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Comparison of bile acid binding to sinusoidal and bile canalicular membranes isolated from rat liver

Kiyoto Yachi [a], Yuichi Sugiyama [a], Tatsuji Iga [a], Yusei Ikeda [a], Gotaro Toda [b] and Manabu Hanano [a]

[a] *Faculty of Pharmaceutical Sciences, and* [b] *The First Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo (Japan)*

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Simon et al. (J. Clin. Invest., 70 (1982) 401) studied cholate binding to crude liver plasma membrane vesicles and suggested that the binding may represent mainly the binding to the receptor (carrier) on the canalicular membrane. This hypothesis was supported by finding a good correlation between the number of cholate binding sites on liver plasma membrane and the maximal rate of biliary secretion (T_m) for taurocholate. We studied bile acid binding to sinusoidal and canalicular membrane vesicles isolated from rat liver by a rapid filtration technique. Scatchard analysis of the saturation kinetics showed both [^{3}H]cholate and [^{3}H]chenodeoxycholate bind to two classes of binding site on each membrane. However, little difference was observed between the binding to sinusoidal and canalicular membrane vesicles for each bile acid (cholate, K_{d1} = 10.4 and 19.8 μM, n_1 = 31.0 and 23.6 pmol/mg protein, K_{d2} = 1.32 and 1.73 mM, n_2 = 13.1 and 23.4 nmol/mg protein; and chenodeoxycholate, K_{d1} = 0.207 and 0.328 μM, n_1 = 36.7 and 27.4 pmol/mg protein, K_{d2} = 1.16 and 2.26 mM, and n_2 = 20.6 and 24.2 nmol/mg protein; numbers show the mean values for sinusoidal and canalicular membrane vesicles, respectively). Chenodeoxycholate binding to sinusoidal membrane vesicles was markedly inhibited by cholate but not by Rose bengal, an organic anion dye. These studies indicate that both membranes (sinusoidal and canalicular membrane vesicles) have two kinds of binding site for bile acids, although no clear difference in the binding properties was observed between the two membranes. Consequently, the cholate binding Simon detected may represent the binding not only to canalicular membrane vesicles but also to sinusoidal membrane vesicles.

Introduction

Bile acids are efficiently extracted from the portal blood across sinusoidal membranes by hepatic parenchymal cells [1,2] and after intracellular translocation are excreted across the canalicular membranes into the bile [3]. The initial hepatic uptake of bile acid is characterized by saturation kinetics, competitive inhibition, and requirement for sodium [4–7]. On the other hand, the canalicular excretion are poorly understood. The canalicular membrane domain is figuratively the blind side of the cell, which gives technical problems in the kinetic study on the biliary excretion of bile acids. However, a high bile-to-liver concentration ratio of bile acids, saturation kinetics, and competitive

Correspondence: T. Iga, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

inhibition with bile acids [3,8,9] also suggest a carrier-mediated process at the canalicular membrane.

Most models for the carrier-mediated transport involve the binding of the transported ligands to specific receptor sites [10]. Specific bile acid binding sites in liver surface membrane fractions have been identified and characterized by Accatino and Simon [11]. The specific binding characteristics have met the requirments for the receptor, including the saturation, specificity, and reversibility [11]. Further support has been obtained by correlating the number of bile acid (cholate) binding sites with the maximal rate of biliary secretion (T_m) for taurocholate [1]. In these studies they used the Neville technique [12] to prepare liver plasma membrane fractions. Although the Neville method provides a highly enriched plasma membrane fraction, it contains both sinusoidal and bile canalicular membranes [4]. However, they suggested that they were probably measuring the putative bile canalicular carrier (receptor) in their binding studies, from the observations that the alteration of the maximal number of binding sites were correlated well with that of T_m (presumably a measure of canalicular secretion capacity) and that the measured bile acid binding did not show sodium dependency [4].

Recently, several methods which separate sinusoidal and canalicular membrane vesicles [13–16] have been reported. However, no reports have been published using these fractions to characterize bile acid binding. The aim of the present study is to compare the binding properties of cholate and chenodeoxycholate between the sinusoidal and bile canalicular membrane fractions. This approach may allow further insights into the carrier system of both sinusoidal and canalicular membranes.

Methods

Preparation of rat liver sinusoidal membrane vesicles

Sinusoidal and canalicular membrane vesicles were prepared from male Sprague-Dawley rat liver according to the method of Blitzer and Donovan [14] and Inoue [15], respectively, and suspended in 10 mM Hepes-Tris buffer (pH 7.4)/0.25 M sucrose/0.2 mM $CaCl_2$/10 mM $MgCl_2$ (standard buffer) and stored in small portions. In order to check the purity of prepared vesicles, $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activity was determined by the method of Schoner et al. [17]. Alkaline phosphatase activities were determined in 5 mM *p*-nitrophenylphosphate/5 mM $MgCl_2$/50 mM 2-amino-2-methylpropanol-HCl (pH 10.0). After 20 or 30 min of incubation at 37 °C, the reaction was terminated by addition of 10% trichloroacetic acid. The denatured protein was removed by centrifugation, the clear supernatant was neutralized by addition of 0.1 M NaOH, and the amount of *p*-nitrophenol released was determined by the absorbacy at 420 nm. Acid phosphatase activity was determined in 5 mM β-glycerophosphate/50 mM acetate-NaOH (pH 5.0). After 30 min of incubation at 37 °C, the reaction was terminated by addition of 10% trichloroacetic acid. Following the removal of denatured protein by centrifugation the amount of inorganic phosphate in the clear supernatant was estimated by the method of Fiske and SubbaRow [18]. Leucine aminopeptidase activities were determined by the method of Goldbarg and Rutenburg [19]. Glucose-6-phosphatase activity was determined by the method of Nordle and Arion [20]. Succinate dehydrogenase activity was determined by the method of Gutman et al. [21]. Protein was determined by using Bio-Rad protein assay kit with bovine serum albumin as standard.

Binding assay

Binding of [^{3}H]cholate (16.0 Ci/mmol, New England Nuclear, Boston, MA) and [^{3}H]chenodeoxycholate (36.8 Ci/mmol, New England Nuclear) was measured by a rapid filtration technique [6]. The incubation medium contained 0.25 M sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM Hepes-Tris (pH 7.4) and varying concentrations of unlabelled cholate and chenodeoxycholate. Other additions are indicated in the individual experiments.

The binding reaction was started by adding membrane veiscle suspension (approximately 20 μg protein) to the incubation medium to complete a reaction volume of 0.11–0.15 ml. The reaction mixture was incubated for 10 min at 37 °C, and the binding reaction was terminated by diluting the mixture with 1 ml of ice-cold buffer compris-

ing 0.25 M sucrose/0.2 mM $CaCl_2$/10 mM $MgCl_2$/10 mM Hepes-Tris (pH 7.4)/0.1 M NaCl. The diluted sample was immediately filtered through a Millipore filter (HAWP, 0.45 μm), which was quickly washed once with 5 ml of the ice-cold buffer to separate vesicle-associated ligands from free ligands. Washed filters were removed from holders with the vacuum on, placed in scintillation vials containing 10 ml liquid scintillation cocktail (0.1 g of POPOP, 4.0 g of DPO, and 500 ml of Triton X-100 per liter of toluene) and the radioactivity was determined in a Packard Tri-Carb counter (model 3255, Packard Instruments Co., Inc., Downers Grove, IL). Quench was determined by automatic external standardization. All values were corrected for the amount of radioactivity found on the filter in the absence of vesicles.

Determination of binding parameters

Binding parameters for the specific bile acid binding were estimated according to the following equation:

$$r = \frac{n_1 \cdot C_f}{K_{d1} + C_f} + \frac{n_2 \cdot C_f}{K_{d2} + C_f}$$

In this equation, r is the amount of bound bile acid per mg of membrane protein at the free bile acid concentration, C_f. The first and the second terms in the equation represent the binding to the primary and secondary binding sites, respectively, where K_d is the apparent dissociation constant and n is the maximal binding capacity for each site. The above equation was fitted to the binding data sets by an iterative non-linear least-squares method using a MULTI program [22] to obtain the estimates of the binding parameters. The input data were weighted as the reciprocal of the square of the observed values, and the algorithm used for the fitting was the Damping Gauss Newton Method in MULTI [22].

Results

Preparation of liver plasma membrane vesicles

The specific activity of $(Na^+ + K^+)$-ATPase, a marker enzyme of sinusoidal membrane, in sinusoidal membrane vesicles increased 39-fold relative to that in the crude homogenate, whereas in canalicular membrane vesicles only 3-fold increase was observed. On the other hand, the specific activities of leucine aminopeptidase and alkaline phosphatase, which are considered markers of the bile canalicular membrane, in canalicular membrane vesicles increased 44- and 50-fold relative to that in the crude homogenate, respectively, whereas in sinusoidal membrane vesicles 3- and 6-fold increases were observed, respectively. The relative enrichments of the specific activities of glucose-6-phosphatase (a marker of microsomes), acid phosphatase (a marker of lysosomes), and succinate dehydrogenase (a marker of mitochondria) were less than 1.2 except for that of acid phosphatase (3.1) in sinusoidal membrane vesicles.

Bile acid binding to membrane vesicles

Typical results for the binding measurements of cholate and chenodeoxycholate to sinusoidal (a) and canalicular (b) membrane vesicles are shown in Figs. 1 and 2, respectively. Bile acids may be transported into vesicles as well as exhibiting binding to membranes. However, the binding ratios (bound/free) normalized by the membrane protein concentrations ranged approx. from 3 to 23 μl/mg protein for cholate (Fig. 1) and from 10 to 150 μl/mg protein for chenodeoxycholate (Fig. 2), and these values are much larger than those in the intravesicular volume (0.5–1 μl/mg protein [6,7]), suggesting the minimum contribution of transport to the membrane binding. This is also supported from the previous study [23] showing that the increasing osmolarity in the medium did not alter the cholate binding to liver plasma membranes. Scatchard plots (Figs. 1 and 2) revealed at least two classes of binding site except for the cholate binding to canalicular membrane vesicles. In the cholate binding to canalicular membrane vesicles, the model of two classes of the binding site yielded little improvement of fit (judged by the weighted residual sum of squares) over that of a single class of binding in two experimental data sets among the three. However, considering the facts that the two classes of binding were observed for the other combinations, the binding parameters of cholate to canalicular membrane vesicles calculated based on the two classes of binding site are listed in Table I. The dissociation constants (K_d) and the

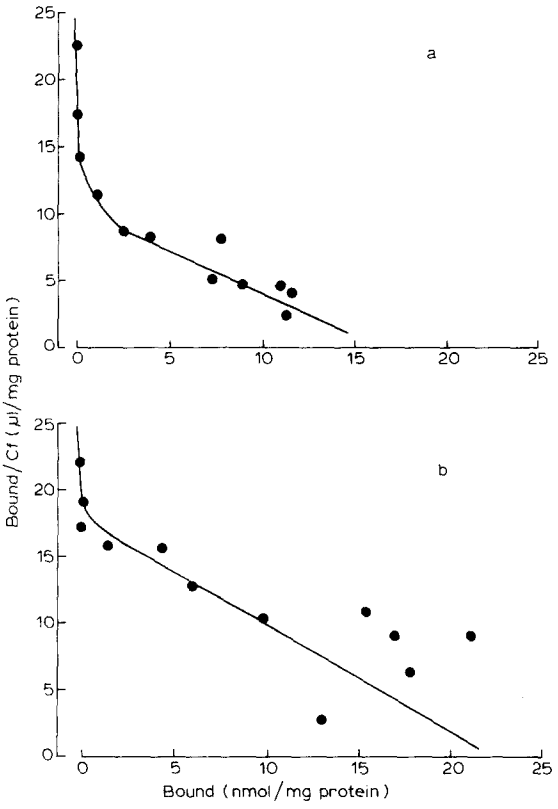

Fig. 1. Typical results for the Scatchard plots of [^{3}H]cholate binding to sinusoidal membrane vesicles (a) and canalicular membrane vesicles (b). The assay mixture contained, in a final volume of 0.11 ml, 0.25 mM sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 10 mM Hepes-Tris (pH 7.4), membrane vesicles (approximately 20 μg proten), and varying concentrations of cholic acid (0.2–5000 μM). After the incubation at 37 °C for 10 min, binding reaction was termined by the dilution of the whole reaction medium with 1 ml of ice-cold buffer. Binding was determined by a rapid filtration technique (see 'Methods'). The line is a theoretical curve calculated by the non-linear least-squares method.

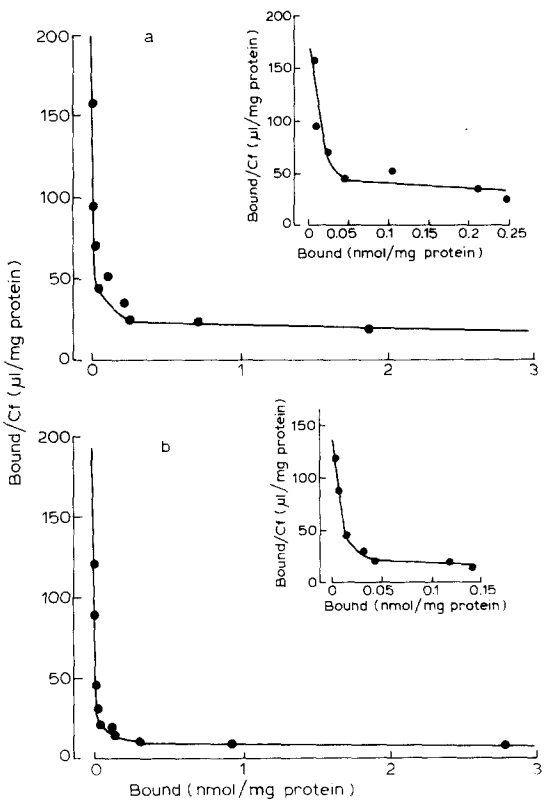

Fig. 2. Typical results for the Scatchard plots of [^{3}H]chenodeoxycholate binding to sinusoidal (a) and canalicular membrane vesicles (b). The assay mixture contained in a final volume of 0.15 ml, buffer described in the legend of Fig. 1, membrane vesicles (approx. 20 μg), and varying concentrations of chenodeoxycholate (0.05–300 μM). Other conditions were the same as described in the legend to Fig. 1. Inset: Scatchard plot of chenodeoxycholate binding at lower concentrations (0.05–10 μM). Lines are the theoretical curves calculated by the non-linear least-squares method.

TABLE I

BINDING PARAMETERS OF CHOLATE AND CHENODEOXYCHOLATE (CDCA) BINDING

Each value represents the mean ± S.E. obtained from three independent preparations. Binding parameters were estimated by an iterative nonlinear least-squares method. SMV and CMV are sinusoidal and canalicular membrane vesicles, respectively.

		High-affinity site		Low-affinity site	
		K_{d1} (μM)	n_1 (pmol/mg protein)	K_{d2} (mM)	n_2 (nmol/mg protein)
Cholate	SMV	10.4 ±5.2	31.0± 4.4	1.32±0.21	13.1±1.8
	CMV	19.8 ±0.9	23.6± 1.0	1.73±0.41	23.4±1.3 *
CDCA	SMV	0.207±0.036	36.7±10.9	1.16±0.35	20.6±2.5
	CMV	0.328±0.079	27.4± 6.5	2.26±0.59	24.2±1.6

* $P < 0.01$.

maximal binding capacities (n) for the binding of cholate to canalicular membrane vesicles calculated based on a single class of binding site model are 1.75 ± 0.32 mM and 25.3 ± 3.2 nmol/mg protein, respectively, which are almost the same as the binding parameters for the lower-affinity site in the two classes of binding site model. Little difference was observed between the binding parameters to sinusoidal and canalicular membrane vesicles for either cholate or chenodeoxycholate, except for the binding capacity of the low-affinity site (n_2) for cholate. The n_2 value for cholate in canalicular membrane vesicles was approximately twice that in sinusoidal membrane vesicles.

In comparison of the binding characteristics between cholate and chenodeoxycholate, a remarkable difference was observed in the dissociation constant for the high-affinity site (K_{d1}), in sinusoidal and in canalicular membrane vesicles. That is, the binding affinity of chenodeoxycholate for the high-affinity site is approx. 50-times as high as that of cholate.

It has been indicated that the hepatocellular uptake of bile acids by the liver occurs predominantly by a process separated from that which takes up organic anions, like bilirubin, sulfobromophthalein and Rose bengal [24,25]. From this point of view, the inhibitory effects of cholate and Rose bengal upon the binding of chenodeoxycholate to sinusoidal membrane vesicles were examined (Fig. 3). Our preliminary experiment showed that the value of K_d for the binding of Rose bengal to sinusoidal membrane vesicles is approx. 0.1 μM (data not shown). The concentrations of cholate (1 mM) and Rose bengal (10 μM) used in the present experiments are approximately two orders higher than the corresponding K_d values, and therefore would be high enough to inhibit the binding of chenodeoxycholate to sinusoidal membrane vesicles, if these compounds share the common binding site with chenodeoxycholate. As shown in Fig. 3, 1 mM cholate inhibited the high-affinity binding of chenodeoxycholate to sinusoidal membrane vesicles. The theoretical line (Fig. 3a), which was drawn assuming that cholate and chenodeoxycholate share the common high-affinity site, is consistent with the experimental data. However, one

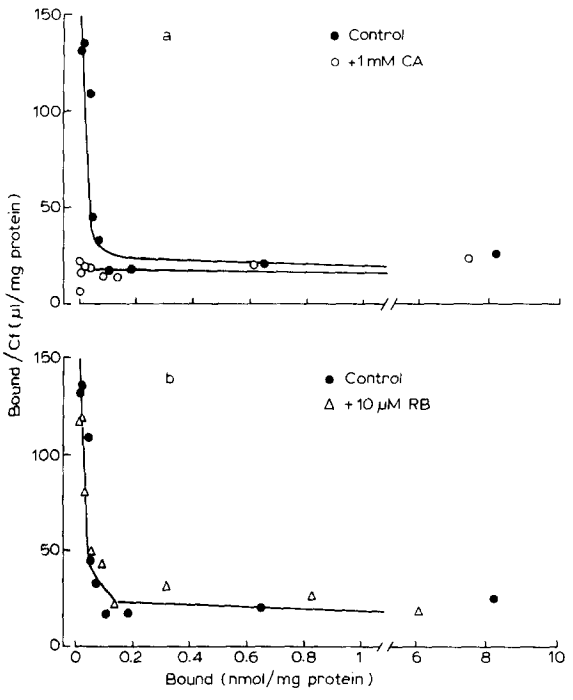

Fig. 3. Typical results for the Scatchard plots of chenodeoxycholate binding to sinusoidal membrane vesicles in the presence (○) or absence (●) of cholate (1 mM) (a) and Rose bengal (10 μM) (b). Lines are theoretical curves calculated by a non-linear least-squares method. The lines in the presence of cholate were calculated assuming that cholate competitively inhibits the high-affinity binding of chenodeoxycholate.

cannot conclude the common binding site between the two bile acids only from these kinetic data, since the degree of inhibition is too large to obtain the precise inhibition constant for cholate. On the other hand, 10 μM Rose bengal shows no inhibition for chenodeoxycholate binding to sinusoidal membrane vesicles (Fig. 3b). These results are consistent with the conventional concept that bile acids and organic anions do not share the common carrier responsible for the hepatic uptake.

Discussion

The apparently specific binding of bile acids to the liver plasma membrane was first reported by Accatino and Simon [11], who proposed the binding sites thus identified were specific membrane receptors. Outside the liver, such specific binding sites had been detected in the ileum and the

kidney, which were known to transport bile acids. They found that the decrease in the binding sites on the liver plasma membrane produced by protein synthesis inhibitors and the increase that followed chronic bile acid feeding paralleled corresponding decreases and increases in the T_m value (presumably a measure of canalicular secretion capacity) [23,26,27]. From these observations together with the lack of sodium dependency of specific cholate binding to the liver plasma membrane, they suggested that they were probably measuring the putative canalicular carrier [23,26]. However, the liver plasma membrane they prepared by the method of Neville appear to include the sinusoidal (basolateral) domain as well as the bile canalicular domain, since the relative enrichments of both $(Na^+ + K^+)$-ATPase and alkaline phosphatase were more than 20 [11,23]. Consequently, we attempted to compare the specific binding activities of bile acids between the sinusoidal and canalicular membrane fractions. A high degree of purification of the two separated liver plasma membrane fractions is crucial for the comparison of the bile acid binding activities between the two domains. A previous study by Meire et al. [13] has shown that the presence of $(Na^+ + K^+)$-ATPase activity in canalicular membrane vesicles is a sensitive criterion to test for the eventual cross contamination with basolateral (or sinusoidal) membrane fraction. The measurement of $(Na^+ + K^+)$-ATPase indicates that the canalicular membrane vesicles preparations are maximally contaminated with sinusoidal membrane components to an extent of approx. 10%. Similarly, based on the specific activities of alkaline phosphatase and leucine aminopeptidase, the sinusoidal membrane vesicles preparations are considered to be contaminated to an extent of approx. 10% with canalicular membrane components. Scatchard analysis of the saturation kinetics showed both cholate and chenodeoxycholate bind to two classes of binding site on each membrane (Figs. 1 and 2). However, little difference was observed between the binding to sinusoidal and canalicular membrane vesicles for either bile acid, except the binding capacity of the low affinity site (n_2) for cholate (Table I) and this difference only in the n_2 value for cholate was too small to suggest that the bile acid binding occurs predominantly to canalicular membrane vesicles. The n_2 value of canalicular membrane vesicles for cholate is approx. 1.8 times that of sinusoidal membrane vesicles. Taking the cross-contamination of each membrane into consideration, the difference in the n_2 values between canalicular and sinusoidal membrane vesicles would become a little larger, as considered below. If we define the apparent and true ratio of the n_2 values (canalicular/sinusoidal) as α_{app} and α, respectively, and if the degree of cross-contamination is 10%, the relationship, $\alpha_{app} = (1 + 9\alpha)/(9 + \alpha)$, may hold. Consequently, the α value of 2.1 can be calculated from the α_{app} value of 1.8. Thus, the difference of the binding parameters between sinusoidal and canalicular membrane vesicles is still small, even if we consider the cross-contamination of each membrane preparation. Therefore, it may be speculated that the specific bindings of cholate and taurocholate Simon et al. [11,23,26] identified represent the bindings not only to canalicular but also to sinusoidal membrane vesicles. If so, good correlations between the binding capacity for cholate and the T_m value they found [23,26] may be accounted for by a hypothesis that the treatment with a protein synthesis inhibitor and chronic bile acid feeding decreased or increased the numbers of transport carriers both on canalicular and sinusoidal membrane vesicles. However, further direct evidence must be obtained to demosntrate the validity of this assumption. The lack of sodium dependency of the specific cholate binding to the liver plasma membrane will not give an evidence that the binding to the putative canalicular carrier is measured [23], since it is well known that the sodium dependency was observed only in the initial uptake of bile acid by sinusoidal membrane vesicles but not in the binding (or uptake) at the equilibrium condition [6,7].

The binding parameters ($K_d = 1$–2 mM, $n =$ 10–20 nmol/mg protein) of cholate for the low-affinity binding sites on both membranes are similar to those ($K_d = 1.3$ mM, $n = 18$ nmol/mg protein) detected by Accatino and Simon [11], although they did not identify the high-affinity binding sites. Multiple binding sites on the liver plasma membrane were also reported by Anwer et al. [28]. It seems to be difficult to determine which binding site corresponds to the transport carrier.

Recently, many studies have been performed to obtain the half-saturation constant (K_m) for the initial uptake of taurocholate and cholate either by isolated hepatocytes [29–32], cultured hepatocyte [33,34] or isolated rat liver plasma membrane vesicles [6,7,36]. When the rate of the diffusion of the ligand-carrier complex across the membrane is assumed to be much slower than the rates of the association and dissociation of the ligand and the carrier (this is usually the case), the K_m represents the dissociation constant for the binding of ligand to the carrier [35]. Consequently, it may be interesting to compare the K_m values obtained from the uptake studies and the K_d values obtained from the binding studies. The K_m values for taurocholate ranged from approx. 4 to 60 μM, and those for cholate from 10 to 70 μM in these studies. In addition, the K_m value for taurocholate in sinusoidal membrane vesicles is comparable with that in canalicular membrane vesicles (approx. 50 μM) [6,7,35]. Thus, the similarity of K_m values of cholate obtained from the transport studies to K_d values (10–20 μM) for the high-affinity binding site obtained from the binding studies (Table I) favors the hypothesis that the high-affinity binding sites correspond to the transport carrier. On the other hand, good correlations between the binding capacities (which correspond to the low affinity binding site in our study) and the maximum transport excretion described above may favor the hypthesis that the low-affinity binding site corresponds to the transport carrier. A possibility that the high- and low-affinity binding sites may correspond to the transport carriers in the sinusoidal and canalicular domain, respectively, cannot be excluded, either.

Recently, lipophilic bile acids such as chenodeoxycholate and lithocholate have been suggested to enter hepatocytes predominantly by nonsaturable, passive diffusion [34,37], and traverse the liver cell presumably after bound to the intracellular binding proteins such as glutathione *S*-transferases, bile-acid binders I and II [38,39]. On the other hand, some studies using isolated hepatocytes have shown that chenodeoxycholate is also taken up by a carrier-mediated process [31,40]. Therefore, we also characterized the binding characteristics of a lipophilic bile acid, chenodeoxycholate to liver plasma membrane. The binding parameters of chenodeoxycholate showed no significant difference between sinusoidal and canalicular membrane vesicles, and the binding affinity of chenodeoxycholate for the higher-affinity site is approx. 50-times as high as that of cholate (Table I), while the binding capacities (n_1, n_2) for chenodeoxycholate are comparable with those for cholate. The comparable binding capacity for the high-affinity site (n_1) between the two bile acids and the inhibition of cholate for the chenodeoxycholate binding to sinusoidal membrane vesicles may suggest that the two bile acids share the common high-affinity binding site. This is contrary to the finding that an organic anion, Rose bengal, did not inhibit the chenodeoxycholate binding to sinusoidal membrane vesicles (Fig. 4). These results support the prevailing concept that bile acids and organic anions are taken up into the hepatocytes by different carriers [24]. The uptake of chenodeoxycholate by isolated rat hepatocytes was previously studied [31,40], and both saturable ($K_m = 33$ μM) and non-saturable passive processes were identified. This K_m value differs from either the K_d value for the high- or the low-affinity binding sites which were detected in the present binding studies. At present, the mechanism for this discrepancy is unclear, but the recognized difficulties of Scatchard analysis in detecting multiple binding sites may account for this discrepancy. In other words, it is difficult to detect the binding site with the K_d value of 33 μM from the Scatchard plots with data points of the present study, even though the corresponding binding component is hidden. Alternatively, the technical difficulties in quantifying the bile acid uptake by isolated hepatocytes or the usage of the different preparations (hepatocytes versus membranes) may be related to this discrepancy.

In conclusion, sinusoidal and canalicular membrane fractions isolated from rat liver have comparable binding properties for bile acids. Consequently, the cholate bindings which Simon et al. [23,26] found to be well correlated with the maximal rate of biliary secretion for taurocholate may represent the binding not only to canalicular but also to sinusoidal membrane vesicles.

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References

1 Glasinovic, J.C., Dumont, M. and Erlinger, S. (1975) J. Clin. Invest. 55, 419–426
2 Reichen, J. and Paumgartner, G. (1975) Gastroenterology 68, 132–136
3 Wheeler, H.O. (1972) Arch. Intern. Med. 130, 533–541
4 Schwarz, L.R., Burr, R., Schwent, M., Praff, E. and Grein, H. (1975) Eur. J. Biochem. 55, 617–623
5 Reichen, J. and Paumgartner, G. (1976) Am. J. Physiol. 231, 732–742
6 Inoue, M., Kinnie, R., Tran, T. and Arias, I.M. (1982) Hepatology 2, 572–579
7 Meier, P.J., Meier-Abt, A. St., Barrett, C. and Boyer, J.L. (1985) J. Biol. Chem. 259, 10614–10622
8 Wheeler, H.O. (1975) in Diseases of liver, (Schiff, L. ed.), pp. 87–110, J.B. Lippincott, Philadelphia
9 Paumgartner, G., Sauter, K., Schwartz, H.P. and Herz, R. (1973) in The liver. Quantitative Aspects of Structure and Function (Paumgartner, G. and Preisig, R., eds.), pp. 337–343, S. Karger, Basel
10 Pardee, A.B. (1986) Science 162, 632–63
11 Accatino, L., and Simon, F.R. (1976) J. Clin. Invest. 57, 496–508
12 Neville, D.M., Jr. (1968) Biochim. Biophys. Acta 154, 540–552
13 Meier, P.J., Sztul, E.S., Reuben, A. and Boyer, J.L. (1984) J. Cell Biol. 98, 991–1000
14 Blitzer, B.L. and Donovan, C.B. (1984) J. Biol. Chem. 259, 9295–9301
15 Inoue, M., Kinne, R., Tran, T., Biempica, L. and Arias, I.M. (1983) J. Biol. Chem. 258, 5183–5188
16 Simon, F.R. Molitoris, B.A. and Sutherland, E. (1984) Gastroenterology 86, 1253
17 Shoner, W., Ilberg, C. Kramer, R. and Seubert, W. (1967) Eur. J. Biochem. 1, 334–343
18 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
19 Goldbarg, J.A., and Rutenburg, A.M. (1958) Cancer 11, 283–291
20 Nordle, R.C. and Arison, W.J. (1966) Methods Enzymol. 9, 619–625
21 Gutamn, M. Kearney, E.B. and Singer, T.P. (1971) Biochem. Biophys. Res. Commun. 44, 526–532
22 Yamaoka, K., Tanigawara, Y., Nakagawa, T. and Uno, T. (1981) J. Pharmacobio. Dyn. 4, 879–885
23 Simon, F.R., Molitoris, B.A., Horton, M. and Sutherland, E. (1984) Falk Symposium 42, Enterohepatic Circulation of Bile Acids and Sterol Metabolism (Paumgartner, G., Stiehl, A. and Gerok, W. eds.), MTP, Lincoln
24 Berk, P.D., and Stremmel, W. (1986) in Progress in Liver Disease. Vol. VIII (Popper, H. and Schaffner, F., eds.), pp. 125–144, Grune & Stratton, Orlando
25 Sugiyama, Y., Kimura, S., Lin, J.H. Izukura, M. Awazu, S. and Hanano, M. (1983) J. Pharm. Sci. 72, 871–876
26 Simon, F.R., Sutherland, E.M. and Gonzalez, M. (1982) J. Clin. Invest. 70, 401–411
27 Gonzalez, M.C., Sutherland, E. and Simon, F.R. (1979) J. Clin. Invest. 63, 684–694
28 Anwer, M.S., Kroker, R., Hegner, D. and Peter, A. (1977) Hoppe-Seyler's Z. Physiol. Chem. 358, 543–553
29 Schwarz, L.R., Burr, R., Schwenk, M., Pfaff, E. and Greim, H. (1975) Eur. J. Biochem. 55, 617–623
30 Ohkuma, S. and Kuriyama, K. (1982) Steroids 39, 7–19
31 Iga, T. and Klaassen, C.D. (1982) Biochem. Pharmacol. 31, 211–216
32 Anwer, M.S. and Hegner, D. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 181–192
33 Scharschmidt, B.F. and Stephens, J.E. (1981) Proc. Natl. Acad. Sci. USA 78, 986–990
34 Van Dyke, R.W., Stephens, J.E. and Scharschmidt, B.F. (1980) Am. J. Physiol. 239, G439–G444
35 Scheler, W. and Blanck, J. (1977) in Kinetics of Drug Action (Van Rossum, J.M., ed.), pp. 3–62, Springer-Verlag, Berlin
36 Inoue, M., Kinnie, R., Tran, T. and Arias, I.M. (1984) J. Clin. Invest. 73, 659–663
37 Duffy, M.C., Blitzer, B.L. and Boyer, J.L. (1983) J. Clin. Invest. 72, 1470–1481
38 Sugiyama, Y., Yamada, T. and Kaplowitz, N. (1983) J. Biol. Chem. 258, 3602–3607
39 Stolz, A., Sugiyama, Y., Kuhleukamp, J. and Kaplowitz, N. (1984) FEBS Lett. 177, 31–35
40 Bartholomew, T.C. and Billing, B.H. (1983) Biochim. Biophys. Acta 754, 101–109