Published in final edited form as:

Compr Physiol. 2014 October ; 4(4): 1715–1735. doi:10.1002/cphy.c140023.

Organic Anion Uptake by Hepatocytes

Allan W. Wolkoff^{1,*}

¹The Herman Lopata Chair in Liver Disease Research, Professor of Medicine and Anatomy and Structural Biology, Associate Chair of Medicine for Research, Chief, Division of Gastroenterology and Liver Diseases, Director, Marion Bessin Liver Research Center, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY

Abstract

Many of the compounds taken up by the liver are organic anions that circulate tightly bound to protein carriers such as albumin. The fenestrated sinusoidal endothelium of the liver permits these compounds to have access to hepatocytes. Studies to characterize hepatic uptake of organic anions through kinetic analyses, suggested that it was carrier-mediated. Attempts to identify specific transporters by biochemical approaches were largely unsuccessful and were replaced by studies that utilized expression cloning. These studies led to identification of the organic anion transport proteins (oatps), a family of 12 transmembrane domain glycoproteins that have broad and often overlapping substrate specificities. The oatps mediate Na⁺-independent organic anion uptake. Other studies identified a seven transmembrane domain glycoprotein, Na⁺/taurocholate transporting protein (ntcp) as mediating Na⁺-dependent uptake of bile acids as well as other organic anions. Although mutations or deficiencies of specific members of the oatp family have been associated with transport abnormalities, there have been no such reports for ntcp, and its physiologic role remains to be determined, although expression of ntcp *in vitro* recapitulates the characteristics of Na⁺-dependent bile acid transport that is seen *in vivo*. Both ntcp and oatps traffic between the cell surface and intracellular vesicular pools. These vesicles move through the cell on microtubules, using the microtubule based motors dynein and kinesins. Factors that regulate this motility are under study and may provide a unique mechanism that can alter the plasma membrane content of these transporters and consequently their accessibility to circulating ligands.

Introduction

The liver plays a major, essential role in removing many endogenous and exogenous compounds from the circulation. For the most part, this function is mediated by specific transporters on the sinusoidal (basolateral) plasma membrane of hepatocytes. Following uptake, these compounds can be metabolized within hepatocytes. Metabolites can be released back into the circulation where they can act upon target cells or be excreted in urine. Alternatively, metabolites can be excreted across the bile canalicular (apical) membrane into bile. This review will focus on hepatocyte uptake of organic anions. These compounds include bile acids and nonbile acid organic anions such as bilirubin, sulfobromophthalein (BSP), and various drugs and hormones.

*Correspondence to allan.wolkoff@einstein.yu.edu.

Hepatic Vascular Anatomy Facilitates Hepatocyte Transport

Most of the organic anionic compounds that are removed from the circulation by hepatocytes are relatively small molecules with molecular weights between 400 and 1000 Da (72,90,179). However, many of these compounds have limited aqueous solubility and circulate by virtue of binding to a carrier protein, most often albumin, a protein with a molecular weight of approximately 68,000 Da (53, 133, 179). Importantly, the design of the liver facilitates interaction of protein bound ligands with hepatocyte basolateral plasma membrane transporters by virtue of having a unique fenestrated sinusoidal endothelium rather than a tight capillary endothelium (57, 201) (Fig. 1). These fenestrae have varied sizes that exist as large (1–3 μm in diameter) and small (0.1 μm in diameter) forms (57). Even the large fenestrae are too small to permit entrance of red cells into the Space of Disse while proteins such as albumin can enter relatively freely. The sinusoidal fenestrae are dynamic structures associated with cytoskeletal elements including actin-based microfilaments, and have been shown to have contractile properties following exposure to compounds such as nicotine, alcohol, and serotonin (16, 43). However, the physiologic function of contraction of fenestrae has not been established. The importance of sinusoidal fenestrae in liver transport function can be appreciated from indicator dilution studies performed in isolated perfused rat liver (50, 206) as seen in Figure 2. In this study, a rat was anesthetized and the liver was perfused in situ in a temperature controlled cabinet at 37°C with bovine erythrocytes in bovine serum albumin (BSA)-containing Krebs-Ringer buffer. Following injection into the portal vein of a mixture of ^{51}Cr -labeled red blood cells, ^{125}I -BSA, and ^3H -Bilirubin, hepatic venous effluent was collected in 2 s fractions over 40 s. Radioactivity was quantified in each fraction and plotted as fractional recovery over time. In this study, recoveries of red cells and albumin were 101% and 106% of what was injected. That is, there was no uptake of either of these labels into the liver. In contrast, recovery of bilirubin was only 53% of that injected, indicating that 47% was taken up in this single pass through the liver. Although red cell and albumin recovery was complete, the shapes of their outflow curves were quite different due to distribution of albumin but not red cells into the space of Disse. Red cells appeared in advance of albumin and their outflow curve had a higher maximum and faster appearance. Correspondingly, the peak of the albumin curve was lower and appeared later than that of the red cells. Appearance of bilirubin paralleled that of albumin to which it was bound tightly. The appearance of its peak was essentially identical to that of albumin, but lower due to uptake by hepatocytes. This ability of albumin-bound compounds to quickly leave the vascular space and enter the extravascular extracellular space of Disse is an important component of the hepatic uptake mechanism. Cirrhosis is characterized by “capillarization” of hepatic sinusoids with loss of fenestrations (76,106). Indicator dilution studies performed in cirrhotic patients revealed a loss of accessibility of albumin to the Space of Disse and corresponding reduced extraction of indocyanine green (ICG) (76).

Clearance of Organic Anions from the Circulation

Evidence for the existence of an organic anion transporter

The hepatocyte efficiently eliminates organic anions from the circulation (150). As much as 50% or more of organic anions such as bilirubin, BSP, and various bile acids, are taken up in

a single pass through the liver (145, 161, 162). Multiple studies have shown that the kinetic characteristics of this uptake process are highly compatible with carrier-mediation. For example, following intravenous injection, bilirubin, BSP, and ICG disappeared quickly with half-lives of 1 to 3 min (150). Studies with increasing doses of each of these ligands revealed that uptake was saturable and that uptake of each of these ligands was mutually competitive by the others (150). Ligand that disappeared from the circulation was recovered in liver and showed a “countertransport” phenomenon, whereby injection of a bolus of unlabeled ligand several minutes after injection of a radiolabeled ligand resulted in efflux of radioactivity from the liver back into the plasma (150). Studies performed in isolated perfused livers using a multiple indicator dilution approach also revealed saturation of the uptake process (52, 140, 203). These studies supported the concept that there was a hepatocyte organic anion transporter, providing a stimulus for studies to discover the molecular basis of organic anion transport.

Role of cytosolic binding proteins in organic anion transport

As noted above, radiolabeled derivatives of organic anions such as bilirubin and BSP disappear rapidly from the circulation and are recovered quantitatively in the liver and bile (51, 52). Computer-based modeling of clearance of these compounds suggested discrete steps of membrane uptake, intracellular storage, and bile canalicular membrane excretion (51, 52). Following uptake, fractionation of radioactivity in the liver revealed that the majority was recovered in the cytosol. Gel chromatography of cytosol containing radiolabeled organic anions identified two protein fractions, originally called Y and Z, that contained most of the radioactivity (100). Y protein was subsequently named ligandin. It had been isolated by three groups of investigators who were studying very different processes. One group identified Y protein based on its binding of organic anions (100). Another group identified a cortisol metabolite binding protein (corticosteroid binder I) in rat liver cytosol (124). The third group isolated a carcinogen binding protein (basic azo dye carcinogen-binding protein) based upon recovery of yellow color covalently attached to protein in rat liver cytosol after injection with the azo dye carcinogen, butter yellow (4-dimethylaminoazobenzene) (86). Subsequent studies showed that these proteins were identical, and the term ligandin was used to refer to them (104). Still another group was studying what appeared to be a totally unrelated system in rat liver, glutathione S-transferase activity, and showed that ligandin was identical to glutathione S (GSH)-transferase B (59). Subsequent studies showed that bilirubin and other organic anions could bind to glutathione S-transferase B as well as the other GSH-transferases as nonsubstrate ligands (85) and this family of proteins was termed ligandins (211). It had been hypothesized that these intracellular binding proteins might represent a major component of the uptake mechanism for organic anions (147), but the studies presented above suggested that they were unlikely to influence initial influx of these compounds, as a ligand in the circulation could not know of the existence of an intracellular transport protein to produce the “carrier-mediated” kinetics that had been observed. In support of this concept is the fact that Evans blue, an organic anion, is minimally taken up by the liver (160), even though it binds avidly to ligandin (100).

Following injection of radiolabeled bilirubin into rats, there is rapid formation of conjugated bilirubin that can be recovered in liver homogenate and cytosol (207). Conjugated bilirubin accumulates in liver and is slowly excreted into bile over time (207). This “storage” of bilirubin and its conjugates in liver (13, 207) suggests that this could be related to binding to ligandins. This relationship was tested for bilirubin uptake using a multiple indicator dilution approach in isolated perfused livers from rats in which ligandin content of liver was increased following administration of phenobarbital or surgical thyroidectomy (206). Analysis of these studies showed that there was no relationship between liver ligandin content and influx of bilirubin into the liver, but a strong inverse relationship with efflux from the liver back into the perfusate. Thus ligandin levels correlate with net bilirubin uptake by functionally sequestering bilirubin that has entered the hepatocyte. Similar studies were performed to examine the role of Z protein in uptake of bilirubin and BSP by isolated perfused livers from rats pretreated with clofibrate, a compound that increases hepatocyte content of Z protein without altering ligandin content (169). In contrast to the ligandin studies, doubling of hepatic Z protein concentration had no effect on influx or efflux of either of these ligands (169). Z protein is now known as liver fatty acid binding protein (L-FABP), a member of the FABP family (136, 157). L-FABP binds a variety of fatty acids and is likely involved in aspects of lipid metabolism, although details are still under study (4, 116, 156).

Several proteins that bind bile acids in liver cytosol have also been described (112, 123, 164, 167, 221). Initial studies using gel filtration of rat liver cytosol to which radiolabeled bile acids were added identified proteins of 33 to 36 kDa in a fraction called Y' (163, 167). Y' proteins were distinct from the GSH-transferases and were subsequently identified as having 3 α -hydroxysteroid dehydrogenase activity, likely serving as a 3-oxo-bile acid reductase (164). Other studies showed that bile acids could bind to the Z protein fraction isolated from rat liver cytosol (87, 168). Although Z protein and other members of the FABP family as well as 3 α -hydroxysteroid dehydrogenase can bind bile acids, physiologic function of these proteins in bile acid transport in hepatocytes remains speculative.

Biochemical characterization of the hepatocyte organic anion transport mechanism

The studies presented above provided strong evidence that hepatocyte organic anion transport is mediated by a specific transporter(s). However, transporter identification proved elusive. It was recognized that the plasma membrane of the hepatocyte offered the initial barrier to entry of organic anions into the cell. It was assumed that this was the site of the carrier and initial investigation focused upon binding of organic anions to purified plasma membrane preparations from the liver. Studies by Cornelius and colleagues showed saturable binding of BSP to isolated liver plasma membranes (28). This binding was inhibited by other organic anions including ICG and flavaspidic acid that were known to compete with BSP for uptake by the liver (28). However, their finding that approximately 200 nmoles of BSP bound per mg membrane protein was much higher than would be expected and suggested that there might be a component of nonspecific (nonsaturable) binding that was not accounted for in these studies. Subsequent studies used ³⁵S-BSP, enabling studies to be performed at lower BSP concentrations (144, 204). These studies provided evidence for high-affinity, low-capacity binding of BSP to liver plasma membrane

preparations, consistent with interaction with a transporter. However, these studies did not provide information regarding the identity of the putative transporter.

Photoaffinity labeling of liver plasma membranes with organic anion derivatives was used as an approach to identify transporters (200, 204). In the course of these studies, it was found that ^{35}S -BSP itself could be photoactivated, and bound covalently to a 55,000 kDa protein in rat liver basolateral (sinusoidal) plasma membrane preparations (204). This protein was purified by affinity chromatography on GSH-BSP agarose gel and named organic anion binding protein (oabp) (204). Antibody to oabp was raised in rabbits (210) and was used to clone a cDNA from a rat liver expression library (47). Subsequent analysis showed that this cDNA encoded the β -subunit of F_1 -ATPase (ATP synthase), a known inner mitochondrial membrane protein, and that oabp and the β -subunit were identical proteins (47). Hepatocyte basolateral plasma membrane and mitochondrial localization of oabp were shown by immunoelectron microscopic techniques (47) and subsequent studies confirmed this bilocalization (113, 138). However, whether oabp plays a role in transport of organic anions remains unknown. A similar sized protein was isolated by Berk and colleagues using BSP-agarose affinity chromatography and termed BSP/bilirubin-binding protein (166). Although preliminary studies suggested that antibody to oabp cross-reacted with this protein (166), this could not be verified later by immunoblot (210) and detailed, more conclusive studies were not performed. These investigators reported that high concentrations of antibody to BSP/bilirubin-binding protein partially inhibited the uptake of bilirubin and BSP (165). However, uptake in these studies was determined at high ligand concentrations in the absence of albumin. Further studies showed that BSP/bilirubin-binding protein was also present on the surface of the human hepatoma-derived cell line, HepG2. These cells are unable to transport BSP in the presence of albumin (120), suggesting that BSP/bilirubin-binding protein does not support high affinity organic anion transport characteristic of hepatocytes.

Tiribelli and colleagues took a different approach and developed an assay to spectrophotometrically measure uptake of BSP by membrane and lipid vesicles (5,141,171). This was based upon the fact that BSP at alkaline pH is purple in color and when it enters a more acidic space within cells or vesicles, there is a reduction in the intensity of this color (171). Using this assay in rat liver plasma membrane vesicles, these investigators described electrogenic transport of BSP (5). They went on to isolate a high-affinity BSP binding protein of 37 kDa from an acetone powder of a crude preparation of rat liver membranes (170). They named this protein bilitranslocase (5, 10, 158, 171). In further studies, monoclonal antibodies raised to a crude plasma membrane extract from rat liver were screened for the ability to inhibit BSP transport in rat liver plasma membrane vesicles. These antibodies recognized a 37 kDa band corresponding to the position of bilitranslocase on gel electrophoresis (171), and were used to clone the protein from a λ gt11 rat liver expression library (10). Of note is the fact that the derived sequence (EMBL Y12178) is 96% identical to the inverse sequence of rat ceruloplasmin. This is an unusual finding without clear explanation. In addition to liver, bilitranslocase has been described as present in vascular endothelium, heart, intestinal epithelium, kidney, as well as in plants including carnation petals and grapes (14, 109). The physiological role of bilitranslocase in organic anion transport will require additional clarification.

Physiological characteristics of organic anion transport by hepatocytes

Although, as presented above, a number of candidate organic anion transporters were identified, their function *in vivo* remained unclear. Further studies were directed at better elucidating the driving forces and cellular requirements for hepatocyte organic anion transport, with the ultimate aim of using this information for more definitive elucidation of transporters.

Role of albumin binding in organic anion uptake—Organic anions, including bilirubin and BSP, circulate bound to albumin. This binding is of high affinity so that even at equimolar concentrations, the unbound fraction of ligand is < 0.1% of total (55, 84). Several studies suggested that this small unbound rather than larger bound fraction determines uptake (9, 24, 54, 134). However, a second model was proposed that included a more direct role for albumin in the uptake process and a hepatocyte cell surface albumin receptor was suggested (38, 197). This “albumin receptor” model was based on kinetic studies as well as assumptions concerning ligand-albumin interaction that included binding affinity, number of binding sites, and dissociation rate constants (161). Although an intriguing hypothesis, it has not been supported by a number of subsequent studies. Single pass indicator dilution studies performed in the isolated rat liver perfused with fluorocarbon showed that over a 200-fold range of bilirubin concentration, uptake did not require the presence of albumin (161). In particular, uptake of bilirubin by the liver did not differ under conditions in which it was injected bound to albumin or to ligandin (161). In addition, there was no delay in albumin transit time through the liver as compared to that of sucrose (161). Similar results were found for plasma disappearance of bilirubin in mutant analbuminemic rats (79). Other studies quantified uptake of dibromosulfophthalein (DBSP) bound to albumin or lactosylated albumin by isolated rat liver under recirculating perfusion conditions (177). These studies also showed a reduced off-rate of DBSP from lactosylated as compared to native albumin that corresponded to reduced hepatic uptake. These data are most consistent with a dissociation-limited ligand uptake process that is not specific for albumin (198, 199, 202).

Cellular requirements for organic anion uptake—Further studies were performed in isolated and short-term cultured hepatocytes. These models facilitated manipulation of the cellular environment, permitting requirements for organic anion uptake to be better characterized. Early studies of BSP transport by isolated hepatocytes revealed saturable uptake that did not have an energy requirement as determined by lack of response following incubation of cells in the metabolic inhibitors antimycin A, rotenone, or carbonylcyanide m-chlorophenylhydrazone (153). In contrast, other studies showed reduced uptake of BSP following incubation of hepatocytes with the metabolic inhibitors KCN and DNP (174). This diversity of results is likely due to the fact that this uptake process has variable sensitivity to metabolic inhibitors for reasons that are not known (152). These studies were performed in hepatocytes in suspension. Subsequently, methods were developed for overnight culture of hepatocytes in uncoated plastic culture dishes (208, 209). In these studies, organic anions such as BSP or bilirubin were incubated with cells in a molar excess of albumin in an attempt to better recapitulate physiologic conditions and to direct ligand to high affinity transport sites. Saturable, temperature-dependent uptake of organic anions in which albumin

remained extracellular and was not taken up or bound by cells was seen (209). Uptake of BSP and bilirubin was not affected by substitution of Na^+ in medium by K^+ or Li^+ , but was markedly reduced by isosmotic substitution of NaCl in medium by sucrose or substitution of Cl^- by HCO_3^- or gluconate (209). This property was termed chloride-dependent organic anion uptake, and was seen in isolated perfused rat liver as well as in hepatocytes (209). Although a Cl^- /organic anion exchange mechanism was hypothesized (209), further studies with ^{36}Cl showed that there was no relationship of Cl^- gradients to BSP transport. Rather, it was the presence of Cl^- that was required (121). This was attributed to an apparent increased affinity of the transporter for ligand in the presence as compared to the absence of Cl^- (121). In contrast to results with Cl^- , there was stimulation of BSP transport with an inside to outside OH^- gradient, consistent with OH^- or HCO_3^- exchange or H^+ cotransport (121, 149).

Identification of hepatocyte organic anion transporters

The studies described above provided several key characteristics of organic anion transport by hepatocytes, namely Cl^- -dependent extraction of ligand from albumin. These studies were facilitated by development of methods to prepare ^{35}S -BSP of high specific activity, enabling quantification of transport with high sensitivity at low ligand concentration (99). Using this uptake assay, expression of Cl^- -dependent extraction of ^{35}S -BSP from albumin was demonstrated in *Xenopus laevis* oocytes that had been injected with 50 ng of rat liver mRNA and cultured for 5 days to allow rat liver protein expression (82). Size fractionation of the mRNA prior to injection revealed that virtually all of the transport activity resided in a fraction containing mRNA of 2 to 3.5 kb (82). An expression library was prepared from this fraction and was used to isolate a single cDNA that was able to confer Cl^- -dependent extraction of ^{35}S -BSP from albumin in oocytes (Fig. 3) (83). This cDNA had an open reading frame of 2010 nucleotides and encoded a protein of 670 amino acids that was originally termed organic anion transporting polypeptide (oatp) now known as oatp1a1 (83). Since the initial identification of oatp1a1, over 20 additional members of the oatp family have been described (64,65,67). These transporters are glycoproteins and best modeled as having 12 transmembrane domains (Fig. 4) (188).

Zonal distribution of oatps in the liver—Immunofluorescence studies have shown that oatps in rat, mouse, and human liver are expressed abundantly on the basolateral (sinusoidal) plasma membrane (12, 97, 143, 190). Although the liver may appear as a homogeneous collection of hepatocytes, differences in protein content and function of hepatocytes have been described between those localized near portal veins (zone 1) as compared to those localized near central veins (zone 3). As sinusoidal blood flows from portal to central zones, hepatocytes in these areas have very different microenvironments with respect to molecules such as oxygen, glucose, and drugs that are in high concentration in zone 1 (portal) and are efficiently extracted as they course through the sinusoid, resulting in a gradient of concentrations from portal (high concentration) to central (low concentration) zones. There have been only limited studies to examine the zonal distribution of oatps in the liver. In the rat, oatp1a1 is distributed homogeneously (12). Although oatp2, now known as oatp1a4, was initially described as being distributed primarily in zone 3 (143) this could not be substantiated in further studies in which it was distributed throughout the

liver in zones 1 and 3, but in an uneven, patchy distribution as seen in low power views (105). Human OATP8, now known as OATP1B3 has also been described as having more immunofluorescence in central as compared to portal hepatocytes (92), but these studies also showed limited areas of the liver and did not include a low power survey. Other studies showed zonal changes in oatp expression following bile duct expression (33). However, based on the limited information at hand, it is difficult to conclude that zonal distribution of oatps is an important determinant of their function, at least under normal conditions.

Function of oatps in the liver—The oatps have broad and overlapping substrate specificities (19,67,71). Transported ligands include many drugs used clinically including statins, macrolide antibiotics, and antihistamines (48,60,64,80,91,154). Although substrate specificity is clear when a single member of the oatp family have been expressed *in vitro*, assigning a role for a specific oatp in drug transport *in vivo* is more difficult likely due to overlapping function with other transporters. To define the role of specific oatps in drug transport, several studies have been performed in mice in which the genes encoding specific Oatps were subjected to targeted disruption, resulting in knockout mouse models. When Oatp1b2 was knocked out, mice had reduced liver to plasma ratios of rifampicin, and lovastatin, but not of pravastatin, simvastatin, rifamycin SV, or cervastatin (20). However, changes in clearance were subtle, presumably due to uptake mediated by other transporters. Another study using a different strain of Oatp1b2 knockout mice showed that they were protected from phalloidin and microcystin hepatotoxicity, presumably due to reduced liver clearance (107). Plasma disappearance of DBSP was delayed in the knockout mice and a small increase of conjugated bilirubin was also noted (107). Studies performed in a third strain of Oatp1b2 knockout mice showed reduced clearance of rifampin and a small increase of conjugated bilirubin (218). Reduced liver to plasma ratio of pravastatin was also noted following continuous infusion to reach steady state, again showing that changes in drug clearance can be subtle, requiring special studies to demonstrate them (218). Studies in mice in which Oatp1a1 or Oatp1a4 were knocked out showed reduced uptake of estradiol-17 β -D-glucuronide, estrone-3-sulfate, and taurocholic acid in primary hepatocytes prepared from both mouse strains (49).

To further clarify the physiologic role of Oatp transporters, mice in which all members of the Oatp1a and Oatp1b families were deleted were prepared (176). This was not an organ specific genetic deletion, and aside from liver, transporters from this family were also knocked out in organs such as small intestine and kidney. Interestingly, these mice had markedly delayed plasma clearance of methotrexate and fexofenadine as well as elevated levels of conjugated bilirubin and unconjugated bile acids (176). There was no change in plasma levels of conjugated bile acids, consistent with the observations noted above in Oatp1a1 and Oatp1a4 knockout mice (49). The elevation of conjugated bilirubin levels is similar to what has been described in patients with the rare disorder known as Rotor syndrome, in which there is chronic elevation of conjugated bilirubin in plasma with otherwise normal routine liver function tests (212). In recent studies, these individuals have been found to have simultaneous null mutations in the genes encoding OATP1B1 and OATP1B3 (175). It was suggested that after conjugation with glucuronic acid in the hepatocyte, bilirubin glucuronides are effluxed from the cell into the circulation

subsequently undergoing OATP-mediated reuptake (175, 176). In the absence of both of these transporters, levels of conjugated bilirubin in the circulation rise as is seen in Rotor syndrome patients and the corresponding knockout mouse model.

Pharmacogenomic studies—The clinical and physiological importance of OATP function have also been inferred from pharmacogenomic studies in which single nucleotide polymorphisms of OATPs have been correlated with occurrence of drug toxicity or altered liver uptake (73, 89, 93, 114, 122, 137, 172). Several studies have pointed out the importance of OATPs in clearance of statins from the circulation. For example, naturally occurring polymorphisms of OATP1B1 have been associated with reduced plasma clearance of pravastatin in European and African-American subjects (73), of rosuvastatin in a Korean population (22), and of pitavastatin in a Japanese population (78). That OATP dysfunction could be associated with drug toxicity became clear from a genome wide association study that examined genetic variants in a group of 85 subjects who developed myopathy while taking simvastatin in a trial involving 12,000 participants (103). A strong association was obtained only with a polymorphism in the gene encoding OATP1B1. Importantly, it was reported that this polymorphism (Val174Ala) encodes a protein variant that traffics poorly to the plasma membrane and accumulates within hepatocytes (172). The myopathy presumably was a result of reduced hepatic uptake of the drug accompanied by elevated serum levels (not determined in this study) which then led to increased uptake by muscle cells resulting in toxicity. Although this correlation was highly significant, the actual clinical presentation again shows the subtlety by which altered OATP function may be manifested, as the cumulative risk of developing myopathy was only 18%, and it characteristically took several months to develop (103).

Transport of bilirubin

Based on experimental evidence that includes mutual competition for uptake, it has been inferred that bilirubin shares an uptake mechanism with other organic anions such as BSP (39, 150, 209). However, studies performed in isolated perfused livers from rats treated with the hypolipidemic agent, nafenopin, suggested that this may not be the case (40). Nafenopin causes hepatocellular proliferation similar to that seen in hepatic regeneration following partial hepatectomy (11, 27, 40). Although uptake of BSP (40, 106) and conjugated bilirubin (40) by isolated perfused livers from nafenopin treated rats was reduced by about 50% as compared to controls, uptake of bilirubin was unchanged (40). These results contrast with reduced bilirubin influx that has been described in regenerating rat liver (41) and suggest that nafenopin administration effectively unmasks differences in the uptake mechanisms between bilirubin and other more water soluble organic anions such as BSP and conjugated bilirubin. Although human OATP1B1 (formerly known as OATP2, OATP-C, and LST-1) was identified as the bilirubin transporter in one study (29), this could not be verified in another study (189). In addition, patients with Rotor syndrome who have a genetic double deletion of this protein as well as OATP1B3 have conjugated not unconjugated hyperbilirubinemia (175). Thus, the search for the bilirubin transporter remains ongoing.

Transport of Bile Acids

Bile acids can be considered as a special subclass of organic anions that are transported by hepatocytes. The liver extracts bile acids from the portal blood very efficiently, and the first-pass clearance can be as high as 90% (25, 45, 146). Unlike studies performed with organic anions such as bilirubin and BSP, uptake of bile acids by hepatocytes has a large Na^+ -dependent component (63, 118, 178). Electrophysiological studies in cells suggest a 2:1 stoichiometry of Na^+ :bile acid (102, 196). Na^+ -dependent uptake of bile acids is thus linked to the cellular outside to inside Na^+ gradient established by activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ on the basolateral (sinusoidal) plasma membrane of hepatocytes (119,155). The Na^+ -independent transport of most bile acids is 20% to 25% of total uptake, but can be higher for unconjugated bile acids (178). The molecular structure of a particular bile acid is an important determinant of the efficiency by which it is transported by hepatocytes (69–71). For example, 6-OH bile acids are preferred ligands for hepatocyte Na^+ -dependent uptake (96), while the 3-OH group is not required for bile acid transport, although hydroxylation at this position is present in all naturally occurring bile acids (8).

Identification of hepatocyte bile acid transporters

Microsomal epoxide hydrolase—Early studies to identify the bile acid transporter(s) utilized a strategy involving photoaffinity labeling of liver cell plasma membrane preparations as well as intact hepatocytes (95, 183, 200). These studies identified several candidate proteins with molecular masses of 48,000 to 54,000 kDa. Other studies utilized radiation inactivation to define the functional mass of the bile acid transport mechanism, revealing it to be 170,000 kDa (36) for Na^+ -dependent transport and 107,000 kDa for Na^+ -independent transport (37). Other studies quantified transport of bile acids by proteoliposomes prepared from protein extracts from rat liver plasma membrane preparations (182). These studies revealed a fourfold increase in Na^+ -dependent uptake of taurocholic acid when a protein fraction that included the 54 kDa region had been incorporated as compared to results using unfractionated liver plasma membrane proteins (182). A panel of monoclonal antibodies were prepared against this liver cell membrane fraction and two antibodies were characterized as possibly interacting with the Na^+ -dependent bile acid transporter (182). One of these antibodies recognized 49 and 54 kDa proteins while the other recognized only a 49 kDa protein. Immunopurification and liposome incorporation of the 49 kDa protein revealed a 20-fold enrichment in bile acid transport as compared to results with total solubilized basolateral membrane proteins (185). Preparation of liposomes from basolateral membrane protein preparations following immunodepletion of this protein resulted in a 90% reduction of bile acid transport (185). As compared to results with hepatocytes, this protein had low expression in hepatoma tissue culture cells (184) and in the HepG2 human hepatoma cell line (110). Expression of this 49 kDa protein correlated with the ability to transport bile acids in fetal and neonatal rat liver (1,184). This protein was purified and sequenced, revealing that it was identical to microsomal epoxide hydrolase (mEH), a protein previously identified as being localized to the endoplasmic reticulum (180). It was suggested that this unexpected finding resulted from insertion of mEH into the endoplasmic reticulum membrane in two orientations, one of which targets this protein to the plasma membrane (219). Although transfection of mEH

cDNA into MDCK cells resulted in expression of Na⁺-dependent bile acid transport in one study (181), another study failed to show Na⁺-dependent bile acid transport in a fibroblast cell line stably transfected with mEH cDNA or in hepatoma cell lines that express mEH (75). In response to these negative studies, it was suggested that mEH is only intracellular and not targeted to the plasma membrane in these cells (186). As of the present time, the potential role of mEH in bile acid transport remains an open question.

Na⁺ taurocholate cotransporting polypeptide—Studies similar to those described above for oatp discovery were performed in which Na⁺ dependent bile acid transport was assayed in *Xenopus laevis* oocytes that had been injected with rat liver mRNA (61). These studies resulted in identification of a single cDNA that, following injection into oocytes, resulted in expression of Na⁺ dependent bile acid transport (68). This cDNA encoded a 39 kDa protein with five potential N-linked glycosylation sites and seven transmembrane domains (111) and was termed Na⁺ taurocholate cotransporting polypeptide (ntcp) (Fig. 5). There is a 77% amino acid homology between the rat and human forms of this protein (62). A truncated form of ntcp, termed ntcp2, has been cloned from mouse liver. This product of alternative splicing has a shorter C-terminal end but also mediates Na⁺-dependent bile acid transport (18). Its functional importance and cellular localization are not known. Ntcp is abundantly localized to the basolateral (sinusoidal) plasma membrane of hepatocytes and is distributed homogeneously through zones 1 to 3 of the liver (159).

Although expression of ntcp confers Na⁺-dependent bile acid transport with characteristics resembling the process in liver (119), there has been no direct demonstration of its physiologic role. There have been no reports of preparation of ntcp knockout or knockdown models. The evidence in favor of ntcp representing the major Na⁺-dependent bile acid transporter resides in the fact that the K_m for bile acid transport in ntcp transfected cells is similar to that in hepatocytes and that the decrease of ntcp expression in liver regeneration and in hepatocytes in culture parallels the decrease of Na⁺ dependent bile acid transport in these models (119). It is possible that these physiologic conditions could affect expression and function of other putative bile acid transporters as well. Thus, a direct connection between changes in ntcp expression and bile acid transport in these models remains elusive. The most conclusive evidence that ntcp plays an important role in bile acid transport came from a study in which *Xenopus laevis* oocytes microinjected with rat liver cRNA in the presence of ntcp antisense RNA, had a 95% reduction in taurocholate uptake as compared to oocytes studied in the absence of the anti-sense construct (66, 119). However, since this study was performed, more complete databases indicate that the antisense oligonucleotide that was used to inhibit ntcp expression was not specific for ntcp and could target other rat and *Xenopus* proteins in addition to ntcp (205). Of great interest is the recent observation that NTCP serves as the hepatocyte receptor for Hepatitis B virus (3,81,101,130,131,192,193,214–216). This unexpected finding is consistent with other studies, described below, that show the presence of ntcp-containing intracellular vesicles that traffic between the hepatocyte basolateral (sinusoidal) plasma membrane and cell interior (148).

Unlike its related ileal apical Na⁺-dependent bile acid transporter (asbt) in which mutations and knockout models have distinctive phenotypes related to altered bile acid transport (30–

32), such models have not been described for ntcp. Although a number of potentially functionally disruptive ntcp genetic polymorphisms have been described, there is no information regarding phenotypic abnormalities in these individuals (74). It has also been observed that in a mouse model of erythropoietic protoporphyria resulting from knockout of the ferrochelatase gene, there was no plasma membrane expression of ntcp or oatp1a1 (117). There was bile duct proliferation and biliary fibrosis accompanied by elevated plasma bile acid levels. Despite these abnormalities, a tracer dose of taurocholic acid was cleared rapidly from the circulation and over 90% of the injected dose was recovered in the liver and bile by 30 min of injection (117). Other studies performed in rats 7 days after bile duct ligation showed a greater than 90% reduction in ntcp expression while hepatocytes isolated from these mice still demonstrated substantial Na^+ -dependent uptake of taurocholate (42). These results were in agreement with other studies in which rats were subjected to 5 days of bile duct obstruction after which the obstruction was removed (26). These rats had markedly elevated levels of bile acids in the serum which returned to normal within 60 to 90 min following relief of the obstruction, accompanied by biliary excretion (26). Plasma clearance of tracer taurocholic acid was normal when the biliary obstruction was removed (26), even though ntcp levels in liver would be expected to be markedly depressed (42, 94). Taken together, these studies indicate that ntcp may not be the only determinant of Na^+ -dependent bile acid transport and that future studies with genetic models such as knockout mice would be very helpful for more definitive assessment of this important pathway.

Cellular Physiology of Transporter Function

It has become clear that subcellular distribution of transporters is an important determinant of their function. For example, if the transporter resides entirely within the cell, it will not serve to remove ligands from the extracellular milieu (Fig. 6). Factors that change the subcellular distribution of transporters may thus serve an important regulatory role. A number of studies have been performed to examine such mechanisms for ntcp and oatps.

Regulation of bile acid transport

Down regulation of bile acid transport by hepatocytes has been noted in cholestasis (98, 173) and liver regeneration (56). As discussed above, in the bile duct ligation model of cholestasis, the changes in bile acid uptake by hepatocytes are modest at best and do not correlate well with ntcp levels, although post-translational changes that might alter activity and subcellular distribution of this protein have not been well studied under these conditions. In contrast, during liver regeneration following two-thirds partial hepatectomy in rats, there is a marked reduction in Na^+ -dependent uptake of taurocholic acid (56). Over the week following partial hepatectomy, there is a large increase in serum bile acid levels (44,187), correlating with a 90% reduction in ntcp protein expression 24 hours after partial hepatectomy (56, 187), returning to normal by 1 week (44). Bile acids are also signaling molecules that have important functions that are mediated via activation of nuclear receptors as is reviewed elsewhere in this series (21).

Regulation of ntcp subcellular distribution

Several studies have shown that $V_{\max}$ for bile acid uptake by hepatocytes is increased within minutes of incubation in cAMP (15, 58). Subsequent studies correlated these changes with redistribution of ntcp from an intracellular pool to the plasma membrane, without synthesis of new transporter (125, 148). Immunoblot analysis with phosphoamino acid specific antibodies indicate that ntcp is a serine/threonine phosphoprotein, and that cAMP incubation results in reduced phosphorylation of ntcp through stimulation of phosphatase activity (126, 127). It has been suggested that this results in its increased retention in the plasma membrane. Additional studies were performed in the HepG2 human hepatoma cell line. These cells do not have endogenous expression of ntcp. Incubation of HepG2 cells with cAMP following transient transfection with GFP-ntcp, resulted in a 40% increase in GFP fluorescence at the plasma membrane within 2.5 min (34). This recruitment of intracellular GFP-ntcp to the cell surface was prevented by cytochalasin D, a disruptor of the actin-based microfilament cytoskeleton. In contrast, perturbation of microtubules following incubation of cells in nocodazole prevented even baseline GFP-ntcp from reaching the plasma membrane. Cells regained plasma membrane fluorescence within 2 h after nocodazole removal. These results suggest a model in which targeting of ntcp to the plasma membrane first requires its microtubule-mediated delivery to a sub-plasma membrane location and then transfer to microfilaments that direct it to the cell surface (34).

Other studies were performed to examine trafficking of ntcp *in vitro* in a cell free system (148). These studies utilized a preparation from rat liver that was enriched in endocytic vesicles (6, 7, 128). Ntcp was present in this preparation as demonstrated by immunoblot and by immunofluorescence (148). These studies utilized a fluorescent microtubule coated microassay chamber with an approximately 5 μ L capacity (129). Vesicles flowed into this chamber attach spontaneously to microtubules and those that contained ntcp were identified following incubation in primary antibody followed by fluorescent secondary antibody (148). These vesicles were seen to move on the microtubules following addition of 50 μ mol/L ATP (Fig. 7). By immunofluorescence it was seen that ntcp-containing vesicles were associated with the minus-end directed microtubule motor dynein, and with the plus-end directed microtubule motors kinesin-1 and kinesin-2 (148).

Previous studies in cells and animals suggested that trafficking of ntcp is regulated at least in part by the PI3 kinase/PKC ζ pathway (115,194,195). A simplified schematic of the PI3 kinase/PKC ζ pathway and its relationship to vesicle motility is shown in Figure 8. Addition of LY294002, an inhibitor of PI3 kinase, resulted in reduced vesicle motility, presumably from reduced formation of PIP3 from vesicle-associated PIP2. Addition of PIP3, an activator of PKC ζ , enhanced vesicle motility. Addition of PIP3 with LY294002 overcame its inhibition, while addition of PKC ζ pseudosubstrate, a potent inhibitor of PKC ζ , in the presence or absence of PIP3 virtually eliminated vesicle motility. These data show that PKC ζ is an important regulator of the motility of ntcp-containing vesicles (148). Interestingly, the pseudosubstrate had no effect on motility of late endocytic vesicles, indicating that this regulatory pathway has specificity for ntcp-containing vesicles (148).

Additional studies were performed to determine whether these findings could be extended to HuH7 cells that had been transiently transfected with rat ntcp-GFP (148). Previous studies

showed that HepG2 cells transfected with a plasmid encoding this recombinant protein gained Na⁺-dependent bile acid transport and that this fluorescent protein translocated to the plasma membrane in response to cAMP (34). In HuH7 cells transfected with unlinked GFP, a diffuse pattern of cellular fluorescence was observed (148). In cells transfected with ntcp-GFP, fluorescence was clearly localized to the plasma membrane as well as intracellular vesicles (148). Movement of these fluorescent intracellular vesicles was studied in living cells using time-lapse epifluorescence microscopy. Microscopy was performed in the presence or absence of a cell permeant inhibitor of PKC ζ . Dose-dependent inhibition of ntcp-GFP containing vesicle motility was observed. There was a nearly 80% reduction in the number of moving vesicles in the presence of 50 μ mol/L inhibitor. That this effect on vesicle movement was specific for ntcp-GFP-containing vesicles was shown by lack of response to this inhibitor by late endocytic vesicles (148). The target of PKC ζ activity as it relates to ntcp trafficking is not as yet known. Additional studies suggested that PKC δ also plays a role in ntcp subcellular trafficking, although this did not seem to be dependent on its kinase activity (3, 139). Additionally, S-nitrosylation of ntcp has been described as reducing plasma membrane localization of ntcp (3, 142, 151). This has been attributed to S-nitrosylation of cysteine 96 of ntcp, but elucidation of the mechanism by which this affects subcellular trafficking remains to be determined (142).

Regulation of nonbile acid organic anion transport

Similar to studies of bile acid transport, modulation of organic anion transport has been described as accompanying several physiological perturbations. For example, in rats, influx of bilirubin and BSP falls by approximately 50% within 6 h of liver regeneration resulting from two-thirds partial hepatectomy, returning to normal by 4 days (41, 135), and correlating with changes in oatp1a1 mRNA and protein levels (220). Transport is also modulated during development as seen by BSP uptake that is 70% lower in hepatocytes prepared from 3-week-old rats as compared to adults (2). This correlates with reduced oatp1a1 protein and mRNA expression in liver for the first month after birth (2, 35). Cholestatic events have also been associated with reduced organic anion transport. For example, uptake of ICG by isolated perfused livers of rats following administration of endotoxin, an agent that causes cholestasis, is reduced by 40% as compared to controls (108). Although oatp1a1 mRNA was unchanged, oatp1a1 protein expression was reduced by 50%.

Other studies showed that uptake of BSP by rat hepatocytes is sensitive to extracellular ATP (17). Within minutes of exposure of cells to ATP, there is an 80% reduction in BSP uptake that correlates with reduced $V_{\max}$ and unchanged K_m (17, 46). This effect appears to be mediated by a purinergic receptor (17). The phosphatase inhibitors okadaic acid and calyculin produced a similar effect (46) and phosphorylation of oatp1a1 was hypothesized. This hypothesis was confirmed in hepatocytes preloaded with inorganic ³²P, by finding that incubation in unlabeled extracellular ATP resulted in serine phosphorylation of oatp1a1 with the appearance of a single major tryptic phosphopeptide (46). Interestingly, BSP uptake in HeLa cells stably expressing oatp1a1 is unaffected by incubation in ATP or phosphatase inhibitors. There is no phosphorylation of oatp1a1 in these cells and it is suggested that they lack the kinase that mediates this effect (46). To establish the site of phosphorylation, tryptic digests of immunoaffinity-purified oatp1a1 were analyzed by mass spectrometry (213). A

single tryptic phosphopeptide with unphosphorylated and singly and doubly phosphorylated forms was discovered near the C-terminus. MS/MS analysis of this peptide revealed that phosphorylation at S634 accounted for all singly phosphorylated peptide, while phosphorylation at S634 and S635 accounted for all doubly phosphorylated indicating that oatp1a1 phosphorylation is an ordered process, in which phosphorylation at S634 precedes that at S635 (213). Studies in transfected cells and primary rat hepatocytes suggest that phosphorylation of oatp1a1 results in its internalization and consequent loss of transport activity (Fig. 9) (23).

Regulation of oatp subcellular distribution

Seven of the oatps that have been described in rat, mouse, or human liver have PDZ consensus binding sites, as defined by their C-terminal 4 amino acid sequences (77, 88, 217). There are over 150 PDZ domain-containing proteins to which a protein with a PDZ consensus site could bind (217). PDZ domain-containing proteins can have multiple binding domains and can form functionally important complexes with their protein ligands (217). It must be noted that for a given protein with a PDZ consensus binding site, there is no way to predict to which, if any, of these PDZ domain-containing proteins, it will bind. Consequently, although many oatps have these consensus domains, their significance is not straightforward. This issue was examined using rat oatp1a1, with the consensus sequence KTKL at its C-terminus, as a prototypical PDZ consensus-binding site containing oatp (190). Using a synthetic peptide corresponding to the C-terminal 16 amino acids of oatp1a1 for affinity isolation, interacting proteins from rat liver cytosol were purified. Protein mass fingerprinting identified PDZK1 as the major interacting protein (190). Using an otherwise identical synthetic peptide, but lacking the C-terminal KTKL that comprises the PDZ consensus binding motif, no interacting proteins were resolved (190). Importantly, PDZK1 and oatp1a1 coimmunoprecipitated from rat liver, indicating that they are bound to each other *in vivo*. Further studies to examine the physiological significance of this interaction were performed in wild type and PDZK1 knockout mice (190). There is an 82% amino acid identity between mouse and rat oatp1a1 and both have the same C-terminal PDZ consensus binding sequence, KTKL. These studies showed that expression of oatp1a1 in the knockout mice was similar to that in the wild type mice. By immunofluorescence, oatp1a1 was primarily localized to the basolateral plasma membrane of hepatocytes in wild-type mouse liver. In contrast, it was located predominantly in intracellular vesicular structures in PDZK1 knockout mouse liver (190). Plasma disappearance of ³⁵S-BSP, a ligand transported by oatp1a1, was reduced in the knockout mice (190).

Further studies performed in HEK 293T cells transiently transfected with GFP-oatp1a1 with or without cotransfection with PDZK1 validated the requirement for interaction of these two proteins for plasma membrane localization of the transporter (Fig. 10) (23). To examine the hypothesis that subcellular trafficking of oatp1a1 required movement of oatp1a1-containing vesicles on microtubules, *in vitro* studies of microtubule-based motility of these vesicles was performed (191). In hepatocytes, microtubules are organized with their plus ends near the cell surface, and minus ends within the cell at a microtubule organizing center (132). Immunofluorescence analysis of endocytic vesicles prepared from wild type mouse liver showed a population of vesicles that contained Oatp1a1. Vesicles were labeled by incubation

in primary antibody to oatp1a1 and fluorescent secondary antibody. Upon addition of 50 $\mu\text{mol/L}$ ATP they moved approximately equally towards the plus and minus microtubule ends (Fig. 11). In contrast, movement of vesicles prepared from livers of PDZK1 knockout mice was highly biased towards the minus end, consistent with movement of vesicles to the cell interior (Fig. 11). The majority of oatp1a1-containing vesicles from wild-type mice colocalized with PDZK1 (Fig. 12) (191). Vesicles were also associated with specific microtubule-based motors. Of note is the fact that vesicles prepared from wild type and PDZK1 knockout mice differed in the distribution of microtubule-based motors (Fig. 12) (191). Kinesin-1, a microtubule-based plus end motor was highly associated with wild-type vesicles, with little associated with PDZK1 knockout vesicles. Dynein, a microtubule-based minus-end motor, was largely associated with knockout vesicles. These studies suggest a novel mechanism in which recruitment of specific motors to oatp1a1-containing vesicles is mediated by PDZK1, thus effectively regulating intracellular trafficking of this transporter. In the absence of PDZK1, kinesin-1 is not recruited to oatp1a1-containing vesicles which associate with dynein as a predominant minus-end directed motor. The mechanism by which specific motor recruitment occurs remains to be established.

Conclusion

The importance of the liver in clearing organic anions from the circulation has been known for many years. However, our understanding of the molecular basis of this process is more recent and ongoing. These compounds circulate tightly bound to albumin, yet are efficiently extracted from this protein carrier by hepatocytes. Studies of their uptake kinetics demonstrated saturation, mutual competition for uptake, and countertransport, properties that are compatible with carrier mediation. Initial investigations used biochemical approaches in an attempt to identify organic anion carriers (transporters). Although a number of candidate proteins were proposed on the basis of these studies, for the most part, their physiologic importance to the transport process has not been substantiated. The advent of newer molecular technologies, especially expression cloning of mammalian proteins in *Xenopus laevis* oocytes resulted in functional identification of the oatp family of Na^+ -independent organic anion transport proteins and ntcp, a Na^+ -dependent organic anion transport protein. Based on many studies including those in knockout mice and transporter mutations in patients, it is clear that the oatps serve an important role in hepatocyte Na^+ -independent uptake of many drugs and endogenous compounds such as conjugated bilirubin and bile acids. A physiologic role for ntcp as being the major Na^+ -dependent bile acid transporter has been imputed from studies in which its expression *in vitro* conferred Na^+ -dependent bile acid transport with characteristics resembling the process in liver (119) (119) (108). However, there has been no direct demonstration of its physiologic role and no clinical abnormalities have been described in patients with ntcp polymorphisms or mutations. It has recently been described as serving as the receptor for Hepatitis B virus in human liver.

Although there has been a great deal learned about hepatocyte organic anion transport mechanisms, important questions remain unanswered. Recent studies have shown that these transporters can traffic between intracellular vesicular pools and the cell surface. Mechanisms regulating these processes are under study and may provide insights into

perturbations of transporter activity that may lead to drug toxicity. Although expression of individual transporters in experimental systems confers the ability to transport ligands, it is not clear that the reconstituted mechanism is as efficient as that seen in hepatocytes. These transporters are hydrophobic proteins that span cell membranes multiple times. It is unlikely that they reside in the cell in isolation, but rather exist complexed to other proteins that may serve to regulate their function. Studies to elucidate components of these complexes are under way and may provide new targets for pharmacogenomic studies of patients with drug toxicity.

Acknowledgments

This was supported by NIH grants DK41296 and DK23026.

References

1. Ananthanarayanan M, Bucuvalas JC, Shneider BL, Sippel CJ, Suchy FJ. An ontogenically regulated 48-kDa protein is a component of the Na⁺-bile acid cotransporter of rat liver. *Am J Physiol.* 1991; 261:G810–G817. [PubMed: 1951700]
2. Angeletti RH, Bergwerk AJ, Novikoff PM, Wolkoff AW. Dichotomous development of the organic anion transport protein in liver and choroid plexus. *Am J Physiol.* 1998; 275:C882–C887. [PubMed: 9730973]
3. Anwer MS, Stieger B. Sodium-dependent bile salt transporters of the SLC10A transporter family: More than solute transporters. *Pflugers Arch.* 2014; 466:77–89. [PubMed: 24196564]
4. Arias IM. Liver function from Y to Z. *J Clin Invest.* 2012; 122:2763–2764. [PubMed: 23024981]
5. Baldini G, Passamonti S, Lunazzi GC, Tiribelli C, Sottocasa GL. Cellular localization of sulfobromophthalein transport activity in rat liver. *Biochim Biophys Acta.* 1986; 856:1–10. [PubMed: 3006767]
6. Bananis E, Murray JW, Stockert RJ, Satir P, Wolkoff AW. Microtubule and motor-dependent endocytic vesicle sorting in vitro. *J Cell Biol.* 2000; 151:179–186. [PubMed: 11018063]
7. Bananis E, Nath S, Gordon K, Satir P, Stockert RJ, Murray JW, Wolkoff AW. Microtubule-dependent movement of late endocytic vesicles in vitro: Requirements for Dynein and Kinesin. *Mol Biol Cell.* 2004; 15:3688–3697. [PubMed: 15181154]
8. Baringhaus KH, Matter H, Stengelin S, Kramer W. Substrate specificity of the ileal and the hepatic Na⁺/bile acid cotransporters of the rabbit. II. A reliable 3D QSAR pharmacophore model for the ileal Na⁺/bile acid cotransporter. *J Lipid Res.* 1999; 40:2158–2168. [PubMed: 10588941]
9. Barnhart JL, Clarenburg R. Factors determining clearance of bilirubin in perfused rat liver. *Am J Physiol.* 1973; 225:497–507. [PubMed: 4722415]
10. Battiston L, Passamonti S, Macagno A, Sottocasa GL. The bilirubin-binding motif of bilitranslocase and its relation to conserved motifs in ancient biliproteins. *Biochem Biophys Res Commun.* 1998; 247:687–692. [PubMed: 9647754]
11. Beckett RB, Weiss R, Stitzel RE, Cenedella RJ. Studies on the hepatomegaly caused by the hypolipidemic drugs nafenopin and clofibrate. *Toxicol Appl Pharmacol.* 1972; 23:42–53. [PubMed: 5071041]
12. Bergwerk AJ, Shi X, Ford AC, Kanai N, Jacquemin E, Burk RD, Bai S, Novikoff PM, Stieger B, Meier PJ, Schuster VL, Wolkoff AW. Immunologic distribution of an organic anion transport protein in rat liver and kidney. *Am J Physiol.* 1996; 271:G231–G238. [PubMed: 8770038]
13. Bernstein LH, Ben Ezzer J, Gartner L, Arias IM. Hepatic intracellular distribution of tritium-labeled unconjugated and conjugated bilirubin in normal and Gunn rats. *J Clin Invest.* 1966; 45:1194–1201. [PubMed: 5967697]
14. Bertolini A, Peresson C, Petrusa E, Braidot E, Passamonti S, Macri F, Vianello A. Identification and localization of the bilitranslocase homologue in white grape berries (*Vitis vinifera* L.) during ripening. *J Exp Bot.* 2009; 60:3861–3871. [PubMed: 19596699]

15. Botham KM, Suckling KE. The effect of dibutyl cyclic AMP on the uptake of taurocholic acid by isolated rat liver cells. *Biochim Biophys Acta*. 1986; 883:26–32. [PubMed: 3015232]
16. Braet F, De Zanger R, Baekeland M, Crabbe E, Van Der SP, Wisse E. Structure and dynamics of the fenestrae-associated cytoskeleton of rat liver sinusoidal endothelial cells. *Hepatology*. 1995; 21:180–189. [PubMed: 7806153]
17. Campbell CG, Spray DC, Wolkoff AW. Extracellular ATP⁴⁻ modulates organic anion transport by rat hepatocytes. *J Biol Chem*. 1993; 268:15399–15404. [PubMed: 8340370]
18. Cattori V, Eckhardt U, Hagenbuch B. Molecular cloning and functional characterization of two alternatively spliced Ntcp isoforms from mouse liver1. *Biochim Biophys Acta*. 1999; 1445:154–159. [PubMed: 10209268]
19. Chang C, Pang KS, Swaan PW, Ekins S. Comparative pharmacophore modeling of organic anion transporting polypeptides: A meta-analysis of rat Oatp1a1 and human OATP1B1. *J Pharmacol Exp Ther*. 2005; 314:533–541. [PubMed: 15845861]
20. Chen C, Stock JL, Liu X, Shi J, Van Deusen JW, Dimattia DA, Dullea RG, de Morais SM. Utility of a novel Oatp1b2 knockout mouse model for evaluating the role of Oatp1b2 in the hepatic uptake of model compounds. *Drug Metab Dispos*. 2008; 36:1840–1845. [PubMed: 18556442]
21. Chiang JY. Bile acid metabolism and signaling. *Compr Physiol*. 2013; 3:1191–1212. [PubMed: 23897684]
22. Choi JH, Lee MG, Cho JY, Lee JE, Kim KH, Park K. Influence of OATP1B1 genotype on the pharmacokinetics of rosuvastatin in Koreans. *Clin Pharmacol Ther*. 2008; 83:251–257. [PubMed: 17568401]
23. Choi JH, Murray JW, Wolkoff AW. PDZK1 binding and serine phosphorylation regulate subcellular trafficking of organic anion transport protein 1a1. *Am J Physiol Gastrointest Liver Physiol*. 2011; 300:G384–G393. [PubMed: 21183661]
24. Clarenburg R, Barnhart JL. Interaction of serum albumin and bilirubin at low concentrations. *Am J Physiol*. 1973; 225:493–496. [PubMed: 4722414]
25. Clayton LM, Gurantz D, Hofmann AF, Hagey LR, Schteingart CD. Role of bile acid conjugation in hepatic transport of dihydroxy bile acids. *J Pharm Exper Ther*. 1989; 248:1130–1137.
26. Cleland DP, Bartholomew TC, Billing BH. Hepatic transport of sulfated and nonsulfated bile acids in the rat following relief of bile duct obstruction. *Hepatology*. 1984; 4:477–485. [PubMed: 6547111]
27. Columbano A, Shinozuka H. Liver regeneration versus direct hyperplasia. *FASEB J*. 1996; 10:1118–1128. [PubMed: 8751714]
28. Cornelius CE, Ben Ezzer J, Arias IM. Binding of sulfobromophthalein sodium (BSP) and other organic anions by isolated hepatic cell plasma membranes in vitro. *Proc Soc Exp Biol Med*. 1967; 124:665–667. [PubMed: 6019914]
29. Cui Y, König J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. *J Biol Chem*. 2001; 276:9626–9630. [PubMed: 11134001]
30. Dawson PA. Role of the intestinal bile acid transporters in bile acid and drug disposition. *Handb Exp Pharmacol*. 2011:169–203. [PubMed: 21103970]
31. Dawson PA, Haywood J, Craddock AL, Wilson M, Tietjen M, Kluckman K, Maeda N, Parks JS. Targeted deletion of the ileal bile acid transporter eliminates enterohepatic cycling of bile acids in mice. *J Biol Chem*. 2003; 278:33920–33927. [PubMed: 12819193]
32. Dawson PA, Lan T, Rao A. Bile acid transporters. *J Lipid Res*. 2009; 50:2340–2357. [PubMed: 19498215]
33. Donner MG, Schumacher S, Warskulat U, Heinemann J, Haussinger D. Obstructive cholestasis induces TNF- α - and IL-1-mediated periportal downregulation of Bsep and zonal regulation of Ntcp, Oatp1a4, and Oatp1b2. *Am J Physiol Gastrointest Liver Physiol*. 2007; 293:G1134–G1146. [PubMed: 17916651]
34. Dranoff JA, McClure M, Burgstahler AD, Denson LA, Crawford AR, Crawford JM, Karpen SJ, Nathanson MH. Short-term regulation of bile acid uptake by microfilament-dependent translocation of rat ntcp to the plasma membrane. *Hepatology*. 1999; 30:223–229. [PubMed: 10385660]

35. Dubuisson C, Cresteil D, Desrochers M, Decimo D, Hadchouel M, Jacquemin E. Ontogenic expression of the Na⁺-independent organic anion transporting polypeptide (oatp) in rat liver and kidney. *J Hepatol.* 1996; 25:932–940. [PubMed: 9007723]
36. Elsner R, Ziegler K. Determination of the apparent functional molecular mass of the hepatocellular sodium-dependent taurocholate transporter by radiation inactivation. *Biochim Biophys Acta.* 1989; 983:113–117. [PubMed: 2758046]
37. Elsner RH, Ziegler K. Radiation inactivation of multispecific transport systems for bile acids and xenobiotics in basolateral rat liver plasma membrane vesicles. *J Biol Chem.* 1992; 267:9788–9793. [PubMed: 1577813]
38. Forker EL, Luxon BA. Albumin helps mediate removal of taurocholate by rat liver. *J Clin Invest.* 1981; 67:1517–1522. [PubMed: 7229036]
39. Gartner U, Goesser T, Wolkoff AW. Effect of fasting on the uptake of bilirubin and sulfobromophthalein by the isolated perfused rat liver. *Gastroenterology.* 1997; 113:1707–1713. [PubMed: 9352876]
40. Gartner U, Stockert RJ, Levine WG, Wolkoff AW. Effect of nafenopin on the uptake of bilirubin and sulfobromophthalein by isolated perfused rat liver. *Gastroenterology.* 1982; 83:1163–1169. [PubMed: 6751925]
41. Gartner U, Stockert RJ, Morell AG, Wolkoff AW. Modulation of the transport of bilirubin and asialoorosomucoid during liver regeneration. *Hepatology.* 1981; 1:99–106. [PubMed: 7026402]
42. Gartung C, Ananthanarayanan M, Rahman MA, Schuele S, Nundy S, Soroka CJ, Stolz A, Suchy FJ, Boyer JL. Down-regulation of expression and function of the rat liver Na⁺/bile acid cotransporter in extrahepatic cholestasis. *Gastroenterology.* 1996; 110:199–209. [PubMed: 8536857]
43. Gatmaitan Z, Varticovski L, Ling L, Mikkelsen R, Steffan AM, Arias IM. Studies on fenestral contraction in rat liver endothelial cells in culture. *Am J Pathol.* 1996; 148:2027–2041. [PubMed: 8669487]
44. Gerloff T, Geier A, Stieger B, Hagenbuch B, Meier PJ, Matern S, Gartung C. Differential expression of basolateral and canalicular organic anion transporters during regeneration of rat liver. *Gastroenterology.* 1999; 117:1408–1415. [PubMed: 10579982]
45. Gilmore IT, Thompson RP. Kinetics of ¹⁴C-glycocholic acid clearance in normal man and in patients with liver disease. *Gut.* 1978; 19:1110–1115. [PubMed: 744496]
46. Glavy JS, Wu SM, Wang PJ, Orr GA, Wolkoff AW. Down-regulation by extracellular ATP of rat hepatocyte organic anion transport is mediated by serine phosphorylation of oatp1. *J Biol Chem.* 2000; 275:1479–1484. [PubMed: 10625701]
47. Goesser T, Nakata R, Braly LF, Sosiak A, Campbell CG, Dermietzel R, Novikoff PM, Stockert RJ, Burk RD, Wolkoff AW. The rat hepatocyte plasma membrane organic anion binding protein is immunologically related to the mitochondrial F1 adenosine triphosphatase beta-subunit. *J Clin Invest.* 1990; 86:220–227. [PubMed: 2142166]
48. Gong IY, Kim RB. Impact of genetic variation in OATP transporters to drug disposition and response. *Drug Metab Pharmacokinet.* 2013; 28:4–18. [PubMed: 23047721]
49. Gong L, Aranibar N, Han YH, Zhang Y, Lecureux L, Bhaskaran V, Khandelwal P, Klaassen CD, Lehman-McKeeman LD. Characterization of organic anion-transporting polypeptide (Oatp) 1a1 and 1a4 null mice reveals altered transport function and urinary metabolomic profiles. *Toxicol Sci.* 2011; 122:587–597. [PubMed: 21561886]
50. Goresky CA. A linear method for determining liver sinusoidal and extravascular volumes. *Am J Physiol.* 1963; 204:626–640. [PubMed: 13949263]
51. Goresky CA. Initial distribution and rate of uptake of sulfobromophthalein in the liver. *Am J Physiol.* 1964; 207:13–26. [PubMed: 14193577]
52. Goresky CA. The hepatic uptake and excretion of sulfobromophthalein and bilirubin. *Can Med Assoc J.* 1965; 92:851–857. [PubMed: 14282938]
53. Goresky CA. Kinetic interpretation of hepatic multiple-indicator dilution studies. *Am J Physiol.* 1983; 245:G1–G12. [PubMed: 6346902]
54. Grausz H, Schmid R. Reciprocal relation between plasma albumin level and hepatic sulfobromophthalein removal. *New Engl J Med.* 1971; 284:1403–1406. [PubMed: 5578322]

55. Gray RD, Stroupe SD. Kinetics and mechanism of bilirubin binding to human serum albumin. *J Biol Chem.* 1978; 253:4370–4377. [PubMed: 659422]
56. Green RM, Gollan JL, Hagenbuch B, Meier PJ, Beier DR. Regulation of hepatocyte bile salt transporters during hepatic regeneration. *Am J Physiol.* 1997; 273:G621–G627. [PubMed: 9316464]
57. Grisham JW, Nopanitaya W, Compagno J, Nagel AE. Scanning electron microscopy of normal rat liver: The surface structure of its cells and tissue components. *Am J Anat.* 1975; 144:295–321. [PubMed: 1211369]
58. Grune S, Engelking LR, Anwer MS. Role of intracellular calcium and protein kinases in the activation of hepatic Na⁺/taurocholate cotransport by cyclic AMP. *J Biol Chem.* 1993; 268:17734–17741. [PubMed: 8394349]
59. Habig WH, Pabst MJ, Fleischner G, Gattaitan Z, Arias IM, Jakoby WB. The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. *Proc Natl Acad Sci U S A.* 1974; 71:3879–3882. [PubMed: 4139704]
60. Hagenbuch B, Gui C. Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. *Xenobiotica.* 2008; 38:778–801. [PubMed: 18668430]
61. Hagenbuch B, Lubbert H, Stieger B, Meier PJ. Expression of the hepatocyte Na⁺/bile acid cotransporter in *Xenopus laevis* oocytes. *J Biol Chem.* 1990; 265:5357–5360. [PubMed: 2318817]
62. Hagenbuch B, Meier PJ. Molecular cloning, chromosomal localization, and functional characterization of a human liver Na⁺/bile acid cotransporter. *J Clin Invest.* 1994; 93:1326–1331. [PubMed: 8132774]
63. Hagenbuch B, Meier PJ. Sinusoidal (basolateral) bile salt uptake systems of hepatocytes. *Semin Liver Dis.* 1996; 16:129–136. [PubMed: 8781018]
64. Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta.* 2003; 1609:1–18. [PubMed: 12507753]
65. Hagenbuch B, Meier PJ. Organic anion transporting polypeptides of the OATP/SLC21 family: Phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch.* 2004; 447:653–665. [PubMed: 14579113]
66. Hagenbuch B, Scharschmidt BF, Meier PJ. Effect of antisense oligonucleotides on the expression of hepatocellular bile acid and organic anion uptake systems in *Xenopus laevis* oocytes. *Biochem J.* 1996; 316:901–904. [PubMed: 8670169]
67. Hagenbuch B, Stieger B. The SLCO (former SLC21) superfamily of transporters. *Mol Aspects Med.* 2013; 34:396–412. [PubMed: 23506880]
68. Hagenbuch B, Stieger B, Foguet M, Lubbert H, Meier PJ. Functional expression cloning and characterization of the hepatocyte Na⁺/bile acid cotransport system. *Proc Natl Acad Sci U S A.* 1991; 88:10629–10633. [PubMed: 1961729]
69. Hardison WG, Bellentani S, Heasley V, Shellhamer D. Specificity of an Na⁺-dependent taurocholate transport site in isolated rat hepatocytes. *Am J Physiol.* 1984; 246:G477–G483. [PubMed: 6720949]
70. Hardison WGM, Heasley VL, Shellhamer DF. Specificity of the hepatocyte Na⁺-dependent taurocholate transporter: Influence of side chain length and charge. *Hepatology.* 1991; 13:68–72. [PubMed: 1988346]
71. Hata S, Wang P, Eftychiou N, Ananthanarayanan M, Batta A, Salen G, Pang KS, Wolkoff AW. Substrate specificities of rat oatp1 and ntcp: Implications for hepatic organic anion uptake. *Am J Physiol Gastrointest Liver Physiol.* 2003; 285:G829–G839. [PubMed: 12842829]
72. Hirom PC, Millburn P, Smith RL. Bile and urine as complementary pathways for the excretion of foreign organic compounds. *Xenobiotica.* 1976; 6:55–64. [PubMed: 1274371]
73. Ho RH, Choi L, Lee W, Mayo G, Schwarz UI, Tirona RG, Bailey DG, Michael SC, Kim RB. Effect of drug transporter genotypes on pravastatin disposition in European- and African-American participants. *Pharmacogenet Genomics.* 2007; 17:647–656. [PubMed: 17622941]
74. Ho RH, Leake BF, Roberts RL, Lee W, Kim RB. Ethnicity-dependent polymorphism in Na⁺-taurocholate cotransporting polypeptide (SLC10A1) reveals a domain critical for bile acid substrate recognition. *J Biol Chem.* 2004; 279:7213–7222. [PubMed: 14660639]

75. Honscha W, Platte HD, Oesch F, Friedberg T. Relationship between the microsomal epoxide hydrolase and the hepatocellular transport of bile acids and xenobiotics. *Biochem J.* 1995; 311:975–979. [PubMed: 7487959]
76. Huet P-M, Goresky CA, Villeneuve J-P, Marleau D. Assessment of liver microcirculation in human cirrhosis. *J Clin Invest.* 1982; 70:1234–1244. [PubMed: 7174791]
77. Hung AY, Sheng M. PDZ domains: Structural modules for protein complex assembly. *J Biol Chem.* 2002; 277:5699–5702. [PubMed: 11741967]
78. Ieiri I, Suwannakul S, Maeda K, Uchimaru H, Hashimoto K, Kimura M, Fujino H, Hirano M, Kusuvara H, Irie S, Higuchi S, Sugiyama Y. SLCO1B1 (OATP1B1, an uptake transporter) and ABCG2 (BCRP, an efflux transporter) variant alleles and pharmacokinetics of pitavastatin in healthy volunteers. *Clin Pharmacol Ther.* 2007; 82:541–547. [PubMed: 17460607]
79. Inoue M, Hirata E, Morino Y, Nagase S, Chowdhury JR, Chowdhury NR, Arias IM. The role of albumin in the hepatic transport of bilirubin: Studies in mutant analbuminemic rats. *J Biochem.* 1985; 97:737–743. [PubMed: 4019432]
80. Iusuf D, van de SE, Schinkel AH. Functions of OATP1A and 1B transporters in vivo: Insights from mouse models. *Trends Pharmacol Sci.* 2012; 33:100–108. [PubMed: 22130008]
81. Iwamoto M, Watashi K, Tsukuda S, Aly HH, Fukasawa M, Fujimoto A, Suzuki R, Aizaki H, Ito T, Koiwai O, Kusuvara H, Wakita T. Evaluation and identification of hepatitis B virus entry inhibitors using HepG2 cells overexpressing a membrane transporter NTCP. *Biochem Biophys Res Commun.* 2014; 443:808–813. [PubMed: 24342612]
82. Jacquemin E, Hagenbuch B, Stieger B, Wolkoff AW, Meier PJ. Expression of the hepatocellular chloride-dependent sulfobromophthalein uptake system in *Xenopus laevis* oocytes. *J Clin Invest.* 1991; 88:2146–2149. [PubMed: 1752967]
83. Jacquemin E, Hagenbuch B, Stieger B, Wolkoff AW, Meier PJ. Expression cloning of a rat liver Na⁺-independent organic anion transporter. *Proc Natl Acad Sci USA.* 1994; 91:133–137. [PubMed: 8278353]
84. Kamisaka K, Listowsky I, Betheil JJ, Arias IM. Competitive binding of bilirubin, sulfobromophthalein, indocyanine green and other organic anions to human and bovine serum albumin. *Biochim Biophys Acta.* 1974; 365:169–180. [PubMed: 4472039]
85. Ketley JN, Habig WH, Jakoby WB. Binding of nonsubstrate ligands to the glutathione S-transferases. *J Biol Chem.* 1975; 250:8670–8673. [PubMed: 1184584]
86. Ketterer B, Ross-Mansell P, Whitehead JK. The isolation of carcinogen-binding protein from livers of rats given 4-dimethylaminoazobenzene. *Biochem J.* 1967; 103:316–324. [PubMed: 4962081]
87. Ketterer B, Tipping E, Hackney JF, Beale D. A low-molecular-weight protein from rat liver that resembles ligandin in its binding properties. *Biochem J.* 1976; 155:511–521. [PubMed: 949315]
88. Kim E, Sheng M. PDZ domain proteins of synapses. *Nat Rev Neurosci.* 2004; 5:771–781. [PubMed: 15378037]
89. Kim RB. Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. *Eur J Clin Invest.* 2003; 33(Suppl 2):1–5. [PubMed: 14641549]
90. Klaassen CD, Watkins JB III. Mechanisms of bile formation, hepatic uptake, and biliary excretion. *Pharmacol Rev.* 1984; 36:1–67. [PubMed: 6144113]
91. König J. Uptake transporters of the human OATP family: Molecular characteristics, substrates, their role in drug-drug interactions, and functional consequences of polymorphisms. *Handb Exp Pharmacol.* 2011:1–28. [PubMed: 21103967]
92. König J, Cui Y, Nies AT, Keppler D. Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J Biol Chem.* 2000; 275:23161–23168. [PubMed: 10779507]
93. König J, Seithel A, Gradhand U, Fromm MF. Pharmacogenomics of human OATP transporters. *Naunyn Schmiedeberg Arch Pharmacol.* 2006; 372:432–443. [PubMed: 16525793]
94. Koopen NR, Wolters H, Müller M, Schippers IJ, Havinga R, Roelofs H, Vonk RJ, Stieger B, Meier PJ, Kuipers F. Hepatic bile salt flux does not modulate level and activity of the sinusoidal Na⁺-taurocholate cotransporter (ntcp) in rats. *J Hepatol.* 1997; 27:699–706. [PubMed: 9365046]

95. Kramer W, Bickel U, Buscher HP, Gerok W, Kurz G. Bile-salt-binding polypeptides in plasma membranes of hepatocytes revealed by photoaffinity labelling. *Eur J Biochem.* 1982; 129:13–24. [PubMed: 7160376]
96. Kramer W, Stengelin S, Baringhaus KH, Enhnen A, Heuer H, Becker W, Corsiero D, Girbig F, Noll R, Weyland C. Substrate specificity of the ileal and the hepatic Na(+)/bile acid cotransporters of the rabbit. I. Transport studies with membrane vesicles and cell lines expressing the cloned transporters. *J Lipid Res.* 1999; 40:1604–1617. [PubMed: 10484607]
97. Kullak-Ublick GA, Ismaier MG, Stieger B, Landmann L, Huber R, Pizzagalli F, Fattinger K, Meier PJ, Hagenbuch B. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology.* 2001; 120:525–533. [PubMed: 11159893]
98. Kullak-Ublick GA, Stieger B, Meier PJ. Enterohepatic bile salt transporters in normal physiology and liver disease. *Gastroenterology.* 2004; 126:322–342. [PubMed: 14699511]
99. Kurisu H, Nilprabhassorn P, Wolkoff AW. Preparation of [35S]sulfobromophthalein of high specific activity. *Anal Biochem.* 1989; 179:72–74. [PubMed: 2757201]
100. Levi AJ, Gatmaitan Z, Arias IM. Two hepatic cytoplasmic protein fractions, Y and Z, and their possible role in the hepatic uptake of bilirubin, sulfobromophthalein, and other anions. *J Clin Invest.* 1969; 48:2156–2167. [PubMed: 4980931]
101. Li H, Zhuang Q, Wang Y, Zhang T, Zhao J, Zhang Y, Zhang J, Lin Y, Yuan Q, Xia N, Han J. HBV life cycle is restricted in mouse hepatocytes expressing human NTCP. *Cell Mol Immunol.* 2014; 11:175–183. [PubMed: 24509445]
102. Lidofsky SD, Fitz JG, Weisiger RA, Scharschmidt BF. Hepatic taurocholate uptake is electrogenic and influenced by transmembrane potential difference. *Am J Physiol.* 1993; 264:G478–G485. [PubMed: 8460701]
103. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, Collins R. SLCO1B1 variants and statin-induced myopathy—a genomewide study. *N Engl J Med.* 2008; 359:789–799. [PubMed: 18650507]
104. Litwack G, Ketterer B, Arias IM. Ligandin: A hepatic protein which binds steroids, bilirubin, carcinogens and a number of exogenous organic anions. *Nature.* 1971; 234:466–467. [PubMed: 4944188]
105. Liu L, Mak E, Tirona RG, Tan E, Novikoff PM, Wang P, Wolkoff AW, Pang KS. Vascular binding, blood flow, transporter, and enzyme interactions on the processing of digoxin in rat liver. *J Pharmacol Exp Ther.* 2005; 315:433–448. [PubMed: 15994370]
106. Lough J, Rosenthal L, Arzoumanian A, Goresky CA. Kupffer cell depletion associated with capillarization of liver sinusoids in carbon tetrachloride-induced rat liver cirrhosis. *J Hepatol.* 1987; 5:190–198. [PubMed: 3693863]
107. Lu H, Choudhuri S, Ogura K, Csanaky IL, Lei X, Cheng X, Song PZ, Klaassen CD. Characterization of organic anion transporting polypeptide 1b2-null mice: Essential role in hepatic uptake/toxicity of phalloidin and microcystin-LR. *Toxicol Sci.* 2008; 103:35–45. [PubMed: 18296417]
108. Lund M, Kang L, Tygstrup N, Wolkoff AW, Ott P. Effects of LPS on transport of indocyanine green and alanine uptake in perfused rat liver. *Am J Physiol.* 1999; 277:G91–G100. [PubMed: 10409155]
109. Maestro A, Terdoslavich M, Vanzo A, Kuku A, Tramer F, Nicolin V, Micali F, Decorti G, Passamonti S. Expression of bilitranslocase in the vascular endothelium and its function as a flavonoid transporter. *Cardiovasc Res.* 2010; 85:175–183. [PubMed: 19706629]
110. Marchegiano P, Carubbi F, Tiribelli C, Amarri S, Stebel M, Lunazzi GC, Levy D, Bellentani S. Transport of sulfobromophthalein and taurocholate in the HepG2 cell line in relation to the expression of membrane carrier proteins. *Biochem Biophys Res Commun.* 1992; 183:1203–1208. [PubMed: 1567398]
111. Mareninova O, Shin JM, Vagin O, Turdikulova S, Hallen S, Sachs G. Topography of the membrane domain of the liver Na+-dependent bile acid transporter. *Biochem.* 2005; 44:13702–13712. [PubMed: 16229460]

112. Martin GG, Atshaves BP, McIntosh AL, Mackie JT, Kier AB, Schroeder F. Liver fatty-acid-binding protein (L-FABP) gene ablation alters liver bile acid metabolism in male mice. *Biochem J*. 2005; 391:549–560. [PubMed: 15984932]
113. Martinez LO, Jacquet S, Esteve JP, Rolland C, Cabezon E, Champagne E, Pineau T, Georgeaud V, Walker JE, Terce F, Collet X, Perret B, Barbaras R. Ectopic beta-chain of ATP synthase is an apolipoprotein A-I receptor in hepatic HDL endocytosis. *Nature*. 2003; 421:75–79. [PubMed: 12511957]
114. Marzolini C, Tirona RG, Kim RB. Pharmacogenomics of the OATP and OAT families. *Pharmacogenomics*. 2004; 5:273–282. [PubMed: 15102542]
115. McConkey M, Gillin H, Webster CR, Anwer MS. Cross-talk between protein kinases Czeta and B in cyclic AMP-mediated sodium taurocholate co-transporting polypeptide translocation in hepatocytes. *J Biol Chem*. 2004; 279:20882–20888. [PubMed: 15007074]
116. McIntosh AL, Atshaves BP, Landrock D, Landrock KK, Martin GG, Storey SM, Kier AB, Schroeder F. Liver fatty acid binding protein gene-ablation exacerbates weight gain in high-fat fed female mice. *Lipids*. 2013; 48:435–448. [PubMed: 23539345]
117. Meerman L, Koopen NR, Bloks V, van Goor H, Havinga R, Wolthers BG, Kramer W, Stengelin S, Muller M, Kuipers F, Jansen PL. Biliary fibrosis associated with altered bile composition in a mouse model of erythropoietic protoporphyria. *Gastroenterology*. 1999; 117:696–705. [PubMed: 10464147]
118. Meier PJ. Molecular mechanisms of hepatic bile salt transport from sinusoidal blood into bile. *Am J Physiol*. 1995; 269:G801–G812. [PubMed: 8572210]
119. Meier PJ, Stieger B. Bile salt transporters. *Annu Rev Physiol*. 2002; 64:635–661. [PubMed: 11826283]
120. Min AD, Goeser T, Liu R, Campbell CG, Novikoff PM, Wolkoff AW. Organic anion transport in HepG2 cells: Absence of the high-affinity, chloride-dependent transporter. *Hepatology*. 1991; 14:1217–1223. [PubMed: 1660021]
121. Min AD, Johansen KJ, Campbell CG, Wolkoff AW. Role of chloride and intracellular pH on the activity of the rat hepatocyte organic anion transporter. *J Clin Invest*. 1991; 87:1496–1502. [PubMed: 2022722]
122. Miura M, Satoh S, Inoue K, Kagaya H, Saito M, Inoue T, Suzuki T, Habuchi T. Influence of SLCO1B1, 1B3, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur J Clin Pharmacol*. 2007; 63:1161–1169. [PubMed: 17906856]
123. Monaco HL. Review: The liver bile acid-binding proteins. *Biopolymers*. 2009; 91:1196–1202. [PubMed: 19441025]
124. Morey KS, Litwack G. Isolation and properties of cortisol metabolite binding proteins of rat liver cytosol. *Biochem*. 1969; 8:4813–4821. [PubMed: 5365784]
125. Mukhopadhyay S, Ananthanarayanan M, Stieger B, Meier PJ, Suchy FJ, Anwer MS. cAMP increases liver Na⁺-taurocholate cotransport by translocating transporter to plasma membranes. *Am J Physiol*. 1997; 273:G842–G848. [PubMed: 9357825]
126. Mukhopadhyay S, Ananthanarayanan M, Stieger B, Meier PJ, Suchy FJ, Anwer MS. Sodium taurocholate cotransporting polypeptide is a serine, threonine phosphoprotein and is dephosphorylated by cyclic adenosine monophosphate. *Hepatology*. 1998; 28:1629–1636. [PubMed: 9828228]
127. Mukhopadhyay S, Webster CRL, Anwer MS. Role of protein phosphatases in cyclic AMP-mediated stimulation of hepatic Na⁺/taurocholate cotransport. *J Biol Chem*. 1998; 273:30039–30045. [PubMed: 9792726]
128. Murray JW, Bananis E, Wolkoff AW. Reconstitution of ATP-dependent movement of endocytic vesicles along microtubules in vitro: An oscillatory bidirectional process. *Mol Biol Cell*. 2000; 11:419–433. [PubMed: 10679004]
129. Murray JW, Bananis E, Wolkoff AW. Immunofluorescence microchamber technique for characterizing isolated organelles. *Anal Biochem*. 2002; 305:55–67. [PubMed: 12018946]
130. Ni Y, Lempp FA, Mehrle S, Nkongolo S, Kaufman C, Falth M, Stindt J, Koniger C, Nassal M, Kubitz R, Sultmann H, Urban S. Hepatitis B and D viruses exploit sodium taurocholate co-

transporting polypeptide for species-specific entry into hepatocytes. *Gastroenterology*. 2014; 146:1070–1083. [PubMed: 24361467]

131. Nkongolo S, Ni Y, Lempp FA, Kaufman C, Lindner T, Esser-Nobis K, Lohmann V, Mier W, Mehrle S, Urban S. Cyclosporin A inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor. *J Hepatol*. 2014; 60:723–731. [PubMed: 24295872]
132. Novikoff PM, Cammer M, Tao L, Oda H, Stockert RJ, Wolkoff AW, Satir P. Three-dimensional organization of rat hepatocyte cytoskeleton: Relation to the asialoglycoprotein endocytosis pathway. *J Cell Sci*. 1996; 109(Pt 1):21–32. [PubMed: 8834787]
133. Ockner RK, Weisiger RA, Gollan JL. Hepatic uptake of albumin-bound substances: Albumin receptor concept. *Am J Physiol*. 1983; 245:G13–G18. [PubMed: 6307061]
134. Oie S, Levy G. Effect of plasma protein binding on elimination of bilirubin. *J Pharm Sci*. 1975; 64:1433. [PubMed: 1151726]
135. Oleaga A, Gonzalez J, Esteller A. Effects of two-thirds hepatectomy on sulfobromophthalein handling by the rat liver. *Comp Biochem Physiol A*. 1987; 87:13–19. [PubMed: 2886252]
136. Ono T, Odani S. Initial studies of the cytoplasmic FABP superfamily. *Proc Jpn Acad Ser B Phys Biol Sci*. 2010; 86:220–228.
137. Oswald S, König J, Lutjohann D, Giessmann T, Kroemer HK, Rimbach C, Roskopf D, Fromm MF, Siegmund W. Disposition of ezetimibe is influenced by polymorphisms of the hepatic uptake carrier OATP1B1. *Pharmacogenet Genomics*. 2008; 18:559–568. [PubMed: 18551036]
138. Park M, Lin L, Thomas S, Braymer HD, Smith PM, Harrison DH, York DA. The F1-ATPase beta-subunit is the putative enterostatin receptor. *Peptides*. 2004; 25:2127–2133. [PubMed: 15572201]
139. Park SW, Schonhoff CM, Webster CR, Anwer MS. Protein kinase Cdelta differentially regulates cAMP-dependent translocation of NTCP and MRP2 to the plasma membrane. *Am J Physiol Gastrointest Liver Physiol*. 2012; 303:G657–G665. [PubMed: 22744337]
140. Paumgartner G, Reichen J. Kinetics of hepatic uptake of unconjugated bilirubin. *Clin Sci Molec Med*. 1976; 51:169–176. [PubMed: 954363]
141. Persico M, Sottocasa FL. Measurement of sulfobromophthalein uptake in isolated rat hepatocytes by a direct spectrophotometric method. *Biochim Biophys Acta*. 1987; 930:129–134. [PubMed: 3620512]
142. Ramasamy U, Anwer MS, Schonhoff CM. Cysteine 96 of Ntcp is responsible for NO-mediated inhibition of taurocholate uptake. *Am J Physiol Gastrointest Liver Physiol*. 2013; 305:G513–G519. [PubMed: 23886862]
143. Reichel C, Gao B, VanMontfort J, Cattori V, Rahner C, Hagenbuch B, Stieger B, Kamisako T, Meier PJ. Localization and function of the organic anion-transporting polypeptide oap2 in rat liver. *Gastroenterology*. 1999; 117:688–695. [PubMed: 10464146]
144. Reichen J, Blitzer BL, Berk PD. Binding of unconjugated and conjugated sulfobromophthalein to rat liver plasma membrane fractions in vitro. *Biochim Biophys Acta*. 1981; 640:298–312. [PubMed: 7213688]
145. Reichen J, Paumgartner G. Kinetics of taurocholate uptake by the perfused rat liver. *Gastroenterology*. 1975; 68:132–136. [PubMed: 1090476]
146. Reichen J, Paumgartner G. Uptake of bile acids by perfused rat liver. *Am J Physiol*. 1976; 231:734–742. [PubMed: 788526]
147. Reyes H, Levi AJ, Gatmaitan Z, Arias IM. Studies of Y and Z, two hepatic cytoplasmic organic anion-binding proteins: Effect of drugs, chemicals, hormones, and cholestasis. *J Clin Invest*. 1971; 50:2242–2252. [PubMed: 5096510]
148. Sarkar S, Bananis E, Nath S, Anwer MS, Wolkoff AW, Murray JW. PKCzeta is required for microtubule-based motility of vesicles containing the ntcp transporter. *Traffic*. 2006; 7:1078–1091. [PubMed: 16734659]
149. Satlin LM, Amin V, Wolkoff AW. Organic anion transporting polypeptide mediates organic anion/HCO₃⁻ exchange. *J Biol Chem*. 1997; 272:26340–26345. [PubMed: 9334206]
150. Scharschmidt BF, Waggoner JG, Berk PD. Hepatic organic anion uptake in the rat. *J Clin Invest*. 1975; 56:1280–1292. [PubMed: 1184749]

151. Schonhoff CM, Ramasamy U, Anwer MS. Nitric oxide-mediated inhibition of taurocholate uptake involves S-nitrosylation of NTCP. *Am J Physiol Gastrointest Liver Physiol.* 2011; 300:G364–G370. [PubMed: 21109590]
152. Schwarz LR, Gotz R, Klaassen CD. Uptake of sulfobromophthalein-glutathione conjugate by isolated hepatocytes. *Am J Physiol.* 1980; 239:C118–C123. [PubMed: 7435550]
153. Schwenk M, Burr R, Schwarz L, Pfaff E. Uptake of bromosulfophthalein by isolated liver cells. *Eur J Biochem.* 1976; 64:189–197. [PubMed: 6268]
154. Shitara Y, Maeda K, Ikejiri K, Yoshida K, Horie T, Sugiyama Y. Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: Their roles in hepatic clearance and intestinal absorption. *Biopharm Drug Dispos.* 2013; 34:45–78. [PubMed: 23115084]
155. Simon FR, Leffert HL, Ellisman M, Iwahashi M, Deerinck T, Fortune J, Morales D, Dahl R, Sutherland E. Hepatic Na(+)-K(+)-ATPase enzyme activity correlates with polarized beta-subunit expression. *Am J Physiol.* 1995; 269:C69–C84. [PubMed: 7631761]
156. Smathers RL, Galligan JJ, Shearn CT, Fritz KS, Mercer K, Ronis M, Orlicky DJ, Davidson NO, Petersen DR. Susceptibility of L-FABP-/-mice to oxidative stress in early-stage alcoholic liver. *J Lipid Res.* 2013; 54:1335–1345. [PubMed: 23359610]
157. Smathers RL, Petersen DR. The human fatty acid-binding protein family: Evolutionary divergences and functions. *Hum Genomics.* 2011; 5:170–191. [PubMed: 21504868]
158. Sottocasa GL, Baldini G, Sandri G, Lunazzi G, Tiribelli C. Reconstitution in vitro of sulfobromophthalein transport by bilitranslocase. *Biochim Biophys Acta.* 1982; 685:123–128. [PubMed: 6895854]
159. Stieger B, Hagenbuch B, Landmann L, Hochli M, Schroeder A, Meier PJ. In situ localization of the hepatocytic Na+/taurocholate cotransporting polypeptide in rat liver. *Gastroenterology.* 1994; 107:1781–1787. [PubMed: 7958692]
160. Stoelinga GB, van Munster PJ. The behaviour of Evans blue (azo-dye T-1824) in the body after intravenous injection. *Acta Physiol Pharmacol Neerl.* 1967; 14:391–409. [PubMed: 5582709]
161. Stollman YR, Gartner U, Theilmann L, Ohmi N, Wolkoff AW. Hepatic bilirubin uptake in the isolated perfused rat liver is not facilitated by albumin binding. *J Clin Invest.* 1983; 72:718–723. [PubMed: 6308060]
162. Stollman YR, Theilmann L, Stockert RJ, Wolkoff AW. Reduced transport of bilirubin and asialoorosomucoid in regenerating rat liver is a microtubule-independent event. *Hepatology.* 1985; 5:798–804. [PubMed: 4029892]
163. Stolz A, Sugiyama Y, Kuhlenkamp J, Kaplowitz N. Identification and purification of a 36 kDa bile acid binder in human hepatic cytosol. *FEBS Lett.* 1984; 177:31–35. [PubMed: 6500050]
164. Stolz A, Takikawa H, Sugiyama Y, Kuhlenkamp J, Kaplowitz N. 3 alpha-hydroxysteroid dehydrogenase activity of the Y' bile acid binders in rat liver cytosol. Identification, kinetics, and physiologic significance. *J Clin Invest.* 1987; 79:427–434. [PubMed: 3468121]
165. Stremmel W, Berk PD. Hepatocellular uptake of sulfobromophthalein and bilirubin is selectively inhibited by an antibody to the liver plasma membrane sulfobromophthalein/bilirubin binding protein. *J Clin Invest.* 1986; 78:822–826. [PubMed: 3745441]
166. Stremmel W, Gerber MA, Glezerov V, Thung SN, Kochwa S, Berk PD. Physicochemical and immunohistological studies of a sulfobromophthalein- and bilirubin-binding protein from rat liver plasma membranes. *J Clin Invest.* 1983; 71:1796–1805. [PubMed: 6190841]
167. Sugiyama Y, Yamada T, Kaplowitz N. Newly identified bile acid binders in rat liver cytosol. Purification and comparison with glutathione S-transferases. *J Biol Chem.* 1983; 258:3602–3607. [PubMed: 6833218]
168. Takikawa H, Kaplowitz N. Binding of bile acids, oleic acid, and organic anions by rat and human hepatic Z protein. *Arch Biochem Biophys.* 1986; 251:385–392. [PubMed: 3789743]
169. Theilmann L, Stollman YR, Arias IM, Wolkoff AW. Does Z-protein have a role in transport of bilirubin and bromosulfophthalein by isolated perfused rat liver? *Hepatology.* 1984; 4:923–926. [PubMed: 6384000]
170. Tiribelli C, Lunazzi G, Luciani M, Panfili E, Gazzin B, Liut G, Sandri G, Sottocasa G. Isolation of a sulfobromophthalein-binding protein from hepatocyte plasma membrane. *Biochim Biophys Acta.* 1978; 532:105–112. [PubMed: 620047]

171. Tiribelli C, Lunazzi GC, Sottocasa GL. Biochemical and molecular aspects of the hepatic uptake of organic anions. *Biochim Biophys Acta*. 1990; 1031:261–275. [PubMed: 2223818]
172. Tirona RG, Leake BF, Merino G, Kim RB. Polymorphisms in OATP-C: Identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem*. 2001; 276:35669–35675. [PubMed: 11477075]
173. Trauner M, Boyer JL. Bile salt transporters: Molecular characterization, function, and regulation. *Physiol Rev*. 2003; 83:633–671. [PubMed: 12663868]
174. Van Bezooijen CFA, Grell T, Knook DL. Bromsulphophthalein uptake by isolated liver parenchymal cells. *Biochem Biophys Res Commun*. 1976; 69:354–361. [PubMed: 1267791]
175. van de Steeg E, Stranecky V, Hartmannova H, Noskova L, Hrebicek M, Wagenaar E, van Esch A, de Waart DR, Oude Elferink RP, Kenworthy KE, Sticova E, al Edreesi M, Knisely AS, Kmoch S, Jirsa M, Schinkel AH. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. *J Clin Invest*. 2012; 122:519–528. [PubMed: 22232210]
176. van de SE, Wagenaar E, van der Kruijsen CM, Burggraaff JE, de Waart DR, Elferink RP, Kenworthy KE, Schinkel AH. Organic anion transporting polypeptide 1a/1b-knockout mice provide insights into hepatic handling of bilirubin, bile acids, and drugs. *J Clin Invest*. 2010; 120:2942–2952. [PubMed: 20644253]
177. van der Sluijs P, Postema B, Meijer DKF. Lactosylation of albumin reduces uptake rate of dibromosulphophthalein in perfused rat liver and dissociation rate from albumin in vitro. *Hepatology*. 1987; 7:688–695. [PubMed: 3610047]
178. Van Dyke RW, Stephens JE, Scharschmidt BF. Bile acid transport in cultured rat hepatocytes. *Am J Physiol*. 1982; 243:G484–G492. [PubMed: 7149031]
179. van Montfort JE, Hagenbuch B, Groothuis GM, Koepsell H, Meier PJ, Meijer DK. Drug uptake systems in liver and kidney. *Curr Drug Metab*. 2003; 4:185–211. [PubMed: 12769665]
180. von Dippe P, Amoui M, Alves C, Levy D. Na⁺-dependent bile acid transport by hepatocytes is mediated by a protein similar to microsomal epoxide hydrolase. *Am J Physiol*. 1993; 264:G528–G534. [PubMed: 8460705]
181. von Dippe P, Amoui M, Stellwagen RH, Levy D. The functional expression of sodium-dependent bile acid transport in Madin-Darby canine kidney cells transfected with the cDNA for microsomal epoxide hydrolase. *J Biol Chem*. 1996; 271:18176–18180. [PubMed: 8663355]
182. von Dippe P, Ananthanarayanan M, Drain P, Levy D. Purification and reconstitution of the bile acid transport system from hepatocyte sinusoidal plasma membranes. *Biochim Biophys Acta*. 1986; 862:352–360. [PubMed: 3778896]
183. von Dippe P, Drain P, Levy D. Synthesis and transport characteristics of photoaffinity probes for the hepatocyte bile acid transport system. *J Biol Chem*. 1983; 258:8890–8895. [PubMed: 6863315]
184. von Dippe P, Levy D. Expression of the bile acid transport protein during liver development and in hepatoma cells. *J Biol Chem*. 1990a; 265:5942–5945. [PubMed: 2318840]
185. von Dippe P, Levy D. Reconstitution of the immunopurified 49-kDa sodium-dependent bile acid transport protein derived from hepatocyte sinusoidal plasma membranes. *J Biol Chem*. 1990b; 265:14812–14816. [PubMed: 2394700]
186. von Dippe P, Zhu QS, Levy D. Cell surface expression and bile acid transport function of one topological form of m-epoxide hydrolase. *Biochem Biophys Res Commun*. 2003; 309:804–809. [PubMed: 13679044]
187. Vos TA, Ros JE, Havinga R, Moshage H, Kuipers F, Jansen PLM, Müller M. Regulation of hepatic transport systems involved in bile secretion during liver regeneration in rats. *Hepatology*. 1999; 29:1833–1839. [PubMed: 10347127]
188. Wang P, Hata S, Xiao Y, Murray JW, Wolkoff AW. Topological assessment of oatp1a1: A 12 transmembrane domain integral membrane protein with three N-linked carbohydrate chains. *Am J Physiol Gastrointest Liver Physiol*. 2008; 294:G1052–G1059. [PubMed: 18308854]
189. Wang P, Kim RB, Chowdhury JR, Wolkoff AW. The human organic anion transport protein SLC21A6 is not sufficient for bilirubin transport. *J Biol Chem*. 2003; 278:20695–20699. [PubMed: 12670950]

190. Wang P, Wang JJ, Xiao Y, Murray JW, Novikoff PM, Angeletti RH, Orr GA, Lan D, Silver DL, Wolkoff AW. Interaction with PDZK1 is required for expression of organic anion transporting protein 1A1 (OATP1A1) on the hepatocyte surface. *J Biol Chem*. 2005; 280:30143–30149. [PubMed: 15994332]
191. Wang WJ, Murray JW, Wolkoff AW. Oatp1a1 requires PDZK1 to traffic to the plasma membrane by selective recruitment of microtubule-based motor proteins. *Drug Metab Dispos*. 2013; 42:62–69. [PubMed: 24115750]
192. Watashi K, Sluder A, Daito T, Matsunaga S, Ryo A, Nagamori S, Iwamoto M, Nakajima S, Tsukuda S, Borroto-Esoda K, Sugiyama M, Tanaka Y, Kanai Y, Kusuhara H, Mizokami M, Wakita T. Cyclosporin A and its analogs inhibit hepatitis B virus entry into cultured hepatocytes through targeting a membrane transporter, sodium taurocholate cotransporting polypeptide (NTCP). *Hepatology*. 2013; 59:1726–1737. [PubMed: 24375637]
193. Watashi K, Urban S, Li W, Wakita T. NTCP and beyond: Opening the door to unveil hepatitis B virus entry. *Int J Mol Sci*. 2014; 15:2892–2905. [PubMed: 24557582]
194. Webster CR, Srinivasulu U, Ananthanarayanan M, Suchy FJ, Anwer MS. Protein kinase B/Akt mediates cAMP- and cell swelling-stimulated Na⁺/taurocholate cotransport and Ntcp translocation. *J Biol Chem*. 2002; 277:28578–28583. [PubMed: 12034724]
195. Webster CRL, Anwer MS. Role of the PI3K/PKB signaling pathway in cAMP-mediated translocation of rat liver ntcp. *Am J Physiol*. 1999; 277:G1165–G1172. [PubMed: 10600813]
196. Weinman SA, Weeks RP. Electrogenicity of Na-coupled bile salt transport in isolated rat hepatocytes. *Am J Physiol*. 1993; 265:G73–G80. [PubMed: 8338174]
197. Weisiger R, Gollan J, Ockner R. Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. *Science*. 1981; 211:1048–1050. [PubMed: 6258226]
198. Weisiger RA. Dissociation from albumin: A potentially rate-limiting step in the clearance of substances by the liver. *Proc Natl Acad Sci USA*. 1985; 82:1563–1567. [PubMed: 3856281]
199. Weisiger RA, Zacks CM, Smith ND, Boyer JL. Effect of albumin binding on extraction of sulfobromophthalein by perfused elasmobranch liver: Evidence for dissociation-limited uptake. *Hepatology*. 1984; 4:492–501. [PubMed: 6724517]
200. Wieland T, Nassal M, Kramer W, Fricker G, Bickel U, Kurz G. Identity of hepatic membrane transport systems for bile salts, phalloidin, and antamanide by photoaffinity labeling. *Proc Natl Acad Sci USA*. 1984; 81:5232–5236. [PubMed: 6591186]
201. Wisse E. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J Ultrastruct Res*. 1970; 31:125–150. [PubMed: 5442603]
202. Wolkoff AW. The role of an albumin receptor in hepatic organic anion uptake: The controversy continues. *Hepatology*. 1987; 7:777–779. [PubMed: 3038726]
203. Wolkoff AW, Bhargava MM, Chung C, Gatmaitan Z. Purification of ligandin by affinity chromatography on sulfobromophthalein-agarose gel. *Proc Soc Exper Biol Med*. 1979; 160:150–153. [PubMed: 419138]
204. Wolkoff AW, Chung CT. Identification, purification, and partial characterization of an organic anion binding protein from rat liver cell plasma membrane. *J Clin Invest*. 1980; 65:1152–1161. [PubMed: 7364942]
205. Wolkoff AW, Cohen DE. Bile acid regulation of hepatic physiology: I. Hepatocyte transport of bile acids. *Am J Physiol Gastrointest Liver Physiol*. 2003; 284:G175–G179. [PubMed: 12529265]
206. Wolkoff AW, Goresky CA, Sellin J, Gatmaitan Z, Arias IM. Role of ligandin in transfer of bilirubin from plasma into liver. *Am J Physiol*. 1979; 236:E638–E648. [PubMed: 375751]
207. Wolkoff AW, Ketley JN, Waggoner JG, Berk PD, Jakoby WB. Hepatic accumulation and intracellular binding of conjugated bilirubin. *J Clin Invest*. 1978; 61:142–149. [PubMed: 618909]
208. Wolkoff AW, Klausner RD, Ashwell G, Harford J. Intracellular segregation of asialoglycoproteins and their receptor: A prelysosomal event subsequent to dissociation of the ligand-receptor complex. *J Cell Biol*. 1984; 98:375–381. [PubMed: 6319424]

209. Wolkoff AW, Samuelson AC, Johansen KL, Nakata R, Withers DM, Sosiak A. Influence of Cl⁻ on organic anion transport in short-term cultured rat hepatocytes and isolated perfused rat liver. *J Clin Invest.* 1987; 79:1259–1268. [PubMed: 3031134]
210. Wolkoff AW, Sosiak A, Greenblatt HC, Van Renswoude J, Stockert RJ. Immunological studies of an organic anion-binding protein isolated from rat liver cell plasma membrane. *J Clin Invest.* 1985; 76:454–459. [PubMed: 3897285]
211. Wolkoff AW, Weisiger RA, Jakoby WB. The multiple roles of the glutathione transferases (ligandins). *Prog Liver Dis.* 1979; 6:213–224. [PubMed: 396556]
212. Wolkoff AW, Wolpert E, Pascasio FN, Arias IM. Rotor's syndrome. A distinct inheritable pathophysiologic entity. *Am J Med.* 1976; 60:173–179. [PubMed: 766621]
213. Xiao Y, Nieves E, Angeletti RH, Orr GA, Wolkoff AW. Rat organic anion transporting protein 1A1 (Oatp1a1): Purification and phosphopeptide assignment. *Biochem.* 2006; 45:3357–3369. [PubMed: 16519530]
214. Yan H, Peng B, He W, Zhong G, Qi Y, Ren B, Gao Z, Jing Z, Song M, Xu G, Sui J, Li W. Molecular determinants of hepatitis B and D virus entry restriction in mouse sodium taurocholate cotransporting polypeptide. *J Virol.* 2013; 87:7977–7991. [PubMed: 23678176]
215. Yan H, Peng B, Liu Y, Xu G, He W, Ren B, Jing Z, Sui J, Li W. Viral entry of hepatitis B and d viruses and bile salts transportation share common molecular determinants on sodium taurocholate cotransporting polypeptide. *J Virol.* 2014; 88:3273–3284. [PubMed: 24390325]
216. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J, Li W. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife.* 2012; 1:e00049. [PubMed: 23150796]
217. Ye F, Zhang M. Structures and target recognition modes of PDZ domains: Recurring themes and emerging pictures. *Biochem J.* 2013; 455:1–14. [PubMed: 24028161]
218. Zaher H, zu Schwabedissen HE, Tirona RG, Cox ML, Obert LA, Agrawal N, Palandra J, Stock JL, Kim RB, Ware JA. Targeted disruption of murine organic anion-transporting polypeptide 1b2 (oatp1b2/Slco1b2) significantly alters disposition of prototypical drug substrates pravastatin and rifampin. *Mol Pharmacol.* 2008; 74:320–329. [PubMed: 18413659]
219. Zhu Q, von Dippe P, Xing W, Levy D. Membrane topology and cell surface targeting of microsomal epoxide hydrolase. Evidence for multiple topological orientations. *J Biol Chem.* 1999; 274:27898–27904. [PubMed: 10488137]
220. Ziegler K, Lins W, Frimmer M. Hepatocellular transport of cycloso-matostatins: Evidence for a carrier system related to the multispecific bile acid transporter. *Biochim Biophys Acta.* 1991; 1061:287–296. [PubMed: 1705441]
221. Zwicker BL, Agellon LB. Transport and biological activities of bile acids. *Int J Biochem Cell Biol.* 2013; 45:1389–1398. [PubMed: 23603607]

Figure 1.

The sinusoidal endothelium of the liver is fenestrated. Large and small fenestrae in the sinusoidal endothelium are observed in these scanning electron micrographs of sections of rat liver. (A) The portal end of a sinusoid is lined with endothelium containing many large fenestrae. Clusters of small fenestrae are also present. $\times 4200$. (B) The hepatic venous end of a sinusoid contains endothelium with only small fenestrae. $\times 5250$. (C) Sinusoidal endothelium showing both large and small fenestrae. (D) Microvilli on the sinusoidal surface of underlying hepatocytes (arrows) are visible through some fenestrae. $\times 19,600$. Reprinted, with permission, from (57) Grisham JW, Nopanitaya W, Compagno J, and Nagel AE. Scanning electron microscopy of normal rat liver: the surface structure of its cells and tissue components. *Am J Anat* 144: 295–321, 1975.

Figure 2.

Representative indicator dilution curves from an isolated perfused rat liver. A rat liver was perfused without recirculation at approximately 15 mL/min at 37°C *in situ* with oxygenated perfusate consisting of 20% (vol/vol) washed bovine erythrocytes in Krebs-Ringer buffer containing 2 g/dL bovine albumin and 100 mg/dL glucose. At time zero, a small bolus containing ^{51}Cr labeled red cells (RBC), ^{125}I -albumin (BSA), and ^3H -bilirubin (BR) was injected into the portal vein and all outflow was collected in aliquots approximately 2-s apart. In this study, recovery of red cells and albumin was essentially identical to what was injected (101% and 106% of injected), indicating that there was no removal during this single pass through the liver. In contrast, only 53% of bilirubin was recovered, indicating that 47% was taken up by the liver. Also of note is the comparison of the shapes of the red cell and albumin curves. Red cells remain in the sinusoids and come out faster, while albumin distributes into the space of Disse and has a more attenuated curve due to its larger volume of distribution.

Figure 3.

Functional expression of oatp cRNA in *Xenopus laevis* oocytes. Oocytes were either not injected or injected with 25 ng of total rat liver mRNA or 0.5 ng of oatp cRNA. Following culture for 3 days, uptake of ^{35}S -BSP was determined over 2 h at 25°C in 100 mmol/L NaCl containing 7.4 $\mu\text{mol/L}$ BSA (BSA/BSP molar ratio, 3.7) or medium in which NaCl was replaced isosmotically by choline chloride, Na gluconate, or sucrose as indicate. Bars represent means $\pm$ SD of 12 to 24 determinations in two separate oocyte preparations. Reprinted, with permission, from (83) Jacquemin E, Hagenbuch B, Stieger B, Wolkoff AW, and Meier PJ. Expression cloning of a rat liver Na^+ -independent organic anion transporter. *Proc Natl Acad Sci U S A* 91: 133–137, 1994. Copyright (1994) National Academy of Sciences, U.S.A.

Figure 4.

Diagram of the computer-generated 12-transmembrane domain model of oatp1a1. Intracellular and extracellular domains are indicated. The four potential N-linked glycosylation sites are indicated by the arrows. Although it is in a consensus site for N-glycosylation, asparagine 62 lies in a transmembrane domain and does not undergo N-glycosylation whereas asparagines 124, 135, and 492 are extracellular and are N-glycosylated. Reprinted, with permission, from (188) Wang P, Hata S, Xiao Y, Murray JW, and Wolkoff AW. Topological assessment of oatp1a1: a 12 transmembrane domain integral membrane protein with three N-linked carbohydrate chains. *Am J Physiol Gastrointest Liver Physiol* 294: G1052–G1059, 2008.

Figure 5.

Models for the seven transmembrane domain organization of ntcp. Data are consistent with two models. Panel A shows the seven membrane inserted sequences, H1, H2, H3, H4, H5, H6, and H9, with H7 and H8 represented as extending away from the membrane. Panel B shows these latter segments as membrane-associated or as re-entrant loops. CHO is an N-linked carbohydrate. Reprinted, with permission, from (111) Mareninova O, Shin JM, Vagin O, Turdikulova S, Hallen S, and Sachs G. Topography of the membrane domain of the liver Na⁺-dependent bile acid transporter. *Biochem* 44: 13702–13712, 2005. Copyright (2005) American Chemical Society.

Figure 6.

Importance of transporter subcellular distribution on ligand clearance. (A) Transporters such as oatps and ntcp can cycle in vesicles between the plasma membrane and intracellular locations. Recruitment and retrieval mechanisms utilize microtubule-based motility of vesicles and regulatory elements that may be unique for each transporter. If all transporter were to be sequestered within the cell, as in panel B, ligand (e.g., drug) in the sinusoidal (portal venous) blood, would bypass the hepatocyte uptake mechanism. This could result in high, potentially toxic, levels of this ligand in the peripheral circulation.

Figure 7. Characterization of bidirectional motility of ntcp-containing vesicles on microtubules (MTs) *in vitro*. (A) Polarity-marked fluorescent MTs (red) were attached to glass chambers, incubated with endocytic vesicles prepared from rat liver and washed. The ntcp-containing, MT-bound vesicles were then visualized with primary and fluorescent secondary antibodies, and 50 $\mu\text{mol/L}$ ATP was added to initiate motility. Time-lapse digital fluorescence images were captured in Cy2 (to detect ntcp) and Cy3 (to detect MTs) channels. Seconds after addition of ATP are indicated at the upper left. The arrows follow two motile ntcp-containing vesicles (bright green dots) moving in opposite directions. Arrowheads show the original location of the vesicles. “+” and “-” indicate the plus and minus ends of the MTs. Bar, 5 μm . (B) Quantification shows that ntcp-containing vesicles moved with approximately equal frequency toward the plus and minus ends. Parentheses indicate the number of vesicles moving in each direction. Reprinted, with permission, from (148) Sarkar S, Bananis E, Nath S, Anwer MS, Wolkoff AW, and Murray JW. PKCzeta is required for microtubule-based motility of vesicles containing the ntcp transporter. *Traffic* 7: 1078–1091, 2006.

Figure 8.

Involvement of the Phosphoinositide 3-Kinase/Protein Kinase C ζ (PI3K-PKC ζ) pathway in ntcp-containing vesicle motility on microtubules (MTs). (A) A simplified PI3K-PKC ζ pathway for regulation of ntcp-containing vesicle motility. PI3K converts PIP2 to PIP3 that then activates PKC ζ , leading to vesicle motility through unknown substrates. LY294002 and PKC ζ PS are inhibitors of PI3K and PKC ζ , respectively. (B) Motility of ntcp-containing vesicles on microtubules (MTs) was scored following incubation with buffer, 50, 100, or 200 μ mol/L LY294002. (C) Microtubule-based motility of ntcp-containing vesicles was scored following incubation with buffer, 10 mmol/L PIP3, 50 mmol/L LY294002 with and without 10 mmol/L PIP3, or PIP3 along with 50 mmol/L PKC ζ PS. * P < 0.005, ** P < 0.0001 compared to buffer control. Parentheses indicate the number of vesicles scored. Reprinted, with permission, from (148) Sarkar S, Bananis E, Nath S, Anwer MS, Wolkoff AW, and Murray JW. PKCzeta is required for microtubule-based motility of vesicles containing the ntcp transporter. *Traffic* 7: 1078–1091, 2006.

Figure 9.

Influence of phosphorylation on internalization of cell surface oatp1a1 in HuH7 cells stably transfected with GFP-oatp1a1 and in overnight-cultured rat hepatocytes. (A and B) HuH7-derived cell lines stably expressing nonphosphorylatable (GFP-oatp1a1^{AA}) or phosphomimetic (GFP-oatp1a1^{EE}) oatp1a1 constructs were prepared. These cells constitutively express PDZK1. Cells were surface biotinylated with membrane-impermeant sulfo-NHS-SS-biotin for 30 min at 4°C and then incubated at 37°C for up to 120 min to allow internalization. After removal of residual biotin from the cell surface by reduction, internalized biotinylated GFP-oatp1a1 was collected on streptavidin-agarose beads and subjected to immunoblot for oatp1a1. (A) Representative experiment. (B) Results of densitometric quantitation of four individual experiments. Data were normalized to total starting cell surface biotinylated oatp1a1. Lines are drawn through means at each time. Open symbols, oatp1a1^{AA}; filled symbols, oatp1a1^{EE}. (C and D) Hepatocytes isolated from rat liver and cultured overnight were surface biotinylated with membrane-impermeant sulfo-NHS-SS-biotin for 30 min at 4°C, and then incubated at 37°C for 10 or 30 min in the absence (–) or presence (+) of 1 mmol/L ATP. Previous studies showed that this short incubation of rat hepatocytes in extracellular ATP stimulates serine phosphorylation of oatp1a1 via activity of a purinergic receptor. After removal of residual biotin from the cell surface by reduction, internalized biotinylated oatp1a1 was collected on streptavidin-agarose

beads and subjected to immunoblot for oatp1a1. (A) Representative study. (B) Densitometric quantitation of three experiments. Data were normalized to total starting cell surface biotinylated oatp1a1. Values are means $\pm$ SE. Reprinted, with permission, from (23) Choi JH, Murray JW, and Wolkoff AW. PDZK1 binding and serine phosphorylation regulate subcellular trafficking of organic anion transport protein 1a1. *Am J Physiol Gastrointest Liver Physiol* 300: G384–G393, 2011.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 10.

Influence of phosphorylation and interaction with PDZK1 on subcellular distribution of oatp1a1. (A) Human embryonic kidney (HEK) 293T cells were transfected with plasmids encoding nonphosphorylatable (AA) or phosphomimetic (EE) oatp1a1 (oatp1a1^{AA} and oatp1a1^{EE}, respectively) linked to green fluorescence protein (GFP) at the NH₂ terminus. Experiments were performed without or with coexpression of PDZK1. After transfection, cells were cultured for 2 days, fixed and permeabilized with 0.1% Triton X-100 in PBS, and incubated with primary antibody to PDZK1 and Cy3-labeled secondary antibody. Distribution of fluorescence was examined by confocal microscopy. Scale bar, 8 μ m. B. HEK 293T cells were transfected with plasmids encoding FLAG-PDZK1 as well as full-length or truncated oatp1a1^{AA} or oatp1a1^{EE} linked to GFP at the NH₂ terminus. Truncated plasmids, indicated as -4, encoded oatp1a1 without its last 4 amino acids (KTKL), which define its PDZ-binding motif. After transfection, cells were cultured for 2 days, fixed and permeabilized with 0.1% Triton X-100 in PBS, and incubated with primary antibody to PDZK1 and Cy3-labeled secondary antibody. Distribution of fluorescence was determined by confocal microscopy. These studies indicate that an intact PDZ-binding motif is required for cell surface expression of oatp1a1 independent of its phosphorylation state. Scale bar, 8 μ m. Reprinted, with permission, from (23) Choi JH, Murray JW, and Wolkoff AW. PDZK1 binding and serine phosphorylation regulate subcellular trafficking of organic anion transport protein 1a1. *Am J Physiol Gastrointest Liver Physiol* 300: G384–G393, 2011.

Figure 11.

Microtubule-based motility of Oatp1a1-associated endocytic vesicles. Oatp1a1-containing endocytic vesicles were prepared from livers of wild type (WT) and PDZK1 knockout (KO) mice and flowed into microchambers that had been coated with polarity-marked fluorescent microtubules. After the binding of vesicles to microtubules, motility was initiated with the addition of 50 $\mu\text{mol/L}$ ATP. (A) Representative images demonstrating minus-end directed movement of an Oatp1a1-containing vesicle prepared from PDZK1 knockout mouse liver. A red microtubule with attached green Oatp1a1-labeled vesicles runs horizontally and contains markings for microtubule polarity. The polarity marks were generated by polymerizing brightly fluorescent tubulin from short, dimly fluorescent microtubule seeds, allowing the growth of long microtubule plus ends. Visible from left to right is the microtubule minus end, a dimly fluorescent seed, and the microtubule plus end (+) to which a green, motile vesicle is bound. The white arrow follows this vesicle as it moves toward the minus end of the microtubule. The yellow arrowhead indicates the starting point for the vesicle. Seconds after addition of ATP are indicated at the top left of each panel. In the 34 seconds of this study, the vesicle moved approximately 18 μm (approximately 0.5 $\mu\text{m/s}$). Scale bar = 10 μm . (B) The percentage of microtubule-bound vesicles that moved following ATP addition is indicated by the bars for vesicles prepared from wild-type (open bars) and PDZK1 knockout (solid bars) mice. (C) The percentage of motile vesicles from the studies in (B) moving toward the plus (closed bars) or minus (open bars) ends of microtubules is indicated. Numbers in parentheses represent the number of motile vesicles that were examined. Error bars represent the mean $\pm$ SEM * $P < 0.0001$ as compared with plus-end motility of wild-type vesicles; ** $P < 0.0001$ as compared with minus-end motility of wild-type vesicles. Reprinted

with permission from (191) Wang WJ, Murray JW, and Wolkoff AW. Oatp1a1 requires PDZK1 to traffic to the plasma membrane by selective recruitment of microtubule-based motor proteins. *Drug Metab Dispos* 42: 62–69, 2013.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 12.

Colocalization of motor proteins and PDZK1 with Oatp1a1-associated vesicles. Endocytic vesicles isolated from wild-type (WT) and PDZK1 knockout (KO) mouse livers were attached to the glass surface of microchambers and immunostained for Oatp1a1 and motor proteins or PDZK1. (A) Representative images are shown in which Oatp1a1 is in red and PDZK1 or motor proteins are in green. Vesicles in yellow represent colocalization of the two. Scale bar = 10 μm. (B) Quantification of protein colocalization with Oatp1a1-containing vesicles. The percentage of Oatp1a1-containing vesicles that colocalized with each of the proteins indicated in the figure is represented by filled (wild-type) or open (PDZK1 knockout) bars. The number of Oatp1a1-associated vesicles examined is in parentheses. Error bars represent the mean ± S.E.M. * $P < 0.0001$ as compared with colocalization in wild-type vesicles. Reprinted, with permission, from (191) Wang WJ, Murray JW, and Wolkoff AW. Oatp1a1 requires PDZK1 to traffic to the plasma membrane by selective recruitment of microtubule-based motor proteins. *Drug Metab Dispos* 42: 62–69, 2013.