Hepatobiliary Transport of a Nonpeptidic Endothelin Antagonist, (+)-(5*S*,6*R*,7*R*)-2-Butyl-7-[2((2*S*)-2-Carboxypropyl)-4-Methoxyphenyl]-5-(3,4-Methylenedioxyphenyl) Cyclopentenol[1,2-b]Pyridine-6-Carboxylic Acid: Uptake by Isolated Rat Hepatocytes and Canalicular Membrane Vesicles

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Purpose. Hepatobiliary excretions of drugs from the blood to the bile include two essential transmembrane processes: uptake into hepatocytes and secretion from hepatocytes. The purpose of this study was to clarify the transport mechanisms underlying these processes for a new non-peptide endothelin antagonist, (+)-(5*S*,6*R*,7*R*)-2-butyl-7-[2((2*S*)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopentenol[1,2-b]pyridine-6-carboxylic acid (J-104132).

Methods. Biliary excretion of J-104132 was assessed in rats after intravenous injection. To evaluate the hepatic uptake process, J-104132 was incubated with freshly isolated rat hepatocytes and the uptake of J-104132 was calculated. To evaluate the biliary secretion process, the uptake of J-104132 into rat canalicular membrane vesicles that were isolated from normal Sprague–Dawley rats or Eisai hyperbilirubinemic rats was measured.

Results. After intravenous injection, J-104132 was recovered from the bile quantitatively (99.7 ± 1.3%) as its intact form. J-104132 was taken up by isolated rat hepatocytes in a time- and temperature-dependent manner. The uptake was saturable with K_m and V_{max} of 5.7 μM and 564 pmol/min/10^6 cells, respectively. The uptake was Na^+ independent and was reduced in the presence of ATP depleters (rotenone and carbonyl cyanide-p-(trifluoromethoxy)-phenylhydrazone), organic anions (dibromosulfophthalein, indocyanine green, BQ-123, and pravastatin), and bile acids (taurecholate and cholate). In Sprague–Dawley rats, J-104132 was taken up by canalicular membrane vesicle ATP-dependently with K_m and V_{max} values of 6.1 μM and 552 pmol/min/mg protein, respectively. However, ATP-dependent uptake disappeared in Eisai hyperbilirubinemic rats.

Conclusions. These data suggest that energy-dependent and carrier-mediated transport systems play important roles in hepatobiliary excretion of J-104132 (both uptake and secretion processes), which is the main excretion route in rats. As for the secretion process of J-104132, an involvement of mrp2 was demonstrated.

KEY WORDS: hepatobiliary excretion; hepatocytes; CMV; cMOAT.

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INTRODUCTION

Many endogenous and exogenous compounds are excreted from the body via the hepatobiliary route. The hepatobiliary transport system includes two essential transmembrane processes, i.e., uptake into hepatocytes via the sinusoidal (basolateral) membrane and secretion from hepatocytes via the bile canalicular (apical) membrane. Recently, it has been reported that many classes of carrier-mediated transporters operate in these transmembrane processes (1). In the hepatic uptake process, the organic anion-transporting polypeptide (OATP) family transports organic anions and a variety of organic amphiphilic compounds including organic cations (1,2). The involvement of Na^+-taurocholate cotransporting polypeptide (NTCP) (1) and organic cation transporter (1) is also well known. In the biliary-secretion process, multidrug resistance transporters (1,3) and multidrug resistance-associated protein (MRP) (1,3,4) are representative transporter families that have been investigated extensively. Above all, the role of MRP2 (canalicular multispecific organic anion transporter, cMOAT) (4,5) in pharmacokinetics has recently been elucidated because hereditarily defective mutant strains, such as EHBR (6) and TR^- rat (7) have become available. It should be noted that these uptake and secretion transport activities determine the systemic clearance of drugs that are mainly excreted into the bile without having been metabolized. For example, *in vivo* hepatic clearance of a peptidic endothelin antagonist, BQ-123, was in accordance with the transport activity of hepatic uptake that was determined *in vitro* (8–10).

Endothelin is a family of endogenous peptides that are involved in the pathophysiology of various diseases, such as hypertension, congestive heart failure, septic shock, and asthma (11). There are at least three endothelins, i.e., ET-1, ET-2, and ET-3 (12), that act on two distinct receptor subtypes, ET_A and ET_B (13,14). (+)-(5*S*,6*R*,7*R*)-2-butyl-7-[2((2*S*)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopentenol[1,2-b]pyridine-6-carboxylic acid (J-104132, Fig. 1) is a potent, nonpeptidic, orally active ET_A/ET_B dual-receptor antagonist that is presently under development (15). The oral bioavailability of J-104132 is approximately 40%, and the elimination of J-104132 from the systemic circulation after intravenous or oral administration is moderate in rats (15). In the preliminary experiments, we noticed that J-104132 was excreted into the bile extensively without oxidative metabolism. J-104132 was chosen as a model compound for this study because it was hypothesized that hepatic carrier-mediated transporter(s) determine its pharmacokinetics. In this study, we examined biliary-excretion profile of J-104132 and then elucidated the mechanism involved in its hepatic transporting process using isolated hepatocytes and canalicular membrane vesicles (CMVs) prepared from Sprague–Dawley rats (SDRs) or Eisai hyperbilirubinemic rats (EHBRs).

MATERIALS AND METHODS

Animals

Male SDRs (250–470 g) and male EHBRs (270–300 g) were purchased from Charles River Japan Inc. (Kanagawa,

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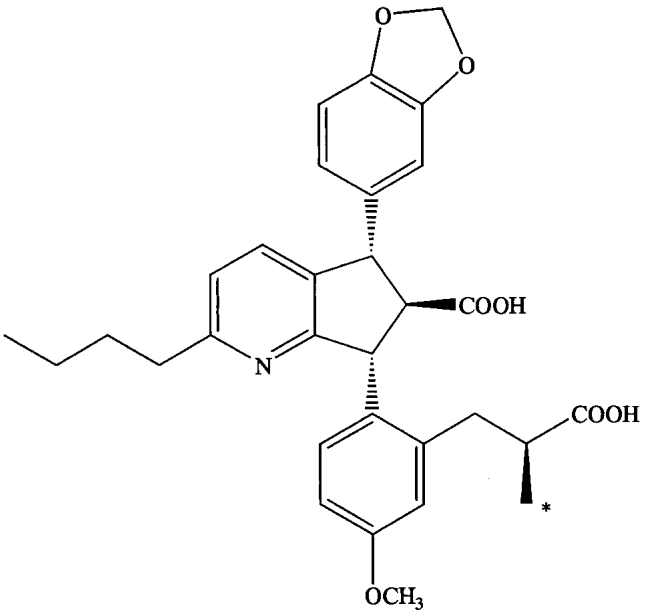

Fig. 1. Chemical structure of J-104132. The position of the [^{14}C]-label is indicated with an asterisk (*).

Japan) and S. L. C Japan Inc. (Shizuoka, Japan), respectively. Rats were housed in a temperature-controlled room (22 ± 2°C) with a 12-h light /dark cycle (lit from 7:00 a.m. to 19:00 p.m.) and had *ad libitum* access to food and water. Animal experiments were performed according to the 'Principles of Laboratory Animal Care' (NIH publication #85-23, revised 1985).

Chemicals and Reagents

[^{14}C]-J-104132 was synthesized by Daiichi Pure Chemical Company, Ltd. (Ibaraki, Japan). The labeled compound had the specific activity of 3.70 MBq/mg (100 μCi/mg). The radiochemical purities determined by thin-layer chromatography and high-performance liquid chromatography (HPLC) were more than 97%. Nonlabeled J-104132 (disodium salt) was synthesized in Banyu Pharmaceutical Co. Ltd.. *S*-(2,4-dinitrophenyl)-glutathione (DNPSG) and [^{3}H]-DNPSG were synthesized according to the method described by Kobayashi *et al.* (16). [^{3}H]-taurocholate (TCA) was purchased from Daiichi Pure Chemical Company (Ibaraki, Japan). Carbonyl cyanide-*p*-(trifluoromethoxy)-phenylhydrazone (FCCP), cholate, *p*-aminohippuric acid (PAH), indocyanine green (ICG), ATP, creatine phosphate, and creatine phosphokinase were purchased from Sigma Chemical (St. Louis, MO, USA). Tris (hydroxymethyl) aminomethane (Tris), rotenone, cimetidine, ouabain, and TCA were purchased from Wako Pure Chemical Industries (Osaka, Japan). *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) was purchased from Dojindo Laboratories (Kumamoto, Japan). Dibromosulfophthalein (DBSP) was purchased from Societé d' Etudes et de Recherches Biologiques (Paris, France). All other chemicals and reagents were commercial products of analytical grade.

In Vivo Biliary Excretion

SDRs (n = 4, body weight 250–350 g) with bile duct cannulation were administered [^{14}C]-J-104132 intravenously (1 mg/kg). After administration, bile was collected at 0–1, 1–2, 2–4, 4–6, 6–8, 8–24, and 24–48 h and stored on ice. Urine was collected at 0–4, 4–8, 8–24, and 24–48 h. Bile and urine were diluted with distilled water and were directly assayed with scintillation cocktail (Hionicfluor, Packard Instrument Company, Inc., Tokyo, Japan). The total radioactivity in each sample was analyzed by a liquid scintillation spectrometer (LSC-903, Aloka Co., Ltd., Tokyo, Japan).

HPLC Analysis of Bile

The bile sample was analyzed for J-104132 and possible metabolites with an HPLC system (Gold series, Beckman Instruments, Inc., Tokyo, Japan) consisting of a pump, interface, autosampler, ultraviolet detector, and radio detector fitted with a solid scintillator flow cell. The analyte was eluted from the column (CAPCELL PAK C18, particle size 5 μm, 250 × 4.6 mm id; Shiseido Inc., Tokyo, Japan) by a gradient method (A: 0.1% trifluoroacetic acid in acetonitrile, B: 0.1% trifluoroacetic acid in water; A 10% for 0–10 min, 25% for 10–15 min, and 25–42.5% for 15–50 min at a flow rate of 1 mL/min).

Uptake by Freshly Isolated Rat Hepatocytes

Hepatocytes were isolated from SDR by a collagenase perfusion method (17). After isolation, the hepatocytes were suspended at 0°C in an albumin-free Krebs-Henseleit buffer with 12.5 mM HEPES (pH 7.4). Cell viability was routinely checked by the trypan blue [0.4% (w/v)] exclusion test; the viability was greater than 85%. To check reproducibility, the uptake experiment was repeated more than three times using different hepatocyte preparations. Uptake was initiated by adding labeled J-104132 (0.4 μM) to the preincubated cell suspension (1×10^6 cells/mL). Na^+ ion in the Krebs-Henseleit buffer was replaced with isotonic choline when necessary. At designated time points, the reaction was terminated by separating the cells from the medium according to the centrifugal filtration method (18), and the radioactivity in the cell and in the medium was determined with a liquid scintillation spectrophotometer. Radioactivity in the cell was converted to intracellular concentration when necessary. The values of 4.3 μL/10^6 cell and 2.0 μL/10^6 cell were used for intracellular space and adherent fluid, respectively (18). Initial uptake velocity (V_0) was calculated from uptakes at 15 and 40 s.

Uptake by Rat CMVs

CMVs were prepared from male SDRs and EHBRs according to Kobayashi *et al.* (16). The prepared CMVs were suspended in 10 mM Tris·HCl buffer (pH 7.4) containing 250 mM sucrose, frozen in liquid nitrogen, and stored at –80°C until use. The uptake by CMVs was measured according to the rapid filtration method (19). The transport medium was composed of 2.5 μM [^{14}C]-J-104132, 250 mM sucrose, 10 mM $MgCl_2$, 5 mM ATP, 10 mM creatine phosphate, and 2 μg creatine phosphokinase in 10 mM Tris·HCl buffer (pH 7.4). A 16-μL aliquot of the transport medium was mixed with 4 μL of vesicle suspension (5 μg of protein) and incubated at 37°C. The uptake reaction was stopped at a designated time (2 min for the kinetic study) by adding 1 mL of ice-cold buffer (referred to as stop buffer) containing 100 mM NaCl, 250 mM sucrose, and 10 mM Tris·HCl (pH 7.4). The reaction mixture

was filtered through a 0.45-μm HAWP filter (Millipore, Bedford, MA, USA) and the filter was washed twice with 5 mL of the stop buffer. Radioactivity that was retained on the filter was measured by liquid scintillation counting. The uptake of J-104132 was normalized with respect to the quality of membrane vesicles in the transport medium. Protein concentration was determined using the Bio-Rad protein assay kit with bovine serum albumin as the standard (20). Reproducibility of the uptake was checked for three experiments. In a control experiment, ATP-dependent uptakes of [^{3}H]-TCA were 298 ± 59 and 299 ± 108 μL/min/mg protein for SDRs and EHBRs, respectively, whereas the uptake of [^{3}H]-DNPSG was markedly low for EHBRs (23 ± 3 μL/min/mg protein) compared with that for SDRs (115 ± 17 μL/min/mg protein).

Calculation of Kinetic Parameters and Statistics

Kinetic parameters for the uptakes by rat hepatocytes and CMVs were estimated according to Eqs. (1) and (2), respectively.

$$V_0 = V_{max} \cdot S / (K_m + S) + P_{dif} \cdot S \quad (1)$$

$$V_0 = V_{max} \cdot S / (K_m + S) \quad (2)$$

where V_0 and S represent the initial uptake velocity and the concentration in the medium (μM), respectively. The K_m, V_{max}, and P_{dif} represent the Michaelis constant (μM) the maximum uptake velocity (pmol/min/10^6 cells or pmol/min/mg protein), and the nonspecific uptake clearance (μL/min/10^6 cells), respectively. The experimental data were fitted into the above equations by an iterative, nonlinear, least-squares method. Student's t test was used to assess the significance of any differences. Data are presented as the mean ± SD.

RESULTS

In Vivo Study

After intravenous administration of [^{14}C]-J-104132 at 1 mg/kg, the radioactivity was excreted into the bile very rapidly; 88.3 ± 1.7% (mean ± SD) of the dose was recovered in the bile within 1 h (Fig. 2). The biliary and urinary excretions up to 48 h were 99.7 ± 1.3% and 0.2 ± 0.1%, respectively. An HPLC analysis revealed that the radioactivity in the bile almost solely consisted of the parent drug, which accounted for more than 98% of dose.

Time Course and Concentration Dependency of Uptake by Rat Hepatocytes

[^{14}C]-J-104132 uptake into isolated rat hepatocytes occurred extensively in a time-dependent manner. At 37°C, the uptake was very rapid for the initial 40 s and thereafter gradually slowed down. The cell-to-medium concentration ratio reached 41 at 180 s (Fig. 3). At 0°C, concentrative uptake was minimal. The uptake was not influenced by replacement of Na^+ with choline. Therefore, J-104132 was taken up into isolated rat hepatocytes in a time- and temperature-dependent manner, but the uptake was Na^+-independent. The uptake process of J-104132 into rat hepatocytes had both saturable and non-saturable components. The substrate-velocity curve and Eadie-Hofstee plots of the uptake data are shown in Fig. 4. The kinetic parameters were as follows: K_m 5.7 μM; V_{max} 564 pmol/min/10^6 cells, and P_{dif} 12.1 μL/min/10^6 cells.

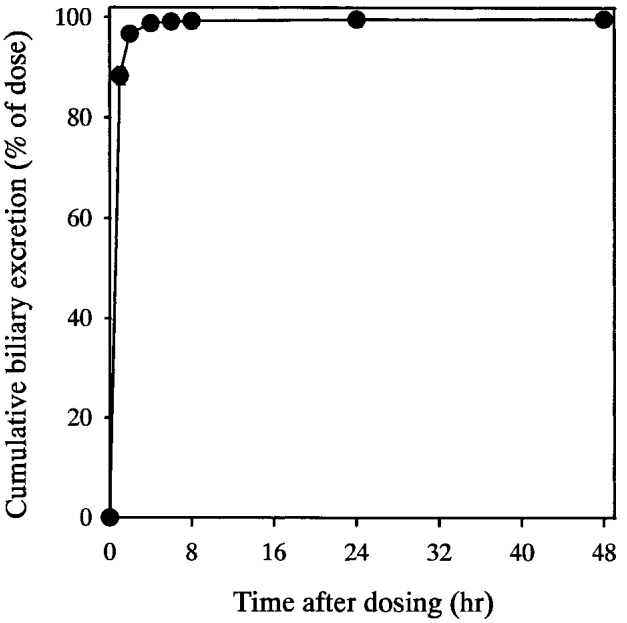

Fig. 2. Cumulative biliary excretion of total radioactivity in SD rats. The time profiles of cumulative biliary excretion of total radioactivity was determined after intravenous administration of [^{14}C]-J-104132 (1 mg/kg). Each point represents the mean ± SD of four animals but most of the error bars are within each circle. An HPLC analysis revealed that radioactivity in the bile almost solely consisted of the parent drug, which accounted for more than 98% of does.

The Effect of Metabolic Inhibitors and Various Compounds on Uptake by Rat Hepatocytes

In the presence of metabolic inhibitors such as rotenone (100 μM) and FCCP (100 μM), the uptake of J-104132 de-

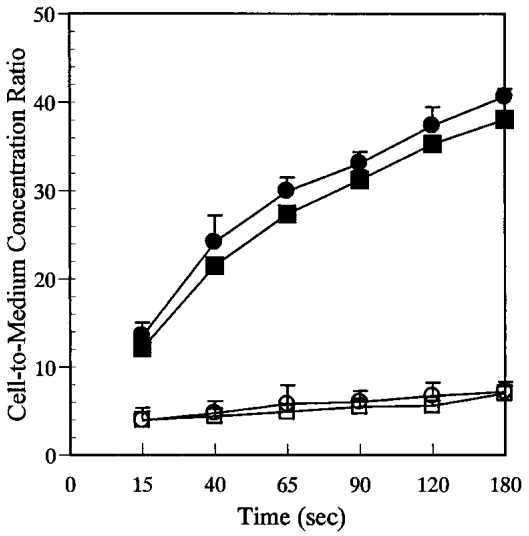

Fig. 3. Time course of uptake of J-104132 by rat hepatocytes. Initial concentrations of [^{14}C]-J-104132 and hepatocytes in the medium were 0.4 μM and 1 × 10^6 cells/mL, respectively. Each point represents the mean ± SD of three determinations of a cell-to-medium concentration ratio in the presence of 142 mM Na^+ (37°C, ●; 0°C, ○) or in the absence of Na^+ (37°C, ■; and 0°C, □).

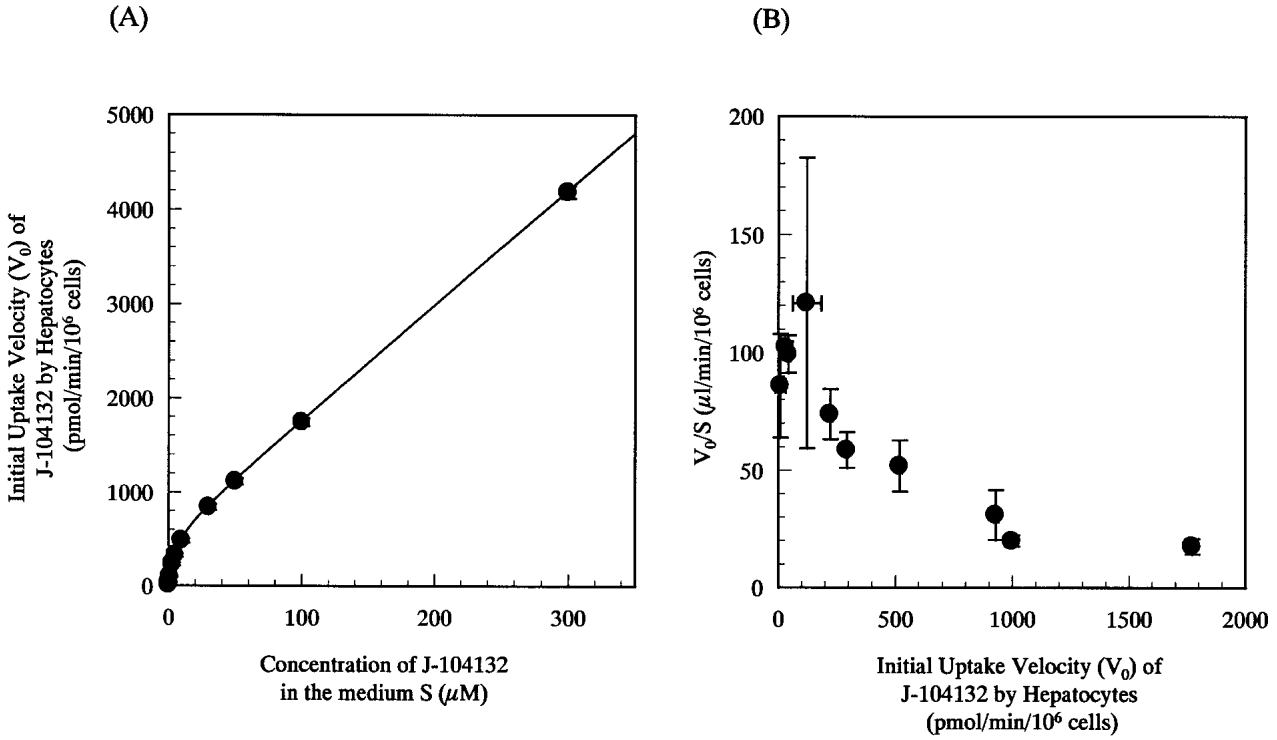

Fig. 4. Concentration dependency of initial uptake velocity (v_0) of J-104132 by rat hepatocytes. (A) Relationship between V_0 and the concentration of [^{14}C]-J-104132 (S) in the medium. (B) Eadie-Hofstee plots of J-104132. The concentration of hepatocytes in the medium was 1×10^6 cells/mL. Each point represents the mean ± SD of three to nine determinations. Data were fitted into Eq. (1). The kinetic parameters were as follows: K_m 5.7 μM; V_{max} 564 pmol/min/10^6 cells; P_{dif} 12.1 μL/min/10^6 cells.

creased to 42 and 62% of the control value, respectively (Fig. 5). To characterize the uptake, the effects of various compounds, which are known as typical substrates of hepatic transporter, were examined. Organic anions (DBSP, ICG, and BQ-123, 100 μM; pravastatin, 200 μM) and bile acids (cholate and TCA, 100 μM) inhibited uptake of J-104132 at the substrate concentration of 0.4 μM. Ouabain (500 μM) slightly reduced uptake, but the change was insignificant. Uptake of J-104132 was little influenced by cimetidine (300 μM) or PAH (300 μM; Fig. 5).

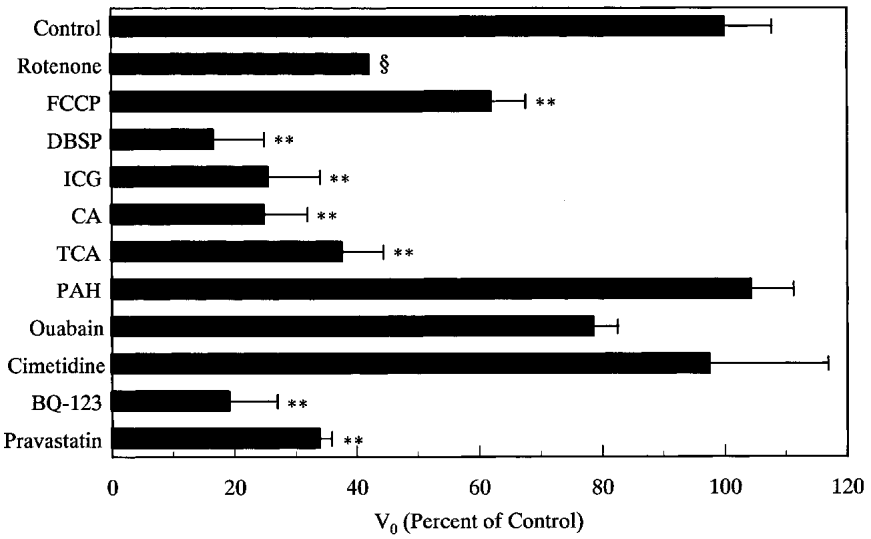

Fig. 5. Effect of various compounds on the uptake of J-104132 by rat hepatocytes. Each value represents the mean ± SD of 3 to 11 determinations except for the uptake in the presence of rotenone (§, mean of two determinations). Asterisks (**) indicate a significant difference from the control ($p < 0.05$). Initial concentrations of J-104132 and hepatocytes in the medium were 0.4 μM and 1×10^6 cells/mL, respectively. Concentration of inhibitors are as follows: rotenone, FCCP, DBSP, ICG, CA (Chorate) TCA, and BQ-123, 100 μM; pravastatin, 200 μM; PAH and cimetidine, 300 μM; ouabain, 500 μM.

Uptake into CMVs Prepared from SDRs and EHBRs

The time course of the uptake of [^{14}C]-J-104132 into CMV was examined in the presence and absence of ATP (Fig. 6). In the presence of ATP, these was an extensive uptake of J-104132 into CMVs prepared from SDRs. ATP-dependent uptake was calculated by subtracting uptake in the absence of ATP from that in the presence of ATP. Because uptake of J-104132 was very rapid, a linear phase of the initial uptake was not assessable. Although some underestimation would be unavoidable, the initial uptake rate was calculated from the data at 2 min after the start of reaction. The data for ATP-dependent uptake were fitted into Eq. (2) to obtain the kinetic parameters (Fig. 7). The values of K_m and V_{max} were estimated to be 6.1 μM and 552 pmol/min/mg protein, respectively, by a nonlinear fitting analysis. ATP-dependent uptake decreased when CMVs prepared from EHBRs were used (Fig. 6).

DISCUSSION

Hepatic sinusoidal membrane is embedded with transporters for a large variety of substrates (1). It has been reported that a family of organic anion-transporting polypeptides (OATP) mediates the uptake of organic amphiphilic compounds in a Na^+-independent manner (1). To our knowledge, four and five members of oatp have been identified in rats (oatp 1-4) (2,21,22) and in humans (OATPA-E) (23), respectively. In rats, oatp1 and oatp2 are abundantly expressed in both liver and kidney, whereas oatp3 and oatp4 are expressed preferentially in kidney and liver, respectively. In humans, OATP-C (OATP-2/LST-1) is abundant in the liver (21,23). OATP-A is expressed in a restricted range of tissues,

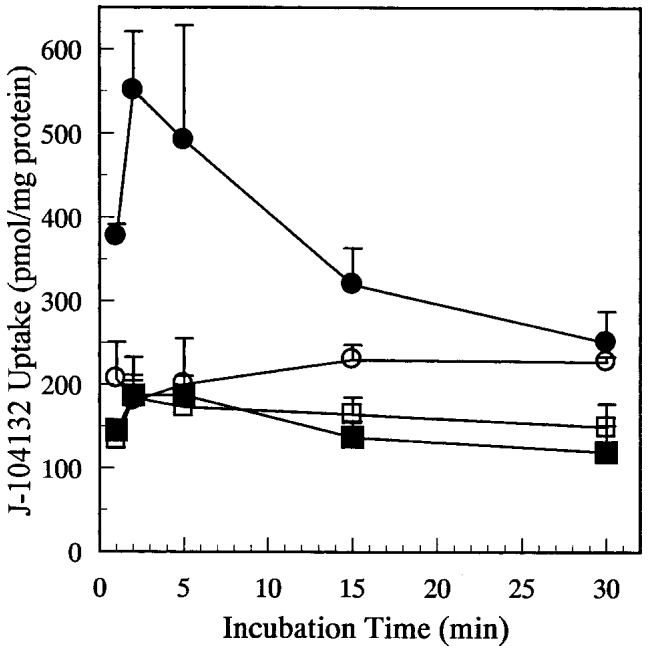

Fig. 6. Time course of uptake of J-104132 by rat CMVs. Initial concentrations of [^{14}C]-J-104132 in the medium was 2.5 μM. CMVs were incubated with (●, ■) or without (○, □) ATP. Circles and squares represent the uptake into CMVs prepared from SDRs and EHBRs, respectively. Each value represents the mean ± SD of three determinations.

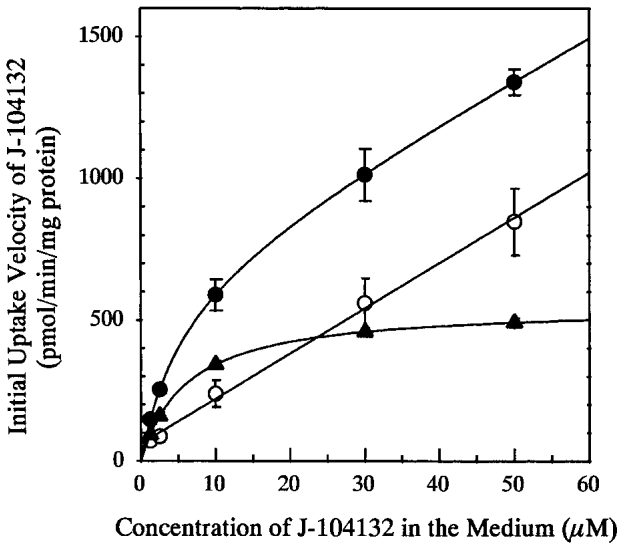

Fig. 7. Concentration dependency of uptake of J-104132 by rat CMVs. ATP-dependent uptake of [^{14}C]-J-104132 (▲) was calculated by subtracting the ATP-independent uptake (○) from the total uptake (●). Each value represents the mean ± SD of 4 to 11 determinations. Triangles were fitted to Eq. (2). The values of K_m and V_{max} were estimated to be 6.1 μM and 552 pmol/min/mg protein, respectively, by a nonlinear fitting analysis.

whereas OATP-B, -D, and -E show broad expression profiles (23). It has been demonstrated that DBSP, ICG, pravastatin, and BQ-123 are substrates of oatps (8,18). In contrast with the broad substrate specificity of OATPS, OCT transports small cations in a more selective manner (1). Cimetidine is a typical inhibitor of OCT (24). NTCP preferentially transport bile acids in a Na^+-dependent manner. It was known that the basolateral uptake of organic anion is mediated by organic anion transporter 1 (oat 1) in the kidney (25). Recently, it has been reported that oat 2 is abundantly expressed in the liver and responsible for the hepatic uptake of organic anions, such as PAH (26).

In this study, we confirmed that the hepatobiliary transport was the main route of excretion for J-104132. Furthermore, data indicated the extensive uptake of J-104132 into isolated rat hepatocytes. The uptake was temperature-dependent and sensitive to metabolic inhibitors (Figs. 3 and 5). These data suggested that energy-dependent transport system(s) were involved in the hepatobiliary transport of J-104132. The uptake of J-104132 was decreased but still detected in the presence of metabolic inhibitors (42% and 62% of the control for rotenone and FCCP, respectively). Because the cellular ATP content was not measured in the present study, it was unclear whether the uptake of J-104132 depends directly on the cellular ATP content. We performed inhibition studies to characterize the transporter responsible for the uptake of J-104132. The uptake of J-104132 was insensitive to the coexistence of Na^+ and PAH, suggesting that contribution of ntcp or oat was minimal. Furthermore, the observation that the uptake of J-104132 was little affected by cimetidine (Fig. 5) appeared to exclude the potential contribution of oct as a transporter for J-104132. This was also consistent with the anionic characteristics of J-104132, which contains two carboxylic acids (Fig. 1). In contrast, the uptake of J-104132 was inhibited by organic anions that are known as typical sub-

strates of oatps (DBSP, ICG, pravastatin, and BQ-123). From these data, it is concluded that member(s) of the oatp transporter family contribute to the hepatic uptake of J-104132 in rats whereas possible involvement of other transporters or opposite (efflux) transport would not be completely ruled out. The hepatic uptake studies using transporter-expressed systems would be helpful to further characterize transporters responsible for the hepatic uptake of J-104132.

The kinetic analysis revealed that hepatic uptake of J-104132 consisted of two components: unsaturable component with P_{dif} of 12.1 μL/min/10^6 cells and saturable component with K_m of 5.7 μM and V_{max} of 564 pmol/min/10^6 cells (Fig. 3). From these parameters, the clearance values for overall and saturable uptake were calculated to be 123 and 111 μL/min/10^6 cells by $V_{max}/K_m + P_{dif}$ and V_{max}/K_m, respectively. The contribution of the saturable (carrier-mediated) uptake to the overall uptake was approximately 90%. Thus, J-104132 would be predominantly taken up into rat liver by a carrier-mediated mechanism at *in vivo* relevant plasma concentrations.

To investigate the mechanism underlying biliary-secretion of J-104132, we used *in vitro* experiments using CMV. Primary active transporters on CMV have been known to be export pumps for their ligands into bile. These ligands undergo the transport mediated by ATP-binding cassette transmembrane transporters (ABC transporters) such as multidrug resistance transporters (1,3) and MRP (1,3,4). The MRP family contains at least nine members (MRP1–9) in rodents and humans (4,27,28). Although the expression of MRP1 in the liver is minimal, MRP2/cMOAT, a homolog of MRP1, is predominantly expressed on bile canalicular membrane (3). Studies in mutant rats (TR^- or EHBRs) having hereditarily-defective MRP2/cMOAT activity (6,7) have shown that the substrate specificity of MRP2 is very similar to that of MRP1 (29,30), which include organic anions such as pravastatin and BQ-123, and glutathione conjugates such as *S*-(2,4-dinitrophenyl) glutathione and leukotriene C_4, and glucuronic acid conjugates (4,9).

In the presence of ATP, J-104132 was taken up extensively into the CMVs of SDRs. The initial and rapid uptake was followed by a gradual decrease (so-called an overshoot phenomenon). The reason why the uptake decreased along the incubation time is not clear because an ATP-generating system was included in the incubation medium. Insufficient supply of ATP and/or secondary inhibitory mechanism(s) during incubation may be involved in this phenomenon. Nevertheless, the transport of J-104132 was driven by the ATP hydrolysis because the uptake was completely lost in the absence of ATP. Although the CMVs prepared in this experiment were a mixture of inside- and outside-out vesicles, we hypothesized that the ATP-dependent uptake into the CMV would occur only in the inside-out vesicles because the ATP-binding sites of efflux transporters are accessible only on inside-out vesicles. Therefore, ATP-dependent uptake into CMV would be regarded as an indication of active biliary secretion from hepatocytes. On the other hand, nonspecific uptake into CMV is difficult to interpret because it is a combination of influx, efflux, and adhesion. ATP-dependent uptake of J-104132 disappeared in the case of CMV prepared from EHBR (Fig. 6) that had genetically defective mrp2 (cMOAT; Ref. 6). These data strongly suggest that mrp2 is the major transporter responsible for the biliary secretion of J-104132 in rats.

In conclusion, this study suggests that hepatobiliary excretion, a key issue in the pharmacokinetics of J-104132 which is excreted into the bile as its intact form, is explained mainly by two distinct, carrier-mediated transport systems present at the entrance and exit of hepatocytes in rats. This information will be helpful to understand pharmacokinetics of this drug in clinical studies. In addition, considering the significant contributions of transporters on pharmacokinetics of J-104132, it is essential to optimize transport profile in pharmacokinetics of new drug candidates in the near future.

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