CARRIER-MEDIATED HEPATIC UPTAKE OF THE CATIONIC CYCLOPEPTIDE, OCTREOTIDE, IN RATS

Comparison between In Vivo and In Vitro

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ABSTRACT:

The plasma concentration and biliary excretion profiles of a cationic cyclic octapeptide, octreotide, were compared between control rats and rats given an intravenous infusion of a bile acid, taurocholate (TCA), and an organic anion, dibromosulfophthalein (DBSP). Both TCA and DBSP reduced the plasma elimination and biliary excretion of octreotide after its intravenous bolus administration. Two mechanisms accounting for this phenomenon were considered a priori: decreased hepatic uptake from blood to liver and decreased biliary excretion from liver to bile. The tissue uptake clearance (CL\textsubscript{uptake}) of octreotide in plasma and several tissues was determined, and extensive uptake of octreotide (0.20 ml/min/g liver) was observed only in liver. The kinetic analysis indicated that CL\textsubscript{uptake} in liver fell to 10% of controls after administration of both TCA and DBSP. To compare CL\textsubscript{uptake} between in vivo and in vitro, the initial velocity of octreotide uptake by isolated hepatocytes and primary cultured hepatocytes was measured. The estimated kinetic parameters K\textsubscript{m} and V\textsubscript{max} were 100 \mu M and 200 pmol/min/10\textsuperscript{6} cells in both systems, respectively. Hepatic uptake clearance estimated from the in vivo data was comparable with that observed in vitro. Biliary excretion of octreotide is reduced in Eisai hyperbilirubinemic rats (EHBs), which have a heredity defect of multispecific organic anion transporter on the bile canalicul membrane, compared with that of Sprague-Dawley rats. The kinetic analysis demonstrated that the hepatic uptake was reduced in EHBs. The uptake study using primary cultured hepatocytes suggested that a high level of unidentified endogenous substrate(s) in EHBR plasma may be responsible for the reduction of hepatic uptake of octreotide in EHBs. In conclusion, we have demonstrated that carrier-mediated hepatic uptake of octreotide is inhibited by TCA and DBSP and that the CL\textsubscript{uptake} obtained in vivo is comparable with the CL\textsubscript{uptake} obtained in vitro in isolated hepatocytes and primary cultured hepatocytes.

Recently, a variety of small peptides—somatostatin analogs (1–3), endothelin antagonists (4, 5), and renin inhibitors (6)—have been developed as therapeutic agents. Despite of their stability to enzymatic degradation in the gastrointestinal tract and liver, the short half-lives in plasma hinder their development as therapeutic agents. More than 70% of an intravenous dose of octreotide is excreted into urine (26). To investigate the initial tissue distribution of peptides in vivo has received little detailed attention. The tissue distribution of drugs is governed by many biological and physiological factors, such as tissue volume, blood flow, protein binding in plasma and tissue, specific transport mechanisms, etc. In particular, there is a specific transport mechanism for organic anions in liver and kidney, organs involved in the metabolism and excretion of exogenous compounds, such as DBSP (liver) and PAH (kidney) (25). A transport system for organic cations is also known to exist in both organs (15).

In this study, analysis of the tissue distribution of the cyclooctapeptide, octreotide, was conducted using it as a model compound for peptides. More than 70% of an intravenous dose of octreotide is excreted into the bile in unchanged form, and 30% of the dose is excreted into urine (26). To investigate the initial tissue distribution of octreotide, the time profiles of [14C]octreotide in plasma and several tissues were determined to calculate the tissue uptake clearance of octreotide in vivo in several tissues, including the liver and kidney. The uptake clearances obtained were compared among the tissues. To...
determine whether specific transport exists in the tissue distribution of octreotide, the effects of organic anions (DBSP and PAH), bile acid (TCA), and unlabeled octreotide on the tissue uptake of [14C]octreotide were examined. The characteristics of octreotide uptake and the magnitude of the uptake and inhibition by another compound were compared in vivo and in vitro using isolated rat hepatocytes and primary cultured hepatocytes. In addition, biliary excretion was determined in EHBRs in which the ATP-driven canalicular transport system (cMOAT) for organic anions such as DBSP and dinitrophe-nylglutathione is genetically defective, whereas the biliary excretion system for bile acids is conserved (27).

Materials and Methods

Chemicals and Reagents. Unlabeled octreotide and [14C]octreotide (specific activity: 46.2 mCi/mmol) were supplied by Sandoz Pharma Ltd. (Basel, Switzerland). [3H]Inulin was purchased from New England Nuclear (Boston, MA). DBSP was from the Société d’Etudes et de Recherches Biologiques (Paris, France). All other chemical and reagents were commercial products of analytical grade.

Analysis of the Time Profiles of the Plasma Disappearance and the Biliary Excretion of [14C]Octreotide. Male SDRs (Nissuiizai, Tokyo, Japan) and EHBRs (Eisai Laboratories, Gifu, Japan) weighing ~220 g were used throughout the experiments. Under light ether anesthesia, both femoral arteries and veins were cannulated with polyethylene catheters (PE-50) for blood sampling and the bolus injection of octreotide and infusion of DBSP or TCA, respectively. The bile duct was cannulated with polyethylene catheters (PE-10) for bile collection.

Thirty minutes before administration of [14C]octreotide (40 μCi/kg), DBSP (1 and 2.5 μmol/min/kg in saline and 25 μmol/kg as a loading dose) or TCA (4 and 10 μmol/min/kg in 3% human serum albumin and 5% glucose) infusions were started and continued throughout the experiment. As a control experiment, an equivalent volume of saline was infused. Bile was collected in preweighed test tubes at 10-min intervals. Plasma was obtained by centrifugation of blood samples (Microfuge, Beckman, Fullerton, CA). Aliquots of plasma (100 μl) and bile (10 μl) were mixed with scintillation cocktail (Clear-sol I, Nacarai Tesque, Tokyo, Japan) and counted (LS 6000SE, Beckman Instruments, Fullerton, CA).

Determination of Kinetic Parameters. Plasma concentration profiles were fitted with the following biexponential equation using the nonlinear iterative least squares method (28).

\[ C_p = A \exp(-\alpha t) + B \exp(-\beta t) \]  

AUC at \( t \) was calculated by integrating eq.1 from 0 to \( t \).

Pharmacokinetic parameters were calculated according to the following equations:

\[ CL_{int} = \frac{dose}{AUC_{(0-\infty)}} \]  

\[ CL_{bile} = \frac{X_{bile}}{AUC_{(0-\infty)}} \]  

where \( CL_{int} \), \( AUC_{(0-\infty)} \), \( CL_{bile} \), and \( X_{bile} \) are the total body clearance of octreotide, the amount of octreotide excrated into bile, and the tissue uptake clearance defined by plasma concentration, respectively.

Analysis of Tissue Uptake after Intravenous Administration of [14C]Octreotide in SDRs and EHBRs. After intravenous bolus administration of [14C]octreotide (8 μCi/kg) via the femoral vein, blood was collected from the femoral artery. At time 1, 3, or 5 min after administration of [14C]octreotide, rats were killed, and liver, kidney, intestine, pancreas, lung, muscle, spleen, and cerebrum were excised immediately, and a portion of each tissue was weighed and radioactivity counted. The tissue uptake rate can be described by the following differential equation during the relatively early phase when the efflux of [14C]octreotide is minimal:

\[ \frac{dX(t)}{dt} = CL_{up} \cdot C_p(t) \]  

where \( X(t) \) is the amount of octreotide in the tissue at time \( t \), \( CL_{up} \) is the tissue uptake clearance, and \( C_p(t) \) is the plasma concentration of octreotide. Integration of eq. 4 gives:

\[ X(t) = CL_{up} \cdot AUC_{(0-\infty)} + X(0) \]  

where \( AUC_{(0-\infty)} \) represents the area under the plasma concentration-time curve from 0 to \( t \).

Equation 5 divided by \( C_p(t) \) gives:

\[ \frac{X(t)}{C_p(t)} = CL_{up} \cdot \frac{AUC_{(0-\infty)}}{C_p(t)} + V_r \]  

where \( V_r \) represents the distribution volume that consists of the plasma space and the volume in which the drug concentration equilibrates instantaneously with that in plasma. The \( CL_{up} \) value can be obtained from the initial slope of a plot of \( X(t)/C_p(t) \) vs. \( AUC_{(0-\infty)}/C_p(t) \), designated as an “integration plot” (12, 29, 30).

Inhibition of Octreotide Uptake by DBSP, TCA, PAH, and octreotide. Thirty minutes before administration of the mixture of [14C]octreotide and [3H]inulin, DBSP (2.5 μmol/min/kg and 25 μmol/kg as loading dose), TCA (10 μmol/min/kg), PAH (23 μmol/min/kg and 700 μmol/kg as loading dose), or octreotide (250 nmol/min/kg) infusions were started and continued throughout the experiment. As a control experiment, an equivalent volume of saline was infused.

Plasma concentration-time profile and the amount in each tissue at 5 min after administration of [14C]octreotide (8 μCi/kg) were determined as described previously. In the case of liver, biopsy was performed at 1 min. For kidney, the tissue uptake clearance determined herein involved glomerular filtration. Thus, to obtain the exact uptake clearance for kidney via the basolateral membrane, a correction had to be made using the values of the unbound fraction of octreotide in plasma and the apparent uptake by GFR, estimated from the integration plot analysis of [3H]inulin as described previously.

Uptake of Octreotide by Isolated Hepatocytes. Hepatocytes were isolated from male SDRs (250–280 g) by the procedure of Baur et al. (31). After isolation, hepatocytes were suspended (5 × 10^6 cells/ml) in Hepses supplemented with 10 mM HEPES (pH 7.4) containing 1% BSA and 250 μmol/l of DBSP. Cell viability was checked by the trypan blue (0.4%) exclusion test.

Uptake of [14C]octreotide (1–1000 μM) was initiated by adding the ligand to the preincubated (37°C for 3 min) cell suspension (2 × 10^6 cells/ml). After 0.5 or 2 min, the reaction was terminated by separating cells from the medium using a centrifugal filtration technique (32). Then 200 μl aliquots were transferred into 0.4 ml centrifuge tubes containing 50 μl of 2 N NaOH, covered by a 100 μl mixture of silicone and mineral oil (density: 1.015). Samples were then centrifuged for 10 sec in a bench microfuge (Beckman Instruments, Fullerton, CA).

Centrifugation drove the pelleted hepatocytes through the oil layer and into the concentrated solution (2 N NaOH). After cells were dissolved in alkaline solution, the tube was sliced with a razor blade and both sections (medium portion and the bottom portion including cells) were transferred to scintillation vials. The bottom portion was neutralized with 50 μl of 2 N HCl. Then, 10 ml of counting solution was added to the vial, and both cell and medium radioactivities were determined in a liquid scintillation spectrophotometer (LS6000SE, Beckman Instruments). The initial uptake velocity was calculated applying linear regression to points taken at 30 and 120 sec.

Uptake of Octreotide by Primary Cultured Hepatocytes. Hepatocytes were prepared from male SDRs (220–280 g) by the procedure of Kato and Sugiyama (33). After isolation, hepatocytes were suspended (5 × 10^6 cells/well) in a 12-well plate (Corning, Conning, NY) in 5% calf serum, 1 mM insulin, and 1 mM dexamethasone containing William’s medium E. Cells were cultured for 4.5 hr at 37°C and 5% CO2. Cell viability was checked by the trypan blue (0.4%) exclusion test.

Hepatocytes cultured for 4.5 hr were preincubated at 37°C for 10 min with 600 μl of Krebs-Henseleit buffer containing 2% BSA or 90% rat plasma. Uptake was started by adding an aliquot of octreotide solution to give a final concentration. Before stopping the uptake, 50 μl of medium was removed to determine the concentration of ligand in the medium. Uptake was stopped by adding cold buffer, and cells were washed. Then, 400 μl of buffer containing 0.4 N NaOH and 0.1% sodium dodecyl sulfate was added and incubated for 30
min at room temperature. Then, 350 μL of the cell solution was removed. Radioactivities in the medium and cells were determined in a liquid scintillation spectrophotometer (LS6000SE, Beckman Instruments). The initial uptake velocity was calculated by subtracting the uptake at 5 min from that at 1 min.

**Estimation of In Vitro Uptake Clearance from In Vitro Data.** The kinetic parameters for octreotide uptake were estimated from the following equation:

\[ V_u = V_{\text{max}} \cdot S / (K_M + S) + P_{\text{diff}} \cdot S. \]  

where \( V_u \) is the initial uptake rate of octreotide (pmol/min/10⁶ cells), \( S \) is the octreotide concentration in the medium (μM), \( K_M \) is the Michaelis constant (μM), \( V_{\text{max}} \) is the maximum uptake rate (pmol/min/10⁶ cells), and \( P_{\text{diff}} \) is the nonspecific uptake clearance (μL/min/10⁶ cells). The aforementioned equation was fitted to the uptake data sets by an iterative nonlinear least squares method using a MULTI program (28) to obtain estimates of kinetic parameters. Input data were weighted as reciprocals of the square of the observed values, and the algorithm used for fitting was the Damping Gauss Newton Method.

The uptake clearance of octreotide in vivo was estimated from the following equation, considering that octreotide does not distribute to blood cells. Venous equilibrium model (34):

\[ CL_{\text{v}} = Q_p \cdot \frac{f_p \cdot PS_{\text{sat}}}{Q_p \cdot fp + PS_{\text{sat}}}. \]  

Sinusoidal perfusion model (35):

\[ CL_{\text{b}} = Q_p \cdot \left(1 - \exp(-fp \cdot PS_{\text{sat}}/Q_p)\right). \]  

where \( Q_p \) is hepatic plasma flow (0.825 mL/min/g) (36), and \( f_p \) is the plasma unbound fraction (0.41). \( PS_{\text{sat}} \) is permeability-surface area product (mL/min/g liver). Based on the value (1 mg protein = 1 × 10⁶ cells and 1 g liver = 1.25 × 10⁷ cells), \( PS_{\text{sat}} \) is calculated from the observed value obtained from isolated hepatocytes (2.57 μL/min/mg protein) and primary cultured hepatocytes (2.46 μL/min/mg protein).

**Results**

**Effect of DBSP and TCA on Plasma Elimination and Biliary Excretion of [14C]Octreotide.** The effects of an organic anion, DBSP, and a bile acid, TCA, on the plasma elimination and biliary excretion of [14C]octreotide were examined (fig. 1). Intravenous infusion of DBSP and TCA into rats was conducted at two infusion rates before and after bolus administration of [14C]octreotide. Biliary excretion rates of TCA after infusion at a rate of 4 or 10 μmol/min/kg were 3.9 or 7.1 μmol/min/kg from 20 to 30 min after initiation of infusion, respectively, and the plasma concentration after 30 min was 100 or 350 μM, respectively. Biliary excretion rates of DBSP after infusion at a rate of 1 or 2.5 μmol/min/kg were 0.9 or 1.7 μmol/min/kg, and plasma concentration was 60 and 300 μM, respectively. Biliary excretion of DBSP and TCA exhibited minimal increase by further increasing the infusion rates. Biliary excretion rates at the infusion rates of 10 μmol/min/kg for TCA and 2.5 μmol/min/kg for DBSP were then estimated as transport maximum (\( T_{\text{max}} \)). At 30 min after starting of infusion of TCA or DBSP, [14C]octreotide was administered by intravenous bolus injection, and plasma and biliary excretion profiles were examined. Plasma elimination and biliary excretion of [14C]octreotide were inhibited by both TCA and DBSP in a dose-dependent manner and the biliary excretion of [14C]octreotide was reduced to <10% of that of controls at the \( T_{\text{max}} \) of each compound. Moreover, the plasma elimination of [14C]octreotide was markedly delayed at \( T_{\text{max}} \) of TCA and DBSP. The biliary excretion clearance (\( CL_{\text{bile,p}} \)) defined with respect to the octreotide concentration in plasma was reduced to 0.5% by TCA and to 6.6% by DBSP, compared with controls (table 1).

**Comparison of Plasma Elimination and Biliary Excretion of [14C]Octreotide between SDRs and EHBRS.** Plasma elimination and biliary excretion of [14C]octreotide in EHBBR after intravenous bolus administration were compared with SDRs (fig. 2). Plasma elimination was delayed, compared with SDRs and the cumulative biliary excretion over 120 min was reduced to 40% of that in SDRs. The \( CL_{\text{bile,p}} \) in SDRs and EHBRRs was 7.4 mL/min/kg and 1.7 mL/min/kg, respectively, and the \( CL_{\text{bile,p}} \) in EHBBRs was reduced to 23% of that in SDRs (table 1).

**Analysis of Early-Phase Tissue Distribution of [14C]Octreotide in SDRs.** The early-phase tissue distribution of [14C]octreotide was examined (fig. 3). The time profiles of [14C]octreotide concentrations...
TABLE 1
Comparison of $CL_{\text{tot}}$ and $CL_{\text{bile,p}}$ of octreotide among SDRs, EHBRS, and treated SDRs

<table>
<thead>
<tr>
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<th>$CL_{\text{tot}}$</th>
<th>$CL_{\text{bile,p}}$</th>
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<tbody>
<tr>
<td></td>
<td>ml/min/kg</td>
<td>ml/min/kg</td>
</tr>
<tr>
<td>Control (SDRs)</td>
<td>12.63 ± 0.56</td>
<td>7.44 ± 0.29</td>
</tr>
<tr>
<td>+ TCA (4 μmol/min/kg)</td>
<td>14.22 ± 0.62</td>
<td>5.69 ± 0.78</td>
</tr>
<tr>
<td>+ TCA (10 μmol/min/kg)</td>
<td>1.42 ± 0.48$^a$</td>
<td>0.04 ± 0.00$^a$</td>
</tr>
<tr>
<td>+ DBSP (1 μmol/min/kg)</td>
<td>7.59 ± 0.78$^a$</td>
<td>2.28 ± 0.61$^a$</td>
</tr>
<tr>
<td>+ DBSP (2.5 μmol/min/kg)</td>
<td>6.25 ± 0.25$^a$</td>
<td>0.49 ± 0.09$^a$</td>
</tr>
<tr>
<td>EHBBR</td>
<td>7.18 ± 0.22$^a$</td>
<td>1.69 ± 0.05$^a$</td>
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$^a$ Significant difference from control ($p < 0.05$).

in plasma and several tissues 1, 3, and 5 min after intravenous bolus injection were determined to calculate $CL_{\text{tot}}$ of octreotide in vivo. The $CL_{\text{tot}}$ values in the liver and kidney were 0.2 ml/min/g and 0.58 ml/min/g, respectively, and were much higher than other organs, such as spleen, pancreas, lung, muscle, intestine, and brain. $K_p$ of $[\text{14C}]$octreotide was similar to that of $[\text{14C}]$inulin as an extracellular space marker in muscle, intestine, and brain, whereas slightly higher $K_p$ values were observed in the spleen, lung, and pancreas (table 2). To determine the contribution of glomerular filtration to the uptake of octreotide by the kidney, the $CL_{\text{tot}}$ of $[\text{14C}]$inulin at 5 min was determined to be 1.02 ± 0.16 ml/min/g kidney. The glomerular filtration clearance of octreotide in kidney estimated using the unbound fraction of octreotide in plasma (0.41) and the $CL_{\text{tot}}$ of inulin (1.02 ml/min/g kidney) was 0.42 ml/min/g kidney, as indicated by a broken line in fig. 3B. This value was similar to the $CL_{\text{tot}}$ of octreotide (0.58 ml/min/g) in the kidney. These results suggest that most of the apparent uptake of octreotide by the kidney is mainly due to the glomerular filtration (fig. 3B).

Inhibition of $[\text{14C}]$Octreotide Hepatic Uptake by Taurocholic Acid and Organic Anions in SDRs. The effects of coinfusion of TCA, DBSP, PAH, and unlabeled octreotide on the initial hepatic uptake clearance of $[\text{14C}]$octreotide were examined 5 min after intravenous bolus injection of $[\text{14C}]$octreotide (fig. 4, table 2). The $CL_{\text{tot}}$ was reduced to 10% of controls by coinfusion of TCA and DBSP, but PAH had no effect. In addition, the $CL_{\text{tot}}$ was reduced to 40% of controls by coinfusion of excess unlabeled octreotide, when the estimated octreotide concentration in plasma was 30 μM. A decrease in $K_p$ value at 5 min after administration, which reflects the early-phase distribution to each tissue, was observed not only in liver but also in the lung and intestine with coinfusion of TCA and DBSP. However, the reduction in the $K_p$ value in the lung and intestine was <15%, compared with that in liver.

Comparison of $[\text{14C}]$Octreotide Hepatic Uptake between SDR and EHBRS. The $CL_{\text{tot}}$ of $[\text{14C}]$octreotide observed in EHBRS under the same condition was 0.05 ml/min/g liver (fig. 4). The $CL_{\text{tot}}$ in EHBRS thus decreased to 25% of SDRs. The $K_p$ value in the liver 5 min after administration of octreotide in EHBRS also decreased to one-third that in SDRs (table 2). A change of $K_p$ value in other tissues was minimal (table 2).

In Vitro Uptake Study. The concentration dependence of octreotide by isolated hepatocytes was investigated (fig. 5 and table 3). A marked concentration dependence was observed and the estimated parameters ($K_M$, $V_{\text{max}}$, and $P_{\text{diff}}$) were $108 ± 37$ μM, $223 ± 75$ pmol/min/10^6 cells, and $0.51 ± 0.14$ μl/min/10^6 cells, respectively. Similar experiments were also conducted using primary cultured hepatocytes, and a similar concentration dependence was observed. The estimated parameters ($K_M$, $V_{\text{max}}$, and $P_{\text{diff}}$) were $97 ± 8$ μM, $185 ± 11$ pmol/min/10^6 cells, and $0.55 ± 0.04$ μl/min/10^6 cells, respectively. The $PS$ product for hepatic uptake ($PS_{\text{influx}}$), which represents the membrane permeability clearance—calculated from the equation, $V_{\text{max}}(K_M + P_{\text{diff}})$ was 2.57 μl/min/10^6 cells for isolated hepatocytes and 2.46 μl/min/10^6 cells for primary cultured hepatocytes.

Effect of Plasma Prepared from SDRs and EHBRS on $[\text{14C}]$Octreotide Uptake by Primary Cultured Hepatocytes. To examine the cause of the reduced $CL_{\text{tot}}$ EHBRS in vivo, the effects of plasma obtained from SDRs and EHBRS on the uptake of $[\text{14C}]$octreotide were investigated using primary cultured hepatocytes prepared from SDRs (fig. 6). The addition of EHBRS plasma reduced the uptake clearance to 75% that prepared from SDRs. Part of the decrease of $CL_{\text{tot}}$ observed in EHBRS is thus supposed to be due to an endogenous substance (not identified yet) in the plasma of EHBRS.

**Discussion**

Recently, cyclic and linear small peptides consisting of 5 to 10 amino acids have been developed as therapeutic agents: the somatostatin analog octreotide (17), endothelin antagonists (4, 5), and renin inhibitors (10). Efficient hepatic uptake followed by rapid biliary excretion of unchanged compound is a common characteristic of these small peptides. This short plasma half-life caused by them has hindered their development as therapeutic agents. Several recent studies have demonstrated that a specific active transport system for the uptake of peptide exists on the sinusoidal hepatocyte membrane using isolated hepatocytes in vitro (17–21). We have already examined the hepatic uptake of an anionic pentapeptide, the endothelin antagonist BQ-123 (23). Pharmacokinetic and biochemical analyses have demonstrated that there is carrier-mediated transport in the hepatic uptake of peptides, and this transport consists of both Na+-dependent and -independent uptake. The former is the same transport system for bile acids and latter shares the uptake system with the multispecific anion transporter (23). Moreover, we have demonstrated that the biliary excretion of BQ-123 from liver to bile is by primary active transport and BQ-123 is excreted by cMOAT (37). Decreased biliary excretion of BQ-123 was demonstrated in EHBRS, which have a defect of cMOAT (27, 38–40).

In this study, the distribution of a peptide into organs, including liver and kidney, was examined in vivo using the cationic octapeptide, octreotide, in contrast to the anionic BQ-123. Moreover, the uptake of octreotide was examined using isolated hepatocytes and primary cultured hepatocytes in vitro, and uptake clearance observed was compared with that obtained in vivo. Previous studies have reported that the uptake of octreotide is by carrier-mediated transport and is competitively inhibited by bile acids and organic anions (16, 17). In this study, the effects of organic anions and bile acids on the hepatic uptake of octreotide were examined, and saturation of the uptake of octreotide was also investigated in vivo. Both the biliary excretion and $CL_{\text{bile,p}}$ of octreotide were reduced to <10% of that of controls by TCA and DBSP at their $T_{\text{max}}$ (fig. 1 and table 1). From a pharmacokinetic point of view, decreased $CL_{\text{bile,p}}$ is brought by the decrease in the hepatic uptake from blood to liver and/or the decrease in biliary excretion from liver to bile. Thus, to determine how far the hepatic uptake, as the first process of hepatobiliary transport, is influenced by TCA and DBSP, the early-phase tissue distribution of octreotide was determined in vivo (figs. 3 and 4). A high $CL_{\text{tot}}$ was observed in the liver and kidney (0.2 ml/min/g liver and 0.58 ml/min/g kidney), compared with other organs (fig. 3). The apparent $CL_{\text{tot}}$ in the kidney involves amount of octreotide remaining in the lumen after being excreted by glomerular filtration. Thus, to obtain the exact uptake clearance for kidney via the basolateral membrane, a correction was made using the values of the unbound fraction of octreotide in plasma [0.41 (26)] and GFR (1.02 ml/min/g) estimated from the renal uptake
The glomerular filtration clearance of octreotide, estimated at 0.42 ml/min/g, demonstrated that 70% of the apparent uptake of octreotide by kidney was accounted for by glomerular filtration. This result demonstrates that octreotide is specifically taken up mostly by the liver during the early distribution phase. The hepatic uptake thus evaluated was reduced to 15% of controls by TCA and DBSP (fig. 4). On the other hand, PAH had no effect on the uptake of octreotide by the liver. Uptake of [14C]octreotide was reduced to 40% of controls by 30 μM octreotide in plasma resulting from infusion of unlabeled octreotide, thus indicating saturation of hepatic uptake of octreotide in vivo. Inhibition by TCA and bromosulfophthalein using isolated hepatocytes. Although a significant but slight decrease in $K_p$ at 5 min was observed in the intestine and lung after DBSP and TCA administration (table 2), the mechanism of the inhibitory effect is not known yet. The $K_p$ value of octreotide at 5 min in the kidney was significantly reduced to 3.8 by PAH, compared with 5.3 in controls. However, the $K_p$ value of inulin in the kidney reflecting glomerular filtration showed a similar decrease from 9.2 to 6.9. Considering that glomerular filtration contributes 70% of the uptake clearance of octreotide in the kidney, the decrease in uptake clearance of octreotide in kidney after administration of excess PAH may not be due to inhibition of the uptake process via basolateral membrane.

To compare the hepatic uptake clearance in vitro and in vivo, the uptake clearance of octreotide was also measured using isolated hepatocytes and primary cultured hepatocytes (fig. 5 and table 3). Similar results were obtained in both experimental systems. The estimated parameters ($K_{in}$, $V_{max}$, and $P_{diff}$) were ~100 μM, 200 pmol/min/10⁶ cells, and 0.5 μl/min/10⁶ cells. A similar uptake clearance under a linear condition ($V_{max}/K_{M} + P_{diff}$) was obtained in both systems: 2.57 μl/min/10⁶ cells (isolated hepatocytes) and 2.46 μl/min/10⁶ cells (primary cultured cell). The uptake clearance for octreotide in vivo was estimated from eq. 8 (venous equilibrium model) and eq. 9 (sinusoidal perfusion model). The uptake clearance in vivo was calculated from in vitro data to be 0.23 ~ 0.26 ml/min/g liver (isolated...
Clearance defined in respect to the concentration in liver. In addition, the concentration in plasma and liver and the biliary excretion rate were determined at 1 and 5 min after administration of [14C]octreotide. Each represents the mean of six determinations. The primary hepatocytes (●) were initiated by adding the ligand to the preincubated (37°C for 3 min) cell suspension (2 × 10⁶ cells/mL). At 0.5 or 2 min, reaction was terminated by separating cells from the medium using a centrifugal filtration technique. Initial uptake velocity \( V_o \) was calculated by linear regression of points taken at 30 and 120 sec. Each point represents the mean ± SEM of six determinations. The primary hepatocytes (○) cultured for 4.5 hr were preincubated at 37°C for 10 min with 600 μL of Krebs-Henseleit buffer. Initial uptake velocity \( V_o \) was calculated by linear regression of points taken at 1 and 5 min. Each point and bar represent mean ± SEM of three determinations.

We have already compared hepatobiliary transport of octreotide in SDRs with that in EHBRs (41). In this study, the steady-state concentration in plasma and liver and the biliary excretion rate were monitored after intravenous infusion. Comparing SDRs and EHBRs, differences were observed in \( Cl_{\text{bile,p}} \) but not in biliary excretion clearance defined in respect to the concentration in liver. In addition, the liver-to-plasma concentration ratio \( K_p \) in EHBRs (1.15) was half that of SDRs (2.20) (41). This result suggests that the decrease in \( Cl_{\text{bile,p}} \) is not caused by a decrease in biliary excretion via the bile canalicular membrane, but rather a decrease in hepatic uptake via the sinusoidal membrane. In fact, we have already determined the primary active ATP-dependent uptake of octreotide by bile canalicular membrane vesicles, which is maintained in EHBRs (41), as well as in SDRs. To determine the mechanism of the reduced \( K_p \) in EHBRs, the hepatic uptake clearance in EHBRs was compared with that in SDRs in vivo using integration plot analysis. As shown in fig. 4, the hepatic uptake clearance of octreotide is significantly lower in EHBRs than in SDRs.

![Graph](image-url)
three experiments.

- **bile acids were investigated**
- **hepatic uptake of octreotide and its inhibition by organic anions and**
- **which is deficient in EHBRs.**
- **ligands in EHBRs that the ligands are excreted into bile by cMOAT,**
- **decreased hepatic uptake in EHBRs. Thus, we should be careful not**
- **ronide, elevated in EHBR plasma may play a role in part of the**
- **suggested that an endogenous substance, such as bilirubin glucu**
- **plasma to 49**
- **prepared from EHBRs was reduced to 75% that from SDRs. The**
- **in EHBRs. To examine the mechanism for the decrease in the hepatic**
- **uptake clearance in EHBRS was ~25% of that in SDR. However,**
- **considering that only the transporter (cMOAT) on the bile canalicu**
- **membrane has been believed to be deficient in EHBRS, it is unlikely**
- **that the transport carrier on the sinusoidal membrane is also deficient**
- **in EHBRS. To examine the mechanism for the decrease in the hepatic**
- **uptake in EHBRS, the uptake of octreotide by primary cultured hepatoc**
- **tocytes from SDRs and EHBRs, and compared with that in the absence**
- **of plasma (fig. 6). The uptake clearance in the presence of plasma**
- **prepared from EHBRS was reduced to 75% that from SDRs. The**
- **bilirubin glucuronide concentration was reported to increase in EHB**
- **R plasma to 49 μM from 1 μM in the normal plasma (38). This**
- **suggested that an endogenous substance, such as bilirubin glucuru**
- **ronide, elevated in EHBRS may play a role in part of the**
- **decreased hepatic uptake in EHBRS. Thus, we should be careful not**
- **to conclude easily from the observation of reduced biliary excretion**
- **of ligands in EHBRS that the ligands are excreted into bile by cMOAT,**
- **which is deficient in EHBRS.**

In conclusion, the existence of a carrier-mediated transport for the hepatic uptake of octreotide and its inhibition by organic anions and bile acids were investigated in vivo. Kinetic analysis demonstrated that the decreased biliary excretion of octreotide observed in EHBRS and the marked decreased biliary excretion caused by bile acids and organic anions were due to reduced hepatic uptake rather than biliary excretion.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>In Vivo</th>
<th>Isolated Cells</th>
<th>Primary Cultured Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>µl/min/mg protein</td>
<td>—</td>
<td>2.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>nl/min/g liver</td>
<td>0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Observed value with isolated hepatocytes ( = V<sub>max</sub>/K<sub>M</sub> + P<sub>diff</sub>).

<sup>b</sup> Observed value with primary cultured hepatocytes ( = V<sub>max</sub>/K<sub>M</sub> + P<sub>diff</sub>).

<sup>c</sup> Observed value obtained from integration plot method (fig. 3).

<sup>d</sup> Calculated based on the sinusoidal perfusion model (eq. 8).

<sup>e</sup> Calculated based on the venous equilibrium model (eq. 9).

**FIG. 6. Uptake of [14C]octreotide by primary cultured rat hepatocytes in the presence of plasma prepared from SDRs and EHBRS.**

Cultured cells were preincubated at 37°C for 10 min with 2% BSA containing buffer (●), 90% (v/v) SDR plasma (●), or 90% (v/v) EHBRS plasma (■). Uptake was started by the addition of [14C]octreotide to give a final concentration of 6 μM. Each point and vertical bar represent mean ± SEM of three experiments.

**References**


