Human organic anion transporter 1B1 and 1B3 function as bidirectional carriers and do not mediate GSH-bile acid cotransport

Chitravina Mahagita,1,2 Steven M. Grassl,2 Pawinee Pyiachaturawat,1 and Nazzareno Ballatori3

1Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand; 2Department of Pharmacology, State University of New York Upstate Medical University, Syracuse; and 3Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, New York

Submitted 12 February 2007; accepted in final form 3 April 2007

Mahagita C, Grassl SM, Pyiachaturawat P, Ballatori N. Human organic anion transporter 1B1 and 1B3 function as bidirectional carriers and do not mediate GSH-bile acid cotransport. Am J Physiol Gastrointest Liver Physiol 293: G271–G278, 2007. First published April 5, 2007; doi:10.1152/ajpgi.00075.2007.—Organic anion transporting polypeptides (OATP/SLCO) are generally believed to function as electroneutral anion exchangers, but direct evidence for this contention has only been provided for one member of this large family of genes, rat Oatp1a1/Oatp1 (Slc10a1). In contrast, a recent study has indicated that human OATP1B3/OATP-8 (SLCO1B3) functions as a GSH-bile acid cotransporter. The present study examined the transport mechanism and possible GSH requirement of the two members of this protein family that are expressed in relatively high levels in the human liver, OATP1B3/OATP-8 and OATP1B1/OATP-C (SLCO1B1). Uptake of taurocholate in Xenopus laevis oocytes expressing either OATP1B1/OATP-C, OATP1B3/OATP-8, or polymorphic forms of OATP1B3/OATP-8 (namely, S112A and/or M233I) was cis-inhibited by taurocholate and estrone sulfate but was unaffected by GSH. Likewise, taurocholate and estrone sulfate transport were trans-stimulated by estrone sulfate and taurocholate but were unaffected by GSH. OATP1B3/OATP-8 also did not mediate GSH efflux or GSH-taurocholate cotransport out of cells, indicating that GSH is not required for transport activity. In addition, estrone sulfate uptake in oocytes microinjected with OATP1B3/OATP-8 or OATP1B1/OATP-C cRNA was unaffected by depolarization of the membrane potential or by changes in pH, suggesting an electroneutral transport mechanism. Overall, these results indicate that OATP1B3/OATP-8 and OATP1B1/OATP-C most likely function as bidirectional facilitated diffusion transporters and that GSH is not a substrate or activator of their transport activity.

membrane transport; anion exchange; driving force; glutathione; organic solute transporters

THE CLEARANCE of endogenous substances and xenobiotics from blood is one of the vital functions of the liver. This elaborate system requires specific uptake, biotransformation, and excretion mechanisms. Hepatic uptake is mediated in large part by three types of transporters: the sodium-taurocholate cotransporter (SLC10) (9), organic anion or cation transporters (SLC22A) (6, 15), and organic anion transporting polypeptides (OATP/SLCO) (12, 20).

Members of the OATP/SLCO family of solute carriers function as transporters for a large variety of amphipathic organic compounds. These proteins mediate Na+- and ATP-independent transport of bile salts, steroids, thyroid hormones, anionic peptides, drugs, and xenobiotics (7, 8). Among the OATP/SLCO family members, OATP1B1/OATP-C (SLCO1B1) and OATP1B3/OATP-8 (SLCO1B3) are expressed selectively in the liver (1, 11, 16) and have also been called liver-specific organic anion transporters (LST-1 and LST-2, respectively).

The current nomenclature for the OATP/SLCO family was introduced in 2004 by Hagenbuch and Meier (7) to prevent confusion and to permit unequivocal, species-independent identification.

SLCO1B1 and SLCO1B3 genes, encoding OATP1B1/OATP-C and OATP1B3/OATP-8 proteins, were identified and cloned in 1999–2000 (1, 11, 16, 17) and are thought to function as the major hepatic uptake systems for endogenous compounds, drugs, and albumin-bound organic anions. More than 20 single-nucleotide polymorphisms (SNPs) of the SLCO1B1 gene have been identified in African-American, European-American, and Japanese people (18), and 3 nonsynonymous SNPs in human blood samples have been reported for SLCO1B3, consisting of 334T>G, encoding OTP1B3/OATP-8-S112A; 699G>A, encoding OTP1B3/OATP-8-M233I; and 1564G>T, encoding OTP1B3/OATP-8-G522C (18, 21). The 334T>G and 699G>A gene mutations are detected frequently in European and American Caucasians (>70% allelic frequency for each), whereas the 1564G>T mutant is seen in ~2% of individuals in these populations (21, 30). The OATP1B3/OATP-8 reference sequence and the S112A and M233I sequences localize correctly to the basolateral membrane of hepatocytes and exhibit similar transport properties (21). In contrast, OTP1B3/OATP-8-G522C is retained inside cells and is thus unable to mediate cellular transport activity (21).

In recent years, numerous studies (4, 10, 13, 24, 26, 30, 32) have focused on the substrates transported by human OATP1B1/OATP-C and OATP1B3/OATP-8, their roles in liver disease, and their regulation of drug disposition. On the other hand, the energy coupling transport mechanisms of human liver OATPs remain poorly understood. OATP/Oatp-mediated transport is independent of sodium, chloride, and potassium gradients, membrane potential, and ATP levels and is generally believed to occur by electroneutral exchange, in which the cellular uptake of organic anions is coupled to the efflux of anions such as HCO3-, GSH, and/or glutathione-S-conjugates (12, 14, 19, 22, 23, 27–29, 33). However, a coupled exchange mechanism has been demonstrated for only one member of this large protein family, namely, rat Oatp1a1 (22, 28). Studies with other members of this family have been
unable to identify the driving force and have also not implicated a role for GSH or HCO₃⁻ in the transport cycle. In contrast with the electroneutral transport observed in these previous studies, a recent study (3) with OATP1B3/OATP-8 suggests that this protein functions as a GSH-bile acid co transporter and thus would presumably result in the net movement of charge across the plasma membrane, although this was not directly examined.

Based on a comparative analysis of OATPs from multiple species, Meier-Abt et al. (25) proposed a common transport mechanism for all OATPs/Oatps, whereby substrates are translocated through a central, positively charged pore in a rockercswitch type of mechanism. However, this analysis did not establish whether transport is coupled (i.e., driven by exchange or cotransport with another molecule) or simply occurs by facilitated diffusion through this putative central pore. The present study aimed to investigate the transport mechanism mediated by OATP1B1/OATP-C and OATP1B3/OATP-8 and to examine whether GSH is required for their energy coupling mechanism. Because OATP1B3/OATP-8 has some common polymorphisms, three of these were also examined (namely, S112A and M233I, and both S112A and M233I) to compare their transport functions. The latter amino acid sequence is the most prevalent among Europeans and Americans (56% allelic frequency) (21), and most of the present experiments were carried out with this sequence (i.e., OATP1B3/OATP-8-S112A&M233I).

MATERIALS AND METHODS

Materials. [³H]estrone sulfate (46 Ci/mmol), [³H]taurocholate (1.19 Ci/mmol), and [³H]GSH (41.5 Ci/mmol) were purchased from NEN Life Science Products (Boston, MA). Other chemicals and reagents were obtained from Sigma-Aldrich Chemical (St Louis, MO) or from J. T. Baker (Phillipsburg, NJ). Mature Xenopus laevis oocytes were purchased from Nasco (Fort Atkinson, WI). Animals were maintained under constant light. SLCO1B1 and SLCO1B3-S112A&M233I cDNAs were kindly provided by Drs. Bruno Stieber and Bruno Hengbuch (Zurich, Switzerland, and Kansas City, MO, respectively). Capped cRNA was transcribed in vitro with T7 RNA polymerase (Ambion, Austin, TX), and cRNA was precipitated with lithium chloride and resuspended in RNase-free water for oocyte injection. Generation of OATP cRNA was verified by gel electrophoresis.

Site-directed mutagenesis of SLCO1B3. Mutations were introduced into the cDNA of SLCO1B3-S112A&M233I, which contains both S344T>G and 699G>A in pSPORT, using the QuickChange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). Primers were purchased from Operon Biotechnologies (Huntsville, AL). For mutano of SLCO1B3-344G>T, the forward primer was 5'-GGG AAC TGG AAG TAT TTT GAC AGC ATG TTT ACC ACA TTT CCT CAT GGG-3' and the reverse primer was 5'-CCC ATG AAG AAA TGT GGT AAA GAT GTC AAA ATA ATC CCA GGT TTC CCC-3'. For the SLCO1B3-699A>G mutation, the forward primer was 5'-GGG ATC TCT GTT TGC TAA GAA GTA CGG TAT TGG ATA AGT G3' and the reverse primer was 5'-CTA CAT ATC CAA TATCCA CGT ACA TTT TAG CAA ACA GAC ACT CC-3'. DNA sequencing confirmed the intended mutations (underlined).

cDNA sequence analysis. cDNAs were sequenced at the DNA Sequencing Facility of the Mount Desert Island Biological Laboratory (Salisbury Cove, ME). cDNA sequences were aligned against the reference sequences (GenBank/EMBL Accession Nos. NM_006446 and NM_019844 for SLCO1B1 and SLCO1B3 genes, respectively).

Xenopus laevis oocyte preparation and injection. Isolation of Xenopus laevis oocytes was performed as described by Goldin (5) and previously employed in our laboratory (2, 22). Frogs were anesthetized by immersion for 15–20 min in ice-cold 0.3% tricane. Oocytes were removed from the ovary and washed with Ca²⁺-free OR-2 solution (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 5 mM HEPES-Tris; pH 7.5) and incubated at room temperature with gentle shaking for 90 min in OR-2 solution containing 2 mg/ml collagenase (type IA, Sigma). Oocytes were transferred to fresh collagenase solution after the first 45 min. Collagenase was removed by a wash with OR-2 solution at room temperature. Defolliculated Stage V and VI oocytes were selected and incubated at 18°C in modified Barth’s solution (pH 7.4–7.5) [88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO₃, 0.82 mM MgSO₄, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, and 20 mM HEPES-Tris (pH 7.5)] or U-medium (100 mM choline chloride, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES; pH was adjusted to 7.0 with Tris) supplemented with gentamycin (0.5 mg/ml). U-medium was used in some experiments, reproducing the conditions used by Briz et al. (3). After 2 h of incubation, oocytes were injected with 50 nl of OATP1B1/OATP-C cRNA, OATP1B3/OATP-8 cRNA (5 ng/oocyte), or sterile water. Injected oocytes were cultured at 18°C with a daily change of modified Barth’s media or U-medium containing gentamycin. Oocytes with a homogeneous brown animal half and distinct equator line were selected for transport experiments after 3 days.

Cis-inhibition of [³H]estrone sulfate and [³H]taurocholate uptake into oocytes. Control or cRNA-injected oocytes were incubated at 25°C in 100 μl of modified Barth’s solution or U-medium in the presence of [³H]estrone sulfate or [³H]taurocholate with or without other substrates. The pH of GSH solutions was adjusted to 7.4 using dilute NaOH. For some experiments, the pH of the modified Barth’s solution was adjusted to 6.5 using dilute HCl and to 8.5 using dilute NaOH. Uptake was stopped by rapid dilution with 2.5 ml of ice-cold modified Barth’s solution or U-medium, and oocytes were washed three times with these solutions. Two oocytes were placed in a polypropylene scintillation vial and dissolved in 200 μl of 10% SDS by vortexing. Five milliliters of Opti-Fluor (Packard Instruments, Downers Grove, IL) scintillation cocktail were added. Samples were left for at least 3 h before being counted in a Beckman 6500 scintillation counter.

Trans-stimulation of [³H]taurocholate and [³H]estrone sulfate uptake into oocytes. Control and cRNA-injected oocytes were reinjected with 50 nl of each of the following solutions: 550 μM estrone sulfate, 550 μM taurocholate, 220 mM GSH, 275 mM NaHCO₃, 55 mM NaSO₄, or 550 μM 17β-estradiol-d-17β-glucuronide (E₂17βG). Oocytes were washed three times at room temperature and then incubated for 20–40 min in ice-cold modified Barth’s solution or U-medium. [³H]estrone sulfate or [³H]taurocholate uptake was measured at 25°C for 30 min or 1 h, respectively.

Efflux of [³H]GSH and [³H]taurocholate from oocytes. Fifty nanoliters of either 20 mM [³H]GSH, 300 μM [³H]taurocholate, or 300 μM [³H]taurocholate with 20 mM unlabeled GSH were injected into control and cRNA-injected oocytes, which were washed three times at room temperature and then allowed to recover for 20–40 min in ice-cold U-medium solution. The pH of the injected solutions was adjusted to ~7.4 using dilute NaOH. Efflux was measured in individual oocytes for 1 h at 25°C in 200 μl of U-medium. Acivicin (0.5 mM) was used in experiments that measured [³H]GSH efflux (2, 22, 23). The medium was removed to terminate efflux and counted separately from the oocyte.

Trans-stimulation of [³H]estrone sulfate efflux from oocytes. Control and cRNA-expressing oocytes were preloaded by a 1-h incubation at 25°C with ~50 nM [³H]estrone. Oocytes were washed, and efflux was measured at 25°C for 30 min in the presence and absence of either 0.1 mM estrone sulfate, 0.1 mM taurocholate, 0.1 μM leukotriene C₄, or 10 mM GSH in 200 μl of modified Barth’s solution. Amounts of [³H]estrone sulfate in the oocytes and the efflux buffer were measured.
Membrane potential measurements. Single oocytes were placed in a small (0.2 ml) Plexiglas chamber and superfused with modified Barth’s solution or a high-K+/H11001 modified Barth’s solution in which the NaCl was replaced with KCl (88 mM). Membrane potential determinations were made as previously described using a custom-made voltage-clamp amplifier (31). Both reference electrodes and intracellular recording electrodes were filled with 3 M KCl, and oocytes were impaled at the vegetal pole. All measurements were made at room temperature (22–24°C).

Statistical analysis. Statistical significance was determined using an unpaired t-test for two means where \( P < 0.05 \) was taken as the limit denoting significant differences. Values are means ± SE.

RESULTS

OATP1B3/OATP-8 and three of its polymorphisms do not mediate GSH-bile acid cotransport. To replicate the finding that OATP1B3/OATP-8 can mediate cotransport of bile acids and GSH (3), the effects of GSH were examined under multiple experimental conditions using four different OATP1B3/OATP-8 sequences: the reference sequence (Refseq), S112A (SLCO1B3-334T>G), M233I (SLCO1B3-699G>A), and a construct containing both S112A and M233I (S112A&M233I). As noted above, the sequence containing both S112A and M233I is the most common sequence found in European and American Caucasians. When expressed in Xenopus laevis oocytes, each of these four constructs was able to mediate taurocholate transport activity and to roughly similar levels (Fig. 1). To examine the inhibitor sensitivity of the four constructs, cis-inhibition of taurocholate uptake was measured (Fig. 2). Surprisingly, GSH and GSSG did not cis-stimulate taurocholate uptake by OATP1B3/OATP-8, as expected based on the results of Briz and coworkers (3). As expected, E217G, taurocholate, and estrone sulfate cis-inhibited the uptake of taurocholate by all four sequences, whereas HCO\(_3^-\) had no significant effect (Fig. 2).

To further examine whether OATP1B3/OATP-8 functions as a GSH-bile acid cotransporter, trans-stimulation of taurocholate uptake was investigated in oocytes expressing OATP1B3/OATP-8 and two of its polymorphic forms. In agreement with the cis-inhibition results (Fig. 2), E217G,
taurocholate, and estrone sulfate trans-stimulated taurocholate uptake with these constructs, albeit at slightly different levels (Fig. 3). However, taurocholate uptake was not affected by GSH or HCO$_3^-$ (Fig. 3), suggesting that GSH is neither cotransported nor countertransported with taurocholate. In the experiments described above, all solutions containing GSH were supplemented with either NaOH or KOH to adjust the pH to the physiological range (i.e., to raise the pH from ~3.7 to 7.4). Interestingly, when the pH was not adjusted, taurocholate uptake was cis-stimulated (Fig. 4). Similar results were noted for GSSG (Fig. 4). However, because these low pH values are well beyond the physiological range, and are possibly toxic to the oocytes, the observed transport stimulation may be of no significance with regard to transport mechanism.

OATP1B3/OATP-8-S112A&M233I-mediated uptake of estrone sulfate is not cis-inhibited or trans-stimulated by GSH. Cis-inhibition and trans-stimulation of another substrate, estrone sulfate, was examined to further test the hypothesis that GSH is not required for OATP1B3/OATP-8-S112A&M233I function. As expected, [3H]estrone sulfate uptake by OATP1B3/OATP-8-S112A&M233I was cis-inhibited by taurocholate, estrone sulfate, dehydroepiandrosterone sulfate (DHEAS), E$_2$17ßG, sulfobromophthalein sodium (BSP), and probenecid but not by GSH, HCO$_3^-$, SO$_4^{2-}$, glutamate, glutarate, lactate, and citrate (Fig. 5A). [3H]estrone sulfate uptake was trans-stimulated by intracellular estrone sulfate and taurocholate but not by GSH, HCO$_3^-$, and SO$_4^{2-}$ (Fig. 5B). These results are in agreement with the findings presented in Figs. 1–3 and also suggest that glutamate, glutarate, lactate, and citrate are unlikely to be substrates of this transporter.

OATP1B3/OATP8-S112A&M233I does not mediate [3H]GSH export, and intracellular GSH does not stimulate [3H]taurocholate efflux. To investigate whether GSH is a direct substrate for OATP1B3/OATP8-S112A&M233I, [3H]GSH efflux and GSH cis-stimulation of [3H]taurocholate efflux was measured. As shown in Fig. 6A, GSH efflux was not enhanced in OATP1B3/OATP8-S112A&M233I-expressing oocytes (~2% release/h), whereas Briz et al. (3) demonstrated an extensive efflux of GSH in both control and OATP1B3/OATP8-S112A&M233I-expressing oocytes (~30% and 60% release/h, respectively). Oatp1a1/Oatp1 was used as a positive control for GSH release, and this transporter did increase GSH efflux (Fig. 6A), as previously reported (22). As shown in Fig. 6B, high intracellular GSH did not promote [3H]taurocholate efflux in OATP1B3/OATP8-S112A&M233I-injected oocytes compared with control, supporting the present conclusion that OATP1B3/OATP-8-S112A&M233I does not function as a GSH-bile acid cotransporter.
OATP1B1/OATP-C and OATP1B3/OATP-8 transport of taurocholate and estrone sulfate is also not cis-inhibited or trans-stimulated by GSH. Similar to OATP1B3/OATP-8, taurocholate and estrone sulfate uptake by OATP1B1/OATP-C was cis-inhibited by taurocholate, DHEAS, E217/H9252 G, BSP, and probenecid but not by GSH, glutamate, glutarate, lactate, and citrate (Fig. 7, A and B). HCO₃⁻ and SO₄²⁻ slightly inhibited taurocholate uptake (Fig. 7A) but had no effect on estrone sulfate uptake (Fig. 7B). [³H]Estrone sulfate efflux was trans-stimulated by unlabeled estrone sulfate and taurocholate but not by GSH (Fig. 7C). Therefore, GSH is also not required for OATP1B1/OATP-C transport activity.

pH and membrane potential insensitivity of OATP1B1/OATP-C and OATP1B3/OATP-8-S112A&M233I. To examine if the energy coupling mechanism involves the pH gradient, estrone sulfate uptake was measured in media of pH 6.5, 7.5, or 8.5 (Fig. 8). However, estrone sulfate uptake was unaffected either at pH 6.5 or 8.5, indicating that H⁺ and OH⁻ are probably not involved. The effect of membrane potential on the transport mechanism of OATP1B1/OATP-C and OATP1B3/OATP-8-S112A&M233I was investigated by isoosmotic substitution of NaCl for KCl in modified Barth’s solution, resulting in a high K⁺ and a depolarization of the membrane potential. Oocyte resting membrane potentials were approximately −40 mV (Fig. 9A). As expected, a marked depolarization was observed upon K⁺ substitution, and this depolarization was reversed upon return to buffer with Na⁺ (Fig. 9A). If transport is mediated by a uniport mechanism or by a cotransport with another anion, it would be electrogenic and thus should be sensitive to membrane potential alteration. However, membrane potential depolarization induced by increased K⁺ had no effect on estrone sulfate uptake by OATP1B1/OATP-C or OATP1B3/OATP-8 (Fig. 9, B and C). Therefore, the transport on both OATPs is independent of membrane potential and...
the Na\(^+\) gradient and appears to be electroneutral. Because estrone sulfate is a monovalent anion, the voltage insensitivity of its uptake suggests that it associated with either the outward movement of a monovalent anion or the uptake of a monovalent cation; however, the identity of these ions remains unknown.

**DISCUSSION**

Despite many years of work by several laboratories, the energetic coupling mechanism for the large family of OATP proteins remains largely unknown. Although OATPs are generally regarded as electroneutral anion exchangers, direct evidence for this contention has only been provided for one member of this large family of proteins, rat Oatp1a1. However, even for this one protein, there is uncertainty as to whether it functions as a GSH exchanger or as a HCO\(_3^-\) exchanger (22, 28). The present data provide support for the hypothesis that human OATP1B1 and OATP1B3 function as bidirectional facilitated diffusion transporters rather than exchangers or cotransporters. That is, transport on OATP1B1 and OATP1B3 appears to be energized by the electrochemical substrate gradient and is not directly coupled to the simultaneous movement of another substrate across the membrane. The evidence for this is as follows: 1) none of a variety of plausible cotransported or countertransported ions tested had any significant effect on the rate of transport; and 2) a modest trans-stimulation of transport was observed only when known organic solute substrates were placed on opposite sides of the plasma membrane. This modest trans-stimulation is consistent with accelerated exchange diffusion (i.e., more rapid movement of the loaded carrier) rather than coupled transport; and 3) OATP-mediated export from the oocytes was observed despite the fact that the cell culture medium consisted of a simple salt solution and thus was presumably devoid of countertransportable organic ions. Nevertheless, these data cannot conclusively rule out the possibility that an as-yet-unidentified ion may have been present and may have energized the transport event. Thus, additional studies are needed to resolve this critical question.

The present results also demonstrate that GSH has no effect on substrate uptake or efflux on OATP1B1/OATP-C and OATP1B3/OATP-8, indicating that GSH is not required for transport activity. These findings contrast with those of Briz et al. (3), who reported that OATP1B3/OATP-8 mediates the cotransport of bile acids with either GSH or GSSG. In an attempt to resolve this discrepancy, the present study characterized the transport properties of both the reference sequence for OATP1B3/OATP-8 and three of its common polymorphisms, since SNPs may alter their transport activity (22). However, the four OATP1B3/OATP-8 sequences examined in this study exhibited comparable transport properties, and none were dependent on GSH for their activity. In addition, exper-
Fig. 9. Membrane potential insensitivity of OATP1B1/OATP-C and OATP1B3/OATP-8 mediated transport. A: membrane potential was measured in oocytes cultured in media in which KCl (K) was substituted for 88 mM NaCl in modified Barth’s solution (MB). Measurements were made in sets of 6 oocytes with bath changes from MB to high K and from high K to MB. Control and cRNA-injected oocytes were incubated at 25°C in MB containing 50 nM and 10 μM [3H]GSH for OATP1B1/OATP-C (B) and OATP1B3/OATP-8 (C), respectively. Values are means ± SE; n = 3–5 experiments in different oocyte preparations. *P < 0.01 compared with the MB control of each group.

ml 10.2 ± 0.3

G277 OATP1B1/OATP-C AND OATP1B3/OATP-8 TRANSPORT MECHANISM

membrane potential was markedly depolarized when Na

either OATP1B1/OATP-C or OATP1B3/OATP-8. Although HCO3

The uptake of estrone sulfate by OATP1B1/OATP-C and

- saturated at a surprisingly high rate (30%/h), and this was further enhanced in oocytes expressing OATP1B3/OATP-8 (60%/h). In contrast, our present (Fig. 6) and previous studies (2, 22) indicated that oocytes release [3H]GSH at rate of 2–3%/h, which is more consistent with cellular GSH turnover rates (2). As shown in Fig. 6A, GSH release was not enhanced in OATP1B3/OATP-8-expressing oocytes, and it was not affected by loading the oocytes with taurocholate (Fig. 6B). It is important to note that, in contrast with GSH, oocytes have an active endogenous taurocholate export mechanism (31) and that the baseline rate of taurocholate export is relatively high (31) (Fig. 6B). This high background rate of taurocholate release by oocytes probably masks any small release that may be mediated by OATP1B3/OATP-8 under these conditions.

Except for known substrates, none of the anions tested altered transport activity on either OATP1B1/OATP-C or OATP1B3/OATP-8. Although HCO3 and SO4 showed significant cis-inhibition of taurocholate uptake by OATP1B1/OATP-C, the magnitude was small compared with that produced by taurocholate, estrone sulfate, BSP, or probenecid (Fig. 7A). Estrone sulfate uptake by OATP1B1/OATP-C was also not cis-inhibited by HCO3 and SO4 (Fig. 7B). Transport of taurocholate and estrone sulfate occurred in both the inward and outward direction, indicating that transport is bidirectional. Furthermore, we found that estrone sulfate uptake was not significantly altered when the pH was changed from 7.5 to 6.5 or 8.5 (Fig. 8).

The uptake of estrone sulfate by OATP1B1/OATP-C and OATP1B3/OATP-8 was also not significantly change after substitution of NaCl for KCl in modified Barth’s solution (Fig. 9, B and C). This Na+-independent transport is in agreement with previous studies (1, 7, 17) indicating that OATP proteins do not require Na+ for their transport function. Because membrane potential was markedly depolarized when Na+ was replaced with K+ (Fig. 9A), these data also argue against an electrogenic GSH-bile acid cotransport mode of transport of OATP1B3/OATP-8. Given that both GSH and taurocholate are anions, cells would lose (or gain) two negative charges with each transport cycle, and thus this process should be sensitive to membrane potential. Although the present and previous results indicate that transport is electroneutral, the nature of the neutralizing ion is unknown.

ACKNOWLEDGMENTS

We thank Albert Koh and Whitney Christian for expert technical assistance and Dr. Ted Begenisich (Department of Pharmacology and Physiology, University of Rochester) for assistance in making electrophysiology measurements.

GRANTS

This work was supported in part by National Institute of Health Grants DK-48823, DK-067214, ES-01247, and ES-07026. Additional financial support for C. Mahagita and P. Piychatorawat was provided by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant PHD/0266/2545).
REFERENCES


