/min/mg protein or pmol/min/μl tissue). P_{dif} represents the uptake clearance corresponding to the
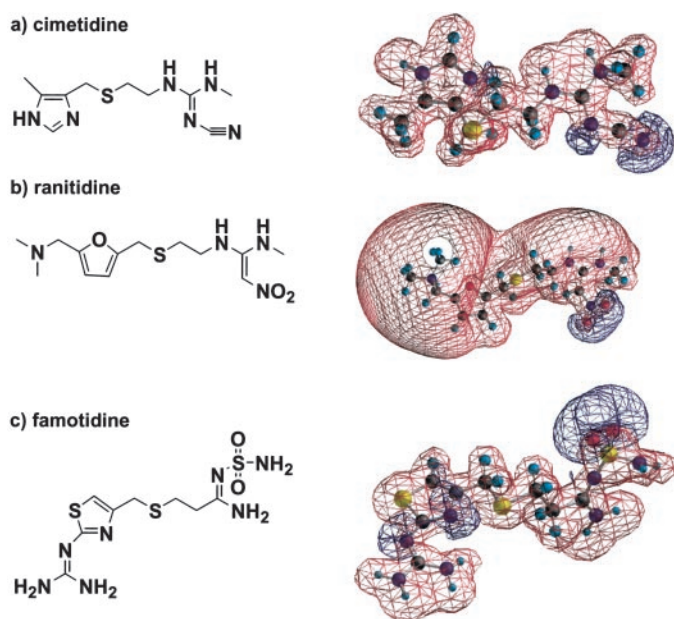
FIG. 1. Molecular structures and molecular electrostatic potential of H$_2$-receptor antagonists. MEP of cimetidine, ranitidine, and famotidine at pH 7.4 were calculated using the MNDO Hamiltonian. The figure illustrates the isoelectric line at 2.8 atomic units. The colors indicate the charge of their electrostatic potential: red is positive and blue is negative.

nonsaturable component (μl/min/mg protein or μl/min/μl tissue). To obtain the kinetic parameters, the equation was fitted to the uptake velocity using a MULTI program (Yamaoka et al., 1981). The input data were weighted as the reciprocals of the observed values, and the Damping Gauss-Newton method algorithm was used for fitting. Inhibition constants (K_i) were calculated by assuming competitive inhibition using the equation $CL_{+inh} = CL/(1 + I/K_i) + P_{dif}$, where CL represents the uptake clearance and $_{+inh}$ represents the value in the presence of inhibitor. I represents the concentration of inhibitor (μM). The substrate concentration was low compared with its K_m value in the inhibition study.

Results

Molecular Electrostatic Potential of H$_2$-Receptor Antagonists. Figure 1 illustrates the chemical structures of the H$_2$-receptor antagonists and their isoelectric lines at 2.8 atomic units calculated at pH 7.4. There was a region showing negative MEP in the chemical structures of H$_2$-receptor antagonists (Fig. 1; indicated by blue in the cyanoimine group for cimetidine, the nitro group for ranitidine, and the imine and sulfonamide groups for famotidine).

Uptake of H$_2$-Receptor Antagonists (Cimetidine, Ranitidine, and Famotidine) by rOat3-LLC. Figure 2 shows the time profiles of the uptake of cimetidine, ranitidine, and famotidine by vector- and rOat3-LLC. The uptake of cimetidine, ranitidine, and famotidine was significantly greater in rOat3-LLC than in vector-LLC, although the absolute value of famotidine uptake was quite small compared with that of cimetidine and ranitidine (Fig. 2). For further analyses, the uptake of cimetidine and ranitidine was determined at the earliest time, both technically and practically (3 min for cimetidine and 5 min for ranitidine). The uptake of cimetidine and ranitidine was saturable (Fig. 3), and kinetic analyses revealed that the K_m and V_{max} values of cimetidine and ranitidine by rOat3-LLC were 79.2 $\pm$ 17.8 and 121 $\pm$ 36 μM and 150 $\pm$ 29 and 367 $\pm$ 95 pmol/min/mg protein, respectively. The uptake clearance corresponding to the saturable component (V_{max}/K_m) for cimetidine and ranitidine was 1.89 $\pm$ 0.30 and 3.03 $\pm$ 0.39 μl/min/mg protein, respectively, whereas that corre-

sponding to the nonsaturable component was 1.07 $\pm$ 0.03 and 0.381 $\pm$ 0.023 μl/min/mg protein, respectively.

Effect of Organic Anions and Cations on the Uptake of Cimetidine by rOat3. Benzylpenicillin and ranitidine inhibited the rOat3-mediated [^{3}H]cimetidine uptake in a concentration-dependent manner (Fig. 4, a and b), whereas TEA had no inhibitory effect at the concentrations examined (0.1 to 10 mM; data not shown). The K_i values of benzylpenicillin and ranitidine for cimetidine uptake by rOat3-LLC were determined to be 76.7 $\pm$ 13.2 and 119 $\pm$ 44 μM, respectively. Famotidine only weakly inhibited the uptake of cimetidine by rOat3-LLC (Fig. 4c).

Effect of Probenecid on the Plasma, Brain, and CSF Concentrations of H$_2$-Receptor Antagonists. Rats were given H$_2$-receptor antagonists by constant infusion with or without probenecid. The plasma concentrations of H$_2$-receptor antagonists reached steady-state within 3 h (data not shown), whereas the plasma concentration of probenecid showed a gradual increase during infusion (355 $\pm$ 107 μM at 1 h, 514 $\pm$ 143 μM at 2 h, and 651 $\pm$ 246 μM at 3h). The unbound plasma concentration and CSF concentration of probenecid at 3 h were 154 $\pm$ 26 and 57.3 $\pm$ 2.0 μM, respectively. Probenecid treatment did not affect the brain and plasma concentrations of the H$_2$-receptor antagonists (Table 1), whereas their CSF concentrations significantly increased (Table 1). The CSF-to-unbound plasma concentration ratio ($C_{CSF}/C_{p,fu}$) significantly increased by 3.7-fold for cimetidine, 4.3-fold for ranitidine, and 2.5-fold for famotidine (Table 1).

Uptake of H$_2$-Receptor Antagonists by the Isolated Rat CP. The time profiles of the uptake of [^{3}H]cimetidine and [^{14}C]TEA by isolated rat CP are shown in Fig. 5a (uptake units: μl/μl CP volume) and those of cimetidine, ranitidine, and famotidine are shown in Fig. 5b (uptake units: μl/mg protein). All the H$_2$-receptor antagonists showed time-dependent accumulation by the isolated rat CP, and their transport activities were in the following order: ranitidine $\approx$ cimetidine > famotidine (Fig. 5b). The uptake of TEA by the isolated rat CP was small compared with that of H$_2$-receptor antagonists (Fig. 5a). The uptake of [^{3}H]cimetidine and ranitidine was saturable, and kinetic analyses revealed that the uptake of [^{3}H]cimetidine and ranitidine consists of one saturable and one nonsaturable component (Fig. 6). The K_m and V_{max} values and uptake clearance corresponding to the nonsaturable component of [^{3}H]cimetidine were 92.7 $\pm$ 46.1 μM, 137 $\pm$ 71 pmol/min/μl tissue, and 0.581 $\pm$ 0.123 μl/min/μl tissue, respectively, whereas the corresponding values for ranitidine were 171 $\pm$ 57 μM, 1250 $\pm$ 360 pmol/min/mg protein, and 1.49 $\pm$ 0.26 μl/min/mg protein. The uptake of famotidine was saturated at high substrate concentrations (Table 2), but 50% of the total uptake remained at the highest concentration examined (1 mM, Table 2).

Effect of Organic Anions and TEA on the Uptake of Cimetidine by Isolated Rat CP. The effect of benzylpenicillin and TEA was examined with regard to the uptake of cimetidine, ranitidine, and famotidine by the isolated rat CP (Table 2). Benzylpenicillin inhibited the uptake of the H$_2$-receptor antagonists in a concentration-dependent manner. TEA did not affect the uptake of H$_2$-receptor antagonists. Furthermore, the effect of estradiol-17β-glucuronide, estrone sulfate, benzylpenicillin, PAH, ranitidine, and famotidine was also examined with regard to the uptake of [^{3}H]cimetidine by isolated rat CP (Fig. 7). All organic anions, ranitidine, and famotidine showed a concentration-dependent inhibition of the uptake of [^{3}H]cimetidine by the isolated rat CP (Fig. 7), and their inhibition constants are summarized in Table 3. The K_i and K_m values for the uptake of benzylpenicillin and cimetidine by the isolated rat CP were comparable (Table 3).
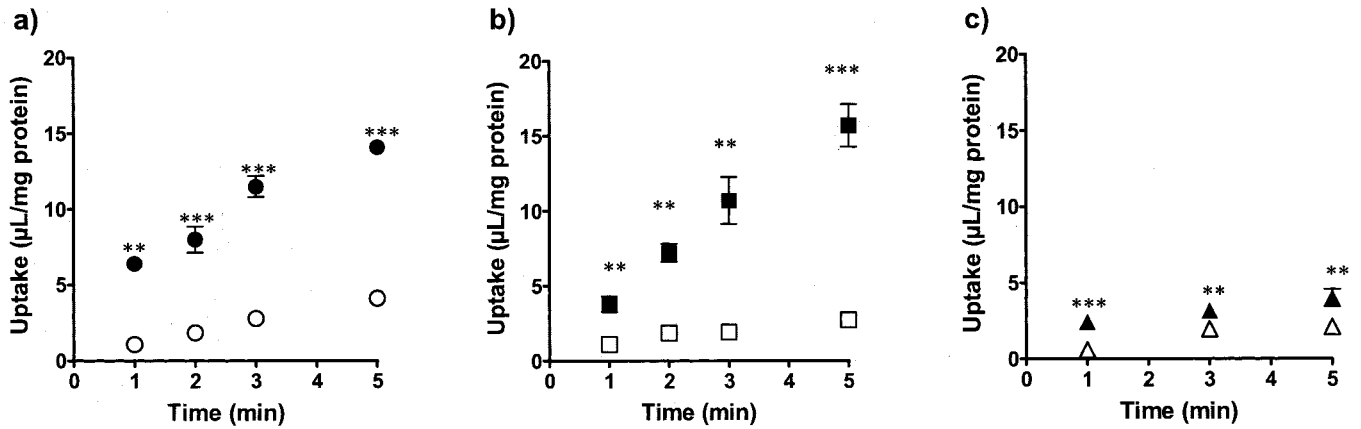
FIG. 2. Uptake of cimetidine, ranitidine, and famotidine by rOat3-LLC. The uptake of [^{3}H]cimetidine (a; circle), ranitidine (b; square) and famotidine (c; triangle) by rOat3-LLC (closed symbol) and vector-LLC (open symbol) was determined. The uptake was initiated by adding ligand ([^{3}H]cimetidine (1 μM), ranitidine (10 μM), and famotidine (10 μM) and terminated at designated times by adding ice-cold buffer. Each point represents the mean $\pm$ S.E. (n = 3–6). **, $p < 0.01$ and ***, $p < 0.001$, significantly different from vector-LLC (unpaired t test).
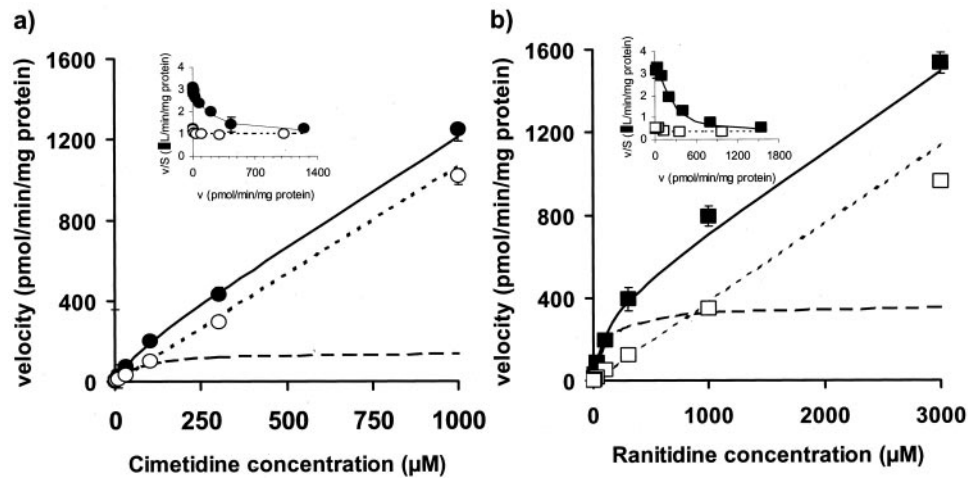

FIG. 3. Concentration dependence of the uptake of cimetidine and ranitidine by rOat3-LLC. The cellular accumulation of [^{3}H]cimetidine (a) and ranitidine (b) by rOat3-LLC for 3 and 5 min was determined at different substrate concentrations. The concentration dependence of the uptake of cimetidine and ranitidine by rOat3-LLC is shown as Eadie-Hofstee plots in the inset. Kinetic analyses revealed that the uptake of [^{3}H]cimetidine and ranitidine consists of one saturable and one nonsaturable component and follows the Michaelis-Menten equation. The solid, dotted, and broken lines represent the fitted line, the clearance of the nonsaturable component, and the clearance of the saturable component obtained by nonlinear regression analysis, respectively. Each point represents the mean $\pm$ S.E. (n = 3–6).
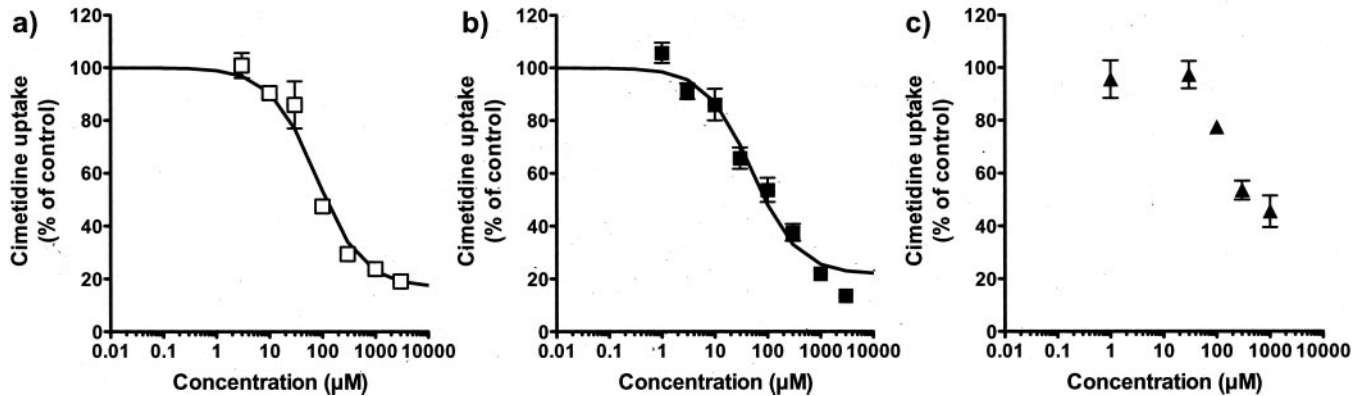

FIG. 4. Effect of organic anions and cations on the uptake of cimetidine by rOat3. The cellular accumulation of [^{3}H]cimetidine (1 μM) by rOat3 for 3 min was determined in the presence and absence of nonradiolabeled compounds at the concentrations indicated. Symbols represent benzylpenicillin (a; $\square$), ranitidine (b; $\blacksquare$), and famotidine (c; $\blacktriangle$). The inhibition constants (K_i) of these compounds were calculated by assuming competitive inhibition. The solid lines represent the fitted line obtained by nonlinear regression analysis. The details of the fitting are described under *Materials and Methods*. Each point represents the mean $\pm$ S.E. (n = 3).

Discussion

In the present study, we reported that rOat3 is involved in the uptake of H$_2$-receptor antagonists (cimetidine, ranitidine, and famo-

tidine) by the isolated rat CP, and that drug-drug interaction causes an increase in the CSF concentration of H$_2$-receptor antagonists without affecting their plasma concentration. rOat3 has been characterized by

TABLE 1

Total plasma, unbound plasma, CSF, and brain concentrations of H_2-receptor antagonists after intravenous infusion in rats treated with or without probenecid

H_2-receptor antagonists (2 mg/kg) were administered to rats by bolus intravenous administration followed by constant infusion (2 mg/h/kg) during 3 h. Probenecid (15 mg/kg) was administered to rats by bolus intravenous administration followed by constant infusion (30 mg/h/kg) during 3 h, and the unbound plasma concentrations and CSF concentrations of probenecid at 3 h were 154 ± 26 and 57.3 ± 2.0 μM, respectively. Each value represents the mean $\pm$ S.D. ($n = 3$).

	Cimetidine		Ranitidine		Famotidine	
	Control	+Probenecid	Control	+Probenecid	Control	+Probenecid
C_p (μM)	3.71 ± 1.20	2.97 ± 0.15	2.65 ± 1.25	2.11 ± 0.18	3.47 ± 1.22	2.79 ± 0.03
$C_{p,fu}$ (μM)	2.25 ± 0.60	1.89 ± 0.11	1.72 ± 0.49	1.52 ± 0.06	2.01 ± 0.72	1.54 ± 0.18
C_{CSF} (nM)	39.1 ± 19.8	$116 \pm 17**$	25.9 ± 12.0	$97.1 \pm 5.2***$	23.1 ± 9.2	$43.9 \pm 4.1*$
C_{brain} (nM)	96.5 ± 27.1	99.9 ± 12.5	85.7 ± 33.8	83.9 ± 10.9	106 ± 30	102 ± 18
$C_{CSF}/C_{p,fu}$ ratio	0.0166 ± 0.005	$0.0615 \pm 0.0121**$	0.0147 ± 0.0046	$0.0639 \pm 0.0046***$	0.0114 ± 0.0027	$0.0289 \pm 0.0057**$

C_p, total plasma concentration; $C_{p,fu}$, unbound plasma concentration; C_{CSF}, CSF concentration; C_{brain}, brain concentration.
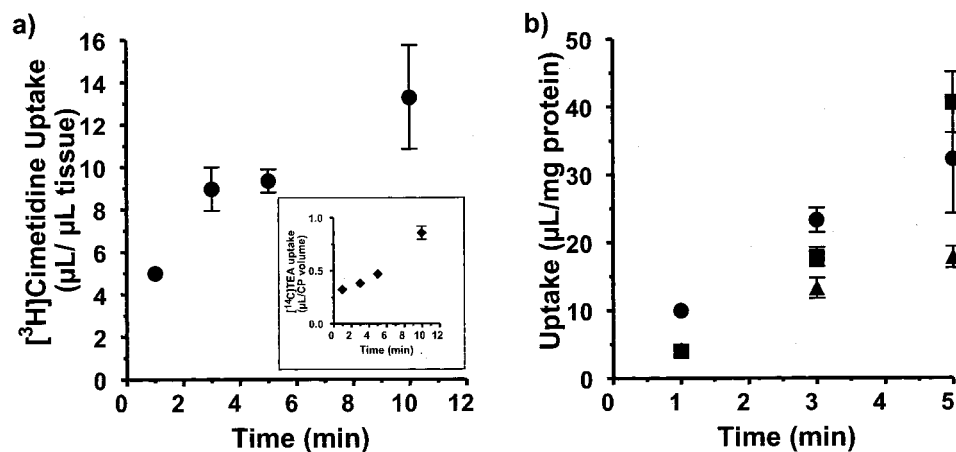$* p < 0.05$, $** p < 0.01$, and $*** p < 0.001$, significantly different from control rats (unpaired t test).

FIG. 5. Uptake of cimetidine ranitidine, famotidine, and TEA by the isolated rat CP. The rat CP was isolated from the lateral ventricles. a, uptake of [^{3}H]cimetidine (●) and [^{14}C]TEA (♦) by the isolated rat CP was determined by the centrifugal filtration method as described under *Materials and Methods*. The tissue-to-medium concentration ratio of [^{3}H]cimetidine and [^{14}C]TEA was calculated with [^{14}C]urea and [^{3}H]water as cell water space markers, respectively, and corrected for the adherent water space. b, uptake of cimetidine (10 μM; ●), ranitidine (10 μM; ■), and famotidine (10 μM; ▲) by the isolated CP was determined by the rapid filtration method followed by quantification using LC-MS. Each point represents the mean $\pm$ S.E. ($n = 3$).
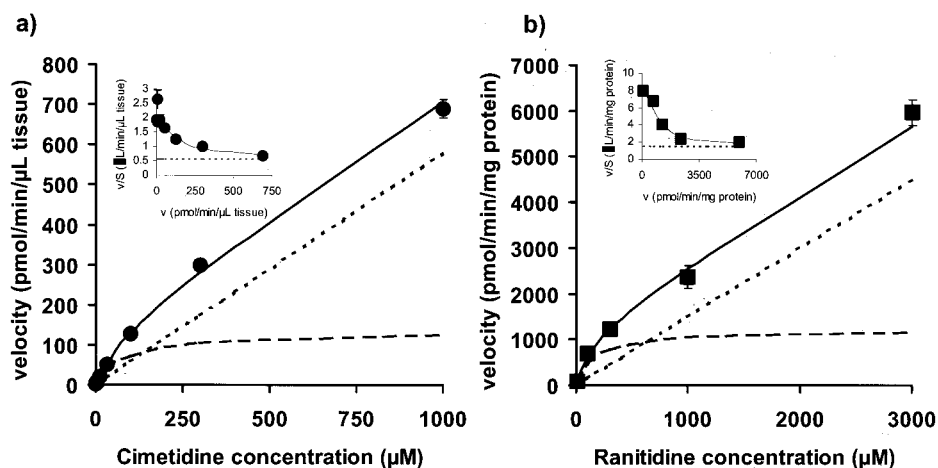

FIG. 6. Concentration dependence of cimetidine and ranitidine accumulation by the isolated rat CP. The accumulation of [^{3}H]cimetidine (a) and ranitidine (b) in the isolated rat CP for 3 and 5 min, respectively, was determined at various substrate concentrations. The concentration dependence for the uptake of [^{3}H]cimetidine and ranitidine is shown as Eadie-Hofstee plots in the inset. Kinetic analyses revealed that the uptake of [^{3}H]cimetidine and ranitidine consists of one saturable and one nonsaturable component and follows the Michaelis-Menten equation. The solid, dotted, and broken lines represent the fitted line, the clearance of the nonsaturable component, and the clearance of the saturable component obtained by nonlinear regression analysis, respectively. Each point represents the mean $\pm$ S.E. ($n = 3$).

its broad substrate specificity for organic anions, from amphipathic to hydrophilic organic anions, and also a weakly basic compound, cimetidine (Kusuhara et al., 1999). In addition to cimetidine, ranitidine was found to be a good substrate of rOat3, whereas famotidine seems

to be a poor substrate of rOat3 (Fig. 2). Because it is one of the clues to understanding the interaction of such weak base or cationic compounds with rOat3, the MEP of the H_2-receptor antagonists was calculated (Fig. 1). The H_2-receptor antagonists contain a region of

Effect of benzylpenicillin and TEA on the uptake of H$_2$-receptor antagonists by the isolated rat CP

The uptake of cimetidine, ranitidine, and famotidine by isolated rat CP was determined in the presence and absence of benzylpenicillin and TEA at the concentrations indicated. The substrate concentration of cimetidine, ranitidine, and famotidine was 10 μM, and the excess amount of unlabeled substrates represents concentrations of 3, 3, and 1 mM for cimetidine, ranitidine, and famotidine, respectively. Each value represents the mean $\pm$ S.E. ($n = 3$).

	Concentration	Uptake (Percentage of Control)		
		Cimetidine	Ranitidine	Famotidine
	mM			
Control		100 $\pm$ 7	100 $\pm$ 12	100 $\pm$ 22
Excess cold		30.4 $\pm$ 1.1	24.8 $\pm$ 1.1	50.5 $\pm$ 3.6
Benzylpenicillin	0.1	72.5 $\pm$ 5.1	76.7 $\pm$ 19	83.8 $\pm$ 8.3
	1	46.3 $\pm$ 1.1	34.9 $\pm$ 3.9	78.6 $\pm$ 6.6
	3	30.4 $\pm$ 1.1	31.8 $\pm$ 3.5	51.6 $\pm$ 1.9
TEA	0.1	99.5 $\pm$ 8.3	94.8 $\pm$ 14	120 $\pm$ 3
	1	87.5 $\pm$ 6.1	97.3 $\pm$ 8.6	110 $\pm$ 14
	3	93.7 $\pm$ 10.5	88.8 $\pm$ 13.6	93.4 $\pm$ 3.6

negative MEP in their chemical structures (Fig. 1). As Ullrich et al. (1993) suggested, this site might play a key role for the substrate recognition of H$_2$-receptor antagonists by rOat3. Suzuki et al. (1987) clearly demonstrated a linear correlation between the lipophilicity and reciprocal number of K_i values of β-lactam antibiotics for the uptake of benzylpenicillin by the isolated rat CP. Lipophilicity is likely to be an important factor for recognition by rOat3 in the case of β-lactam antibiotics. In contrast, the kinetic parameters of cimetidine and ranitidine for their uptake by rOat3 were comparable, although they had different cLogD values. Lipophilicity may not be a determinant factor in the case of the H$_2$-receptor antagonists.

Probenecid has previously been reported to inhibit the apical-to-basal side of azidodeoxythymidine (corresponding to the efflux transport from the CSF side to the blood side under physiological conditions), resulting in an increase in the basal-to-apical transport (Strazielle et al., 2003). Simultaneous administration of probenecid caused a significant increase in the CSF concentration of all the H$_2$-receptor antagonists examined (Table 1). Since it did not affect the brain and unbound plasma concentrations, it is likely that the effect of probenecid is due to the inhibition of the efflux transport of H$_2$-receptor antagonists across the CP (Table 1). Strazielle et al. (2003) also found that benzbromarone had an inhibitory effect for the apical-to-basal transport of azidodeoxythymidine. It is possible that benzbromarone treatment also causes an increase in the CSF concentration of H$_2$-receptor antagonists. Since the brain-to-unbound plasma concentration ratio of the H$_2$-receptor antagonists was well below unity (Table 1), it is possible that the efflux transport across the brain capillaries limits their brain distribution; however, probenecid had no effect on the brain concentration. Further studies are necessary to investigate whether the H$_2$-receptor antagonists undergo the efflux across the brain capillaries and whether organic anion transporters, including rOat3, contribute to this efflux.

The uptake, an initial process for elimination from the CSF, was investigated using the isolated rat CP. Time-dependent uptake of the H$_2$-receptor antagonists was detected in the isolated rat CP (Fig. 5). The saturable component accounts for a large part of the total uptake of cimetidine and ranitidine (Fig. 6). The uptake of cimetidine by the isolated rat CP was markedly inhibited by benzylpenicillin with a K_i value similar to its own K_m value for uptake by the isolated rat CP (Nagata et al., 2002). Conversely, the K_m value of cimetidine for the uptake by the isolated rat CP was similar to its K_i value for the uptake
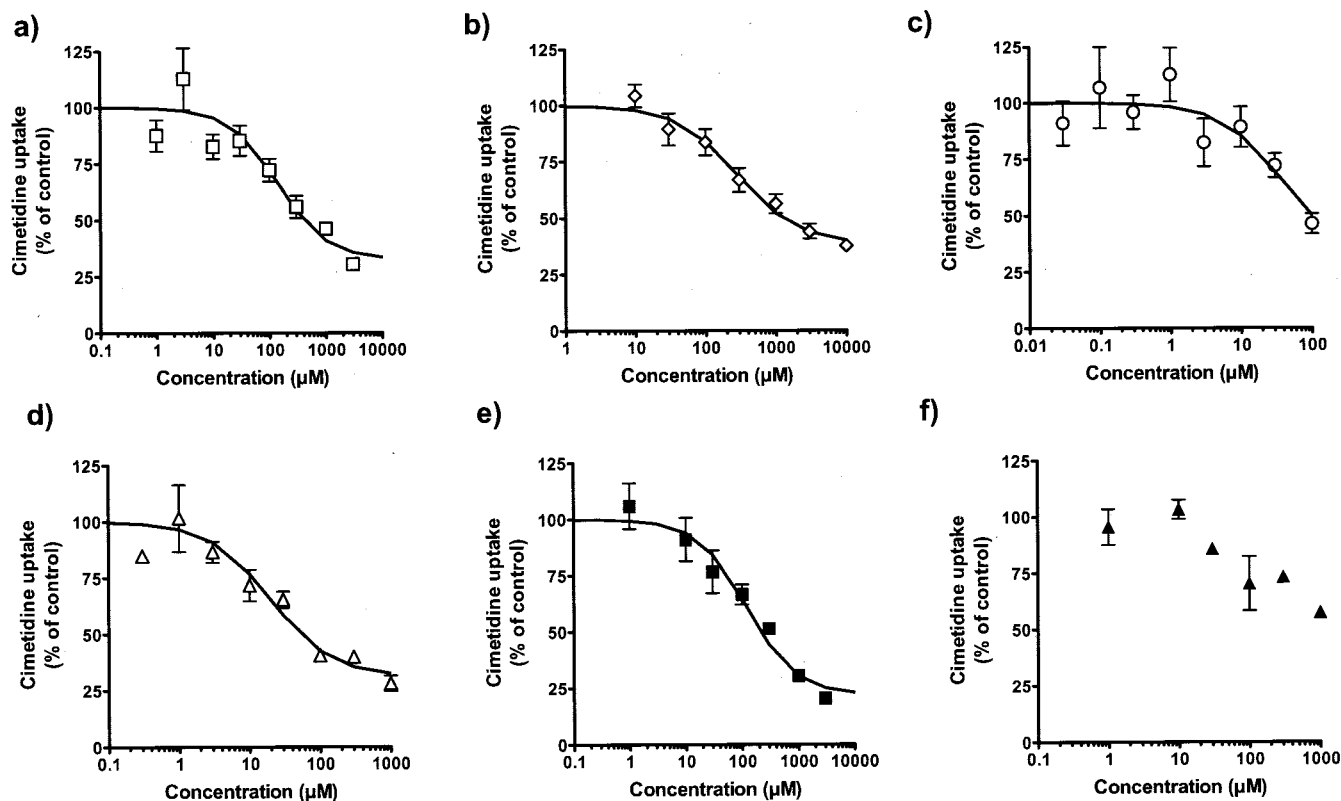

FIG. 7. Inhibitory effect of organic anions and cations on the uptake of cimetidine by the isolated rat CP. The accumulation of [^{3}H]cimetidine (1 μM) by the isolated rat CP for 3 min was determined in the presence and absence of nonradiolabeled compounds at the concentrations indicated. Symbols represent benzylpenicillin (a; $\square$), PAH (b; $\diamond$), estradiol-17β-glucuronide (c; $\bigcirc$), estrone sulfate (d; $\triangle$), ranitidine (e; $\blacksquare$), and famotidine (f; $\blacktriangle$). The solid lines represent the fitted line obtained by nonlinear regression analysis. The details of the fitting are described under *Materials and Methods*. Each point represents the mean $\pm$ S.E. ($n = 3$).

TABLE 3

K_i and K_m values for the uptake of cimetidine, ranitidine, and benzylpenicillin by the isolated rat CP

The effect of estrone sulfate, cimetidine, estradiol-17β-glucuronide (E$_2$17βG), PAH, benzylpenicillin, and ranitidine was examined with regard to uptake by the rat isolated CP. The K_i and K_m values were determined by nonlinear regression analysis as described under *Materials and Methods*. Data are taken from Fig. 7. Each value represents the mean ± S.E. ($n = 3$–6).

	K_i and K_m values (μM)		
	Benzylpenicillin[a]	Cimetidine	Ranitidine
Estrone sulfate	22.3	19.5 ± 8.8	
Cimetidine	44.4	92.7 ± 46.1[b]	
E$_2$17βG	33.0	36.3 ± 15.7	
PAH	406	281 ± 74	
Benzylpenicillin	111[b]	140 ± 97	
Ranitidine		49.6 ± 15.1	171 ± 57[b]

[a] Parameters cited from Nagata et al. (2002).
[b] K_m value.

of benzylpenicillin by the isolated CP (Table 3; Nagata et al., 2002). Furthermore, the inhibition constants of the compounds listed in Table 3, which are substrates of rOat3, are similar for the uptake of cimetidine and benzylpenicillin by the isolated rat CP (Table 3). These results suggest that the same organic anion transporter, namely, rOat3, is responsible for the uptake of cimetidine by the isolated rat CP. Since the K_i value of ranitidine for the uptake of cimetidine was close to its K_m value (Table 3) and benzylpenicillin showed similar inhibition potency (Table 2), rOat3 is presumably also involved in the uptake of ranitidine. Since the uptake of famotidine was saturable and inhibited by benzylpenicillin (Table 2), the involvement of a transporter was suggested, although the fraction of the saturable component could not be precisely estimated due to its limited solubility. This is consistent with the in vivo result. Since famotidine is a poor substrate of rOat3, the benzylpenicillin-sensitive fraction of famotidine uptake may at least partly be accounted for by rOat3; however, it is possible that other transporters distinct from rOat3 and organic cation transporter may play a major role in the uptake of famotidine by the isolated rat CP. Villalobos et al. (1997) demonstrated the presence of a membrane potential-sensitive uptake mechanism for hydrophilic organic cations at the primary cultured CP epithelial cells. Reverse transcription-polymerase chain reaction analyses detected mRNA expression of Oct2 and Oct3 in the CP, and this may account for the uptake of hydrophilic organic cations in the CP (Sweet et al., 2001). The expression level of rOct2 in the CP was considerably lower than that in the kidney, whereas that of rOct3 was the same for all the tissues examined, although the absolute value was low (Choudhuri et al., 2003). The low expression of Oct mRNA may account for the lower uptake of TEA by the isolated rat CP compared with that of the H$_2$-receptor antagonists (Fig. 5). Although cimetidine is a substrate of rOct2 (Grundemann et al., 1999), the effect of TEA on the uptake of the H$_2$-receptor antagonists by the isolated rat CP was minimal, even at a concentration sufficient to saturate TEA uptake by primary cultured choroid epithelial cells (Table 2) (Villalobos et al., 1997). Therefore, the contribution of rOcts to the total uptake of H$_2$-receptor antagonists by the isolated rat CP is minimal, although they are involved.

rOat3 has been shown to be expressed on the basolateral membrane of the proximal tubules and involved in the uptake of organic anions (Hasegawa et al., 2002, 2003). However, probenecid treatment did not affect the steady-state plasma concentration of H$_2$-receptor antagonists, although the unbound plasma concentration was sufficient to inhibit rOat3-mediated uptake (Table 1). This result is in a good agreement with the previous report by Boom and Russel (1993). They examined the uptake of cimetidine by freshly isolated rat proximal

tubular cells and demonstrated that the major fraction of cimetidine uptake (approximately 50%) was inhibited by TEA (Boom and Russel, 1993). Probenecid was only a weak inhibitor, with an IC$_{50}$ value (700 μM) greater than the unbound plasma concentration employed in this study. Organic cation transporter(s) will play a major role in the renal uptake of cimetidine, whereas the uptake by the isolated rat CP is totally accounted for by the organic anion transporter. This unique phenomenon is entirely due to the unique nature of the bisubstrate, which is a substrate of both organic anion and cation transporters.

The results of the present study suggest the possibility of drug-drug interactions between the H$_2$-receptor antagonists and organic anions that cause an increase in the CSF concentration of H$_2$-receptor antagonists without affecting their plasma concentration profiles, as in the case of probenecid. The risk of mental confusion with cimetidine in patients with renal or hepatic dysfunction is higher than that in normal patients (Schentag et al., 1981). Furthermore, Schentag et al. (1979) reported that the concentration of cimetidine in the CSF is related to the mental status. The $C_{CSF}/C_{p,fu}$ ratio in patients with hepatic dysfunction was 2-fold greater than normal patients, whereas the plasma clearance by both types of patients was not significantly different (Schentag et al., 1979, 1981). These phenomena might be attributed to the inhibition of the efflux transport across the CP by endogenous compounds accumulated in the body due to hepatic dysfunction. Indeed, some organic anions (e.g., quinolinic acid) are known to be elevated in the CSF of patients with hepatic dysfunction (Moroni et al., 1986).

In conclusion, cimetidine and ranitidine are good substrates for rOat3, whereas famotidine is a poor substrate for this transporter. The efflux transport across the CP plays an important role in regulating the CSF concentration of H$_2$-receptor antagonists. rOat3 is the most likely candidate transporter for the uptake of H$_2$-receptor antagonists by the isolated rat CP.

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