Organic anion transporting polypeptide 2B1 and breast cancer resistance protein interact in the transepithelial transport of steroid sulfates in human placenta

Markus Grube, Sebastian Reuther, Henriette Meyer zu Schwabedissen, Kathleen Köck, Katrin Draber, Christoph A Ritter, Christoph Fusch, Gabriele Jedlitschky, Heyo K. Kroemer

From the Research Center of Pharmacology and Experimental Therapeutics, Department of Pharmacology (M.G., S.R., K.K., K.D., C.A.R., G.J., H.K.K); Department of Neonatology (C.F.), Department of Pharmacy (C.A.R.), Ernst-Moritz-Arndt-University Greifswald, Germany; Department of Pharmacology, Vanderbilt University, Nashville, USA (H.M.z.S)
OATP2B1 and BCRP in placenta

Heyo K. Kroemer, PhD

Department of Pharmacology

Friedrich Loefflerstr. 23d

17487 Greifswald

Germany

Phone: +49 3834 865630

FAX: +49 3834 865631

Email: kroemer@uni-greifswald.de

BCRP, breast cancer resistance protein; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulfate; E3S, estrone-3-sulfate; MDCK, madin darby canine kidney; MRP, multidrug resistance associated protein; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; SLC, solute carrier
Abstract

The human placenta has both protective and nurturing functions for the fetal organism. Uptake and elimination of xenobiotics and endogenous substances are facilitated by various transport proteins from the SLC- and ABC-families, respectively. A functional interaction of uptake and elimination, which is a prerequisite for vectorial transport across cellular barriers, has not been described for placenta. In this study we examined expression of OAT4 (SLC22A11), OATP2B1 (SLCO2B1, OATP-B) and BCRP (ABCG2) in human placenta (n=71), since all three proteins are involved in transmembranal transfer of estrone-3-sulfate (E3S; metabolic product) and dehydroepiandrosterone-sulfate (DHEAS; precursor molecule). On mRNA level we found a significant correlation of between OATP2B1 and BCRP (R² = 0.534; P < 0.01) but not between OAT4 and BCRP (R² = -0.104; P > 0.05). Localization studies confirmed basal expression of OATP2B1 and apical expression of BCRP. To study functional interactions between OATP2B1 and BCRP we developed an MDCKII cell model expressing both transport proteins simultaneously (OATP2B1 and BCRP in the basal and apical membrane, respectively). Employing this cell model in a transwell system resulted in a significantly increased basal to apical transport of both E-3-S and DHEAS, when both transporters were expressed with no change of transfer in the apical to basal direction. Taken together these data demonstrate the potential for a functional interaction of OATP2B1 and BCRP in transepithelial transport of steroid sulfates in human placenta.
Introduction

The hormonal system is essential for the maintenance of a wide variety of physiological functions. In addition to peptidic or amino acid based structures many hormones are steroids. Various tissues like the adrenal gland, the ovaries, the testes or the placenta are involved in the synthesis of steroid hormones. However, not all of them express the entire set of proteins required for this process and therefore, are dependent on precursor molecules. An interesting tissue in this context is the human placenta, which is involved in the production of gestagens and estrogens during pregnancy. This function is a pivotal prerequisite for maintaining pregnancy and for physiological functions such as fetal growth. While the syncytiotrophoblast as an important functional cell in placenta expresses all enzymes necessary for the production of progesterone, the placental estrogen synthesis dependents highly on the supply of C-19 steroids, since the 17β-hydroxylase/17-20-lyase enzyme is missing (Voutilainen and Miller, 1986; Kallen, 2004). An important source of these precursor molecules is dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), which are produced in the fetal adrenal gland and are available in large amounts in the fetal circulation (up to 10 µM) (Mesiano and Jaffe, 1997). In the placenta, DHEAS undergoes deconjugation by steroid sulfatases, which have been found in high levels in the syncytiotrophoblast (Salido, et al., 1990). The resulting DHEA is used for the placental estrogen synthesis (Kallen, 2004). Another important steroid sulfate is estrone-3-sulfate (E3S), which is synthesized by the fetal liver (Lanoux, et al., 1985). The E3S represents a metabolite of estrogen but can also function as a biological inactive pool for this hormone (Miki, et al., 2002).

To fulfill their physiological role these substances have to enter the syncytiotrophoblast and later the products of hormone synthesis have to exit the cell. The uptake of steroid sulfates into the syncytiotrophoblast has been characterized to be mediated by the organic anion transporting polypeptide 2B1 (OATP2B1) and the organic anion transporter 4 (OAT4), which have been described to transport DHEAS and E3S. Both transporters are expressed in the fetal
facing membrane of the syncytiotrophoblast suggesting an important function in the uptake of fetal derived sulfates (Ugele, et al., 2003; St Pierre, et al., 2002). Besides these uptake mechanism there are also several efflux transporter of the ABC-family (e.g. P-glycoprotein, several MRPs and BCRP), which are mainly expressed in the maternal facing membrane of the syncytiotrophoblast (Nakamura, et al., 1997; Cordon-Cardo, et al., 1990; Maliepaard, et al., 2001; Zu Schwabedissen, et al., 2006). At present, however, these efflux transporters are regarded as placental mechanism to protect the growing fetus against drugs or xenobiotics from the maternal circulation and to remove endogenous compounds from the fetal blood circulation (Young, et al., 2003). Aside from their protective function these proteins may be involved in the regulation of the hormone synthesis by controlling the intracellular and fetal concentration of precursor molecules like DHEAS and E3S, thereby interacting with various uptake transporters.

While uptake and efflux transporter in human placenta are well characterized in terms of their individual function, information regarding their interaction on functional level is scanty. We therefore examined the placental expression of BCRP and the two uptake transporter OATP2B1 and OAT4, which are all characterized to transport steroid sulfates (Cha, et al., 2000; Tamai, et al., 2001; Nozawa, et al., 2004; Suzuki, et al., 2003a). Moreover, OATP2B1 and OAT4 are shown to be expressed in the basal membrane of the syncytiotrophoblast, while BCRP is localized in the apical maternal facing membrane (Maliepaard, et al., 2001; St Pierre, et al., 2002; Ugele, et al., 2003). We determined the mRNA expression of BCRP, OATP2B1 and OAT4 in a large sample of human placentas to examine a possible coregulation of BCRP and a corresponding uptake transporter. Moreover, we studied localization of OATP2B1 and BCRP in the placental syncytiotrophoblast using confocal laser scanning microscopy. Finally, to elucidate their functional interaction, we developed a model system based on OATP2B1, BCRP and OATP1B2/BCRP overexpressing MDCKII cells. Using this system, we analyzed the transepithelial transport of E3S, DHEAS and 17β-estradiolglucuronide.
Methods

Cell Culture and Substances. After declaration of consent human placenta tissue samples were obtained from women undergoing caesarian sections or normal birth. Madin-darby-canine kidney (MDCKII) cells were grown in DMEM medium containing 10% fetal calf serum, 2 mM L-glutamine, 1% MEM nonessential amino acids and penicillin/streptomycin (0.5U/ml and 150 µg/ml). Cells were incubated at 37°C in an air atmosphere containing 5% CO₂.

[^3H]estone-3-sulfate (E3S) (specific activity: 50 Ci/mmol), [^3H]dehydroepiandrosterone-3-sulfate (DHEAS) (specific activity: 60 Ci/mmol), [^3H]17β-estradiolglucuronide (specific activity: 53 Ci/mmol) and [14C]inulin (specific activity: 2.5 mCi/mmol) were obtained from Hartmann Analytic, Braunschweig, Germany).

RNA isolation and analysis. RNA was isolated from 71 placentas (45 term, 26 premature placentas) using RNeasy Mini Extraction Kit (Qiagen, Frankfurt, Germany) according to the manufacturer’s instructions. The integrity of the RNA was checked by ethidium bromide staining in formaldehyde containing 1% agarose gel.

The isolated RNA was reverse transcribed using random hexamer primers and the TaqMan® reverse transcription (RT) kit (Applied Biosystems, Foster City, CA). The resulting cDNA (10 ng/µl reverse transcribed RNA) was amplified by real-time PCR for the human transport proteins BCRP, OATP2B1 and OAT4 as well as for the 18S rRNA (18S endogenous control assay (Applied Biosystems)) using the ABI Prism 7700 Sequence Detector System (Applied Biosystems). Real-time quantitative PCR for BCRP was performed using forward primer 5’-CTGGGAACATGATTAGGAAGC-3’, reverse primer 5’-GAGGATTTCCAGAGCCGAC-3’ and the probe 5’-6FAM-CAGTCCGAGATGTGAACCTGGACAT-XTp. Primer and probe oligonucleotides were designed based on the cDNA sequence (Accession No. AF098951.2) using the primer express software (Applied Biosystems). The OATP2B1 real-time PCR was performed as described by St-Pierre et al. (St Pierre, et al., 2002) and for OAT4 an assay on
demand (Hs00218486_m1, Applied Biosystems) was used. For all real-time PCR applications a PCR mastermix (45 mM TrisHCl (pH 8.4), 115 mM KCl, 7 mM MgCl2, 460 µM dNTPs, 9% glycerol, 2.3% ROX reference dye and 0.035U/ml Platinum Taq DNA polymerase (the ROX dye and the polymerase were purchased from Invitrogen, Carlsbad, CA, USA)) was used. For quantification of BCRP, OATP2B1 and 18S rRNA, cloned PCR products were used to prepare a dilution series and to calculate a standard curve.

Cloning and Transfection. The full length OATP2B1 and BCRP cDNA were amplified using reverse transcribed RNA from placenta (OATP2B1: forward primer: 5´-AGCTCACTGCACTCCAGCAGTCATGG-3´; reverse primer: 5´-AAAGGACTCAGAGGAGGTACTGCTGTGGCTGC -3´; BCRP forward primer: 5´-CTGAGATCCTGAGCCTTTGGTTAA-3´; reverse primer: 5´-TGATGGYAAGGGYYCAGAAAAAC-3´) and cloned into the mammalian expression vectors pcDNA3.1 and pcDNA3.1/hygro (both Invitrogen). After verifying the sequence by cycle sequencing and matching against the reference sequences (accession No. AB026256.1 for OAPT2B1 and AF098951.2 for BCRP) MDCKII cells were single transfected with OATP2B1 and BCRP using the FuGENE 6 Transfection Reagent (Roche, Penzberg, Germany). Cells were selected for antibiotic resistance using 0.6 mg/ml hygromycin B (Invitrogen). Resistant cell clones were characterized for OATP2B1 and BCRP expression using western blot analysis and immunofluorescence staining. Next, positive BCRP transfected cells were transfected with pcDNA3.1 vector containing the OATP2B1 cDNA. Here, selection was performed using hygromycin B and neomycin together. Resistant cells were characterized for OATP2B1 and BCRP as described above.

OATP2B1-antibody. The anti-OATP2B1 antibody was raised in rabbits against the 15 amino acids at the carboxy terminus of the deduced OATP2B1 Sequence (LLVSGPGKKPEDSRV).
The peptide was synthesized automatically and then coupled to maleimide-activated keyhole limpet hemocyanin (Peptide Specialty Laboratories GmbH, Heidelberg, Germany). New Zealand white rabbits (Charles River, Sülzfeld, Germany) were immunized with this conjugate as described previously (Grube, et al., 2005).

**Preparation of Apical and Basal Membrane Fractions.** Apical and basal membrane fractions from two term and two preterm placentas were isolated and characterized as described previously (Meyer Zu Schwabedissen, et al., 2005; Grube, et al., 2005). In brief, 20 g placental cotyledons were washed with ice-cold PBS and reduced to small pieces. Following, tissue was homogenized in incubation buffer (250 mM sucrose, 10 mM Tris/HCl, and 1 mM EDTA, pH 7.4) supplemented with protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 0.3 µM aprotinin, and 0.1 µM of pepstatin) by 20 strokes (1,000 rpm) using the Potter S homogenizer (B. Braun Biotech, Melsungen, Germany) and incubated on ice for 1 h under continuous stirring. After centrifugation at 9,000 x g, the supernatant was centrifuged at 100,000 x g and the resulting membrane pellets were resuspended in incubation buffer and homogenized with a loose-fitting Dounce B homogenizer. For separation of basal and apical membranes, MgCl₂ was added to a final concentration of 10 mM and centrifuged at 2,000 x g after 10 min incubation on ice. The separated membranes, apical fraction (supernatant) and the basal fraction (pellet), were resuspended in incubation buffer and homogenized with a tight-fitting Dounce B homogenizer. After centrifugation at 100,000 x g, the membranes were homogenized using the tight-fitting Dounce B homogenizer and centrifuged at 100,000 x g. After that, the pellets of both membrane fractions were resuspended in 1 ml of homogenization buffer, frozen and stored in liquid nitrogen. An aliquot of the resuspended membrane fractions was used to determine protein concentration by the BCA method.
Immunoblot analysis. Crude membrane fractions were loaded onto a 10% sodium dodecylsulfate-polyacrylamid gel after incubation in sample buffer at 95°C for 10 min. Immunoblotting was performed using an ice-cooled tank blotting system (BioRad, Hercules, CA, USA) at 370 mA for 1.5 h. After a protein control staining with PonceauS, the Blots were blocked over night with 5% milk in TBST (TBS containing 0.05% Tween 20) at 4°C. The OATP2B1 and BCRP primary antibodies were diluted in TBST containing 5% bovine serum albumin (BSA) and the incubation of the blots took place for 2 hours at room temperature. The plots were washed with TBST (3 x 5 min and 2 x 10 min) and blocked for another hour with 5% BSA in TBST at room temperature. Secondary horse-radish peroxidase-conjugated goat anti-rabbit antibody (BioRad) was used at a 1:2000 dilution (2 h, room temperature). Finally, the immobilized antibodies were stained using an enhanced chemiluminescence (ECL) system (Amersham Biosciences, Freiburg, Germany) and exposed to X-ray films.

Immunofluorescence Microscopy. Protein localization was investigated by confocal laser scanning immunofluorescence microscopy in the human placenta as well as in overexpressing cell lines. For the placental staining 5 µm cryo-slices were used. After drying at 37°C on a heating plate for 10 min, sections were fixed in 100% Ethanol at -20°C for 10 min and then washed in phosphate-buffered saline (PBS) (pH 7.3). Blocking was performed in 5% FCS in PBS for 45 min, followed by incubation with the primary antibodies at 4°C overnight. After three washes with PBS, the sections were stained. OATP2B1 staining with a polyclonal antibody (rabbit, dilution 1:200) was performed as described before (Grube, et al., 2005). For BCRP staining the monoclonal mouse antibody BXP-21 (dilution 1:25) (Alexis Biochemicals, Gruneberg, Germany) was used. The detection was carried out with fluorescence labeled secondary antibodies Alexa Flour® 488 IgG (anti mouse, dilution 1:100) and Alexa Flour® 568 IgG (anti rabbit, dilution 1:200) (Molecular Probes, Eugene, Oregon, USA). Nuclei counterstaining with TOTO®-3-iodide dye (dilution 1:1000) (Molecular Probes) was
performed within the Fluorescent Mounting Medium (DakoCytomation, Carpinteria, CA, USA).

For staining of the transfected cells, cells were cultured on cover slides and grown to confluence. Before fixation, cells were incubated for 24 h with 2.5 mM butyrat to induce protein expression. After washing with PBS cells were fixed for 10 min with ethanol (100 %); next, cells were washed with PBS again before permeabilisation with Triton-X 100 (0.1%) for 5 min. After following washings with PBS, cells were blocked with 5 % fetal calf serum and processed as described above.

Transport studies. Transport studies were performed in a 24 well transwell system. Non-transfected, OATP2B1, BCRP and OATP2B1/BCRP transfected MDCKII cells were seeded in transwell plates and cultured to confluence. One day before the experiment the medium in apical and basal compartment was changed and cells were incubated with 2.5 mM butyrate for 24 h. The transepithelial transport of radiolabelled E3S, DHEAS, 17β-estradiol-glucuronide and inulin was performed by adding 300 µl incubation buffer (140 mM NaCl, 5 mM KCl, 1 mM KH2PO4, 1.5 mM CaCl2, 5 mM glucose and 12.5 mM Hepes (pH 7.3)) to the basal and 200 µl to the apical compartment. Radiolabelled substances were added to the apical compartment to determine apical to basal transport and to the basal compartment to study the basal to apical transport (in each case 1 µCi/ml incubation buffer). The inulin transport was performed to determine the leakage of the cell layer; therefore, inulin was added to the basal compartment (0.4 µCi/ml incubation buffer). After 0.5, 1, 2 and 4 hours of incubation 10 µl were taken from the opposite compartment and mixed with 2 ml scintillation cocktail (Rotiszint, Roth, Karlsruhe, Germany) and measured in a scintillation beta-counter (type 1409, LKB-Wallac, Turku, Finland).
**Statistical methods and software.** Values are represented as means ± SD. Graphs and calculation were prepared using Microsoft excel, Graph-Pad Prism 3.0 (Graph-Pad Software, San Diego, CA, USA) and SPSS (SPSS, Chicago, IL, USA) software. Student’s t-test and Mann-Whitney-U test were performed to determine statistical significance. For correlation analysis of OATP2B1, OAT4 and BCRP the Pearson’s test was used. Differences were considered significant at p < 0.05. 3D analysis of the transfected cells was done using the volocity software (Improvision, Lexington, MA, USA).
Results

**OATP2B1 and BCRP mRNA expression.** Expression of OATP2B1, OAT4 and BCRP mRNA and 18S rRNA was determined in all placenta samples using real-time PCR technique. Expression of all three transport proteins were normalized to 18S rRNA levels for further calculations. In all 71 samples mRNA of OATP2B1 and BCRP could be detected with similar levels; however, there was a wide interindividual range (OATP2B1/18S: median = 4.5 x 10^{-6} (25% and 75% percentil: 2.0 – 8.6 x 10^{-6}); OAT4/18S: median = 11.5 x 10^{-6} (25% and 75% percentil: 11.0 – 12.1 x 10^{-6}), BCRP/18S: median = 4.1 x 10^{-6} (25% and 75% percentil: 2.1 – 7.9 x 10^{-6})). The expression of OATP2B1 and BCRP exhibited a lognormal distribution, while mRNA levels of OAT4 were normal distributed (Fig. 1A). Moreover, BCRP and OATP2B1 mRNA expression levels were positive correlated (correlation: 0.534, p < 0.01), an observation missing between BCRP and OAT4 (correlation: -0.104, p > 0.05), (Fig. 1B).

**Localization of OATP2B1 and BCRP.** Localization of both OATP2B1 and BCRP was studied by western blot analysis on enriched apical or basal placental membrane fractions and immunofluorescence on placenta slices. The results of the western blot analysis demonstrated a strong signal in the enriched apical membrane fractions and weak band in the basal one for BCRP (72 kDa), for OATP2B1 (84 kDa) the western plot exhibited the opposite result. These signals were comparable with the expected bands in the respective overexpression cells (Fig. 2).

The immunofluorescence staining confirmed the expression of both transporters in the placental syncytiotrophoblast, revealing an apical localization of BCRP and a basal one for OATP2B1. The nuclei counterstaining (blue fluorescence) indicates the localization of the multinuclear syncytiotrophoblast (Fig. 3, A - F).

The staining of BCRP, OATP2B1 or BCRP/OATP2B1 transfected MDCKII cells demonstrates also a localization of BCRP in the apical and OATP2B1 in the basolateral membrane of the MDCKII cells (Fig. 3, G – I).
Transepithelial Transport studies. The characterization of the transfected cells with regard to the results of the western blot analysis and immunofluorescence staining were already mentioned before. Using these four cell lines in a transwell system, transport studies with E3S, DHEAS and 17-estradiol-glucuronide were performed. For all experiments inulin transport measurements were carried out to make sure that there was no leakage. Cells were considered to be tight if the inulin transport was below 1 % per hour (data not shown). All three substances, E3S, DHEAS and 17β-estradiol-glucuronide were tested for apical to basal as well as basal to apical transport. For all three compounds neither a remarkable apical to basal nor a basal to apical transport could be observed in non-transfected MDCKII during 4 h of incubation. While a transport of 17β-estradiol-glucuronide was also not observed in the other three cell lines, all transfected cells demonstrated an altered transport of E3S and DHEAS. With regard to E3S there was no substantial difference between the apical to basal or vice versa transport in the OATP2B1 transfected cells; however, the transport velocity in both directions was much higher compared to non-transfected and BCRP transfected ones. In contrast, the OATP2B1/BCRP double-transfected cells exhibited a strongly enhanced basal to apical transport of E3S, which was 2.1 fold higher compared to OATP2B1-transfected and more than 11 fold compared to the non- and BCRP transfected cells after 4 h of incubation. Moreover, the double-transfected cells showed nearly no apical to basal transport compared with the control cells (ratio of basal to apical transport after 4 h: MDCK: 1.60, OATP2B1 transfected: 1.56, BCRP transfected: 0.47 and OATP2B1/BCRP transfected: 0.04). The observations for the DHEAS transport were similar to the results for E3S (Fig. 4).
In the present study we investigated expression and function of the uptake transporters OATP2B1 and OAT4 as well as the efflux transporter BCRP in human placenta and examined functional interaction of OATP2B1 and BCRP in an in vitro system.

The mRNA expression of all proteins could be detected in each placenta sample; however, the mRNA levels of OATP2B1 and BCRP demonstrate wide interindividual variability following a lognormal distribution for both proteins. In contrast the expression of OAT4 shows a normal distribution. Unlike OAT4 the mRNA expression levels of OATP2B1 and BCRP show a significant correlation, thereby indicating a co-regulation or interaction of both genes on the transcriptional level. While regulatory effects for BCRP expression have been described (for example a regulation through placental hormones like progesterone and estradiol or the epithelial growth factor (EGF) (Wang, et al., 2006; Zu Schwabedissen, et al., 2006)), data on regulation of OATP2B1 expression have not been reported.

In addition to these transcriptional studies we examined OATP2B1 and BCRP on protein level. Here, a strong expression for BCRP was present in the apical membrane of the placental syncytiotrophoblast, while OATP2B1 could be detected in the basal membrane. These results were confirmed both by western blot analysis and immunofluorescence microscopy and are in line with findings of previous studies (St Pierre, et al., 2002; Maliepaard, et al., 2001). The localization of OATP2B1 to the fetal and BCRP to the maternal membrane of the syncytiotrophoblast and the positive correlation of both transporters on mRNA expression level indicated a functional coupling. Such cooperation would be of functional importance since compounds like sulfate-conjugates from the fetal circulation could enter the syncytiotrophoblast via OATP2B1 and are subsequently eliminated into the maternal blood by BCRP. To test this hypothesis we developed a BCRP/OATP2B1 overexpressing MDCKII cell line and respective controls, demonstrating a syncytiotrophoblast like localization of OATP2B1 and BCRP. Therefore, this in vitro model may simulate the placental syncytiotrophoblast with regard to the expression and localization.
of BCRP and OATP2B1. In addition, a system like this can be used to study transporter mediated transepithelial transport processes (Cui, et al., 2001). Using this model we could demonstrate a strongly increased basal to apical transport of the sulfated steroids E3S and DHEAS in BCRP/OATP2B1 double-transfected cells, while Mock- or BCRP-transfected cells showed almost no transport. The expression of OATP2B1 alone resulted in an increase in basal to apical as well as apical to basal transport. In contrast, for glucuronide conjugates like 17β-estradiol-glucuronide, which is transported by BCRP but not by OATP2B1 (Chen, et al., 2003; Suzuki, et al., 2003a; Tamai, et al., 2001), neither an enhanced basal to apical nor an apical to basal transport could be observed. Taken together, these findings demonstrate a functional interaction between the uptake transporter OATP2B1 and the ABC-transporter BCRP in transepithelial transport of E3S and DHEAS. Moreover, our results indicate that the expression of an efflux transporter like BCRP is necessary to get a directional transport across this epithelium, because OATP2B1 alone seems to work in both directions, this observation is in line with previous reports of functional coupling of transport proteins (Cui, et al., 2001; Kopplow, et al., 2005).

With regard to the placenta these results are of potential physiological relevance, because of the important role of this organ in steroid hormone synthesis during pregnancy (for review see Kallen, 2004). In this context, the placenta is strongly dependent on the uptake of precursor molecules like DHEAS for the estrogen synthesis, as the placenta is lacking the 17β-hydroxylase/17-20-lyase (CYP17) (Voutilainen and Miller, 1986). A possible source of DHEAS is therefore the fetal adrenal gland (Seron-Ferre, et al., 1978). There are some data supporting the idea that the uptake of this molecule to the placental syncytiotrophoblast as hormone synthesizing cell is mediated by the organic anion uptake transporters OATP2B1 and OAT4. Both proteins are localized in the basal, fetal facing membrane of the syncytiotrophoblast (Ugele, et al., 2003; St Pierre, et al., 2002). Moreover, DHEAS, which is converted to estradiol by the aromatase pathway, represents the main source of precursor
molecules for estrogen synthesis. E3S is another endogenous precursor molecule for estradiol synthesis (Santen, et al., 1986). E3S represents the major circulating plasma estrogen and can be formed by sulfation of estrone (Falany, 1997). The formation of the sulfate is mediated by the estrogen sulfotransferase (EST), while the desulfation towards a biological active estrogene is catalyzed by the steroid sulfatase (STS). Again, both enzymes are expressed in the syncytiotrophoblast of the placenta (Miki, et al., 2002; Suzuki, et al., 2003b). In this context, the expression of uptake transporter like OATP2B1 in the basal membrane of the syncytiotrophoblast seems to be responsible for the uptake of fetal derived sulfated precursor molecules (E3S and DHEAS) for the steroid synthesis. The apical expression of BCRP, however, may be involved in the elimination of sulfates like DHEAS or E3S from the fetal circulation into the maternal blood. In addition, BCRP can regulate the placental estrogen synthesis by modifying intracellular E3S concentration, which may function as a pool for inactive estrogen. In this context, it is interesting that BCRP accepts E3S but not estrogen as substrate (Imai, et al., 2003). The BCRP expression may also regulate the availability of maternal DHEAS as a precursor for the placental estrogen synthesis, which in return points to the importance of the fetal DHEAS for the steroid hormone synthesis. Finally the apical BCRP expression may protect the fetal organism from maternal E3S and xenobiotics (Allen and Schinkel, 2002).

Relevance of this functional cooperation is not restricted to the placenta, because OATP2B1 and BCRP mRNA are expressed in significant levels in many tissues like liver, intestine, mammary gland, ovary and testes (Tamai, et al., 2000; Pizzagalli, et al., 2003; Maliepaard, et al., 2001).

In conclusion, our data demonstrate an interaction between OATB2B1 and BCRP on expression and functional level. Moreover, we have evidences that vectorial transport of substrates of OATP2B1, which is mainly expressed in the basal membrane of the
syncytiotrophoblast, requires coexpression of an efflux transporter like BCRP in the corresponding apical membrane.
We thank Karen May for helpful discussions and support with the experiments and acknowledge the excellent technical assistance by Tina Brüggmann, Bärbel Uecker and Kerstin Böttcher Dept. of Pharmacology, Greifswald.
References


Footnotes

a)

This work was supported by the Karl und Lore Klein-Stiftung, Oy-Mittelberg, Germany and by a grant of the Deutsche Krebshilfe (grant # 502735).

b)

Heyo K. Kroemer, PhD
Department of Pharmacology
Friedrich Loefflerstr. 23d
17487 Greifswald
Germany
Phone: +49 3834 865630
FAX: +49 3834 865631
Email: kroemer@uni-greifswald.de

c)

None
Figure 1. mRNA expression of BCRP, OAT4 and OATP2B1 in 71 placenta samples. mRNA expression of all transporters was measured using real-time PCR and normalized to expression of 18S rRNA. A. distribution of the expression levels between the placenta samples. B. correlation of the normalized OATP2B1/OAT4 mRNA and BCRP mRNA expression.

Figure 2. Western blot analysis of OATP2B1 and BCRP in apical and basal placental membrane preparations as well as non-, BCRP-, OATP2B1- and double-transfected MDCKII cells. OATP2B1 and BCRP were detected using the OATP2B1 rabbit antiserum and the BXP-21 anti-BCRP antibody.

Figure 3. Immunolocalization of BCRP and OATP2B1 in the human placenta as well as in BCRP-, OATP2B1- and double-transfected MDCKII cells. Placenta section were stained with the BXP-21 anti-BCRP antibody (A and D, green fluorescence) and the rabbit serum against OATP2B1 (B and E, red fluorescence). C and F show the merge. The nuclei were counterstained with TOTO®-3-iodide (A to F, blue fluorescence). The BCRP-, OATP2B1- and double-transfected MDCKII transfected cells were stained in the same way. Panel G (BCRP), H (OATP2B1) and I (double-transfected) show a top view and a cross section of each cell type.

Figure 4. Transepithelial transport studies using non-, BCRP-, OATP2B1- or double-transfected MDCKII cells in a transwell system. Cells were incubated with incubation buffer containing 1 μCi/ml of the respective tritium-labelled substance in the apical or basal compartment and apical to basal (dotted line) as well as basal to apical (continuous line) transport were determined. After indicated times 10 μl of the incubation buffer were taken from the opposite compartment and radioactivity was determined. The data represent the
calculated concentration of the respective substance (E3S, DHAES and 17β-estradiol-glucuronide) (mean ± SD, n = 3).
Figure 1

A

- Frequency distribution of expression of OATP2B1 mRNA/18S rRNA x 10^5
- Frequency distribution of expression of BCRP mRNA/18S rRNA x 10^5
- Frequency distribution of expression of OAT4 mRNA/18S rRNA x 10^5

B

- Correlation: 0.534, p < 0.01 between OATP2B1 and BCRP
- Correlation: -0.104, p > 0.05 between OAT4 and BCRP
Figure 2
Figure 4

This article has not been copyedited and formatted. The final version may differ from this version.