Substrate specificities of rat oatp1 and ntcp: implications for hepatic organic anion uptake

Soichiro Hata,1 Pijun Wang,1 Nicole Eftychiou,1 Meenakshisundaram Ananthanarayanan,2 Ashok Batta,3 Gerald Salen,3 K. Sandy Pang,4 and Allan W. Wolkoff5

1Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Bronx 10461; 2Department of Pediatrics, Mt. Sinai School of Medicine, New York, New York 10029; 3Department of Medicine, Division of Gastroenterology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark 07103; Gastrointestinal Research Laboratory, Veterans Affairs Medical Center, East Orange, New Jersey 07018; and 4Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 2S2

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Bile acids are a group of amphipathic compounds synthesized from cholesterol within the hepatocyte, conjugated with the amino acids glycine or taurine, and excreted into bile (19). Conjugation reduces the acidic dissociation constants of bile acids and increases their aqueous solubilities at physiological pH (11). The majority of bile acids that are excreted into bile are reabsorbed by the apical Na+/dependent bile acid transporter (asbt) from the terminal ileum (33) into the portal circulation from which they are taken up by hepatocytes (11, 46). This recovery process is so efficient that only 5% or less of the bile acid pool needs to be synthesized each day to maintain homeostasis (11). Besides their role as physiologically important detergents that are essential for biliary cholesterol secretion and intestinal absorption of lipid components, bile acids interact with specific nuclear orphan receptors and regulate cholesterol catabolic pathways (10, 11, 13).

Transport of bile acids by the hepatocyte has been a subject of intense study for many years (41, 46). Both Na+/dependent and -independent transporters of bile acids have been described in the liver. According to studies in the isolated perfused rat liver (35, 36) as well as in isolated rat hepatocytes (22, 41, 46), it appears that ~75% of the uptake of taurocholic acid (TCA) is Na+/dependent. A Na+/dependent bile acid transporter Na+/taurocholate cotransporting polypeptide (ntcp) was subsequently expressed cloned from rat liver cDNA (17, 29). A structurally unrelated transporter termed organic anion transporting polypeptide 1 (oatp1) was shown to mediate Na+/independent transport of TCA through an electroneutral ion exchange mechanism (20, 37, 42). Although it has been suggested that these rat liver transporters have broad specificities for other bile acids (30, 43), much of this information is limited to only a few bile acids: cholic, TCA, glycocholic (GCA), tauroursodeoxycholic, and taurochenodeoxycholic (12, 23, 30, 38). As discussed below, in recent studies, Kramer and colleagues (2, 25) examined rabbit ntcp-mediated transport of a large variety of bile acid derivatives. In contrast to ntcp, little is known regarding the bile acid specificity of oatp1.

In the present study, we prepared HeLa cell lines that were stably transfected with ntcp or oatp1 under regulation of a metallothionein promoter, by using techniques we described previously (34, 42). We uti-
lized these stably transfected HeLa cells to quantify the abilities of rat ntcp and oatp1 to mediate transport of cholic, TCA, GCA, taouroursodeoxycholic, and tauro-
chenodeoxycholic acids as well as 18 other conjugated and unconjugated bile acids of varied hydroxylation patterns and side-chain lengths (Fig. 1A and Table 1). We then tested the hypotheses that different patterns exist for the uptake of di- and trihydroxy bile acids and their amidates and that transporter specificities are similar between ntcp and oatp1.

MATERIALS AND METHODS

Radiolabeled ligands. Structures of bile acids used in this study are shown in Fig. 1A and Table 1. Norcholic acid was prepared according to Schteingart and Hofmann (39). Trihydroxyprostanoic acid was obtained from the bile of Alligator mississippiensis (4). Lagodeoxycholic acid was prepared according to Batta et al. (3). All other bile acids were purchased from Steraloids (Wilton, NH). Unconjugated bile acids were labeled with $^3$H via sodium $^3$H-borohydride reduction of the respective o xo compounds prepared according to published methods and purified by preparative thin-layer chromatography (TLC) in a solvent system of chloroform/methanol/acetic acid, 40:4:2 vol/vol/vol, or chloroform/methanol/acetic acid, 40:2:1 vol/vol/vol (3). The $^3$H glycine- and tauine-conjugated bile acids were prepared from the respective $^3$H labeled unconjugated bile acids by the method of Tseng et al. (45) and purified by preparative TLC (5). The radiolabeled bile acids had specific activities of ~10–200 µCi/µmol and were >98% pure as judged by TLC zonal radiolabel scanning. $[^3]$Sulfobromophthalein ([$^3]$S)BSP was prepared at a specific activity of ~6,000 µCi/µmol as described previously (26).

Preparation of HeLa cell lines stably transfected with Rat ntcp or oatp1. HeLa cells stably transfected with rat oatp1 under regulation of a metallothionein promoter were prepared as we previously described (42). Similar methods were used to prepare HeLa cells that were stably transfected with rat ntcp. In brief, full-length rat ntcp, cloned in the Blue script KS(+) plasmid (1), was first digested with Sac1, and the two ends filled in by the Klenow procedure. The ntcp insert was then excised from the plasmid with XhoI and gel purified. The zinc-inducible vector pMEP-4 (Invitrogen) was digested with BamH1, and the two ends were filled in by the Klenow procedure. pMEP-4 was then digested with XhoI and was ligated with the ntcp insert. HeLa cells were transfected with pMEP-4-ntcp by using Lipofectamine Plus. Cells were cultured in selective medium containing 550 µM of hygromycin. After 14–18 days, resistant colonies were selected and expanded in selective medium. Expression of ntcp was induced by culture of cells in 100 µM ZnSO4 for 24 h, followed by the further addition of 50 µM ZnSO4 for the final 18–20 h before use. Without zinc exposure, transporters were not expressed, and these cells served as controls to assess the ability of HeLa cells to transport compounds in the absence of exogenous transporter expression.

Immunoblot analysis. HeLa cells were extracted with 0.1 M Na2CO3 to enrich the yield of integral membrane proteins (6). Basolateral plasma membrane was prepared from rat liver as described previously (8, 18). Proteins were resolved on 10% SDS-PAGE, and immunoblots were performed by a chemiluminescence procedure by using rabbit antibody to oatp1 prototypic substrate [35S]BSP is a substrate of ntcp (18) as we have described previously (6). In some studies, HeLa cell proteins or basolateral plasma membranes were N deglycosylated by using N-glycosidase F (New England Biolaboratories, Beverly, MA) according to the manufacturer’s instructions.

Transport studies. Uptake of [3H] bile acids by HeLa cells transfected with either ntcp cDNA or oatp1 cDNA was quantified as described previously (37, 42). The possibility that the oatp1 prototypic substrate [35S]BSP is a substrate of ntcp was also examined. In brief, noninduced and zinc-induced cells were initially washed three times with serum-free medium (SFM) consisting of (in m) 135 NaCl, 1.2 MgCl2, 0.81 MgSO4, 27.8 glucose, 2.5 CaCl2, and 25 HEPES, pH 7.2, and incubated at 37°C or 4°C. SFM (1 ml) containing ~50,000 to 100,000 cpm of radiolabeled ligand was then added. At appropriate times, uptake was stopped by removal of the incubation solution and washing of the cells five times with SFM at 4°C. The third wash contained 5% BSA and was allowed to stand for 5 min at 4°C. Cells were then harvested, and radioactivity was determined. On plotting the cell-associated counts vs. time, the linear portion of the profile was used to determine the initial uptake velocity. The concentrations of the ligands used were 1 µM for BSP and some C24 bile acids of high specific activities (~200 µCi/µmol), 4 µM for GCA, and 20 µM for cholic acid, chenodeoxycholic acid, ursodeoxycholic acid, the C23-norcholic acid, the C27-trihydroxyprostan
toic and their tauro- and glycylen conjugates, compounds of low specific activities (10 µCi/µmol). Preliminary studies revealed that the incubation times for linear velocity existed within 5 min for transport studies conducted at 1 to 4 µM and
15 min for transport studies conducted at 20 μM. Cell protein was determined in replicate plates by the BCA assay (Pierce) according to the manufacturer’s instructions by using BSA as the standard. The accumulation of substrates within the zinc-induced and noninduced cells was normalized with time and protein concentration to yield the rates (pmol·min⁻¹·mg protein⁻¹), and the fold change was estimated as the ratio of induced/noninduced cells. In studies that were performed to examine the Na⁺ dependence of uptake, Na⁺ in SFM was replaced by K⁺, as we have described previously (49). Saturation uptake studies were further conducted for taurodeoxycholic acid (TDCA), taurohyodeoxycholic acid (THDCA), hyodeoxycholic acid (HDCA), TCA, and BSP (see Fig. 1) for 5 min with HeLa cells stably transfected with ntcp or oatp1 cDNAs. The concentrations of the bile acids varied from 0.5 to 200 μM, and from 0.25 to 120 μM for BSP.

Inhibition studies. Inhibition studies were conducted in HeLa cells transfected with ntcp or oatp1 cDNA. TCA or BSP was used as the substrate, with the alternate (BSP or TCA) used as the inhibitor in parallel incubations. Substrate concentrations varied from 0.5 to 200 μM for TCA, and from 0.25 to 100 μM for BSP. Inhibitor concentrations for BSP varied from 0, 1, 10, and 50 μM, whereas those for TCA were 0, 1, 10, and 100 μM.

### Data fitting

Uptake data from zinc-induced, transfected HeLa cells were fit to Eq. 1

\[ u_{\text{uptake}} = \frac{V_{\text{max}}[S]}{K_m + [S]} + k[S] \]  

(1)

where \( u_{\text{uptake}} \) represents initial uptake, with \([S]\) denoting the concentration of ligand, \( K_m \) and \( V_{\text{max}} \) denoting the Michaelis-Menten constant and the maximum velocity, respectively, and \( k \) representing noncarrier-mediated diffusion uptake (15).

Because the noncarrier-mediated uptake rates for TCA and BSP by HeLa cells stably transfected with ntcp or oatp1 were zero, transport data in the absence and presence of inhibitor of concentration (II), were fit simultaneously to Eq. 2 or 3 for competitive and noncompetitive inhibition, respectively

\[ v_{\text{comp \_uptake}} = \frac{V_{\text{max}}[S]}{K_m + \frac{1}{(K_i + [S])}} \]  

(2)

\[ v_{\text{noncomp \_uptake}} = \frac{V_{\text{max}}}{(1 + \frac{[S]}{K_i})} \]  

(3)

### Table 1. Summary of ntcp- and oatp1-mediated transport of bile acids and BSP

<table>
<thead>
<tr>
<th>Bile Acids</th>
<th>Induced Uptake</th>
<th>Noninduced Uptake</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurocholic acid (C24)</td>
<td>16.8 ± 2.6</td>
<td>13.1 ± 0.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Glycocholic acid (C24)</td>
<td>46.4 ± 9.7</td>
<td>36.0 ± 1.5</td>
<td>12.9</td>
</tr>
<tr>
<td>23-Nor-cholic acid (C23)</td>
<td>7.7 ± 1.3</td>
<td>7.0 ± 0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Hydroxydeoxycholic acid (C24)</td>
<td>20.7 ± 5.8</td>
<td>0.44 ± 0.1</td>
<td>47.1</td>
</tr>
<tr>
<td>Cholic acid (C24)</td>
<td>33 ± 3.7</td>
<td>26.8 ± 1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Murocholic acid (C24)</td>
<td>51 ± 6.4</td>
<td>36.0 ± 1.2</td>
<td>14.1</td>
</tr>
<tr>
<td>Taurodeoxycholic acid (C24)</td>
<td>38 ± 2.2</td>
<td>32.7 ± 3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Glycochenodeoxycholic acid</td>
<td>40.5 ± 13.8</td>
<td>2.8 ± 1.4</td>
<td>14.4</td>
</tr>
<tr>
<td>Glycochenodeoxycholic acid</td>
<td>23.4 ± 2.4</td>
<td>2.5 ± 0.6</td>
<td>24.5</td>
</tr>
<tr>
<td>Glycochenodeoxycholic acid</td>
<td>9.2 ± 1.3</td>
<td>3.0 ± 0.9</td>
<td>3.1</td>
</tr>
<tr>
<td>Glycochenodeoxycholic acid</td>
<td>12.6 ± 2.4</td>
<td>2.5 ± 0.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Glycochenodeoxycholic acid</td>
<td>9.3 ± 1.4</td>
<td>1.0 ± 0.5</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Values are means ± SE of 3 or 4 rats. R₁–R₄ corresponds to the positions on the diagram of bile acids in Fig 1A; ntcp, Na⁺/taurocholate cotransporting polypeptide; oatp1, organic anion transporting polypeptide 1; BSP, sulfoconjugated bile acids to be transported.
where $K_i$ is the inhibition constant. It is generally acknowledged that the $K_i$ for competitive inhibition is the $K_m$ for the inhibitor. The data for experiments performed in the absence of the inhibitor were also utilized for fitting with Eq. 1. Simple fitting was conducted with SigmaPlot (version 6.1; SPSS, Chicago, IL) or Scientist (MicroMath Scientific Software, Salt Lake City, UT), and complex fitting was conducted with the program SCIENTIST version 2 (MicroMath Scientific Software). Three weighting schemes (unity, $1$/predicted, and $1$/predicted$^2$) were used. The optimized fit was found with a weighting of unity that was associated with the minimum sum of squared residuals, the highest value of the model selection criterion (MSC), and the least coefficient of variation (standard deviation of the parameter estimate/value of estimate). The resultant $K_m$ for substrate was compared with that obtained for the $K_i$ when the substrate was employed as an inhibitor. The appropriateness of the model (competitive or noncompetitive inhibition) was provided in the comparison of $K_m$ and $K_i$, and further appraised by the standard deviation of the parameter estimates, the MSC, the residual plot, and the residual sum of squares.

**Statistical analysis.** Data are presented as means ± SE or means ± SD, where indicated, and the means were compared by ANOVA or the paired Student’s $t$-test statistic, accordingly. The level of significance was set at $P = 0.05$.

**RESULTS**

**Characterization of stably transfected cell lines.** In the absence of zinc induction of HeLa cells stably transfected with oatp1 under regulation of a zinc-inducible promoter, expression of oatp1 was undetectable by immunoblot analysis (data not shown), as demonstrated previously (42). Similarly, there was no expression of ntcp in the absence of zinc-induction in HeLa cells that had been stably transfected with ntcp under regulation of the same promoter (Fig. 2). After induction of these cells with zinc, ntcp was expressed; its molecular weight as determined by immunoblot was similar to that in rat liver (~49 kDa). After N-deglycosylation, the molecular weight of this protein decreased to ~33 kDa in both HeLa cells and basolateral liver cell plasma membrane (Fig. 2). This observation is identical to the previously described size of N-deglycosylated ntcp in rat liver (18). As reported previously, there is no effect of zinc on uptake by control or vector-transfected HeLa cells (42). As shown in Table 1, ntcp mediated the uptake of 1 μM TCA and BSP. As seen in Fig. 3, ntcp-mediated transport of these compounds was sodium dependent.

**Fig. 2.** Immunoblot analysis of ntcp expression in transfected HeLa cell lysates and basolateral liver cell plasma membrane (bLPM). Total protein (50 μg) was applied to each lane. In HeLa cells stably transfected with Na$^+$-taurocholate cotransporting polypeptide (ntcp) cDNA, ntcp was not detectable in the absence of zinc induction. Moreover, substantially higher ntcp expression was obtained in the zinc-induced cells compared with bLPM. Molecular weight of the expressed ntcp was similar to that in bLPM. After N-deglycosylation, the molecular sizes of the proteins in both HeLa cells and bLPM decreased to ~33 kDa.

**Fig. 3.** Na$^+$ dependence of the transport of 1 μM [3H]taurocholic acid ([3H]TCA) (A) and 1 μM [35S]sulfobromophthalein ([35S]BSP) (B) in HeLa cells stably transfected with ntcp cDNA and induced to express the protein with 48-h zinc incubation (filled bars). Controls without zinc incubation in which ntcp was not expressed were also studied (open bars). Dependency on sodium was shown by the much higher uptake rates of TCA and BSP in buffer containing Na$^+$ buffer vs. that in which Na$^+$ was replaced with an equivalent amount of K$^+$. 

$\text{G832 OATP1- AND NTCP-MEDIATED ORGANIC ANION TRANSPORT}$
Studies of chain length, hydroxylation position, and patterns on bile acid transport mediated by ntcp and oatp1. In the uptake studies that used these cell lines, any difference in ligand transport between zinc-induced and noninduced cells could be attributed to transporter expression, and the ratio (zinc-induced/noninduced) of uptake rates provides the proclivity of transporter function. The composite results on the transport of the bile acid derivatives are summarized in Table 1. The striking observation was the much poorer, and insignificant uptake of nonamidated compounds compared with their tauroconjugates [compare deoxycholic acid and TDCA, lagodeoxycholic acid and TLDCA, HDCA, and THDCA, and murocholic acid and tauromurocholic acid (TMCA)] for both ntcp and oatp1 at low bile acid concentrations (Table 1). The configuration of the hydroxyl group (α or β) at position 6 (compare THDCA and TMCA) or 12 (compare TDCA and TLDCA) did not influence uptake. The trihydroxy C24 bile acids [cholic acid (CA), TCA, and GCA] are all substrates of ntcp and oatp1. Interestingly, ntcp-mediated uptake of the conjugates of cholic acid (trihydroxy) was four- to eight-fold below that of the dihydroxy bile acid conjugates (Table 1). Aside from better transport of trihydroxy bile acids by ntcp, the only other substantial difference between oatp1- and ntcp-mediated transport was seen with the unconjugated dihydroxy bile acid, hyodeoxycholate (3α, 6α-OH), in which uptake was enhanced by oatp1 expression but not by ntcp expression (Table 1).

Due to low specific activities (10 μCi/μmol) and restricted quantities, uptake studies of additional bile acids were confined to oatp1-mediated transport over 15 min at 20 μM. Although this concentration is relatively high, it is well within the range that is found in portal blood (11). Studies included C24-dihydroxy (chenodeoxycholic and ursodeoxycholic) bile acids, and trihydroxy bile acids of varying chain lengths (C23-nor-CA, C24-CA, and C27-trihydroxypropionoic acid) and their glyco- and tauroconjugates (Fig. 1A). As seen in Table 1, oatp1-mediated uptake of the conjugates of these trihydroxy bile acids was highest for C27-trihydroxypropionoic acid (8-carbon side chain), intermediate for C23-norcarnic acid (4-carbon side chain), and lowest for C24-cholic acid (5-carbon side chain). Oatp1-mediated uptake of two dihydroxy bile acids with 5 carbon side chains (chenodeoxycholate and ursodeoxycholate) and their glycine and taurine conjugates was also examined. Compared with their trihydroxy counterpart (cholic acid), there was no facilitation of uptake of either of these unconjugated bile acids by oatp1 expression (Table 1). In contrast, uptake of the conjugates of these bile acids was approximately double that of the cholic acid conjugates (Table 1).

Saturation kinetics of transport mediated by ntcp and oatp1. Both TCA and BSP exhibited saturable transport by ntcp and oatp1 (Figs. 5, A and B, Table 2). BSP transport mediated by ntcp and oatp1 was of high affinity with Kₘs of 3–4 μM (Table 2). The Kₘs for TCA transport were slightly higher (11–20 μM; Table 2).
Fig. 5. Saturable uptake of TCA (A) and BSP (B) in the presence of Na⁺ was demonstrated in HeLa cells stably transfected with ntcp cDNA and induced to express the protein with 48-h zinc incubation. The fitted data (Eq. 1) revealed a $K_m$ of 8.2 $\mu$M and maximum velocity ($V_{max}$) of 299 pmol·min⁻¹·mg protein⁻¹ for TCA, and a $K_m$ of 3.3 $\mu$M and $V_{max}$ of 87.3 pmol·min⁻¹·mg protein⁻¹ for BSP. C: influence of albumin (BSA) on oatp1- and ntcp-mediated uptake of 1 $\mu$M [³⁵S]BSP was determined by performing the uptake assay in the presence or absence of 0.025% BSA.
These relatively low $K_m$ values suggest that TCA and BSP are good substrates for each of these transporters. The $K_m$s of two representative taurine-conjugated dihydroxy bile acids, TDCA (3α, 12α-OH) and THDCA (3α, 7α-OH), were also of similar orders of magnitude for both oatp1 and ntcp (Table 2). In contrast, transport of HDCA (hyodeoxycholate) by ntcp-expressing cells did not differ from transport by nonexpressing cells (Table 1) and was not saturable, demonstrating linear uptake with a slope of $29.2 \pm 6.7 \mu l/min\cdot mg^{-1}$.
transport by oatp1-expressing cells was saturable, with a $K_m$ of 17.5 $\mu$M (Table 2). These results indicate that although HDCA is transported by oatp1, it is not transported by ntcp.

**Ntcp and oatp1 transport BSP in the presence of albumin.** Previous studies in isolated perfused rat liver and rat hepatocytes were performed in the presence of BSA. To determine whether albumin inclusion would alter the ability of ntcp or oatp1 to transport BSP, oatp1- and ntcp-mediated BSP transport was quantified in the presence and absence of 0.025% BSA. As seen in Fig. 5C, uptake mediated by either transporter was reduced to $\sim$40% of control levels by albumin inclusion, as we have described previously for oatp1-transfected HeLa cells (42).

**Mutual inhibition by BSP and TCA of their ntcp- and oatp1-mediated uptake.** Because the preceding studies indicated that BSP and TCA are both substrates of ntcp and oatp1, we then determined whether these compounds would be mutual competitors for transport in the ntcp and oatp1 expression systems. The resulting data of the competition experiments (Figs. 6–9), when fit to the competitive (Eq. 2) and noncompetitive (Eq. 3) models, yielded similar coefficient of variation, residual sum of squares, and MSC. However, for both the ntcp (Table 3) and oatp1 (Table 4) uptake studies, the competitive model provided $K_i$ values similar to the corresponding $K_m$ values of the inhibitory compound. The $K_i$ for competitive inhibition of TCA by BSP with ntcp (17 $\mu$M) was similar to the $K_m$ for BSP transport (7.2 and 8.9 $\mu$M) within the same data set (Table 3) and other data sets (3.7 $\mu$M, Table 2); the $K_i$ for noncompetitive inhibition (100 $\mu$M) was considerably higher. Similarly, the $K_i$ for competitive inhibition of BSP by TCA with ntcp (39 $\mu$M) was similar to the $K_m$ for TCA transport (17 and 21 $\mu$M) within the same data set (Table 3) and other data sets (10.5 $\mu$M; Table 2); the $K_i$ for noncompetitive inhibition (182 $\mu$M) was considerably higher. The comments also hold for oatp1 transport (Table 4). The $K_i$ for competitive inhibition of TCA by BSP with oatp1 (8.5 $\mu$M) was similar to the $K_m$ for BSP transport (5.5 and 5.2 $\mu$M) within the same data set (Table 4) and other data sets (3.3 $\mu$M, Table 2); the $K_i$ for noncompetitive inhibition (31 $\mu$M) was considerably higher. Similarly, the $K_i$ for competitive inhibition of BSP by TCA with oatp1 (30 $\mu$M) was similar to the $K_m$ for TCA transport (30 and 22 $\mu$M) within the same data set (Table 4) and other data sets (19.4 $\mu$M; Table 2); the $K_i$ for noncompetitive inhibition (207 $\mu$M) was considerably higher. Discrimination of the fit was afforded by the correspondence between $K_i$ and $K_m$.

**Fig. 8.** Fits of inhibition of TCA uptake in HeLa cells stably transfected with oatp1 cDNA by BSP (0, 1, 10, and 50 $\mu$M) to competitive (A) or noncompetitive (B) models. Initial uptake was determined over 5 min in buffer containing Na+. The competitive inhibition curve model (Eq. 2) fitted the data better than the noncompetitive inhibition model (Eq. 3).

**Fig. 9.** Fits of inhibition of BSP uptake in HeLa cells stably transfected with oatp1 cDNA by TCA (0, 10, 50, and 100 $\mu$M) to competitive (A) or noncompetitive (B) models. Initial uptake was determined over 5 min in buffer containing Na+. The competitive inhibition curve model (Eq. 2) fitted the data better than the noncompetitive inhibition model (Eq. 3).
Table 3. Summary of fitted results from uptake studies of TCA and BSP in the presence of the inhibitor in HeLa cells transfected with ntcp (in the presence of 154 mM sodium ion)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>$K_m$, μM</th>
<th>$V_{max}$, pmol·min$^{-1}$·mg$^{-1}$</th>
<th>$K_i$, μM</th>
<th>MSC</th>
<th>RSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSP</td>
<td>TCA</td>
<td>7.22 ± 2.85</td>
<td>422 ± 336</td>
<td>38.9 ± 18.4</td>
<td>2.74 ± 0.8</td>
<td>25,762 ± 31,807</td>
</tr>
<tr>
<td>(0.25 to 120)</td>
<td>(0, 1 or 10, 50, and 100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSP</td>
<td>TCA</td>
<td>10.4 ± 4.3</td>
<td>468 ± 374</td>
<td>182* ± 52</td>
<td>2.70 ± 0.34</td>
<td>24,965 ± 30,488</td>
</tr>
<tr>
<td>BSP</td>
<td>TCA</td>
<td>17.0 ± 4.8</td>
<td>1,519 ± 481</td>
<td>17.3* ± 12.9</td>
<td>2.77 ± 0.48</td>
<td>379,293 ± 337,143</td>
</tr>
<tr>
<td>(0.25 to 200)</td>
<td>(0.1, 10, and 50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; $n = 3$ results. Composite data were force fit simultaneously to Eqs. 3 or 4 for competitive and noncompetitive inhibition, respectively. Model selection criteria (MSC) were similar as were the residual sum of squares (RSS) data. Only the set of data obtained in absence of inhibitor ($[I] = 0$) was used for fitting. *Significantly different from $K_m$ (composite data of study); †significantly different from $K_m$ (data in absence of inhibitor); ‡not significantly different from $K_m$ (derived from data in absence of inhibitor).

DISCUSSION

Oatp1 and ntcp are Na$^+$-independent and -dependent bile salt transporters, respectively, that are present on the basolateral (sinusoidal) plasma membrane of rat hepatocytes (17, 20, 29, 37, 42). Previous studies revealed that they share the capacity to transport TCA (22), although they are unrelated by amino acid sequence, predicted secondary structure, or topology. Transport mediated by each of these proteins is also mechanistically distinct. Oatp1-mediated uptake of TCA is accomplished by efflux of an anion such as HCO$_3^-$ or GSH, and the net process is electroneutral (27, 37). By contrast, ntcp-mediated uptake of TCA is electrogenic and is accomplished by cotransport of two Na$^+$ for each bile acid molecule (31).

Bile acids represent a diverse class of amphipathic compounds derived from cholesterol. Diversity exists regarding the number and pattern of hydroxyl groups on the steroid rings as well as regarding the length of the side chain and whether the molecule is conjugated with glycine or taurine. Although rat ntcp and oatp1 are thought to be physiologically important transporters of bile acids, previous studies regarding bile acid structure as it relates to transport (12, 23, 30, 38) have been limited to a single trihydroxy bile acid (cholate) and its taurine and glycine conjugates as well as taurine conjugates of two dihydroxy bile acids (ursodeoxycholate and chenodeoxycholate). The present investigation, using HeLa cell lines that were stably transfected with rat oatp1 or ntcp under a zinc-inducible promoter, extends these studies to include a group of pathophysiologically relevant bile acids of varied structures. Kramer and colleagues (2, 25) examined substrate specificity of rabbit ntcp for a number of bile acid derivatives. Compared with the rat and human ntcp counterparts, the rabbit transporter is only 75% and 82% identical, respectively. In that study, rabbit ntcp-mediated uptake of various bile acids was normalized to uptake of TCA (25). Three bile acids (GCA, TDCA, and THDCA) that were used in both studies showed notable differences in uptake between ntcp of the rabbit and rat. Specifically, uptake of GCA was 148% vs. 73%, TDCA was 225% vs. 138%, and THDCA was 249% vs. 165%.

Table 4. Summary of fitted results from uptake studies of TCA and BSP in the presence of the inhibitor in HeLa cells transfected with oatp1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>$K_m$, μM</th>
<th>$V_{max}$, pmol·min$^{-1}$·mg$^{-1}$</th>
<th>$K_i$, μM</th>
<th>MSC</th>
<th>RSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSP</td>
<td>TCA</td>
<td>5.46 ± 3.52</td>
<td>327 ± 251</td>
<td>30.3* ± 4.7</td>
<td>2.44 ± 0.38</td>
<td>22,164 ± 27,192</td>
</tr>
<tr>
<td>(0.25 to 120)</td>
<td>(0, 1 or 10, 50, and 100)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BSP</td>
<td>TCA</td>
<td>7.83 ± 5.51</td>
<td>357 ± 289</td>
<td>207* ± 62</td>
<td>2.37 ± 0.38</td>
<td>17,985 ± 17,628</td>
</tr>
<tr>
<td>BSP</td>
<td>TCA</td>
<td>29.8 ± 28.5</td>
<td>121 ± 51</td>
<td>8.5* ± 12.7</td>
<td>2.38 ± 0.54</td>
<td>2,374 ± 1,160</td>
</tr>
<tr>
<td>(0.25 to 200)</td>
<td>(0.1, 10, and 50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSP</td>
<td>TCA</td>
<td>43.3 ± 33.5</td>
<td>132 ± 54.1</td>
<td>30.91 ± 35.4</td>
<td>2.29 ± 0.66</td>
<td>3,234 ± 2,307</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n = 3$ results. Composite data were force fit simultaneously to Eqs. 2 or 3 for competitive and noncompetitive inhibition, respectively. MSC data were similar as were the RSS data. Only the set of data obtained in absence of inhibitor ($[I] = 0$) was used for fitting. *Significantly different from $K_m$ (composite data of study); †significantly different from $K_m$ (data in absence of inhibitor); ‡not significantly different from $K_m$ (derived from data in absence of inhibitor).
the rat and rabbit proteins could result in significant functional differences.

As seen in Table 1, oatp1 transports conjugated dihydroxy bile acids as well as does ntcp, but is less effective at transport of trihydroxy bile acids. Oatp1 was first identified by its ability to transport \[^{35}S\]BSP. Although previous studies performed in Xenopus oocytes suggest that BSP is a poor substrate for ntcp-mediated transport (16, 30), the present study indicates that this is not the case. As seen in Table 2, both ntcp and oatp1 mediate high-affinity transport of BSP, showing that ntcp has broader substrate specificity than has been previously suspected. In addition, our studies show that, for both transporters, uptake of each of these compounds is competitively inhibited by the other. The relative contributions of oatp1 and ntcp to hepatic transport in vivo have been estimated in the past by indirect means (22, 23). The present results in transfected HeLa cells, in which both ntcp and oatp1 mediate high-affinity transport of BSP and bile acids, contrast with earlier observations in hepatocytes in which BSP transport was found to be fully Na\(^{+}\) independent (32, 49), suggesting no role for ntcp. These results indicate that appropriate caution must be exercised when extrapolating physiological function from in vitro transport data. The reasons for this apparent deviation of in vitro from in vivo data are unexplained, but could result from differences in transporter expression levels as well as the presence of, and possible interactions with, other Na\(^{+}\)-independent and -dependent transport proteins (31, 47).

These data also have important implications for understanding activation of nuclear bile acid binding proteins such as farnesyl nuclear receptor (FXR) and pregnane nuclear receptor (PXR) (9, 13, 28, 40, 48). Bile acids are physiological ligands for FXR that, on binding with bile acids, activates transcription of specific genes (13). Expression of this protein appears to be limited to the liver, kidney, intestine, and adrenals (13, 14). Other data indicate that bile acids also activate another nuclear receptor, PXR (40). PXR is selectively localized to the liver and intestine (21) in which it is essential for regulation of cytochrome P-450 gene expression (21, 50). Bile acid activation of transcription via these nuclear receptors is dependent on the specific bile acid as well as its ability to enter the cell and bind to these proteins (48). The data in the present study indicate that both ntcp and oatp1 have relatively broad specificities for transport of all conjugated bile acids that were examined, although some conjugates are transported more efficiently than others (Fig. 4 and Table 1). In the absence of these transporters, little bile acid conjugate enters the cell, and thus there would be minimal binding to these nuclear receptors. In contrast, our data show that, with the exception of cholic, C23-nor-cholic, and HDCAs, unconjugated bile acids readily enter cells in the absence of these transporters, and that transporter expression has little influence on their cellular accumulation (refer to uptake data in absence of zinc-induction, Table 1). Interestingly, ursoadexycholic acid, one of the bile acids that is taken up by cells in the absence of oatp1 or ntcp expression, is a poor ligand for FXR (48) but a potent ligand for PXR (40). This bile acid is used therapeutically in various hepatobiliary disorders associated with cholestasis (7, 24). Although there have been suggestions that conjugates of ursodeoxycholate would be efficacious, our data show that they require transporter activity for cellular uptake. Because expression of these transporters appears to be downregulated in cholestasis (44), the physiologically beneficial effects of these bile acid conjugates may be substantially muted.

**DISCLOSURES**

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**REFERENCES**


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32. Min AD, Johansen KJ, Campbell CG, and Wolkoff AW. Role of chloride and intracellular pH on the activity of the rat hepato-