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LOCALIZATION OF ORGANIC ANION TRANSPORTING POLYPEPTIDE 4 (Oatp4) IN RAT LIVER AND COMPARISON OF ITS SUBSTRATE SPECIFICITY WITH Oatp1, Oatp2 AND Oatp3

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Adapted from Pfluger's Archive, 2001 (in press).

ABSTRACT

Organic anion transporting polypeptides (rodents: Oatps; human: OATPs) are involved in the absorption and elimination of a wide variety of structurally unrelated amphipathic organic compounds. Several members of this protein family mediate the uptake of substrates across the basolateral membrane of hepatocytes as the first step in hepatic elimination. In contrast to the well-characterized Oatp1 and Oatp2, the localization and substrate specificity of the recently cloned Oatp4 have not been investigated in detail. Therefore, we raised an antibody against the C-terminal end of Oatp4 and localized this 85-kDa protein to the basolateral membrane of rat hepatocytes. Similar to Oatp1 and Oatp2, Oatp4 is a multispecific transporter with high affinities for bromosulfophthalein, dehydroepiandrosterone sulfate, leukotriene C_4, and anionic peptides. In addition, we compared the substrate specificity of Oatp4 to that of Oatp3, which so far has mainly been shown to mediate intestinal bile acid transport. Oatp3 had a similar broad substrate specificity, but in general much lower affinities than Oatp4. Thus, while Oatp4 seems to work in concert with Oatp1 and Oatp2 in the basolateral membrane of rat hepatocytes, Oatp3 is a multispecific transport system in the small intestine.

INTRODUCTION

A major function of the mammalian liver is to remove endogenous and exogenous amphipathic organic compounds from portal blood plasma. The first step of this hepatic clearance process is mediated to a large extent by active transport systems at the sinusoidal (basolateral) hepatocyte plasma membrane (1). These transport systems include the organic anion transporting polypeptides (rat/mouse: Oatps; human: OATPs), which are classified within the *SLC21A* gene family of solute carriers (http://www.gene.ucl.ac.uk/nomenclature/). Oatps/OATPs mediate sodium-independent uptake of a large variety of amphipathic organic compounds including bile salts, steroids and steroid-conjugates, thyroid hormones, anionic peptides, and numerous drugs (2, 3). Oatps/OATPs that are expressed in the liver include rat Oatp1 (gene symbol: *Slc21a1*) (4, 5), rat Oatp2 (*Slc21a5*) (6), rat Oatp3 (*Slc21a7*) (7, 8), rat Oatp4 (*Slc21a10*) (9), human OATP-B (*SLC21A9*) (3), human OATP-C (also called LST-1 or OATP2; *SLC21A6*) (10, 11, 12) and human OATP8 (*SLC21A8*) (13). While Oatp1, Oatp2, OATP-B, OATP-C, and OATP8 have all been localized to the basolateral membrane of hepatocytes (3, 5, 6, 11, 13, 14), the exact cellular and subcellular localization of the "liver specific" Oatp4 has not yet been investigated.

The functional data available so far for rat Oatp4 demonstrate that it represents a multispecific transport system with similar affinities for the organic anion bromosulfophthalein (BSP) (1.1 µM) and the bile salt taurocholate (27 µM) (9) as Oatp1 and Oatp2 (4, 15). However, without knowing additional K_m values it is impossible to make any predictions about the physiological relevance of Oatp4 in the liver as compared to the other Oatps.

Similarly, for Oatp3 K_m values for transport of the bile salt taurocholate (18 µM) (7, 8) and the thyroid hormones T_3 (7 µM) and T_4 (5 µM) (7) are known. In addition, Walters et al. (8) demonstrated that all tested bile salts are substrates of Oatp3. However, the few additional Oatp compounds tested were not transported by Oatp3. Its broad tissue distribution, which extends beyond the liver and the small intestine to

the brain, kidney, lung, and retina for example (7, 8), suggests that besides bile salts and thyroid hormones additional compounds could be transported and Oatp3 could also be a real multispecific organic anion transporter.

Therefore, we wanted to identify the exact subcellular localization of Oatp4 and, in order to be able to compare Oatp3 and Oatp4 with Oatp1 and Oatp2, we determined the K_m values of several Oatp substrates.

This study demonstrates that Oatp4 is also selectively localized to the basolateral plasma membrane of rat hepatocytes, and that Oatp4 exhibits similar, but not identical, polyspecific transport kinetics as Oatp1 and Oatp2. In addition, the results identify Oatp3 as a further non-bile salt organic anion and low-affinity peptide transporter that might be involved in the intestinal absorption of amphipathic nutrients and drugs apart from endogenous bile salts.

MATERIALS AND METHODS

Chemicals

Bromosulfophthalein (BSP), dehydroepiandrosterone-3-sulfate, estrone-3-sulfate, estradiol-17-ß-glucuronide, leukotriene C_4, triiodothyronine (T_3), and Cyclo[D-Trp-D-Asp-L-Pro-D-Val-L-Leu] (BQ-123) were purchased from Sigma (St.Louis, MO, USA). [D-Penicillamine^{2,5}]enkephalin (DPDPE) was obtained from Bachem (Bubendorf, Switzerland), ouabain from Roche Molecular Biochemicals (Basel, Switzerland), thyroxine (T_4) from Fluka (Buchs, Switzerland), and prostaglandin E_2 was purchased from Calbiochem (La Jolla, CA, USA). All other chemicals were of analytical grade and were readily available from commercial sources.

Radiochemicals

[^{35}S]-BSP (2.5 Ci/mmol) was synthesized as described (16). [^{3}H(G)]-taurocholate (2.0 Ci/mmol), [1,2,6,7-^{3}H(N)]-dehydroepiandrosterone-3-sulfate (60 Ci/mmol), [6,7-^{3}H(N)]-estrone-3-sulfate (53 Ci/mmol), [6,7-^{3}H(N)]-estradiol-17ß-glucuronide (44 Ci/mmol), [^{3}H(N)]-leukotriene C_4 (158 Ci/mmol), [^{3}H(N)]-prostaglandin E_2 (200 Ci/mmol), L-[^{125}I]-triiodothyronine (T_3, 348 Ci/mmol), L-[^{125}I]-thyroxine (T_4, 343 Ci/mmol), [^{3}H]-deltorphin II (50 Ci/mmol), [tyrosyl-2,6-^{3}H(N)]-D-penicillamine-enkephalin (DPDPE; 46 Ci/mmol), [^{3}H]-ouabain (30 Ci/mmol), [^{3}H]-digoxin (15 Ci/mmol), [^{3}H]-*p*-aminohippurate (5 Ci/mmol), and [^{14}C]-tetraethylammonium bromide (5 mCi/mmol) were obtained from NEN Life Science Products (Boston, MA, USA). [^{3}H]-Methotrexate (8 Ci/mmol) and prolyl-3,4(N)-[^{3}H]-BQ-123 (43 Ci/mmol) were purchased from Amersham-Pharmacia Biotech (Uppsala, Sweden). [^{14}C]-Rocuronium (54 mCi/mmol) was a kind gift of Organon International BV (Oss, The Netherlands). [^{3}H]-*N*-Methyl-quinidine (85 Ci/mmol) was synthesized as described elsewhere (17).

Animals

Female *Xenopus laevis* were purchased from the African *Xenopus* facility c.c., Noordoek, R. South Africa. Male Sprague-Dawley rats were purchased from RCC, Füllinsdorf, Switzerland. All studies were performed in accordance with the Swiss Federal regulations concerning animal care.

Expression of Oatp1, Oatp2, Oatp3, and Oatp4 and uptake experiments in *Xenopus laevis* oocytes

Oatp-cRNAs were synthesized in vitro from *Not*I-linearized cDNA (plasmid: pSport1, Gibco Life Technologies, Rockville, MD, USA) using the mMESSAGE mMACHINE T7 kit (Ambion, Austin, TX, USA) as described (9). *Xenopus laevis* oocytes were injected either with 50 nl water or with 50 nl (5 ng) of Oatp1, Oatp2, Oatp3 (the Oatp3 cDNA was kindly provided by Dr. Paul A. Dawson, Dept. of Internal Medicine, Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157, USA), or Oatp4 cRNA and incubated for three days in modified Barth's solution [88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 2.4 mM Na_2HCO_3, 0.41 mM $CaCl_2$, 0.3 mM $Ca(NO_3)_2$, pH 7.6] containing 50 mg/l gentamycin. Oatp protein expression was routinely controlled for by uptake measurements of 5 µM radiolabeled taurocholate for 20 minutes as described (9). Substrate uptakes were performed using choline uptake solution [100 mM choline chloride, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES adjusted to pH 7.5 with Tris] as described (9). Kinetic transport parameters were determined with a non-linear curve fitting program (SigmaPlot 5.00, SPSS, Chicago, IL, USA) using a simple Michaelis-Menten model $v = V_{max} \cdot [S]/(K_m+[S])$.

Production and characterization of a polyclonal Oatp4 antiserum

Polyclonal Oatp4 antibodies were raised in rabbits against a synthetic peptide consisting of the 14 C-terminal amino acids of Oatp4 (amino acids 675–687) coupled to keyhole limpet haemocyanine at its C-terminus via an additional N-terminal tyrosine residue (Neosystem, Strasbourg, France). Rabbits were immunized as described previously (18). The specificity of the raised antiserum was characterized on Western blots with isolated basolateral and canalicular rat liver plasma membranes and with membranes isolated from Oatp1-, Oatp2-, Oatp3-, and Oatp4-expressing oocytes. Rat liver plasma membrane vesicles were isolated as described elsewhere (19). Oocytes were resuspended in 3 ml homogenization buffer (10 mM HEPES, 83 mM NaCl, 1 mM $MgCl_2$, 0.5 mM PMSF, pH 7.9, 5 µg/ml antipain and leupeptin) and homogenized with 20 strokes in a glass-Teflon homogenizer. The homogenates were centrifuged at 1000 *g* for 10 min and floating lipids removed with a cotton stick. Plasma membranes were pelleted by recentrifugation of the supernatant at 1000 *g* for 10 min followed by centrifugation at 10'000 *g* for 25 min (20, 21). The final pellet was resuspended in 150 µl homogenization buffer. Samples containing 100 µg of membrane protein (BCA protein determination kit of Pierce, Rockford, IL, USA) were separated on a 7.5% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane (Schleicher&Schuell, Dassel, Germany).

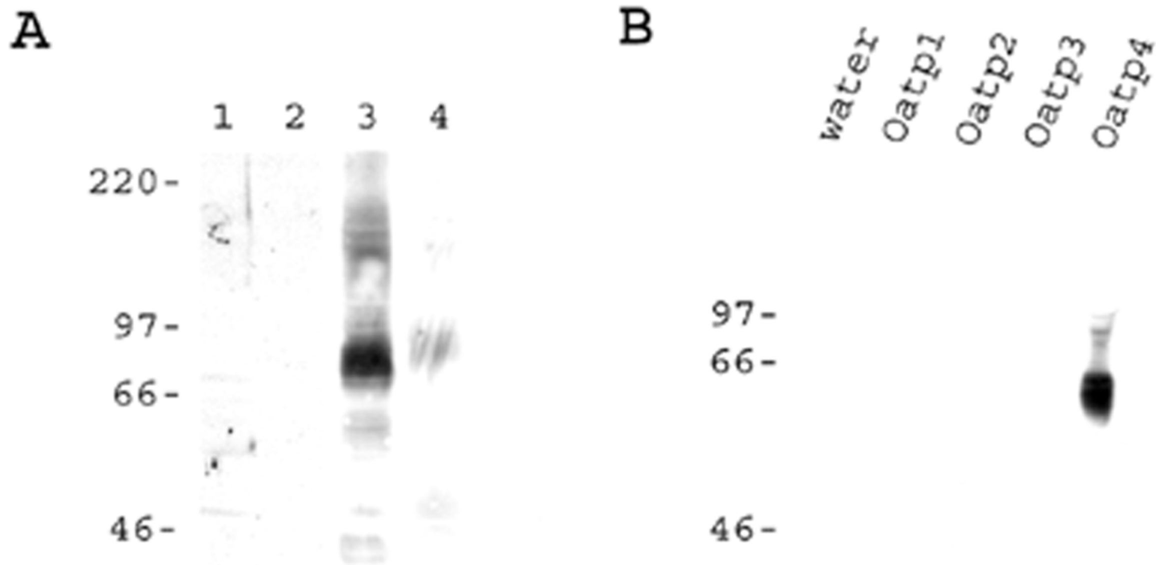

Figure 1A, B. Specificity of the polyclonal organic anion transporting polypeptide 4 (*Oatp4*) antiserum. **A** Western blot of basolateral (*lanes 1* and *3*) and canalicular (*lanes 2* and *4*) rat liver plasma membranes (100 µg protein each) with preimmune (*lanes 1* and *2*) or Oatp4 antiserum (*lanes 3* and *4*). Molecular size markers are indicated on the *left* (kDa). **B** *Xenopus laevis* oocytes were injected with either water or with cRNAs of Oatp1, Oatp2, Oatp3, and Oatp4. After 3 days in culture, the oocytes were homogenized and plasma membranes isolated by differential centrifugation (see Materials and methods). The membranes were subjected to Western blot analysis with the Oatp4 antiserum.

For immunodetection, the blots were washed for 5 minutes in TBS (20 mM Tris, 150 mM sodium chloride, pH 7.3) and blocked for 60 min in TBST (TBS with 1% Triton X-100) containing 5% skim milk powder. After three 5-min washes with TBST the blots were incubated for 1.5 h with preimmune serum or Oatp4 antiserum at a 1:1000 dilution in TBST containing 5% skim milk powder. Following an additional wash step (three times 5 min) with TBST, they were incubated for 1 hour with HRP-conjugated donkey-anti-rabbit IgG antibodies (Amersham-Pharmacia Biotech) at a 1:13'000 dilution. The final wash steps were twice for 5 min in TBST followed by twice 20 min in TBS. Immunoreactive material was detected using the ECL Plus Western blotting detection system (Amersham-Pharmacia Biotech) according to the manufacturer's instructions.

Immunolocalization of Oatp4 in rat liver

Rat livers were fixed by perfusion and tissue sections (0.5-1 µm) were incubated with the Oatp4 antisera as described previously (18). Micrographs were taken with a Zeiss Axiophot epifluorescence microscope (Zeiss, Oberkochen, Germany).

RESULTS

Cellular and subcellular localization of Oatp4 in rat liver

As illustrated in Fig. 1, the polyclonal antiserum against a C-terminal peptide of Oatp4 reacted selectively with a basolateral rat hepatocyte plasma membrane antigen of an apparent molecular mass of approximately 85 kDa (Fig. 1A, lane 3). The Oatp4-antiserum did not react with rat liver canalicular membrane proteins (Fig. 1A, lane 4), nor were any positive reactions observed with the preimmune serum (Fig. 1A, lanes 1 and 2). The specificity of the Oatp4-antiserum was tested in Western blots loaded with Oatp1-, Oatp2-, Oatp3-, and Oatp4-expressing oocyte plasma membranes. As illustrated in Fig. 1B, the Oatp4-antiserum gave a positive immunoreaction only with Oatp4-expressing oocyte membranes. The absence of any positive reaction with Oatp1-, Oatp2-, and Oatp3-expressing oocyte membranes could not be accounted for by low protein expression, since these Oatps could be easily detected on Western blots using specific Oatp1, Oatp2, and Oatp3 antisera (data not shown). Finally, the molecular mass of Oatp4 in oocytes (~ 65 kDa, Fig. 1B) was lower as compared to basolateral liver plasma membranes (~ 85 kDa, Fig. 1A, lane 3). This was consistent with the generally decreased apparent molecular mass of Oatp1, Oatp2 and Oatp3 when expressed in oocytes (data not shown), and might be related to a different glycosylation pattern of the proteins in this expression system (22). Hence, the data strongly indicate that the raised antiserum is specific for Oatp4 and therefore can be used for in situ localization of Oatp4 in rat liver. As illustrated in Fig. 2, expression of Oatp4 is selectively confined to the basolateral plasma membrane of rat hepatocytes. Thereby, Oatp4 expression levels increased from periportal (Fig. 2B) to perivenous (Fig. 2C) hepatocytes, which is similar to Oatp2 (6) with the exception that endogenous Oatp4 expression also includes the innermost layer of perivenous hepatocytes. No Oatp4 expression was observed at the liver endothelium or in bile duct epithelial cells.

Functional transport studies in *Xenopus laevis* oocytes

Figure 3 illustrates that in addition to taurocholate and BSP (9), Oatp4 also mediated saturable uptake of dehydroepiandrosterone sulfate (DHEAS) (K_m ~ 5.3 µM), estrone-3-sulfate (K_m ~ 37 µM), estradiol-17ß-glucuronide (K_m ~ 32 µM), leukotriene C_4 (K_m ~ 6.5 µM), prostaglandin E_2 (K_m ~13 µM), the anionic cyclopentapeptide BQ-123 (K_m ~14 µM), and the opioid peptide DPDPE (K_m ~ 22 µM). Although similar concentration-dependent saturabilities were observed, the apparent K_m values of Oatp3-mediated polyspecific substrate transport were, for most substrates, considerably higher as compared to Oatp4 (Fig. 4). In particular, 7- to 30-fold lower affinities of Oatp3 compared to Oatp4 were found for BSP, DHEAS, estrone-3-sulfate, BQ-123, and DPDPE. However, in contrast to Oatp4, Oatp3 mediated low affinity transport of ouabain (Fig. 4). These data characterize Oatp4 as the third multispecific bile salt carrier of rat liver besides Oatp1 and Oatp2. Furthermore, they demonstrate that the intestinal Oatp3 exhibits similar polyspecific substrate transport as the other Oatps albeit with somewhat lower substrate affinities. For better comparison, the transport properties of all four rat Oatps, which have been

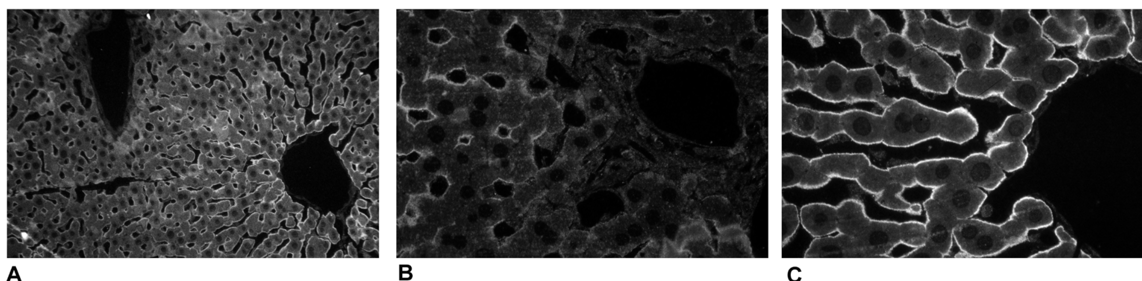

Figure 2A-C. Immunofluorescent localization of Oatp4 in rat liver. Semi-thin cryosections of rat liver were used for indirect immunofluorescence (see Materials and methods) and probed with the antiserum against Oatp4. Oatp4 expression shows a heterogeneous distribution (**A**) with the least expression in the periportal region (**B**) and the greatest expression in central perivenous hepatocytes (**C**).

shown to be expressed within the enterohepatic circulation, are summarized in Table 1.

DISCUSSION

The present study localizes Oatp4 to the basolateral plasma membrane of rat hepatocytes and compares its kinetic transport properties with the other Oatps expressed in the liver (i.e., Oatp1, Oatp2, and Oatp3). In addition, Oatp3, which was previously shown to be expressed along the small intestine (8), is identified as a multispecific transport system which besides bile salts and thyroid hormones also transports the steroid hormone conjugates DHEAS, estrone-3-sulfate and estradiol-17ß-glucuronide, the organic anion BSP, leukotriene C_4, prostaglandin E_2, the cardiac glycosides digoxin and ouabain, the cyclic peptides BQ-123 and DPDPE and even the type II cation rocuronium. Although the affinities of different Oatps for various substrates may differ to some extent, the data provided in this study further support the concept that the members of the *Oatp/OATP* gene family of membrane transporters play an important role in the intestinal absorption and hepatic elimination of a wide variety of endogenous and exogenous organic compounds.

The identification of Oatp4 as the third basolateral organic anion transporting polypeptide raises the question as to its specific role in comparison to the similarly localized Oatp1 and Oatp2 (6) in rat liver. In this regard it is important to note that Oatp4 is selectively expressed in rat liver (9, 23, 24) and exhibits the highest amino acid sequence identities with the human-liver-specific OATP-C (64%) and OATP8 (66%) (3). OATP-C preferentially transports amphipathic organic anions including taurocholate, BSP, steroid conjugates, and unconjugated bilirubin (3, 25). While OATP8 shares extensive overlapping substrate specificity with OATP-C, it is unique among the human OATPs for transport of digoxin and shows especially good transport activities for the anionic peptides DPDPE and BQ-123 (3). As illustrated in Table 1, Oatp4 shares the good organic anion transport activity with OATP-C and the good peptide transport activity with OATP8. However, despite numerous attempts we could not find any bilirubin (data not shown) or digoxin (Table 1) transport by Oatp4.

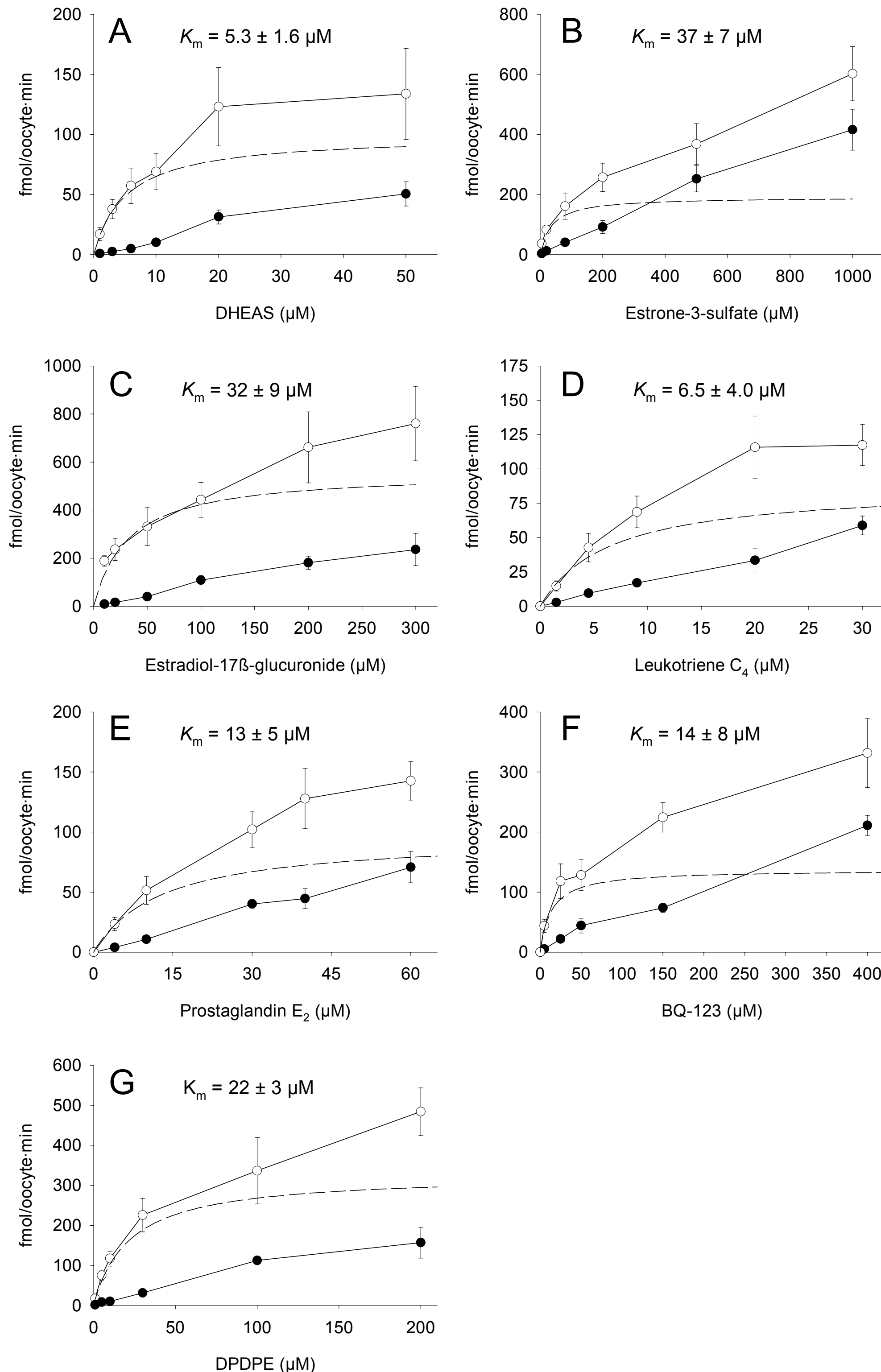

Figure 3A-G. Saturation kinetics of Oatp4-mediated substrate uptake in *Xenopus laevis* oocytes. Uptakes were measured at 20 minutes (linear uptake phase) in the presence of increasing concentrations of the various substrates: **A** [1,2,6,7-^{3}H(N)]-dehydroepiandrosterone-3-sulfate (DHEAS), **B** [6,7-^{3}H(N)]-estrone-3-sulfate, **C** [6,7-^{3}H(N)]-estradiol-17ß-glucuronide, **D** [^{3}H(N)]-leukotriene C_4, **E** [^{3}H(N)]-prostaglandin E_2, **F** prolyl-3,4(N)-[^{3}H]-BQ-123, and **G** [tyrosyl-2,6-^{3}H(N)]-DPDPE. The *dashed line* represents uptake differences between Oatp4-cRNA (*open circles*) and water-injected (*filled circles*) oocytes. Data are expressed as mean ± S.D. of 8 to 10 oocyte uptake measurements.

While the bilirubin uptake system in rat liver remains unknown, high-affinity uptake of digoxin into rat hepatocytes is mediated by Oatp2 (Table 1) (6, 15). Since Oatp2 exhibits a 73% amino acid sequence identity with OATP-A and shares only a 45% amino acid sequence identity with the human liver digoxin transporter OATP8 (3), the data support the concept that rat and human liver Oatps/OATPs do not represent “sensu strictu” orthologous gene products. Thus, although Oatp4 belongs into the same subfamily as OATP-C and OATP8, its spectrum of transport substrates is nevertheless somewhat different from the human liver OATP-C and OATP8. In rat liver, Oatp4 shares overlapping substrate specificities with Oatp1 and/or Oatp2, although the affinities for some substrates vary to some extent (Table 1). Interestingly, among the Oatps tested, Oatp4 was unique in mediating transport of both leukotriene C_4 and prostaglandin E_2 although its affinities for these substrates are considerably lower as compared to Oatp1 (Table 1) and the rat prostaglandin transporter (26), respectively. Furthermore, Oatp4 exhibits similar peptide transport activities as Oatp2 (Table 1) and OATP8 (3), suggesting that Oatp4 might be specifically involved in the hepatic clearance of certain endogenous peptide hormones. This assumption is supported by preliminary experiments indicating selective Oatp4-mediated hepatic uptake of the cyclic heptapeptide microcystin (27) and the gastrointestinal peptide hormone cholecystokinin 8 (CCK-8) (28). Further studies are required to define more exactly the possible liver-specific functions of Oatp4 besides its overall contribution to organic anion uptake in rat liver.

In contrast to Oatp1, Oatp2, and Oatp4, the hepatic expression level of Oatp3 is lower (7, 8). However, recent studies indicate that Oatp3 is, besides brain and lung, also expressed at the brush border membrane of small intestine where it is involved in the sodium-independent reabsorption of unconjugated and conjugated bile salts (8). Our studies now demonstrate that Oatp3 also represents a multispecific bile salt transporter that can mediate transport of a variety of non-bile salt organic compounds including BSP, steroid conjugates, eicosanoids, anionic peptides (albeit with low affinity), cardiac glycosides and even the type II organic cation rocuronium (Table 1). Thus, the spectrum of Oatp3 substrates is close to the substrate spectrum of Oatp1 (Table 1) and of human OATP-A (3). These similarities, together with the 72% amino acid identities between rat Oatp3 and human OATP-A and with the syntenic chromosomal localization of the mouse *Oatp3* (chromosome 6) and the human *OATP-A* (chromosome 12p12) genes, support the view that the rodent Oatp3 is an orthologous gene product to the human OATP-A (8). Based on its strategic localization at the brush border membrane of small intestinal epithelial cells, Oatp3 could play an important role in the intestinal reabsorption of amphipathic nutrients, drugs, and bile salts.

In conclusion, the basolateral localization and multispecificity of Oatp4 suggest that at least three different multispecific organic anion transporting polypeptides with distinct but overlapping substrate specificities are required for efficient elimination of amphipathic organic compounds by rat liver. In addition, Oatp3 exhibits a broad substrate specificity and can mediate intestinal absorption of numerous amphipathic organic compounds in addition to bile salts. Hence, the studies support the concept that different members of the *Oatp/OATP*-gene family of membrane transporters play an important role in the overall transport of numerous amphipathic organic compounds across the various membrane barriers within the enterohepatic circulation.

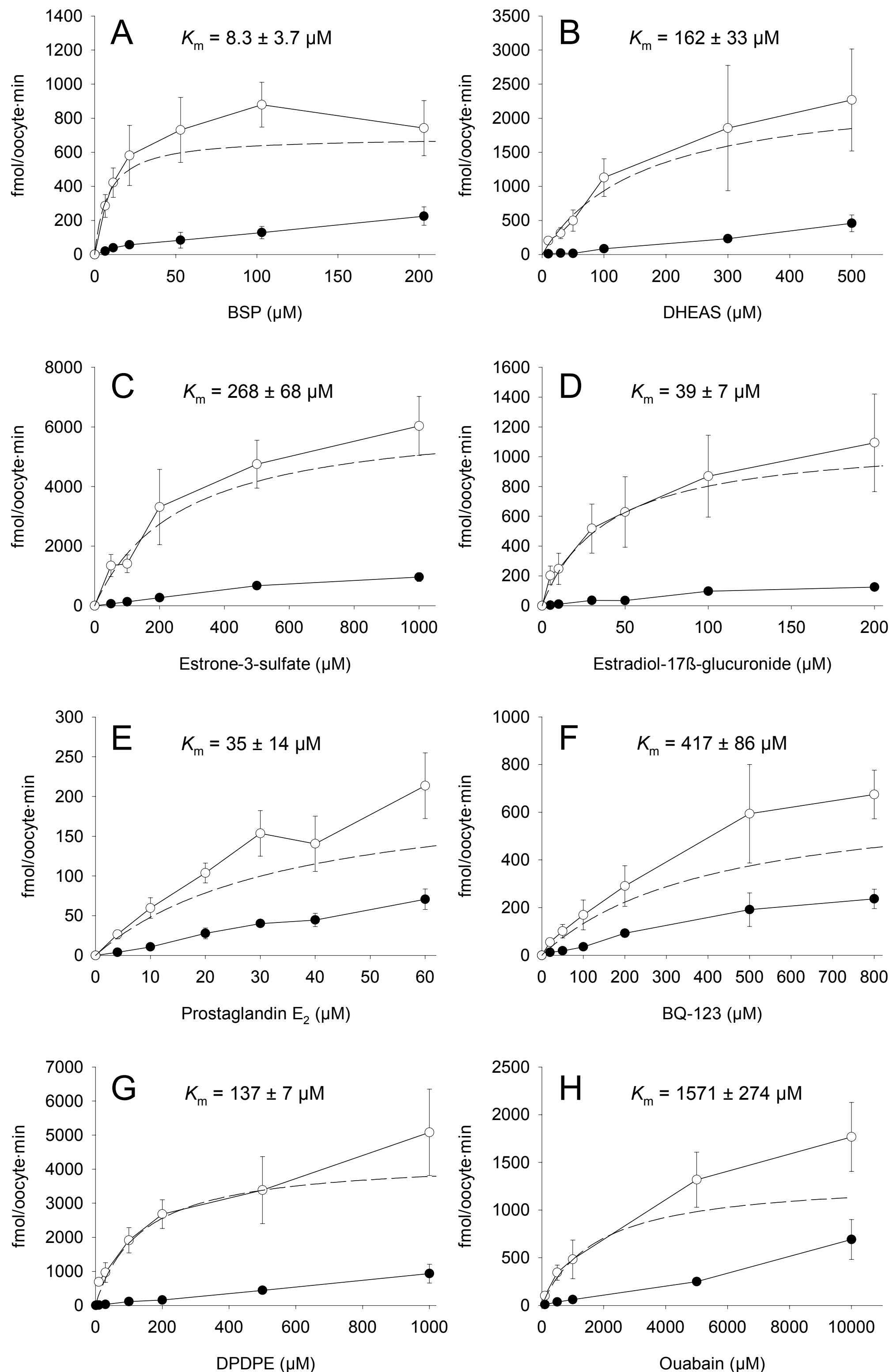

Figure 4A-H. Saturation kinetics of Oatp3-mediated substrate uptake *Xenopus laevis* oocytes. Uptakes were measured at 20 minutes (linear uptake phase) in the presence of increasing concentrations of the various substrates: **A** [^{35}S]-BSP, **B** [^{3}H]-dehydroepiandrosterone-3-sulfate (DHEAS), **C** [6,7-^{3}H(N)]-estrone-3-sulfate, **D** [6,7-^{3}H(N)]-estradiol-17ß-glucuronide, **E** [^{3}H(N)]-prostaglandin E_2, **F** prolyl-3,4(N)-[^{3}H]-BQ-123, **G** [tyrosyl-2,6-^{3}H(N)]-DPDPE, and **H** [^{3}H]-ouabain. The *dashed lines* represent uptake differences between Oatp3-cRNA (*open circles*) and water-injected (*filled circles*) oocytes. Data are expressed as mean ± S.D. of 8 to 10 oocyte uptake measurements.

Table 1. Comparison of substrate specificities of Oatp1, Oatp2, Oatp3 and Oatp4 in the *Xenopus laevis* oocyte expression system. [a)] Numbers indicate the K_m-values (µM) for the various substrates and transporters. [b)] -, substrate not transported. [c)] +, substrate transported but determination of the K_m values was not possible because apparent low affinities exceeded the solubility limits of the respective compounds.

Substrate	Oatp1	Oatp2	Oatp3	Oatp4	References
Taurocholate	19-50[a)]	35	18	27	(5, 7, 9, 14, 15, 29, 30, 31, 32)
BSP	1.5-3.3	-[b)]	8.3	1.1	this study, (4, 5, 6, 9, 33)
DHEAS	5	17	162	5.3	this study, (5, 6)
Estrone-3-sulfate	4.5-11	11	268	37	this study, (5, 15, 34)
Estradiol-17ß-glucuronide	3-11	3	39	32	this study, (5, 15, 29, 34, 35)
Leukotriene C_4	0.27	-	+[c)]	6.5	this study, (6, 15, 36)
Prostaglandin E_2	-	-	35	13	this study, (37)
T_3	+	5.9	7.3	+	this study, (7, 9, 38)
T_4	+	6.5	4.9	+	this study, (7, 9, 38)
BQ-123	600	30	417	14	this study, (6)
DPDPE	48	19	137	22	this study, (39)
Deltorphin II	137	-	-	-	this study, (39)
Ouabain	1700-3000	470	1571	-	this study, (5, 15, 34)
Digoxin	-	0.24	+	-	this study, (6, 9, 14)
N-methyl-quinine	+	-	-	-	this study, (17)
Tetraethylammonium	-	-	-	-	this study, (17)
Rocuronium	+	+	+	-	this study, (17)
Methotrexate	-	-	-	-	this study, (15)
p-aminohippurate	-	-	-	-	this study, (7, 37, 40)

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