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Research Article

Hepatic Uptake of Octreotide, a Long-Acting Somatostatin Analogue, *via* a Bile Acid Transport System

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The hepatic transport mechanism of octreotide (Sandostatin®), a somatostatin analogue, was studied using freshly prepared rat hepatocytes. The initial uptake rate of octreotide represented exclusively a saturable transport process. The half-saturation constant, K_t, and the maximum uptake-rate, J_{max}, for the uptake of octreotide were 91.1±28.4 μM and 104.6±19.7 pmol/mg protein/min, respectively. An energy requirement was demonstrated for [^{14}C]octreotide uptake since metabolic inhibitors (DNP, rotenone, antimycin and NaCN) significantly reduced the initial uptake rate. [^{14}C]octreotide uptake was also significantly inhibited by ouabain. [^{14}C]octreotide uptake was reduced in the absence of Na^+ in the uptake medium. [^{14}C]octreotide uptake was significantly inhibited by bile acids, iodipamide, d-tubocurarine, whereas it was not inhibited by bilirubin, TEMA and insulin. Competitive inhibition of taurocholic acid was observed for octreotide uptake with the inhibition constant, K_i, of 82±17 μM. Moreover, a significant inhibitory effect of octreotide was observed for the Na^+ dependent uptake of [^{14}C]taurocholic acid. These results suggest that octreotide is transported into hepatocytes via a bile acid carrier-mediated system.

KEY WORDS: octreotide; sandostatin®; hepatic transport; bile acid; biliary secretion; carrier-mediated transport.

INTRODUCTION

In order to develop pharmacologically active peptide-drugs, their elimination mechanisms need to be clarified. Enzymatic stability of peptide drugs is difficult to overcome. Octreotide (Sandostatin®) is an analogue of somatostatin (Fig. 1), used in the treatment of acromegaly (1) and pancreatic endocrine tumors (2). Compared to somatostatin, octreotide has a relatively long half-life in the body after parenteral administration and is stable against enzymatic metabolism (3). Interestingly, more than 70% of the intravenously administered dose of octreotide is secreted in the bile in the intact form (4). Moreover, hepatic extraction of octreotide is approximately 70% (4), indicating significant hepatic uptake at the sinusoidal membrane of hepatocytes. However, the transport mechanism of this hepatic uptake has not been elucidated yet.

Although some recent findings suggest the presence of specific somatostatin receptors in liver cell membranes (5), it is generally reported that hepatic plasma membranes do not have such receptors (6). Therefore, it is difficult to assume that octreotide is transported by receptor-mediated endocytosis in the liver. Regarding peptide transport in the liver, we have previously reported that β-lactam antibiotics, dipeptide drugs, are transported by a carrier-mediated transport system being common to that of probenecid (7,8). Moreover, several investigators have reported that a carrier-mediated transport system of bile acid could transport some peptide-drugs, such as antamanide (9), cyclolinopeptide (10), cholecystokinin (11) and renin-inhibiting peptides (12,13). Accordingly, the possibility for octreotide to be recognized by either the bile acid transport system or an organic anion transport system in the liver still exists.

The objective of the present study was to elucidate the hepatic transport mechanism of octreotide. For this purpose freshly prepared hepatocytes of rat were used in uptake studies.

MATERIALS AND METHODS

Chemicals

Octreotide (Sandostatin®), (-)-D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[(1R,2R)-2-hydroxy-1-(hydroxy-methyl)propyl]-L-cysteinamide cyclic (2→7) disulfide diacetate and [^{14}C]oc-

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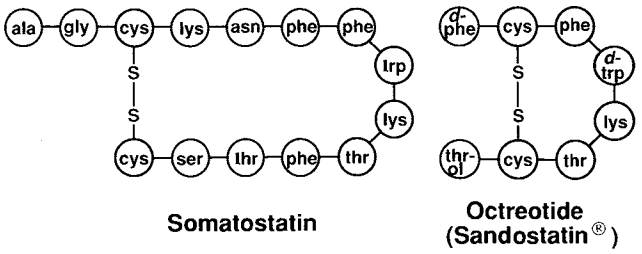

Fig. 1. The primary structures of octreotide (Sandostatin®) and somatostatin.

treotide (41.2 μCi/mg) were kindly supplied from Sandoz Pharma Ltd., (Basel, Switzerland). Tauro[carbonyl-^{14}C] cholic acid (cholyltaurine), sodium salt (60 mCi/mmol) was purchased from Amersham International (Buckinghamshire, England). Inulin-methoxy [methoxy-^{14}C] (9.8 mCi/g) was from New England Nuclear (Boston, MA). All the isotopes were stored at −20 °C until use. Collagenase H and antimycin A were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Ethylene glycol bis-(aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) and rotenone were purchased from Nacalai Tesque Co. (Kyoto, Japan). Bovine serum albumin (BSA, Fraction V), heparin sodium salt and crystalline porcine insulin were purchased from Sigma Chemicals Co. (St. Louis, MO).

Sodium cyanide (NaCN), 2,4-dinitrophenol (DNP) and 4,4-diisothiocyanostilbene 2,2′-disulfonic acid, disodium salt (DIDS) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Ouabain (g-Strophanthin) and 3,3′-adipoyldiiminobis(2,4,6-triiodobenzoic acid) (Iodipamide) were purchased from Tokyo Kasei Co. (Tokyo, Japan). Silicon oil (d = 1.05) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Ebiratide (H•Met(O_2)-Glu-His-Phe-(D)Lys-Phe-NH$(CH_2)_8NH_2$) (Hoechst Japan Ltd., Kawagoe, Japan) and E-2078, a dynorphin-like analgesic peptide (CH_3-Tyr-Gly-Gly-Phe-Leu-Arg-CH_3Arg-(D)-Leu-NH_2) (Eisai Co. Ltd., Tokyo, Japan) were kindly supplied from the cited companies. All other chemicals were of reagent grade and commercially available.

Isolation of Rat Hepatocytes

Hepatocytes from male Wistar rats (Sankyo Laboratory Co. Ltd., Toyama, Japan) aged 7–8 weeks, fed on standard chow, were isolated according to the method previously reported (8). The liver was flushed in situ at 37 °C with 200 ml medium A (138 mM NaCl, 53 mM KCl, 8.1 mM $MgSO_4$, 3.4 mM $Na_2HPO_4 \cdot 12H_2O$, 4.4 mM KH_2PO_4, 26 mM $NaHCO_3$, 0.6 mM EGTA, 13 mM 4-(2-hydroxyethyl)-l-piperazine ethanesulfonic acid (HEPES) and 2% of BSA) in order to remove blood. Thereafter, the liver was perfused for 6 min at 37 °C with 0.12% of collagenase in 100 ml medium B (EGTA and BSA free and containing additionally 4 mM $CaCl_2$ in medium A). After dissociation of the cells, hepatocytes were separated from cell debris by centrifugation at 4 °C at 400 g for 2 min in medium C (Krebs-Henseleit buffer containing 118 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2HPO_4, 13 mM HEPES and 2% of BSA). The cells were kept at 4 °C in an incubation medium (medium C containing 10 mM glucose). Cell viability was tested by trypan blue exclusion and LDH-latency test immediately after the cell isolation. More than 82–95% of the cells were viable as indicated by trypan blue exclusion and LDH-latency test. In order to prevent the increase of nonspecific leakage of drug penetration through the liver cell membrane, uptake experiments were performed within 3 hr after the isolation of cells.

Uptake Studies of [^{14}C]Octreotide

The uptake of the radiolabeled compound by isolated rat liver cells was measured using 200 μl of a liver cell suspension containing 5 mg of cellular proteins. A 0.5 μM concentration of [^{14}C]octreotide was used. Protein content was determined according to the method of Lowry (14) using bovine serum albumin as a standard. The suspension was preincubated at 37 °C for 5 min in incubation medium equilibrated with carbogen (95% O_2, 5% CO_2). Uptake experiments were initiated by the addition of various concentrations of drugs dissolved in 200 μl of incubation medium. The initial uptake rate was determined at 90 sec after the initiation of the uptake reaction. 200 μl aliquots of cell suspension were removed and placed in 0.5 ml microcentrifuge tubes containing 100 μl of silicon oil and 50 μl of 3 M KOH. The uptake reaction was terminated by separating cells from medium using silicon oil centrifugation technique (8). After the cells were dissolved into the alkaline solution, the tube was sliced off and the bottom side containing the cells was neutralized with 50 μl of 3 M acetic acid. Thereafter the radioactivity of this cell precipitate was measured in a liquid scintillation counter LSC-3,500 (Aloka Co. Ltd., Tokyo, Japan). Forty μl aliquots of cell suspension were taken from each incubated cell suspension remaining and analyzed for drug concentration after solubilization in 300 μl of 1 N NaOH. The extracellular fluid contamination in the cell pellet was corrected by subtracting the measured [^{14}C]inulin uptake from the total uptake.

Disposition studies performed in rats (4) indicated a fairly good metabolic stability of octreotide. After intravenous administration of radiolabelled octreotide, parent drug accounts for most radioactivity found in plasma, urine, bile and liver. These previous results clearly support the use of total radioactivity measurements in *in vitro* uptake studies.

According to the results of the uptake study performed in a large concentration range (0.25 μM to 1.0 mM) of octreotide, the extent of nonsaturable uptake was considered to be negligible. In order to estimate the kinetic parameters of octreotide uptake by isolated rat hepatocytes, the uptake rate (J) was fitted to the following equation by using the nonlinear least-square regression analysis program MULTI (15):

$$J = J_{max} \cdot S/(K_t + S) \quad (1)$$

where J_{max} is the maximum uptake rate for carrier-mediated process, S is the concentration of substrate and K_t is the half-saturation constant.

To examine the effect of various compounds, including taurocholate, on the uptake of [^{14}C]octreotide, the uptake study was performed at 37 °C for 90 sec in the presence of various concentrations of these compounds in the uptake medium.

To examine the energy dependency of [^{14}C]octreotide uptake, the uptake studies were performed as follows: isolated rat hepatocytes were preincubated for 5 min at 37 °C in the glucose free incubation medium in the absence or presence of metabolic inhibitors (10 μM antimycin A, 25 μM rotenone, 1 mM NaCN, 1 mM DNP and 1 mM ouabain), then the uptake rate was determined.

To examine the sodium dependency of [^{14}C]octreotide uptake, the isolated hepatocytes were prepared by using sodium free medium. To prepare sodium free uptake medium, choline Cl, choline HCO_3 and KH_2PO_4 were employed to replace sodium salts in the Krebs–Henseleit buffer. After preincubation at 37 °C for 5 min, the uptake rate was determined.

Uptake Studies of [^{14}C]Taurocholate

The initial reuptake rate of [^{14}C]taurocholate was determined at 37 °C for 45 sec using 200 μl of liver cell suspension containing 0.5 mg of cellular proteins. The other conditions were the same as described in the uptake studies of [^{14}C]octreotide.

RESULTS

Time Courses of Octreotide Uptake

The time courses of [^{14}C]octreotide (0.5 μM) uptake at 37 °C and 4 °C by isolated rat hepatocytes are illustrated in Fig. 2. Uptake of [^{14}C]octreotide at 37 °C was linear over the 120 sec incubation period. Thus, 90 sec of uptake time was employed, hereafter, to determine the initial uptake rate of [^{14}C]octreotide. The uptakes extrapolated to "zero time" at 37 °C and 4 °C were almost negligible, showing no strong binding of [^{14}C]octreotide to the plasma membrane of hepatocytes.

Concentration Dependency of Octreotide Uptake

Figure 3 represents the relationship between the initial uptake rate and the concentration of octreotide (0.25 to 1000 μM). Since uptake rate became constant at concentrations higher than 200 μM, equation (1) was used to relate uptake rate and substrate concentration. Using a nonlinear least-squares regression analysis of these data, the following kinetic parameters (mean ± S.D.) were estimated for the uptake of octreotide: J_{max} was 104.6±19.7 pmol/mg protein/min and K_t was 91.1±28.4 μM. A single transport process was confirmed for the uptake of octreotide when the data shown in Fig. 3 were re-analyzed by the Eadie-Hofstee plot (not shown).

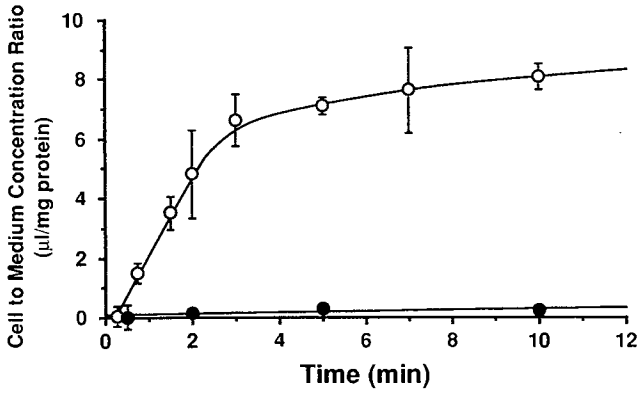

Fig. 2. Time courses of [^{14}C]octreotide uptake by isolated rat hepatocytes at 37 °C (*opened circles*) and at 4 °C (*closed circles*). Uptakes of [^{14}C]octreotide at the concentration of 0.5 μM were measured by incubating the isolated rat hepatocytes in Krebs Henseleit buffer (pH 7.4). Each point represents the mean±S.E. of three experiments. When the S.E. was small, it was included in the symbol.

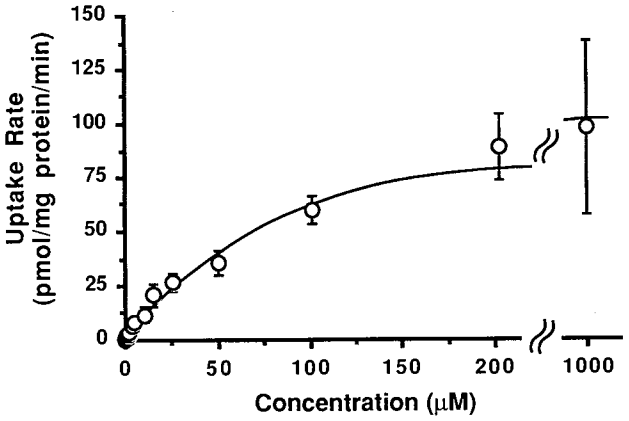

Fig. 3. Concentration dependence of the uptake rate of [^{14}C]octreotide by isolated rat hepatocytes. The incubation conditions at 37 °C were identical to those described in the legend of Fig.2. Initial uptake rate of octreotide was measured in the concentration range from 0.25 μM to 1.0 mM. Each point represents the mean±S.E. of three experiments. When the S.E. was small, it was included in the symbol. The solid line represents saturable uptake rate generated from Eq. (1), using the MULTI fitted parameters (the mean): K_t =91.1 μM, J_{max}=104.6 pmol/mg protein/min.

Effects of Metabolic Inhibitors and Sodium Replacement on the Uptake of Octreotide

As shown in Table I, the uptake rate of [^{14}C]octreotide was significantly reduced by the presence of metabolic inhibitors, i.e., 1 mM DNP, an uncoupler of oxidative phosphorylation, or 25 μM rotenone, 1 mM sodium cyanide and 10 μM antimycin-A, both respiratory chain inhibitors. Inter-

Table I. Effect of Metabolic Inhibitors on the Uptake Rate of [^{14}C]Octreotide

Inhibitors	Concentration	Relative Uptake[a]
	mM	%
Control		100.0 ± 7.6
DNP	1.0	0.6 ± 8.5*
Rotenone	0.025	15.5 ± 5.0*
NaCN	1.0	14.6 ± 3.7*
Antimycin A	0.01	40.6 ± 2.5*
Ouabain	1.0	6.5 ± 6.3*
Choline[b]	141	4.9 ± 5.3*

[a] Each value represents the mean±S.E. of four or five experiments.
[b] The uptake rate was determined in the incubation medium by replacing sodium with choline.
* Significantly different from the control value by the Student's t test ($p<0.001$).

estingly, the uptake rate of [^{14}C]octreotide was significantly reduced by the replacement of sodium with choline in the incubation solution, or by the presence of 1 mM ouabain, a $Na^+ - K^+$ ATPase inhibitor, thus indicating that octreotide was transported by hepatocytes via a sodium dependent carrier-mediated system.

Effects of Various Compounds on the Uptake of Octreotide

Effects of various compounds on the uptake of [^{14}C]octreotide were examined and the results are summarized in Table II. The uptake of [^{14}C]octreotide was significantly inhibited by bile acids, i.e., 100 μM of taurocholate and cholate, and by the anion exchange inhibitor, 4,4′-diisothiocyanostilbene 2,2′-disulfonic acid, disodium salt (DIDS). The anionic dye, sulfobromophthalein (BSP) reduced significantly the uptake of [^{14}C]octreotide in a concentration dependent manner. Iodipamide (100 μM) also reduced the uptake of [^{14}C]octreotide significantly. In contrast to these inhibitory effects of organic anions, bilirubin and triethylmethylammonium (TEMA) did not exhibit a significant effect on the uptake of [^{14}C]octreotide whereas d-tubocurarine reduced this uptake. Oligo peptides, ebiratide, E-2078 and insulin did not change the uptake of [^{14}C]octreotide.

Effect of Taurocholate on the Uptake of Octreotide

Figure 4 illustrates the Lineweaver-Burk plots for the initial uptake rate of octreotide showing the inhibition by taurocholate at a concentration of 100 μM. No significant difference was observed for J_{max} in the presence or absence of taurocholate which was performed for the same preparation of hepatocyte. Although apparent J_{max} value shown in Fig. 4 was different from that of Fig.3, this difference would be caused by the differential cell preparation. The result demonstrates that taurocholate inhibited competitively the initial uptake rate of octreotide. The inhibition constant of taurocholate K_i, was calculated to be 81.7±17.0 μM for the uptake of octreotide.

Table II. Effect of Various Compounds on the Uptake of [^{14}C]Octreotide

Inhibitors	Concentration	Relative Uptake[a]
	mM	%
Control		100.0 ± 7.6
Taurocholate	0.1	50.2 ± 8.4**
Cholate	0.1	59.2 ± 8.1*
Iodipamide	0.1	52.9 ± 8.2***
d-Tubocurarine	1.0	43.6 ± 5.6*
DIDS	0.1	63.5 ± 5.1***
BSP	0.01	58.1 ± 6.4***
BSP	0.05	23.9 ± 7.9*
Bilirubin	0.05	111.9 ± 4.7
TEMA	1.0	104.6 ± 11.0
Insulin	0.01	106.1 ± 11.1
E-2078	1.0	73.1 ± 10.4
Ebiratide	1.0	90.8 ± 13.7

[a] Each value represents the mean±S.E. of four or five experiments.
* Significantly different from the control value by the Student's t test ($p<0.001$).
** Significantly different from the control value by the Student's t test ($p<0.01$).
*** Significantly different from the control value by the Student's t test ($p<0.05$).

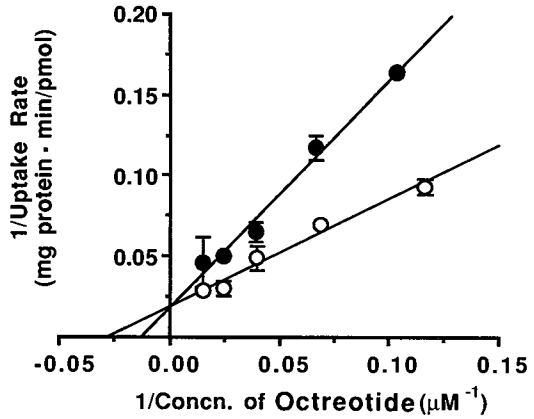

Fig. 4. Lineweaver-Burk plots of octreotide uptake rate by isolated rat hepatocytes in the absence (*opened circles*) and presence (*closed circles*) of 100 μM taurocholate. The incubation conditions were identical to those described in the legend of Fig. 2. Initial uptake rate of octreotide (8.5 μM − 65 μM) was measured at 37 °C for 90 sec. Taurocholate was simultaneously added at the initiation of [^{14}C]octreotide uptake. Each point represents the mean of three or four experiments. The inhibition constant, K_i of taurocholate was estimated to be 81.7±17.0 μM.

Effect of Sodium Replacement and Various Inhibitors on the Uptake of Taurocholate

The effects of various inhibitors on the uptake of [^{14}C]taurocholate were examined and the results summarized in Table III. Replacement of sodium ion with choline in the uptake medium significantly reduced the uptake of [^{14}C]taurocholate. Moreover, the uptake of [^{14}C]taurocholate was decreased by 0.5 mM unlabeled taurocholate to

Table III. Effect of Octreotide and Various Inhibitors and Na^+ Replacement on the Uptake of [^{14}C]Taurocholate

Inhibitors or Replacement	Concentration	Relative Uptake[a]
	mM	%
Control	—	100.0 ± 8.1
Octreotide	0.02	105.3 ± 23.6
	0.1	67.3 ± 17.3
	0.5	20.5 ± 2.4*
Taurocholate	0.5	4.0 ± 1.1*
TEMA	1.0	125.5 ± 18.5
Choline[b]	143	10.1 ± 2.3*

[a] As a control study, the uptake rate of taurocholate was determined to 81.6± 6.59 μL/mg protein/45 sec at the tracer concentration (1 μM, 60 nCi/ml). Each value represents the mean±S.E. of three or four experiments.
[b] The uptake rate was determined in the incubation medium replaced by sodium with choline.
* Significantly different from the control value by the Student's t test ($p<0.001$).

4.0% of the total uptake. These results demonstrate that the sodium dependent bile acid transport-system was functioning in the prepared hepatocytes. Very interestingly, the uptake of [^{14}C]taurocholate was significantly reduced in the presence of 0.5 mM octreotide to 20%, while no effect was shown for the uptake of [^{14}C]taurocholate in the presence of 0.02mM of octreotide. No significant effect was obtained by the addition of 1 mM TEMA.

DISCUSSION

The mechanism of membrane transport and organ distribution for peptide drugs such as β-lactam antibiotics (7,8) and insulin (16) was previously described; these studies demonstrated that carrier-mediated transport systems and receptor-mediated endocytosis play a very important role in the elimination pathways of peptide-drugs from the body. Since hepatic elimination is the major pathway of octreotide in the body and since most octreotide is excreted in bile as an intact form after systemic administration (4), hepatic uptake mechanism of octreotide is of biochemical and pharmacokinetic interest to understand the elimination mechanisms in the body.

Significant inhibitory effects by addition of ouabain, a typical $Na^+ - K^+$ ATPase inhibitor, or by replacement of sodium ion with choline in the incubation medium (Table I) suggest that temperature-, concentration- and energy-dependent uptake of octreotide (Figs. 2, 3, Table I) is governed by a sodium dependent and carrier-mediated process. However, we cannot exclude the possibility that the significant reduction by the replacement of choline (Table I) might be attributed to the involvement of cationic transport system which could also be postulated from the inhibition effect of d-tubocurarine (Table II). Based on the kinetic analysis from Fig. 3 which does not consider nonsaturable uptake process, one can not assume that octreotide passes through the hepatic plasma membrane by a passive diffusion process. Regarding the driving force of peptide-drug transport into the liver, it has been recently reported that the cyclopeptide c(Phe-Thr-Lys-Trp-Phe-D-Pro)(008), an analogue of somatostatin with retro sequence, is taken up by hepatocytes in a membrane potential dependent manner instead of sodium dependent manner (17). On the other hand, phalloidin, a cyclic peptide, was reported to be taken up by hepatocytes in a partially sodium-gradient and membrane-potential dependent manner (18). Although there would be some possibility that the uptake of octreotide might be influenced by the membrane-potential difference, these reported carrier-mediated systems may be different from the transport system of octreotide shown in the present study. Further elaborate study would be necessary to clarify the membrane-potential effect on the octreotide uptake.

The competitive inhibitory effect of taurocholic acid on the uptake of octreotide (Fig. 4) suggests that octreotide is taken up into hepatocytes via the common transport system of taurocholic acid in the liver. The inhibition constant of taurocholic acid obtained was 81.7±17.0 μM (Fig. 4) and was in the same order of magnitude than the reported half-saturation constant of taurocholic acid transport rate (15±0.9 μM) (19). Since iodipamide was reported to be a competitive inhibitor of bile acid transport in the liver (20), the significant inhibitory effect of this compound (Table II) supports this assumption of octreotide transport system being common to that of bile acids. Moreover, a significant inhibitory effect of octreotide (500 μM) on the uptake of [^{14}C]taurocholic acid was obtained (Table III). Assuming a competitive inhibition by octreotide of the [^{14}C]taurocholic acid uptake, the inhibition constant of octreotide was estimated to be 129 μM which is very similar to the half-saturation constant of octreotide uptake rate (91.1±28.4 μM) (Fig.4). These results suggest a sodium dependent octreotide transport and are in accordance with previously reported results showing that a sodium dependent carrier-mediated transport system is one of the important pathways of taurocholic acid in the liver (19). Although EMD 51921, a linear peptide with renin-inhibiting activity, has been reported to be transported by a multispecific bile acid transport system in the liver, the membrane potential but no Na^+ gradient was a driving force for this transport which was not inhibited by phalloidin and iodipamide(13). These characteristics were different from those of octreotide, therefore the octreotide transport system is suggested to be different from that of EMD 51921 in the liver. Additional studies on the structure-transport relationship of bile acid transport carriers may enable us to increase the biological half-life of cyclic peptide drugs such as somatostatin analogues and also of linear peptide drugs such as renin inhibitors.

As shown in Fig. 2, the apparent steady state C/M ratio of octreotide was 3-fold greater than the intracellular volume of hepatocytes, 2.5 μL/mg protein(21), whereas the value was 40-fold smaller than that of taurocholic acid, 300 μL/mg protein(22). One of the possible explanations for the difference of C/M ratio between octreotide and taurocholic acid may be the difference of intracellular binding.

Hepatic Na^+/bile acid cotransport system has been well characterized by several investigators (19–25). A 48-kDa glycoprotein has been identified as the carrier protein of the transport system (26). A recent study of functional expression cloning revealed that the carrier protein of Na^+/bile acid cotransport system consists of 362 amino acids whereas the uptake rate of taurocholic acid can be inhibited by several non-bile acid organic compounds including BSP, an amphipathic organic anion (25). Our results showing a significant inhibitory effect of BSP on [^{14}C]octreotide uptake into isolated hepatocytes (Table II) appear to be in well accordance with the results reported previously (25). Considering the fact that BSP inhibited the hepatic Na^+ dependent taurocholic acid transport in a non-competitive manner (23), the inhibitory effect of BSP on [^{14}C]octreotide uptake would not mean conclusively that octreotide is transported via an organic anion transport system of BSP. As DIDS, an anion exchange inhibitor has been reported to inhibit hepatic uptake of taurocholic acid in a non-competitive manner (27), the inhibitory effect of DIDS on the transport of octreotide (Table II) may be ascribed to a similar non-competitive effect. No inhibition effect of bilirubin on the octreotide uptake (Table II) suggests that octreotide uptake system is different from that of bilirubin which was taken up by hepatocytes in a Na^+ independent manner (28). Recently, we have revealed that cationic oligopeptides, E-2078 and ebiratide are transported by brain capillary endothelial cells via an absorptive-mediated endocytosis (29,30). The absence of inhibitory ef-

fects of E-2078 and ebiratide on [^{14}C]octreotide uptake as shown in Table II suggests that absorptive-mediated endocytosis would not play a role for the octreotide transport in the liver cells. Additionally, TEMA, an organic cation, did not inhibit [^{14}C]octreotide uptake (Table II), supporting the idea that an organic cation transport system would not play a role for the octreotide transport. In contrast, d-tubocurarine, which is also an organic cation, inhibited the octreotide uptake (Table II). As it was reported that bulky amphipathic organic cation such as vecuronium is transported via multispecific uptake system for taurocholate (25), the inhibitory effect of d-tubocurarine might be attributed to the bile acid transport system instead of the organic cation transport system.

In conclusion, the present studies demonstrate that octreotide is transported via a carrier-mediated transport system which is common to that of taurocholic acid in the liver. Although the transport mechanism of octreotide at the bile canalicular membrane has not been clarified yet, the present findings would provide an invaluable insight into the understanding of elimination mechanisms of octreotide in the body after drug administration.

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