0026-895X/00/020335-06$3.00/0
MOLECULAR PHARMACOLOGY

MOL 58:335–340, 2000 /68/842056

Oatp2 Mediates Bidirectional Organic Solute Transport: A Role for Intracellular Glutathione

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Received February 4, 2000; accepted May 8, 2000

This paper is available online at http://www.molpharm.org

ABSTRACT

One member of the OATP family of transporters, rat Oatp1, functions as an anion exchanger that is driven in part by the glutathione (GSH) electrochemical gradient, indicating that other OATP-related transporters may also be energized by this mechanism. The present study examined whether rat Oatp2 is also an anion exchanger, and, if so, whether it is energized by the GSH electrochemical gradient. As with Oatp1, uptake of 10 μM [^{3}H]taurocholate in Oatp2-expressing *Xenopus laevis* oocytes was *trans*-stimulated by intracellular 0.2 mM unlabeled taurocholate, indicating bidirectional transport. Interestingly, [^{3}H]taurocholate uptake in Oatp2-expressing oocytes was also *trans*-stimulated when oocytes were preloaded with GSH, *S*-methylglutathione, *S*-sulfobromophthalein-glutathione, *S*-dinitrophenyl glutathione, or ophthalmic acid (a GSH analog) but not by glutarate or *N*-acetylcysteine, suggesting that GSH derivatives and conjugates may function as intracellular substrates for Oatp2. Support for this hypothesis was provided by the demonstration of enhanced [^{3}H]GSH and [^{3}H]*S*-(2,4-dinitrophenyl)-glutathione efflux in Oatp2-expressing oocytes. However, in contrast to Oatp1, extracellular GSH failed to *cis*-inhibit uptake of [^{3}H]taurocholate or [^{3}H]digoxin in Oatp2-expressing oocytes, indicating that the stimulatory effect of high intracellular GSH concentrations is not due to a coupled exchange mechanism. Taken together, the results indicate that Oatp2 mediates bidirectional transport of organic anions by a GSH-sensitive facilitative diffusion mechanism and suggest that this transporter may play a role in cellular export of specific organic molecules.

To date, two major families of ATP-independent organic anion transporters (OATs) have been identified. The OAT family includes Oat1 (or Roat1), Oat2, and Oat3 (Sekine et al., 1997; Sweet et al., 1997; Wolff et al., 1997; Hosoyamada et al., 1999; Sweet and Pritchard, 1999; Tojo et al., 1999). Cellular uptake of organic anions on Oat1 was recently demonstrated to be directly coupled to efflux of α-ketoglutarate (Sekine et al., 1997; Sweet et al., 1997), whereas Oat2 and Oat3 do not appear to be anion exchangers (Sekine et al., 1998; Kusuhara et al., 1999). The transport mechanisms for Oat2 and Oat3 have not yet been defined.

The second family consists of the OATP-related transporters and includes rat Oatp1 (Jacquemin et al., 1994), Oatp2 (Noe et al., 1997), Oatp3 (Abe et al., 1998), Oat-k1 (Saito et al., 1996), Oat-k2 (Masuda et al., 1999a), human OATP-A (Kullak-Ublick et al., 1995; Meier et al., 1997), OATP-C/LST1 (Abe et al., 1999), and the prostaglandin transporter Pgt (Kanai et al., 1995; Lu et al., 1996; Lu and Schuster, 1998). The transporters in this family exhibit relatively high amino acid identity, although there are major differences in substrate specificity. For example, Oatp1 and Oat-k1 share 72% amino acid identity, yet Oat-k1 is unable to transport either taurocholate or leukotriene (LT)C_4 (Saito et al., 1996), whereas Oatp1 accepts both as substrates (Li et al., 1998). The driving force for uptake on this family of transporters has not been identified, although Oatp1 is believed to function as an anion exchanger. Recent evidence suggests that Oatp1 is a reduced glutathione (GSH) exchanger (Li et al., 1998), although a role for bicarbonate has also been proposed (Satlin et al., 1997).

The present study examined whether Oatp2 also mediates organic anion exchange and, if so, whether it might be influenced by the GSH electrochemical gradient. Oatp1 and Oatp2 share many common features: they exhibit 77% predicted amino acid identity (Noe et al., 1997), are both localized to the basolateral membrane of hepatocytes (Kakyo et al., 1999; Reichel et al., 1999), and share many common substrates (Kullak-Ublick, 1999). However, these two transporters exhibit differences in their tissue distribution, overall substrate specificity, and regulation by physiological and pharmacological agents (Kullak-Ublick, 1999). For example, Oatp1 is distributed homogeneously across the liver acinus,

This work was supported in part by National Institutes of Health Grants DK48823 and ES06484, National Institute of Environmental Health Sciences Center Grant ES01247, and Swiss National Science Foundation Grant 31-45536.95.

ABBREVIATIONS: OAT, organic anion transporter; GSH, reduced glutathione; BSP-SG, glutathione *S*-conjugate of bromosulfophthalein; CSF, cerebrospinal fluid; DNP-SG, *S*-(2,4-dinitrophenyl)-glutathione; LTC_4, leukotriene C_4.

whereas Oatp2 is expressed predominantly in perivenous hepatocytes. In the choroid plexus, Oatp1 is localized exclusively to the apical plasma membrane (Angeletti et al., 1997), whereas Oatp2 is localized to the basolateral membrane (Gao et al., 1999). The functional significance of this polarized distribution in the choroid plexus is unknown. *Oatp1* and *Oatp2* genes also appear to have different regulatory elements, based on differential changes in expression in response to certain stimuli. For example, in acute models of cholestasis, there is significant down-regulation of Oatp1 expression, whereas Oatp2 expression is unaffected (Kullak-Ublick, 1999). The down-regulation of Oatp1 may limit hepatic uptake of potentially toxic bile salts and other compounds that accumulate as a result of the impaired biliary excretion, whereas the preserved expression of Oatp2 during cholestasis may function to facilitate export of these compounds from hepatocytes into blood plasma.

The present results support this suggestion by demonstrating that Oatp2 can mediate either net uptake or efflux of organic solutes, depending on the imposed electrochemical gradient. Our results also indicate that GSH is an intracellular substrate for Oatp2 and a stimulator of organic anion uptake, although the precise mechanism by which it acts remains to be identified.

Experimental Procedures

Materials and Animals. [*glycine*-2-^{3}H]Glutathione (50 Ci/mmol), [^{3}H(G)]taurocholic acid (3.47 Ci/mmol), [^{3}H(G)]digoxin (19 Ci/mmol), and [14,15,19,20-^{3}H(N)]LTC_4 (165 Ci/mmol) were purchased from NEN Life Science Products (Boston, MA). [^{3}H]DNP-SG was synthesized enzymatically from [^{3}H]GSH and 1-chloro-2,4-dinitrobenzene as previously described (Ballatori and Truong, 1995). Unlabeled *S*-(2,4-dinitrophenyl)-glutathione (DNP-SG) and *S*-sulfobromophthalein-glutathione (BSP-SG) were synthesized and purified as described previously (Whelan et al., 1970; Hinchman et al., 1991). Other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO), Aldrich Chemical Co. (Milwaukee, WI), or J. T. Baker (Philipsburg, NJ). Mature *Xenopus laevis* were purchased from Nasco (Fort Atkinson, WI). Animals were maintained under a constant light cycle at a room temperature of 18°C.

Synthesis of Capped cRNA. Oatp1 and Oatp2 cDNAs were prepared as previously described (Noe et al., 1997; Li et al., 1998). Capped cRNA was transcribed in vitro with T3 RNA polymerase (Ambion, Austin, TX); the cRNA was precipitated with lithium chloride, and resuspended in RNase-free water for oocyte injection.

***X. laevis* Oocyte Preparation and Microinjection.** Isolation of *X. laevis* oocytes was performed as described by Goldin (1992) and previously used in our laboratory (Ballatori et al., 1996; Li et al., 1998). Frogs were anesthetized by immersion for 15 min in ice-cold water containing 0.3% tricaine (Sigma Chemical Co., St. Louis, MO). Oocytes were removed from the ovary and washed with Ca^{2+}-free OR-2 solution (containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM 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 of 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 V and VI defolliculated oocytes were selected and incubated at 18°C in modified Barth's solution [containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 20 mM HEPES-Tris, pH 7.5] supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). After 3 to 4 h of incubation, healthy oocytes were injected with 50 nl of either Oatp1 or Oatp2 cRNA (0.5–10 ng/oocyte). Control oocytes were injected with a corresponding volume of sterile H_2O. 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.

Uptake of DNP-SG and LTC_4 into Oocytes. Uptake studies were performed 3 days postmicroinjection of cRNA. Oocytes were pretreated with 0.5 mM acivicin for 30 min at room temperature to inhibit γ-glutamyl transpeptidase activity. For uptake measurements, from six to eight oocytes were incubated at 25°C for 1 h in 100 μl of modified Barth's solution in the presence of 1 μCi of [^{3}H]DNP-SG or [^{3}H]LTC_4 and 0.5 mM acivicin (Ballatori et al., 1996; Li et al., 1998). 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 polypropylene 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).

Taurocholate and Digoxin Uptake into Oocytes. Uptake of 1 and 10 μM taurocholate or 0.57 μM digoxin into oocytes injected with either 5 ng of Oatp1 or Oatp2 cRNA, or water for controls, was determined at 25°C in 100 μl of modified Barth's solution supplemented with either 0.35 μCi of [^{3}H]taurocholate or 1.1 μCi of [^{3}H]digoxin. Uptake was terminated by the addition of ice-cold stop solution, and samples were processed as described obove for other uptake studies. The stop solution for the taurocholate experiments contained 1 mM unlabeled taurocholate to reduce unspecific binding of tracer taurocholate.

GSH and DNP-SG Efflux in Oocytes. Oocytes injected with either water or Oatp2 cRNA were reinjected with 50 nl of [^{3}H]GSH or [^{3}H]DNP-SG (0.5–1 μCi/μl) and allowed to recover for 30 min in modified Barth's solution with 0.5 mM acivicin. Oocytes were washed twice with 2.5 ml of modified Barth's solution before efflux studies. Efflux was measured at 25°C in 200 μl of modified Barth's solution in the presence or absence of various compounds in the extracellular medium. Efflux was terminated after 15 min by removing the medium and counting it separately from the oocytes.

Altering Intracellular GSH Concentration in Oocytes. The endogenous GSH concentration in oocytes is approximately 2.5 mM (Ballatori et al., 1996). 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). After injection, 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 2 mM hydrogen peroxide for 1 h; they were washed with modified Barth's solution and incubated at 25°C for 30 min before they were used in the experiment. GSH content of the oocyte was measured as previously described (Griffith, 1980).

Results

Oatp1 and Oatp2 Mediate Bidirectional Solute Transport. To test the possibility of bidirectional transport on Oatp2, *X. laevis* oocytes expressing either Oatp2 or Oatp1 were loaded with 0.2 mM unlabeled taurocholate by microinjection, and uptake of [^{3}H]taurocholate was measured for 15 min at 25°C (Fig. 1). Although uptake of [^{3}H]taurocholate was accelerated by unlabeled taurocholate in both Oatp1- and Oatp2-expressing oocytes, the *trans*-stimulatory effect was especially high in Oatp2-expressing oocytes, indicating bidirectional transport.

Role of GSH in Oatp2-Mediated Uptake of Taurocholate and Digoxin. To test whether Oatp2-mediated uptake is driven by the outwardly directed GSH electrochemical gradient, initial studies measured uptake of [^{3}H]taurocholate

and [^{3}H]digoxin under conditions where the physiologic GSH gradient was gradually diminished by the addition of 1 to 20 mM GSH to the extracellular medium. However, extracellular GSH had only minimal effects on [^{3}H]taurocholate and [^{3}H]digoxin uptake even at a concentration of 20 mM (Table 1), indicating that transport is not directly coupled to GSH efflux. Taurocholate uptake in Oatp2-expressing oocytes was *cis*-inhibited by ouabain, as expected (Noe et al., 1997; Reichel et al., 1999), and by bromosulfophthalein and DNP-SG (Table 1).

In contrast to Oatp1, Oatp2 was unable to mediate uptake of the glutathione *S*-conjugates LTC_4 and DNP-SG (Table 2), demonstrating a different extracellular substrate specificity. Digoxin was a preferred substrate for Oatp2, and taurocholate was a substrate for both Oatp1 and Oatp2 (Table 2), supporting previous findings (Noe et al., 1997; Reichel et al., 1999).

To further evaluate whether intracellular GSH modulates Oatp2-mediated transport, uptake of 1 μM [^{3}H]taurocholate and 0.57 μM [^{3}H]digoxin was measured in oocytes loaded with differing intracellular GSH concentrations (Fig. 2). GSH levels were lowered by preincubating the oocytes with 2 mM H_2O_2 for 1 h at 25°C, a maneuver that lowered GSH levels from the normal 2.5 mM to 0.5 ± 0.2 mM, and were raised by microinjection of concentrated GSH stock solutions. Uptake of [^{3}H]taurocholate and [^{3}H]digoxin was decreased to 60 and 72% of control after GSH depletion, respectively, whereas uptake was stimulated when oocytes were loaded with GSH concentrations of 10 to 40 mM (Fig. 2). Uptake of taurocholate and digoxin reached maximum values at 10 to 20 mM intracellular GSH and was not further accelerated at higher intracellular GSH concentrations. Taurocholate uptake was enhanced by 10 mM intracellular GSH even when an equal concentration of GSH was present in the extracellular medium (data not shown), indicating that the stimulation of taurocholate uptake is due to the presence of high intracellular GSH concentrations rather than to its transmembrane gradient.

One interpretation of these data is that GSH is itself an intracellular substrate for Oatp2 and that the outward movement of GSH on Oatp2 accelerates transition of the carrier to

TABLE 1

Cis-inhibition of 10 μM [^{3}H]taurocholate and 0.57 μM [^{3}H]digoxin uptake by GSH and some organic anions in Oatp2-expressing oocytes

Oocytes injected with Oatp2 cRNA (5 ng/oocyte) were incubated with either 10 μM [^{3}H]taurocholate or 0.57 μM [^{3}H]digoxin for 10 min at 25°C. Control uptakes were 127 ± 36 and 193 ± 46 fmol · oocyte^{-1} · 10 min^{-1} for [^{3}H]taurocholate and [^{3}H]digoxin, respectively. Values are mean ± S.D. (n = 4–6).

Inhibitor	Concentration	[^{3}H]Taurocholate	[^{3}H]Digoxin
	mM	*% of control*	
Control		100 ± 28	100 ± 24
GSH	20	95 ± 18	86 ± 16
Ouabain	1	12 ± 2*	14 ± 2*
DNP-SG	1	66 ± 3*	62 ± 8*
BSP	0.1	11 ± 4*	
LTC_4	0.5×10^{-3}	92 ± 13	
BSP-SG	0.5	82 ± 14	

* Significantly different from control, $P < .05$ by Student's t test.

TABLE 2

Comparison of the ability of Oatp1 and Oatp2 to mediate uptake of taurocholate, digoxin, and glutathione *S*-conjugates when expressed in *X. laevis* oocytes

Oocytes were injected with either Oatp1 cRNA (0.5 ng/oocyte), Oatp2 cRNA (5 ng/oocyte), or 50 nl of water. Uptake was measured for 1 h under conditions described in *Experimental Procedures*. Values are mean ± S.D. of at least three experiments, each performed in quadruplicate.

Substrate	Concentration	H_2O	Oatp1	Oatp2
	μM	*fmol · oocyte^{-1} · h^{-1}*		
Taurocholate	1	3 ± 2	400 ± 45*	41 ± 15*
Digoxin	0.57	63 ± 8	80 ± 4	690 ± 118*
DNP-SG	50	64 ± 2	668 ± 110*	74 ± 10
LTC_4	0.01	0.23 ± 0.03	0.60 ± 0.22*	0.28 ± 0.06

* Significantly different from water-injected oocytes, $P < .05$ by Student's t test.

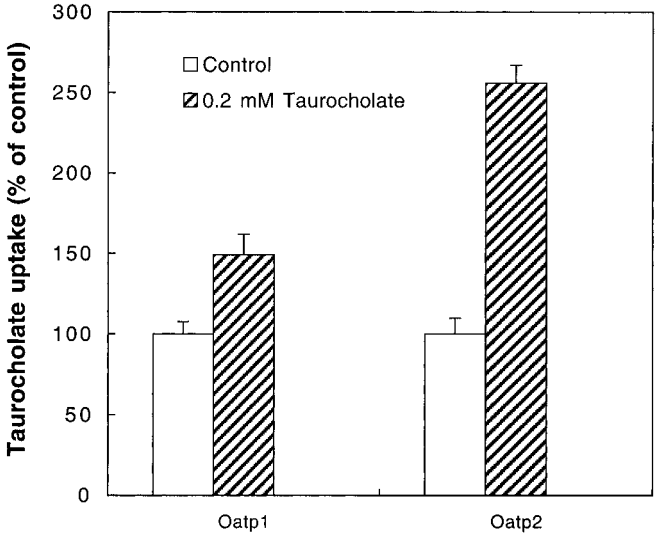

Fig. 1. *Trans*-stimulation of 10 μM [^{3}H]taurocholate uptake in Oatp1- or Oatp2-expressing oocytes by intracellular taurocholate. Oocytes were injected with 5 ng of Oatp1 or Oatp2 cRNA and cultured for 3 days. To load with 0.2 mM taurocholate, oocytes were injected with 50 nl of a 2.2 mM taurocholate stock solution at 30 min before uptake measurements. Oocytes were washed in modified Barth's solution, and uptake of 10 μM [^{3}H]taurocholate was measured at 25°C for 15 min. Values are mean ± S.E. (n = 5–7).

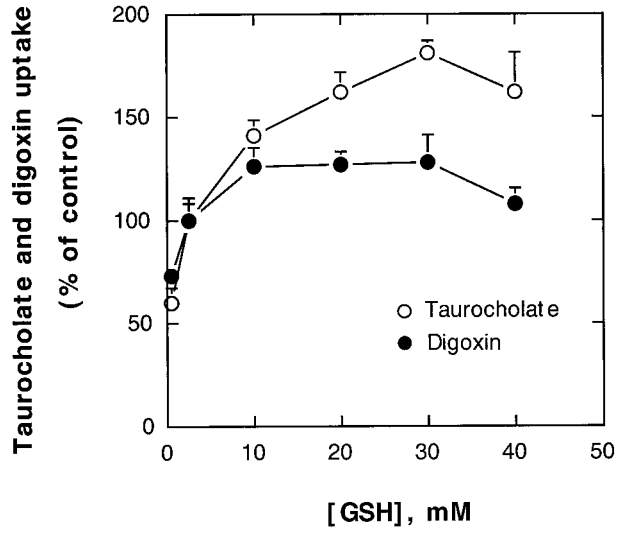

Fig. 2. Intracellular GSH concentration dependence of 1 μM [^{3}H]taurocholate and 0.57 μM [^{3}H]digoxin uptake in Oatp2-expressing oocytes. Oocytes were injected with 5 ng of Oatp2 cRNA. Intracellular GSH was raised or lowered as described in *Experimental Procedures*. [^{3}H]Taurocholate and [^{3}H]digoxin uptake was measured at 25°C for 15 min in the indicated intracellular GSH concentrations. The normal (endogenous) GSH concentration is approximately 2.5 mM. Uptake measurements were started 30 min after the addition of 0.5 mM acivicin to the culture medium. Values are mean ± S.E. (n = 4–6).

an outward-facing mode, enabling accelerated uptake of extracellular substrates. If this hypothesis were correct, Oatp2 should mediate [^{3}H]GSH efflux, and this efflux might be accelerated by the addition of extracellular substrates for Oatp2. To examine this possibility, Oatp2-expressing oocytes were microinjected with a tracer concentration of [^{3}H]GSH (2.5 mM endogenous GSH concentration), and efflux was measured in the presence and absence of various organic anions in the extracellular medium. [^{3}H]GSH efflux was higher in Oatp2-expressing oocytes (1.45 ± 0.09%/15 min) compared with control (water-injected) oocytes (1.24 ± 0.05%/15 min), which is consistent with the possibility that GSH is an intracellular substrate for Oatp2; however, GSH efflux was not further stimulated by extracellular substrates of Oatp2 (data not shown). This lack of effect of extracellular substrates may be explained by the fact that the Oatp2-stimulated [^{3}H]GSH efflux rate is quite high (0.21 ± 0.05%/15 min, or ~2.6 pmol · oocyte^{-1} · 15 min^{-1}), compared with the rate at which Oatp2 mediates uptake of substrates such as taurocholate or digoxin. Taurocholate is taken up at a rate of only 40 fmol · oocyte^{-1} · h^{-1}at 1 μM (Table 2) or approximately 0.1 pmol · oocyte^{-1} · 15 min^{-1}at 10 μM, whereas digoxin is taken up at a rate of approximately 0.3 pmol · oocyte^{-1} · 15 min^{-1} at 1 μM (Table 2). This relatively slow rate of organic anion uptake by Oatp2 may not significantly influence the much larger GSH efflux rate.

Oatp2-Mediated [^{3}H]Taurocholate Uptake Is Also Stimulated by High Intracellular Concentrations of Glutathione *S*-Conjugates and a GSH Analog. Interestingly, taurocholate uptake into Oatp2-expressing oocytes was also stimulated in oocytes that were preloaded with ophthalmic acid (a GSH analog) and the glutathione *S*-conjugates *S*-methylglutathione, DNP-SG, and BSP-SG but not by *N*-acetylcysteine or glutarate (Fig. 3), suggesting that some GSH derivatives may also be substrates for Oatp2. DNP-SG had no effect on Oatp2-mediated [^{3}H]taurocholate uptake at an intracellular concentration of 0.1 mM, but uptake was gradually increased at higher DNP-SG concentrations, reaching a maximum value at 0.5 mM DNP-SG (Fig. 4). Taurocholate uptake was nearly doubled in Oatp2-expressing oocytes loaded with 0.5 mM DNP-SG. In contrast to the effects of DNP-SG on Oatp2-mediated uptake, this glutathione *S*-conjugate did not affect Oatp1-mediated taurocholate uptake, at concentrations up to 1 mM (Figs. 3 and 4).

Accelerated [^{3}H]DNP-SG Efflux in Oatp2-Expressing Oocytes. To directly test the hypothesis that DNP-SG is an intracellular substrate for Oatp2, oocytes were microinjected with 0.5 mM [^{3}H]DNP-SG and efflux was measured in control (water-injected), and Oatp1- and Oatp2-expressing oocytes. Because oocytes have an endogenous ATP-dependent DNP-SG efflux mechanism (Ballatori et al., 1996), the background rate of efflux is quite high (22.7 ± 0.6 pmol · oocyte^{-1} · 15 min^{-1}). Nevertheless, Oatp2-expressing oocytes exported [^{3}H]DNP-SG at a higher rate than control oocytes (Fig. 5), indicating that DNP-SG is an intracellular substrate for Oatp2. In contrast, DNP-SG efflux was unaffected in Oatp1-expressing oocytes (Fig. 5). The Oatp2-stimulated [^{3}H]DNP-SG efflux rate was 2.3 pmol · oocyte^{-1} · 15 min^{-1}, a value comparable to that measured for [^{3}H]GSH efflux in Oatp2-expressing oocytes (2.6 pmol · oocyte^{-1} · 15 min^{-1}).

Discussion

Several ATP-independent organic solute transporters have been identified at the molecular level; however, the functional characterization of these remains incomplete. A major unresolved question relates to the driving force for transport.

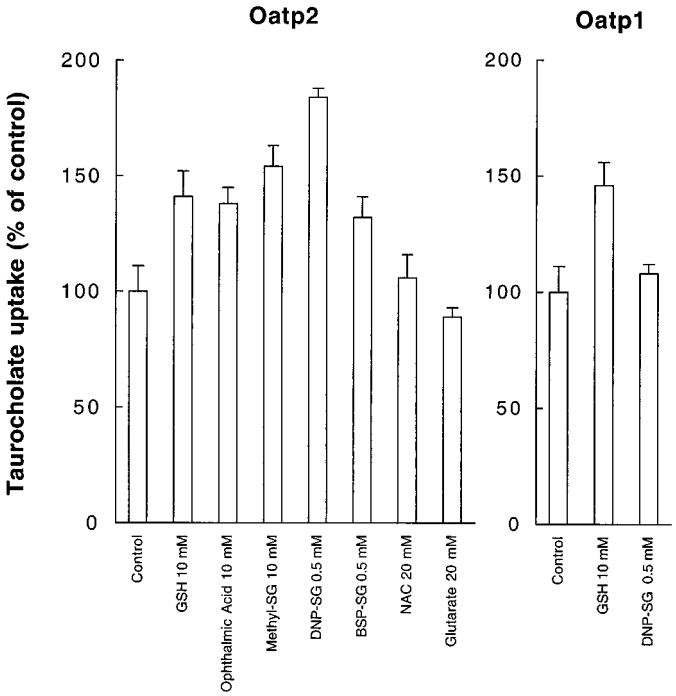

Fig. 3. Effect of intracellular GSH, glutathione *S*-conjugates, and other compounds on [^{3}H]taurocholate uptake in Oatp1- or Oatp2-expressing oocytes. Oatp1- and Oatp2-expressing oocytes were injected with 50 nl of either 110 mM or 220 mM stock solutions of GSH, *S*-methylglutathione, ophthalmic acid, *N*-acetylcysteine (NAC), or glutarate or 5.5 mM stock solutions of DNP-SG or BSP-SG. Oocytes were allowed to recover for 30 min in modified Barth's solution, and 1 μM [^{3}H]taurocholate uptake was measured at 25°C for 15 min. Values are mean ± S.E. (n = 3–6).

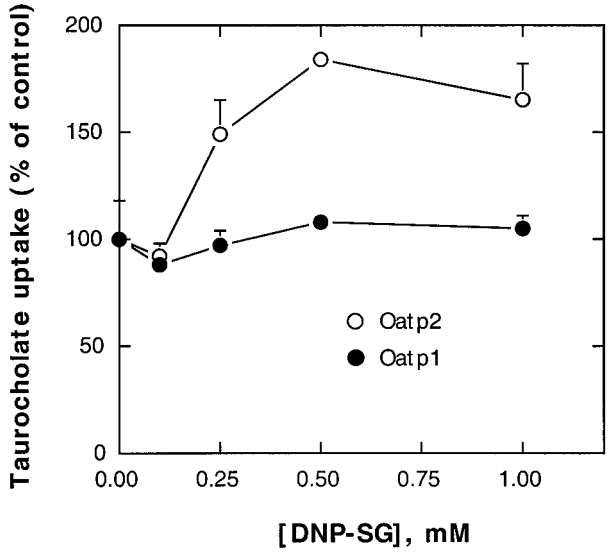

Fig. 4. Relation between intracellular DNP-SG concentration and 1 μM [^{3}H]taurocholate uptake in Oatp1- or Oatp2-expressing oocytes. Intracellular DNP-SG was raised by injecting 50 nl of DNP-SG stock solutions, and oocytes were allowed to recover for 30 min in modified Barth's solution with 0.5 mM acivicin. Uptake of 1 μM [^{3}H]taurocholate was measured at 25°C for 15 min. Values are mean ± S.E. (n = 4).

For example, although there is good evidence that rat kidney Oat1 functions as an uptake transporter that is energized by exchange with intracellular α-ketoglutarate (Sekine et al. 1997; Sweet et al. 1997), the transport mechanism for other members of the OAT family appears to be different. Recent studies indicate that in contrast to Oat1, the Oat2 and Oat3 transporters do not function as α-ketoglutarate exchangers (Sekine et al., 1998; Kusuhara et al., 1999).

The transport mechanism for the OATP transporters also remains undefined. Data on driving forces have been reported for only one member of this family, Oatp1, but these data are discordant. One study indicates that Oatp1 may function as a GSH exchanger (Li et al., 1998), whereas another study suggests a role for bicarbonate (Satlin et al., 1997). The present study tested the hypothesis that another member of the OATP family, Oatp2, functions as a GSH exchanger.

To directly test this hypothesis, initial experiments examined [^{3}H]taurocholate uptake in oocytes incubated in medium containing high GSH concentrations to dissipate the GSH gradient. However, the addition of GSH to the extracellular medium had minimal effects on taurocholate uptake, indicating that Oatp2 is not an obligate GSH exchanger. These results, however, do not exclude the possibilities that transport on Oatp2 either is highly asymmetric or is stimulated indirectly by intracellular GSH. For example, GSH may stimulate transport by a kinetic rather than a catalytic mechanism, as discussed further later. Alternatively, if GSH binding affinity and/or transport rate was high from the intracellular surface of the protein but low on the extracellar surface, this would allow for net outward movement of GSH even with a minimal GSH electrochemical gradient. Conversely, substrate (e.g., taurocholate) binding affinity may be high on the extracellular surface and low in the cytosol, favoring net uptake. If both GSH and extracellular substrate displayed such asymmetric transport kinetics, this may allow for net transport despite seemingly unfavorable chemical gradients. The issue of asymmetric transport kinetics is difficult to evaluate in most experimental systems, especially in intact cells, because intracellular concentrations of substrates are not readily altered or measured. A second limitation relates to the difficulty in distinguishing substrate-induced GSH transport activity from the large basal GSH transport that is observed in all cells that have been examined, including *X. laevis* oocytes (Ballatori et al., 1996; Ballatori and Rebbeor, 1998).

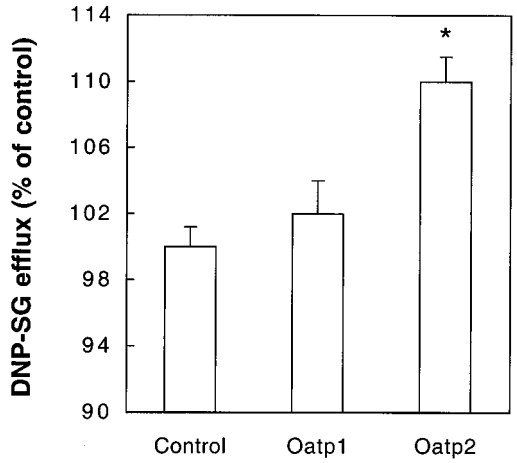

Fig. 5. Efflux of [^{3}H]DNP-SG in Oatp1- or Oatp2-expressing oocytes. Oatp1- or Oatp2-expressing oocytes were reinjected with 50 nl of [^{3}H]DNP-SG to achieve an intracellular concentration of 0.5 mM DNP-SG. Oocytes were allowed to recover for 30 min, and efflux was measured for 15 min in 200 μl of modified Barth's solution. The control rate of DNP-SG efflux was 22.7 ± 0.6 pmol · oocyte^{-1} · 15 min^{-1}. Values are mean ± S.D. (n = 6). *, significantly different from control, $P < .05$ by Student's t test.

Because of these limitations, additional experiments were performed to further explore the role of intracellular GSH in Oatp2-mediated transport in *X. laevis* oocytes. As previously reported for Oatp1 (Li et al., 1998), there was a positive correlation between intracellular GSH concentration and organic anion uptake in Oatp2-expressing oocytes (Fig. 2), consistent with a role for GSH in organic anion uptake. Oatp2-expressing oocytes also demonstrated higher [^{3}H]GSH efflux compared with water-injected oocytes, but GSH efflux was not further stimulated by extracellular substrates of Oatp2 (data not shown). As described in *Results*, this lack of effect of extracellular substrates may be explained by the relatively slow rate at which Oatp2 mediates uptake of organic anions compared with the much larger Oatp2-stimulated GSH efflux rate.

One interesting difference between Oatp1 and Oatp2 relates to their interaction with glutathione *S*-conjugates. [^{3}H]DNP-SG and [^{3}H]LTC$_4$ are taken up by Oatp1-, but not Oatp2-, expressing oocytes, indicating differences in extracellular substrate specificity. Conversely, DNP-SG *trans*-stimulates [^{3}H]taurocholate uptake into Oatp2-, but not Oatp1-, expressing oocytes, indicating differences in intracellular substrate specificity. The demonstration of enhanced [^{3}H]DNP-SG efflux in Oatp2-expressing oocytes provides further strong evidence that this glutathione *S*-conjugate is an intracellular substrate and supports the suggestion that Oatp2-mediated transport is asymmetric. Additional studies in a well-defined (polarized) isolated membrane system are needed to examine this possibility.

Additional studies are also needed to elucidate the precise role of GSH in Oatp2-mediated transport. Our data indicate that GSH is an intracellular substrate for Oatp2, as evidenced both by the ability of GSH to *trans*-stimulate uptake of organic anions and the ability of Oatp2 to stimulate [^{3}H]GSH efflux. However, in contrast to Oatp1, extracellular GSH failed to *cis*-inhibit organic anion uptake, indicating that GSH may facilitate uptake by a kinetic rather than a catalytic mechanism. That is, GSH efflux on Oatp2 may accelerate the transition of the carrier from an inward-facing to an outward-facing mode, which in turn could stimulate uptake of extracellular substrate. Because transport is also stimulated by glutathione *S*-conjugates, the effects of GSH are probably not due to a redox-type reaction.

Taken together, the present findings indicate that Oatp2 can mediate bidirectional transport of organic anions and suggest that under certain conditions, Oatp2 may function primarily as an export carrier. The direction of transport across the cell membrane is most likely determined by the electrochemical gradients and the relative binding affinities of individual substrates. This interpretation is consistent with the previous suggestion that Oatp2 may function as an efflux transporter in cholestasis (Kullak-Ublick, 1999). Oatp1 and Ntcp are down-regulated in cholestasis, as might be expected for uptake transporters, whereas Oatp2 expres-

sion is unaltered, indicating that it may not function as an uptake mechanism for cholephilic compounds. Additional evidence that Oatp2 may function for efflux comes from the polarized distribution of Oatp1 and Oatp2 in the choroid plexus: Oatp1 is localized to the apical membrane, whereas Oatp2 is localized to the basolateral membrane (Angeletti et al., 1997; Gao et al., 1999). Organic anions are eliminated from cerebrospinal fluid (CSF) after i.c.v. administration or during ventriculocisternal perfusion in animals (Suzuki et al., 1997; Nishino et al., 1999), but the transporters involved have not been identified. Our observations that Oatp1 functions a GSH exchanger (Li et al., 1998) and that Oatp2 mediates bidirectional transport suggest a possible mechanism by which these carriers may work in tandem to transport compounds from the CSF into blood. That is, the apically localized Oatp1 may facilitate uptake of organic solutes from the CSF in exchange for intracellular GSH, and basolateral Oatp2 would export the same compounds down their electrochemical gradients into blood plasma. Because both Oatp1 and Oatp2 transport bidirectionally, they may also produce net transport in the opposite direction, under appropriate transepithelial gradient conditions. Additional studies are needed to evaluate these possibilities. Interestingly, another member of the OATP family, Oat-k1, was recently shown to function bidirectionally (Masuda et al., 1999b), lending support to the suggestion that some OATP transporters may function for efflux of organic solutes from cells.

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