Identification of Glutathione as a Driving Force and Leukotriene C₄ as a Substrate for oatp1, the Hepatic Sinusoidal Organic Solute Transporter*

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oatp1 is an hepatic sinusoidal organic anion transporter that mediates uptake of various structurally unrelated organic compounds from blood. The driving force for uptake on oatp1 has not been identified, although a role for bicarbonate has recently been proposed. The present study examined whether oatp1-mediated uptake is energized by efflux (countertransport) of intracellular reduced glutathione (GSH), and whether hydrophobic glutathione S-conjugates such as leukotriene C₄ (LTC₄) and S-dinitrophenylglutathione (DNP-SG) form a novel class of substrates for oatp1. Xenopus laevis oocytes injected with the complementary RNA for oatp1 demonstrated higher uptake of 10 nM [3H]LTC₄ and 50 mM [3H]DNP-SG, and higher efflux of [3H]GSH (2.5 mM endogenous intracellular GSH concentration). The oatp1-stimulated LTC₄ and DNP-SG uptake was independent of the Na⁺ gradient, cis-inhibited by known substrates of this transport protein and by 1 mM GSH, and was saturable, with apparent Kᵦ values of 0.27 ± 0.06 and 408 ± 95 μM, respectively. Uptake of [3H]taurocholate, an endogenous substrate of oatp1, was competitively inhibited by DNP-SG. Of significance, oatp1-mediated taurocholate and LTC₄ uptake was cis-inhibited and trans-stimulated by GSH, and [3H]GSH efflux was enhanced in the presence of extracellular taurocholate or sulfobromophthalein, indicating that GSH efflux down its large electrochemical gradient provides the driving force for uptake via oatp1. The stoichiometry of GSH/taurocholate exchange was 1:1. These findings identify a new class of substrates for oatp1 and provide evidence for GSH-dependent oatp1-mediated substrate transport.

A major hepatic function is the clearance of a multitude of metabolic products and foreign compounds (xenobiotics) from the blood. Hepatic uptake of organic solutes across the sinusoidal membrane is mediated by multiple transport systems, and four transporters have now been cloned. Ntcp mediates Na⁺-coupled transport of bile acids (1), oct1 mediates H⁺-coupled uptake of organic cations (2), and oatp1 and oatp2 are related multispecific transporters for which driving force has not yet been identified (3–5). A recent study indicates that HCO₃⁻ efflux may be involved in oatp1-mediated transport (6); however, because intracellular pH is lower than extracellular pH, the HCO₃⁻ gradient is unlikely to energize transport of organic anions on oatp1. Indeed, Satlin and co-workers (6) demonstrated that oatp1-mediated cellular uptake of taurocholate was accelerated only when the pH gradient was reversed from the normal physiological condition. Under all other pH gradient conditions examined by these investigators, taurocholate uptake was lower than that seen in controls, contradicting the hypothesis that the bicarbonate gradient energizes oatp1-mediated transport.

oatp1-mediated transport is independent of ATP and of transmembrane Na⁺, K⁺, or H⁺ gradients (3–5). Transport is also independent of the Cl⁻ gradient when measured in the absence of albumin (3). However, oatp1-mediated transport is bidirectional (7), suggesting that solute uptake may be energized by efflux of an intracellular solute. Whether oatp1 actually functions as an antiporter (exchanger) and which intracellular solute may be involved remain to be established.

One solute that could function as an intracellular substrate for oatp1 and related organic anion transporters is reduced glutathione (GSH). This anionic tripeptide is present in high concentrations in virtually all cells, including hepatocytes (~10 mM), whereas plasma concentrations are only about 10 μM (8–10). The high GSH chemical gradient coupled with the negative intracellular membrane potential could provide a substantial driving force for uptake via oatp1.

Export of GSH into the extracellular space is the initial and perhaps limiting step in the turnover of the tripeptide in all mammalian cells (8–10); however, the transport system or systems that mediate GSH efflux have not been identified. The previously reported cloning of sinusoidal and canalicular GSH transporters has not been confirmed (14) and appears to be an artifact (10, 14). Rates of GSH efflux differ between cell types and are roughly equivalent to the rates of synthesis under physiological conditions. The liver is quantitatively the major site of synthesis in the body, and the major supplier of plasma GSH (8–10). Studies in intact hepatocytes and isolated sinusoidal plasma membrane vesicles indicate that GSH efflux is mediated by a low affinity, ATP-independent, electrogenic carrier (11–14). Transport is bidirectional, although under physiological conditions unidirectional transport down its concentration gradient is favored, i.e. efflux of GSH from hepatocytes (11–14).

The present study also examined whether leukotriene C₄ (LTC₄) is a substrate for oatp1. The cysteinyl leukotrienes are

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1 The abbreviations used are: LTC₄, leukotriene C₄; BSP, bromosulphophthalein; BSP-SG, glutathione S-conjugate of BSP; DNP-SG, S-(2,4-dinitrophenyl)-glutathione; cRNA, complementary RNA.
potent mediators of inflammatory responses (15), and the liver is the major site of their removal from the blood (15–18). The mechanism for their hepatic uptake is unknown, although evidence from isolated hepatocytes and perfused rat liver studies indicate that uptake is mediated by oatp1 (17–19). LTC4 uptake into isolated rat hepatocytes and plasma membrane vesicles is independent of the Na\(^+\) gradient, is inhibited by substrates of oatp1, and exhibits an apparent Km of 0.20 μM (19). Once taken up into hepatocytes, LTC4 is transported across the canalicular membrane into bile by the ATP-dependent transporter mrp2 (which is also called cMRP or cMOAT). Hepatic canalicular membrane into bile by the ATP-dependent translocation of glutathione 1-conjugates, the present study measured LTC4 and DNP-SG uptake in Xenopus laevis oocytes injected with oatp1 complementary RNA (cRNA). The results demonstrate that LTC4 and DNP-SG are substrates for this multispecific organic solute transporter, and that hepatic uptake is energized by countertransport of intracellular GSH.

**EXPERIMENTAL PROCEDURES**

**Materials and Animals**—[14,15,19,20-H]Leutokrine C\(_4\) (165 Ci/ mmol), [γ-32P]-3H-glutathione (50 Ci/mmol), and [3H(G)]-taurocholate (3.47 Ci/mmol) were purchased from NEN Life Science Products. Chemicals and reagents were obtained from Sigma, Aldrich, or J. T. Baker. Mature X. laevis were purchased from Nasco, Fort Atkinson, WI. Animals were maintained under a constant light cycle at a room temperature of 18 °C.

Unlabeled DNP-SG and S-sulfobromophthalein-glutathione (BS-PG) were synthesized and purified as described previously (21, 22). [3H]DNP-SG was synthesized enzymatically from [3H]GSH and 1-chloro-2,4-dinitrobenzene as described previously (23). It was purified on a 1 × 8-cm DEAE-Sephadex A25 column (Amersham Pharmacia Biotech), eluted with 25 ms Tris (Tris-Hcl, pH 7.4) at a flow rate of 43 ml/h. Fractions containing [3H]DNP-SG were concentrated on a vacuum evaporator (Jouan, Winchester, VA). The final concentration of Tris buffer in the isothe solution was ~150 mM after concentration. Purity of [3H]DNP-SG was confirmed with the high performance liquid chromatography method of Farris and Reed (24).

Synthesis of oatp1 and Ntcp cRNA—Total RNA from rat liver was prepared using the guanidinium thiocyanate/cesium chloride method (25), and mRNA was purified by chromatography with oligo(dT)-cellulose using an mRNA purification kit (Amersham Pharmacia Biotech).

**Preparation and Microinjection of X. laevis Oocytes**—Isolation of X. laevis oocytes was performed as described by Guldin (26) and as described previously by our laboratory (27). Frogs were anesthetized by immersion for 15 min in ice-cold water containing 0.3% tricaine (Sigma). Oocytes were removed from the ovary and washed with Ca2\(^+\) free OR-2 solution (in mM: 82.5 NaCl, 2 KCl, 1 MgCl\(_2\), and HEPES-Tris, pH 7.5) and incubated at room temperature with gentle shaking for 90 min in OR-2 solution supplemented with 2 mg/ml collagenase (Sigma type IA). Oocytes were transferred to fresh collagenase solution after the first 45 min of incubation. Collagenase was removed by extensive washing in OR-2 solution at room temperature. Stage VI and V1Idefolliculated oocytes were selected and incubated at 18 °C in modified Barth’s solution (in mM: 88 NaCl, 1 KCl, 2.4 NaHCO\(_3\), 0.82 MgSO\(_4\), 0.33 Ca(NO\(_3\))\(_2\), 0.41 CaCl\(_2\), and 20 HEPES-Tris, pH 7.5), supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml). After 3–4 h of incubation, healthy oocytes were injected with 50 nl of oatp1 cRNA (0.5–10 ng/oocyte), 50 nl of Ntcp cRNA (0.5 ng/oocyte), or 50 ng of rat liver mRNA. Control oocytes were injected with a corresponding volume of sterile H\(_2\)O. Injected oocytes were cultured at 18 °C with a daily change of modified Barth’s medium. Healthy oocytes with a clean brown animal half and a distinct equator line were selected for experiments.

**Transport Measurements: DNP-SG and LTC4 Uptake**—Uptake studies were performed 2–3 days after microinjection. Oocytes were pre-treated with 0.5 mM acivicin for 30 min at room temperature to inhibit γ-glutamyl transeptidase activity. For uptake measurement, 6–8 oocytes were incubated at 25 °C for 1 h in 100 μl of modified Barth’s solution containing 1 μCi of [3H]DNP-SG and 0.5 μCi of [3H]taurocholate (27). The uptake was stopped by adding 2.5 ml of ice-cold modified Barth’s solution and oocytes washed three times each with 2.5 ml of ice-cold modified Barth’s solution. Two oocytes each were dissolved in a polypyrrole scintillation vial with 0.2 ml of 10% sodium dodecyl sulfate, and counted in a Packard model 4530 scintillation spectrometer after addition of 5 ml of Opti-Fluor (Packard Instruments, Downers Grove, IL).

**Tuorocholate Uptake into Oocytes**—Oocytes were injected with either water or oatp1 cRNA, and incubated at 25 °C in 100 μl modified Barth’s solution supplemented with 2.1–3.5 μCi of [3H]taurocholate. Uptake was measured with the incubation medium containing 1 μCi of [3H]taurocholate. The uptake was terminated at 5, 20, or 65 min by removing the medium and counting it separately from the oocytes. The 5-min values were used as a background correction and were subtracted from the 20- and 65-min values to give 15- and 60-min efflux rates.

**Changing Intracellular GSH Concentrations in Oocytes**—To increase GSH, oocytes were injected with 50 nl of different GSH stock solutions (e.g., 220 mM GSH stock, for an increase of ~20 mM in the oocytes). Oocytes were incubated at 25 °C for approximately 30 min before they were used in the experiment. To decrease GSH, oocytes were incubated in modified Barth’s solution containing 5 mg bithionium sulphoxide and 5 μM 1-chloro-2,4-dinitrobenzene for 18 h prior to uptake experiments. GSH content of the oocyte was measured as described previously (27).

**RESULTS**

**X. laevis oocytes injected with the cRNA for either oatp1 or Ntcp, the Na\(^+\)-taurocholate cotransporting polypeptide, showed enhanced uptake of taurocholate.** (Fig. IA), confirming previous results (1, 3, 4). Oocytes injected with oatp1 cRNA also demonstrated enhanced uptake of 10 nM LTC4 and 50 μM DNP-SG, when compared with oocytes injected with water or total liver mRNA (Fig. IB); however, the extent of stimulation of LTC4 and DNP-SG transport was lower than that of taurocholate. In contrast, oocytes injected with Ntcp-cRNA did not show enhanced transport of these glutathione S-conjugates (Fig. IB).

The oatp1-mediated uptake of [3H]LTC4 and [3H]DNP-SG increased with time over a 4-h interval (Fig. 2), and was independent of extracellular Na\(^+\) and Cl\(^-\) concentrations (Fig. 3). Uptake was unaffected when Na\(^+\) in the culture medium was replaced with choline\(^+\), or when Cl\(^-\) was replaced with gluconate\(^-\) (Fig. 3). Isomotic replacement of NaCl with sucrose also failed to affect LTC4 and DNP-SG uptake. These results with LTC4 and DNP-SG are similar to those reported previously for taurocholate and BSP transport on oatp1 (3, 4).

The concentration dependence of uptake was examined in oocytes incubated with increasing concentrations of LTC4 (10 nM to 1 μM) and DNP-SG (1 μM to 1 mM) (Fig. 4). It was not possible to examine lower concentrations of LTC4, because of limitations imposed by the specific activity of the isotope, nor was it feasible to examine higher LTC4 concentrations due to its limited solubility in aqueous medium. Uptake of LTC4 and DNP-SG was saturable, with apparent Km values of 0.27 ± 0.06 and 408 ± 95 μM (mean ± S.D.), and V\(_{max}\) values of 40 ± 35 fmol/oocyte h\(^{-1}\) and 4.7 ± 0.9 pmol/oocyte h\(^{-1}\), respectively (Fig. 4). This Km value for LTC4 in oatp1-expressing oocytes is
comparable to that measured in isolated rat hepatocytes, 0.20 μM (19), consistent with a role for oatp1 in mediating LTC4 uptake. Inhibition experiments revealed that BSP, taurocholate, and bilirubin ditaurate, substrates for oatp1, are strong inhibitors of LTC4 and DNP-SG transport in oocytes injected with oatp1 cRNA (Table I). Conversely, DNP-SG inhibited [3H]taurocholate uptake, and the inhibition was competitive in nature (Fig. 5). The $K_m$ for taurocholate uptake in the absence of DNP-SG was 38 ± 6 μM (mean ± S.D., n = 3; Fig. 5), a value comparable to that reported previously (3, 4). The $K_m$ was increased to 88 ± 18 μM in the presence of 100 μM DNP-SG, whereas the $V_{max}$ was unchanged (3.5 ± 0.4 and 4.7 ± 1.5 pmol/oocyte $\cdot$ h$^{-1}$, respectively; Fig. 5), indicating competitive inhibition. Mutual inhibition of transport provides strong evidence for a shared transport system.

4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid, ouabain, and other glutathione S-conjugates such as BSP-SG (0.5 mM) and S-(p-chlorophenacyl)-glutathione (1 mM) also inhibited uptake of LTC4 and DNP-SG (Table I). LTC4 uptake was decreased to 43% and 23% of control in the presence of BSP-SG and S-(p-chlorophenacyl)-glutathione, respectively. DNP-SG uptake was also inhibited by these glutathione S-conjugates, but the extent of inhibition was lower (Table I). These data indicate that bulky glutathione S-conjugates may be substrates for oatp1.

Of significance, 1 mM GSH $cis$-inhibited LTC4 and DNP-SG uptake, suggesting that GSH itself may be a substrate for oatp1 (Table I). Extracellular GSH (1 mM) also inhibited [3H]taurocholate uptake by 36 ± 16% (mean ± S.D.; n = 6). However, GSSG (1 mM) did not have any significant effect (Table I).

To test the possibility that GSH is a substrate for oatp1, [3H]GSH efflux was measured in oocytes expressing oatp1. The endogenous GSH transport rate in oocytes was high (Table II), as reported previously (27), but was even higher in oatp1-expressing oocytes (Table II), consistent with the hypothesis that GSH is transported on oatp1. The presence of 50 mM unlabeled GSH in the efflux medium further accelerated [3H]GSH efflux in both control and oatp1-expressing oocytes (Table II).

If [3H]GSH efflux is linked to oatp1-mediated uptake of organic solutes, inclusion of oatp1 substrates in the culture medium should accelerate [3H]GSH efflux. Indeed, Table II shows that inclusion of 50 μM taurocholate or 1 μM BSP in the culture medium stimulated [3H]GSH release in oatp1-expressing oocytes, but not in water-injected controls. After correction for the amount of [3H]GSH released by water-injected oocytes, a total of 0.27 ± 0.04% of intracellular GSH was released during a 15-min interval by oocytes incubated in modified Barth’s medium. This value was higher in the presence of taurocholate or BSP (0.46 ± 0.10% and 0.43 ± 0.07%, respec-
respectively; Table II), consistent with GSH/organic anion exchange on oatp1.

To estimate the stoichiometry of GSH/organic anion exchange on oatp1, the amount of taurocholate-stimulated [3H]GSH efflux (Table II) was compared with the amount of [3H]taurocholate taken up under similar conditions. Measurements of [3H]taurocholate uptake were carried out in parallel with the studies illustrated in Table II, using similar batches of oocytes that were incubated under nearly identical conditions. As shown in Table II, inclusion of 50 μM unlabeled taurocholate in the incubation medium stimulated the release of an additional 0.19% of intracellular [3H]GSH (i.e. 0.46 – 0.27 = 0.19%), or approximately 2.4 pmol/oocyte·15 min⁻¹ [(0.19%/15 min) (0.5 μl/oocyte) (2.5 mM GSH)]. This value is nearly identical to the amount of [3H]taurocholate taken up during the same time interval, 2.5 ± 0.6 pmol/oocyte·15 min⁻¹, indicating a 1:1 exchange on oatp1.

Additional evidence for GSH/organic anion exchange on oatp1 was provided by studies that measured taurocholate and LTC₄ uptake in oocytes with differing intracellular GSH concentrations (Fig. 6). Taurocholate uptake in oocytes with low (~0.3 mM) intracellular GSH was 46% of control oocytes (~2.5 mM endogenous GSH; Fig. 6A). When the intracellular GSH concentration was raised to ~20 mM there was a significant trans-stimulation of taurocholate uptake to 155% of control values, suggesting that GSH is a driving force for oatp1. In contrast, oatp1-expressing oocytes loaded with 20 mM amounts of either N-acetylcysteine, L-glutamate, or glutarate did not demonstrate enhanced uptake of [3H]taurocholate (Fig. 6A), indicating that trans-stimulation is selective for GSH. Uptake of LTC₄ in oatp1-expressing oocytes was also inhibited by GSH depletion, and trans-stimulated by high intracellular GSH (Fig. 6B).

The relation between oatp1-mediated taurocholate uptake and intracellular GSH concentration is illustrated in Fig. 7. Taurocholate uptake reached a maximum at about 10 mM GSH and was not further accelerated at higher intracellular GSH concentrations.
cis-Inhibition of LTC₄ and DNP-SG uptake into X. laevis oocytes expressing oatp1

Oocytes were injected with either water or oatp1 cRNA (5 ng/oocyte for LTC₄ and 0.5 ng/oocyte for DNP-SG). Uptake was measured under conditions described in “Experimental Procedures.” Values are means ± S.D. of three or four oocyte preparations, and are corrected for water-injected oocytes. All results are significantly different from control (p < 0.01, using unpaired Student’s t test), except GSSG.

| Test compound          | Amount of compound | [³H]LTC₄ (10 nM) | [³H]DNP-SG (50 µM) |
|------------------------|--------------------|-----------------|-------------------|
| Organic anions         |                    |                 |                   |
| DIDS                   | 250                | 25 ± 16         | 39 ± 15           |
| BSP                    | 250                | <2              | <2                |
| Bilirubin ditaurate    | 500                | 4 ± 2           | 6 ± 10            |
| Taurocholate           | 250                | 11 ± 2          | 15 ± 13           |
| GSH and glutathione S-conjugates |            |                 |                   |
| GSH                    | 1000               | 55 ± 4          | 76 ± 19           |
| GSSG                   | 1000               | 85 ± 15         | 113 ± 23          |
| LTC₄                   | 0.5                | 54 ± 15         | 72 ± 12           |
| DNP-SG                 | 1000               | 15 ± 7          | 29 ± 9            |
| p-Chlorophenacyl-SG    | 1000               | 23 ± 20         | 31 ± 18           |
| BSP-SG                 | 500                | 43 ± 6          | 70 ± 21           |
| Neutral compound       |                    | 52 ± 11         | 61 ± 9            |

FIG. 5. DNP-SG competitively inhibits taurocholate uptake into oatp1-expressing oocytes. Oocytes were injected with either oatp1 cRNA (0.5 ng/oocyte) or water. Taurocholate uptake (10, 20, 30, 50, and 70 µM) was measured in modified Barth’s solution in the presence or absence of 100 µM DNP-SG. Kᵣ and Vₘₐₓ values for taurocholate uptake in the absence and presence of DNP-SG were 38 ± 8 and 88 ± 18 µM, and 3.5 ± 0.4 and 4.7 ± 1.5 pmol/oocyte⁻¹h⁻¹, respectively (mean ± S.D., n = 3).

DISCUSSION

The present study demonstrates that LTC₄ and DNP-SG are substrates for the hepatic sinusoidal organic solute transporter oatp1, substantiating previous findings in rat hepatocytes and isolated liver plasma membrane vesicles (18–20). Uptake of these glutathione S-conjugates into oatp1-expressing oocytes was saturable, inhibited by known substrates of oatp1, and was independent of the Na⁺/Cl⁻ gradients. The apparent Kᵣ for LTC₄ uptake in oatp1-expressing oocytes, 0.27 µM (Fig. 4), is similar to that measured in hepatocytes, 0.20 µM (19), consistent with hepatic uptake on oatp1. The transport characteristics of DNP-SG in oocytes expressing oatp1 are also similar to those reported in perfused rat liver; uptake was comparatively low, Na⁺-independent, and inhibited by substrates for oatp1 (20). LTC₄ and DNP-SG may of course also be substrates for additional hepatic solute transporters, including other members of the oatp family, and this will need to be evaluated.

The present findings with LTC₄ and DNP-SG differ from previous studies with oatp1 (3, 4), which failed to find an effect of LTC₄ and DNP-SG on [³H]BSP uptake in oatp1-expressing oocytes. The reason for this apparent discrepancy is most likely related to the kinetics of transport of BSP versus the glutathione S-conjugates on oatp1. BSP is one of the best substrates for this transporter, with a Kᵣ of 1.5 µM, whereas DNP-SG has both a lower affinity (Kᵣ of 408 µM) and transport capacity (Vₘₐₓ of 4.7 pmol/oocyte⁻¹h⁻¹) for DNP-SG (Fig. 4B), versus 9 pmol/oocyte⁻¹h⁻¹ for BSP (3)). Thus, high concentrations of DNP-SG are needed to elicit significant inhibition of BSP uptake, but these concentrations were not used in previous studies. In contrast, LTC₄ has a higher affinity (Kᵣ of 0.27 µM), but its use as an inhibitor is limited by its very low water solubility. Maximum LTC₄ concentrations in aqueous medium are on the order of 0.5 µM, a concentration that is expected to have minimal effects on BSP uptake via oatp1.

Our results also provide direct evidence that intracellular GSH is a driving force for solute uptake on oatp1. First, the observation that glutathione S-conjugates are substrates for cystathionine are effective trans-inhibitors of sinusoidal GSH efflux, and dithiothreitol a trans-stimulator of GSH efflux (13, 29–32). However, these agents had no effect on oatp1-mediated taurocholate uptake (data not shown), indicating that oatp1 is not sensitive to these agents and therefore may not be the major mechanism of GSH release from hepatocytes.
To examine the effects of taurocholate, GSH, or BSP on organic anion transport, oocytes were re-injected with 50 nl of [3H]taurocholate or [3H]GSH and efflux measured for 15 min in 200 nl of modified Barth’s solution in the presence or absence of 50 μM taurocholate or 1 μM BSP. Values are means ± S.D. (n = 5). *p < 0.05, significantly different from control (p < 0.05). To examine the effects of taurocholate or BSP, oocytes injected with either water or 10 ng of oatp1 cRNA were re-injected with 50 nl of [3H]GSH, and efflux measured for 15 min in 200 nl of modified Barth’s solution in the presence or absence of 50 μM taurocholate or 1 μM BSP. Values are means ± S.D. (n = 5). **p < 0.01, significantly different from control (p < 0.01).

| Efflux media           | [3H]GSH released in 1 h | [3H]GSH released in 15 min |
|------------------------|-------------------------|---------------------------|
|                        | H2O-injected            | cRNA-injected             | cRNA-H2O                   |
| Modified Barth’s solution | 3.35 ± 0.21             | 4.38 ± 0.48*              | 1.03 ± 0.15                |
| +50 mM GSH             | 4.39 ± 0.59             | 5.66 ± 0.44*              | 1.27 ± 0.24                |
| Modified Barth’s solution | 0.98 ± 0.03             | 1.26 ± 0.06               | 0.27 ± 0.04                |
| +50 μM taurocholate    | 0.94 ± 0.04             | 1.40 ± 0.04               | 0.46 ± 0.10**              |
| +1 μM BSP              | 0.76 ± 0.07             | 1.19 ± 0.09               | 0.43 ± 0.07**              |

**TABLE II**

| Efflux media       | [3H]GSH released in 1 h | [3H]GSH released in 15 min |
|--------------------|-------------------------|---------------------------|
|                    | H2O-injected            | cRNA-injected             | cRNA-H2O                   |
| Modified Barth’s solution | 3.35 ± 0.21             | 4.38 ± 0.48*              | 1.03 ± 0.15                |
| +50 mM GSH         | 4.39 ± 0.59             | 5.66 ± 0.44*              | 1.27 ± 0.24                |
| Modified Barth’s solution | 0.98 ± 0.03             | 1.26 ± 0.06               | 0.27 ± 0.04                |
| +50 μM taurocholate| 0.94 ± 0.04             | 1.40 ± 0.04               | 0.46 ± 0.10**              |
| +1 μM BSP          | 0.76 ± 0.07             | 1.19 ± 0.09               | 0.43 ± 0.07**              |

**FIG. 6. GSH-dependent uptake of 1 μM [3H]taurocholate (A) and 10 nm [3H]LTC4 (B).** Intracellular GSH was raised or lowered as described under “Experimental Procedures,” and 20 mM intracellular N-acetylcysteine (NAC), l-glutamate, or glutarate was achieved by injecting 50 nl of 220 mM stocks of these compounds at 30 min prior to uptake measurements. [3H]taurocholate uptake (0.5 ng of oatp1 cRNA/oocyte) was measured at 25 °C for 1 h in 200 μl of modified Barth’s solution in the presence of extracellular taurocholate, BSP, and high concentrations of GSH, providing strong evidence for GSH/taurocholate exchange. The relation between intracellular GSH and oatp1-mediated taurocholate uptake (Fig. 7) indicates that transport is maximal at GSH concentrations that are comparable to those of intact hepatocytes, ~10 mM, and gradually declines at lower GSH concentrations. Thus, oatp1 function may be compromised by GSH availability if GSH levels are significantly decreased. Fourth, [3H]GSH efflux was higher in oatp1-expressing oocytes, and was further accelerated in the presence of extracellular taurocholate, BSP, and high concentrations of GSH, providing strong evidence for GSH/organic anion exchange on oatp1. Fifth, a comparison of the amount of GSH released and taurocholate taken up on oatp1 indicates one-for-one exchange of these monovalent anions.

Additional evidence for GSH/organic solute exchange on oatp1 comes from previous studies of GSH transport in rat liver sinusoidal membrane vesicles (11). GSH transport in sinusoidal vesicles was cis-inhibited and trans-stimulated by BSP-SG, a substrate for oatp1, suggesting that GSH efflux may drive the uptake of organic anions into hepatocytes (11). Studies with canalicul membrane vesicles have also demonstrated trans-stimulation between GSH and certain organic solutes (33, 34), suggesting that GSH/organic solute exchange may be a general mechanism.

To date, two major families of ATP-independent organic anion transporters have been identified. One family comprises the organic anion/dicarboxylate exchangers, and includes kidney oat1 (or rot1) (35–37). Cellular uptake of organic anions...
Oocytes were injected with 0.5 ng of oatp1 cRNA. Values are means ± S.E. of four oocyte preparations.

on kidney oat1 was recently demonstrated to be directly coupled to efflux of α-ketoglutarate (35, 36). The second family comprises the oatp-related transporters, and includes liver oatp1 and oatp2 (3–5), kidney-specific oat-k1 (38), and the prostaglandin transporter pgt (39). The transporters in this family exhibit approximately 30–80% amino acid identity (40). The driving force for uptake on the oatp-related transporters has not been identified.

The present findings with oatp1 raise the possibility that GSH exchange may be a common mechanism for this family of transporters. However, it is important to note that, despite the high sequence homology among the oatp-related transporters, there are major differences in substrate specificity and possibly in energy coupling. For example, oatp1 and oatp2 share 72% amino acid identity, yet oat-k1 is unable to transport either methionine or cystathionine, which are potentially toxic (Fig. 8).

The overall contribution of oatp1 to sinusoidal GSH efflux is unknown, but it is unlikely to be the predominant mechanism of GSH release in hepatocytes. Studies characterizing sinusoidal GSH efflux reveal that methionine and cystathionine trans-inhibit GSH efflux in hepatocytes (29, 30, 32), and dithiothreitol trans-stimulates GSH efflux (13, 31). In the present study, methionine, cystathionine, and dithiothreitol did not affect oatp1-mediated taurocholate transport, indicating that this putative GSH efflux mechanism is not altered. Taken together, these findings are consistent with the presence of at least two GSH transport systems in the sinusoidal membrane (Fig. 8): a putative GSH transporter (gsht) that is sensitive to methionine, cystathionine, and dithiothreitol; and oatp1, which is unaffected by these compounds. trans-Stimulation of GSH efflux by BSP or BSP-SG may occur through oatp1, whereas cis-inhibition may occur through both systems. It is plausible that other oatp1-like transporters that have recently been cloned may also be contributing to GSH efflux (5), and this possibility is currently being examined in our laboratory.

Previous studies have shown that sinusoidal GSH release in perfused rat liver is inhibited by BSP, rose bengal, indocyanine green, and bilirubin (41), substrates of oatp1. Inhibition occurs from an intracellular site and is reversible (41); inhibition is overcome when these anions are cleared from the cell by transport across the canalicular membrane into bile. This cis-inhibition of the putative sinusoidal GSH transporter by some

FIG. 7. Relation between 1 μM [3H]taurocholate uptake and intracellular GSH concentration in oatp1-expressing oocytes. Oocytes were injected with 0.5 ng of oatp1 cRNA. Values are means ± S.E. of four oocyte preparations.

The driving force for uptake on the oatp-related transporters is high sequence homology among the oatp-related transporters, and includes liver oatp1 and oatp2 (3–5), kidney-specific oat-k1 (38), and the prostaglandin transporter pgt (39). The transporters in this family exhibit approximately 30–80% amino acid identity (40). The driving force for uptake on the oatp-related transporters has not been identified.

The present findings with oatp1 raise the possibility that GSH exchange may be a common mechanism for this family of transporters. However, it is important to note that, despite the high sequence homology among the oatp-related transporters, there are major differences in substrate specificity and possibly in energy coupling. For example, oatp1 and oatp2 share 72% amino acid identity, yet oat-k1 is unable to transport either methionine or cystathionine, which are potentially toxic (Fig. 8). The overall contribution of oatp1 to sinusoidal GSH efflux is unknown, but it is unlikely to be the predominant mechanism of GSH release in hepatocytes. Studies characterizing sinusoidal GSH efflux reveal that methionine and cystathionine trans-inhibit GSH efflux in hepatocytes (29, 30, 32), and dithiothreitol trans-stimulates GSH efflux (13, 31). In the present study, methionine, cystathionine, and dithiothreitol did not affect oatp1-mediated taurocholate transport, indicating that this putative GSH efflux mechanism is not altered. Taken together, these findings are consistent with the presence of at least two GSH transport systems in the sinusoidal membrane (Fig. 8): a putative GSH transporter (gsht) that is sensitive to methionine, cystathionine, and dithiothreitol; and oatp1, which is unaffected by these compounds. trans-Stimulation of GSH efflux by BSP or BSP-SG may occur through oatp1, whereas cis-inhibition may occur through both systems. It is plausible that other oatp1-like transporters that have recently been cloned may also be contributing to GSH efflux (5), and this possibility is currently being examined in our laboratory.

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GSH, LTC₄, and DNP-SG Transport by oatp1

FIG. 8. Schematic representation of possible interactions between oatp1, a putative GSH transporter (gsht), and mrp2. oatp1 and gsht mediate GSH efflux in the sinusoidal membrane of the hepatocyte. gsht is trans-inhibited by methionine, stimulated by dithiothreitol, and cis-inhibited by BSP and other organic anions that are substrates for oatp1. oatp1-mediated uptake of organic anions, including BSP, is energized by GSH efflux. mrp2, which is located on the canalicular membrane, mediates ATP-dependent efflux of organic anions into the bile canaliculus. mrp2 is stimulated by intracellular GSH.

oatp1 substrates may be of physiologic significance in that it would maximize intracellular GSH concentrations for organic anion uptake on oatp1 (Fig. 8). Interestingly, transport of these same organic anions into bile is mediated by mrp2, a transporter that also appears to function optimally when intracellular GSH concentrations are high (42–44). Thus, the inhibition of sinusoidal GSH efflux by oatp1 substrates, and the resulting higher cellular GSH concentrations, may accelerate both hepatic uptake and biliary excretion of these compounds. The net effect is faster hepatobiliary clearance of chemicals, many of which are potentially toxic (Fig. 8).

In conclusion, the present study demonstrates that GSH is a driving force for oatp1 and that glutathione S-conjugates are a novel class of substrates for this transporter. oatp1 may therefore contribute to sinusoidal GSH release and the regulation of GSH turnover in hepatocytes. The data are consistent with the presence of multiple GSH efflux mechanisms, and explain several observations regarding GSH efflux characteristics in intact hepatocytes. However, confirmation of this model awaits the molecular characterization of additional GSH transport proteins.

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