Characterization and identification of steroid sulfate transporters of human placenta

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Submitted 11 June 2002; accepted in final form 16 October 2002

Ugele, Bernhard, Marie V. St-Pierre, Monika Pihusch, Andrew Bahn, and Peer Hantschmann. Characterization and identification of steroid sulfate transporters of human placenta. Am J Physiol Endocrinol Metab 284: E390–E398, 2003. First published October 29, 2002; 10.1152/ajpendo.00257.2002.—Human trophoblasts depend on the supply of external precursors, such as dehydroepiandrosterone-3-sulfate (DHEA-S) and 16α-OH-DHEA-S, for synthesis of estrogens. The aim of the present study was to characterize the uptake of DHEA-S by isolated mononucleated trophoblasts (MT) and to identify the involved transporter polypeptides. The kinetic analysis of DHEA-S uptake by MT revealed a saturable uptake mechanism (Km = 26 μM, Vmax = 428 pmol·mg protein−1·min−1), which was superimposed by a nonsaturable uptake mechanism (diffusion constant = 1.2 μL·mg protein−1·min−1). Uptake of [3H]DHEA-S by MT was Na+ dependent and inhibited by sulfobromophthalein (BSP), steroid sulfates, and probenecid, but not by steroid glucuronides, unconjugated steroids, conjugated bile acids, ouabain, p-aminohippurate (PAH), and bumetanide. MT took up [35S]BSP, [3H]estrone-sulfate, but not [3H]labeled ouabain, estradiol-17β-glucuronide, taurocholate, and PAH. RT-PCR revealed that the organic anion-transporting polypeptides OATP-B, -D, -E, and the organic anion-transporting polypeptide OATP-C, -8, -A, OAT-3, and Na+-taurocholate cotransporting polypeptide (NTCP) are not or are only lowly expressed in term placental tissue and freshly isolated and cultured trophoblasts. Immunohistochemistry of first- and third-trimester placenta detected OAT-4 on cytотrophoblast membranes and at the basal surface of the syncytiotrophoblast. Our results indicate that uptake of steroid sulfates by isolated MT is mediated by OATP-B and OAT-4 and suggest a physiological role of both carrier proteins in placental uptake of fetal-derived steroid sulfates.

In primates, plasma concentrations of unconjugated estrone, estradiol, and estriol increase linearly with advancing gestation. After approximately week 9 of human pregnancy, the placenta becomes the main source of maternal estrogens. However, because the placenta lacks the enzyme system 17α-hydroxylase/17–20-lyase, or CYP17, and thus is unable to convert cholesterol into estrogen, its estrogen synthesis is highly dependent on the supply of C-19 steroids for their conversion into estrogens. It was demonstrated that sulfated C-19 steroids of fetal and maternal origin serve as precursors for the placental estrogen biosynthesis and that the resulting estrogens are mainly released into the maternal blood (for review see Refs. 2, 20, and 32).

Dehydroepiandrosterone-3-sulfate (DHEA-S) of maternal and fetal origin contributes about equally to the placental formation of estrone and estradiol, whereas 16α-hydroxydehydroepiandrosterone-sulfate (16α-OH-DHEA-S) supplied by the fetus contributes to >90% of placental estriol synthesis. Therefore, the concept of a functional fetal placent al unit for estrogen synthesis was established, providing the basis for maternal estriol measurement to assess fetal well-being, placental function, and/or uteroplacental blood flow (for review see Ref. 20).

The conversion of sulfated C-19 steroid precursors to estrogens involves the action of four enzymes located predominantly intracellularly in the syncytiotrophoblast (7), which is formed from mononucleated cytotrophoblasts by cell fusion and differentiation. Therefore, the substrates must enter the cells. The mechanism of their uptake by trophoblast cells is unknown. Recently, we have shown that uptake of DHEA-S by isolated mononucleated trophoblasts (MT) is very likely mediated by one or more carriers that are not functional in choriocarcinoma cell lines (38). In the present study, we examined the kinetic characteristics of this transport activity. Because the results obtained for MT share properties with those identified in hepatocytes, kidney, and other tissues (16, 17), we investigated the expression of these organic anion carriers in human placental tissue and freshly isolated MT by RT-PCR. Furthermore, we studied the expression of these carrier...
ers during cultivation of the MT, which have been shown to fuse within 4 days to syncytia (13, 35, 37).

MATERIALS AND METHODS

Materials. The sodium salts of estradiol-17β-sulfate and estradiol-3, 17β-disulfate were obtained from Leo (Helsingborg, Sweden). Indocyanine green was purchased from ICN Biomedicals (Eschwege, Germany) and ouabain (G-strophanthin) from Boehringer (Mannheim, Germany). All other substrates and inhibitors were purchased from Sigma (Deisenhofen, Germany). Materials used for isolation and cultivation of trophoblasts and uptake studies have been described previously (35, 36, 38).

Radiochemicals. [7-3H(N)]dehydroepiandrosterone sulfate sodium salt, [3H(G)]taurocholic acid, [6,7-3H(N)]estrone sulfate ammonium salt, [6,7-3H(N)]estradiol-17β-d-glucuronide, [3H(G)]ouabain, and p-[glycyl-2-3H]aminohippuric acid were purchased from NEN DuPont (Bad Homburg, Germany). Dehydroepiandrosterone [35S]sulfate and [35S]sulfobromophthalein were synthesized as described earlier (8, 19).

Isolation of MTs. Human term placentas from uncomplicated pregnancies were obtained after spontaneous vaginal delivery and elective caesarean sections and immediately processed. Human MTs were isolated from placental villous tissue, purified, and cultured as described earlier (13, 35, 36).

Transport studies. Cell suspensions, obtained as described above, were washed one time in transport buffer containing (in mM) 142.9 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.8 CaCl2, and 20 HEPES, pH 7.4, and were suspended to 1.5 × 109 cells/ml in cold transport buffer and kept on ice. For Na+-free transport buffer, NaCl was replaced by an equimolar concentration of choline chloride; for SO42−-free transport buffer, MgSO4 was omitted. The uptake of the 3H- and/or 35S-labeled substrates by isolated trophoblasts in suspension was studied by use of the silicone oil centrifugation technique with 22 cycles in the first PCR reaction and 22 (OATP-4), 25 (OATP-2, LST-1, SLC21A6, GenBank accession no. NM_006446) cycles, respectively, in the second PCR reaction. OATP-B, -D, and -E, the organic anion transporters used in the present study were as follows.

OATP-A: (SLC21A3, GenBank accession no. NM_005075)

external: 5’-ctg cat tgg atg tgt ggt tat agg c-3’

antisense nt 765-740

0.5U/µl AmpliTaq Gold DNA polymerase (Applied Biosystems, Weiterstadt, Germany). For amplification of the organic anion-transporting polypeptides (OATP)-A, -C, and -8, the organic anion transporter (OAT)-3, and Na+-taurocholate cotransporting polypeptide (NTCP) transcripts, a nested PCR technique was used with 22 cycles in the first PCR reaction and 22 (OATP-A), 25 (OATP-B, -8, 24 (NTCP), and 30 (OATP-C, OAT-3) cycles, respectively, in the second PCR reaction. OATP-B, -D, and -E, OAT-4, and β-actin were amplified using one single PCR reaction with 28, 32, 28, and 18 cycles, respectively. PCR products were separated on an agarose gel and visualized under ultraviolet light by ethidium bromide staining. Primers used in the present study were as follows.

NTCP: (SLC10A1, GenBank accession no. L21893)

external: 5’-gca cca tgg atg tca gca aga tca agg c-3’

(sense nt 213-240)

5’-ctg cat tgg atg tgt ggt tat agg c-3’

antisense nt 846-820

5’-ctg cat tgg atg tgt ggt tat agg c-3’

antisense nt 1508-1534

5’-ctg cat tgg atg tgt ggt tat agg c-3’

antisense nt 1886-1869

(21)
RESULTS

Uptake of DHEA-S by isolated trophoblasts. Kinetic analysis of all substrates was performed. All substrates were linear to 0.3 s, and the initial uptake velocity (v) was estimated by linear regression analysis of substrate uptake after 0.25, 0.5, 0.75, and 1.0 min. To take care of different uptake mechanisms and carriers, the data were fitted to different equations, ranging from simple saturable Michaelis-Menten (2 parameters, Eq. 1), Michaelis-Menten in the presence of a nonsaturable system, denoted by P_{uni} (3 parameters, Eq. 2), and two Michaelis-Menten components (4 parameters, Eq. 3). A fitting to equations with five or more parameters (e.g., 2 Michaelis-Menten components in the presence of a nonsaturable system) did not converge. Fitting was completed with a weighted nonlinear least square fit computer program (SigmaPlot; SPSS, Chicago, IL), on the basis of the Marquard-Levenberg algorithm. The weighting factor was w = 1/variance.

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

$$v = \frac{V_{max}[S]}{K_m + [S]} + P_{uni} [S] \quad (2)$$

$$v = \frac{V_{max1}[S]}{K_{m1} + [S]} + \frac{V_{max2}[S]}{K_{m2} + [S]} \quad (3)$$

Statistics. An F-test (23, 29) and Akaike’s information criterion (12) were used to select the appropriate model equation(s). The quality of the fitting was scrutinized by analyzing the residuals with the RUNS test (23, 31). To test the significance of differences between data sets, double-sided, paired t-tests were performed.

OAT-3: (SLC22A6, GenBank accession no. AB042505)

external: 5'-tac ctc atc tat atg gtc ttc c (sense nt 641-662)

$$5' - tta ctt acc ccc ata cct gtt gtc-3'$$

(antisense nt 1473-1450)

internal: 5'-ccg tca tct tga atg tgg (sense nt 705-722)

$$5' - tgt aga gga aga ggc agc$$

(antisense nt 1422-1404)

OAT-4: (SLC22A7, GenBank accession no. NM_018484)

5'-ccg gtg get gat tat taa ggc-3' (sense nt 892-912)

$$5' - tgt ggag cac gga gg-3'$$

(antisense nt 1310-1291)

β-Actin: (ACTB, GenBank accession no. X00351.1)

5'-ggt cac cat tgg caa tga ggg-3' (sense nt 779-802)

$$5' - tgt gtg cac tgt gga gag cac tgt g$$

(antisense nt 1521-1498)(25)

The primer sequences were obtained from references as indicated or designed by us, partly by use of the computer program OMIGA (Oxford Molecular).

Immunohistochemistry. Paraffin-embedded sections from three first- and third-trimester placentas were processed for immunohistochemistry. Sections were deparaffinized in xylene, rehydrated through a graded series of ethanol, and pre-treated in 100 mM sodium citrate buffer, pH 6.0, for 5 min in a pressure cooker. The affinity-purified polyclonal rabbit anti-human OAT-4 antiserum raised against an 18-amino acid residue peptide near the cytoplasmic COOH terminus of OAT-4 (Alpha Diagnostic, San Antonio, TX) was diluted 1:50, and the tissue sections were incubated 45 min at room temperature. For visualization, the avidin-biotin peroxidase detection method (Vectastain ABC kit, Vector Laboratories) and 3-amino-9-ethylcarbazole (Sigma, Munich, Germany) as chromogenic substrate were used. Controls were performed by preadsorption of the antiserum with 1 μM antigenic peptide (Alpha Diagnostic, San Antonio, TX).

Protein determinations. Cellular protein was determined using a modification of the method described by Lowry et al. (22).

Calculation of kinetic parameters and statistics. Uptake of all substrates was linear up to 0.6 s, and the initial uptake velocity (v) was estimated by linear regression analysis of substrate uptake after 0.25, 0.5, 0.75, and 1.0 min. To take care of different uptake mechanisms and carriers, the data were fitted to different equations, ranging from simple saturable Michaelis-Menten (2 parameters, Eq. 1), Michaelis-Menten in the presence of a nonsaturable system, denoted by $P_{uni}$ (3 parameters, Eq. 2), and two Michaelis-Menten components (4 parameters, Eq. 3). A fitting to equations with five or more parameters (e.g., 2 Michaelis-Menten components in the presence of a nonsaturable system) did not converge. Fitting was completed with a weighted nonlinear least square fit computer program (SigmaPlot; SPSS, Chicago, IL), on the basis of the Marquard-Levenberg algorithm. The weighting factor was $w = 1/variance$.

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

$$v = \frac{V_{max}[S]}{K_m + [S]} + P_{uni} [S] \quad (2)$$

$$v = \frac{V_{max1}[S]}{K_{m1} + [S]} + \frac{V_{max2}[S]}{K_{m2} + [S]} \quad (3)$$

Fig. 1. Kinetics of dehydropiandros-terone [35S]sulfate (DHEA-35S) uptake by isolated mononucleated tropho-blast. Suspended cells (1.5 × 10^6 cells/ml) were incubated in the presence of increasing substrate concentrations. After 15, 30, 45, and 60 s, aliquots were taken, and the cells were centrifuged through silicone oil. The radioactivity of the pellet was measured, and the initial slope of the specific amount of DHEA-S, which was sedimented with the cells, was calculated by linear regression analysis. Each point represents the arithmetic mean of the values of 5 cell preparations. The curve through the data points (dashed line) represents the nonlinear regression analysis, as described in MATERIALS AND METHODS.

AJP-Endocrinol Metab • VOL 284 • FEBRUARY 2003 • www.ajpendo.org
values of five cell preparations were, for the apparent

\[ K_m = 26 \pm 7.6 \ \mu M; \quad V_{\text{max}} = 428 \pm 98 \ \text{pmol} \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}; \quad \text{and for} \quad P_{\text{diff}} = 1.2 \pm 0.5 \ \text{l} \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}. \]

Uptake of [\text{H}]DHEA-S by MT was significantly \( (P < 0.01) \) decreased in \( \text{Na}^+ \)-free transport buffer (12.5 \pm 21.1\% of control, \( n = 5 \)) but was not changed in \( \text{SO}_4^{2-} \)-free transport buffer (92.5 \pm 31.6\% of control, \( n = 3 \)).

Uptake of [\text{H}]DHEA-S by MT was strongly inhibited by BSP, steroid sulfates (DHEA-S, estrone sulfate, estradiol-17\( \beta \)-sulfate, estradiol-3,17\( \beta \)-disulfate, vitamin D\( _3 \)-sulfate), and probenecid (see Figs. 2 and 3). Uptake of [\text{H}]DHEA-S by MT was not inhibited by indocyanine green, \( p \)-aminohippurate (PAH), bumetanide, steroid glucuronides (estradiol-17\( \beta \)-glucuronide, estrone-glucuronide), unconjugated steroids (DHEA, 16a-OH-DHEA, dexamethasone), conjugated bile acids (taurocholate, tauroursodeoxycholate), and ouabain (see Figs. 2 and 3).

Uptake of different organic anions and ouabain by isolated trophoblasts.

The initial uptake rate of \( ^{[35S]} \text{BSP} \) was about three to five times higher than the uptake rate of [\text{H}]DHEA-S and estrone sulfate (E\( _1 \)S). Initial uptake rate of BSP was decreased by 100 \( \mu M \) rifamycin and low temperature (data not shown). In contrast, there was no significant initial uptake velocity detectable for \( ^{3} \text{H} \)-labeled estradiol-17\( \beta \)-glucuronide (E\( _2 \)17\( \beta \)G), ouabain, taurocholate, and PAH (see Table 1).

Detection of steroid sulfate transporter mRNAs by use of RT-PCR.

OATP-B was highly expressed in term placental tissue and freshly isolated MT. During cultivation, expression levels decreased after 1 day and increased significantly after 4 days, in parallel with...
Table 1. Slope of initial uptake of different radiolabeled organic anions and ouabain by isolated mononucleated trophoblasts

<table>
<thead>
<tr>
<th>Substrate (1 µM)</th>
<th>Slope of initial uptake, pmol/mg protein min⁻¹</th>
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<tbody>
<tr>
<td>[35S]sulfobromophthalein</td>
<td>134.8 ± 35.6</td>
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<tr>
<td>[3H]DHEA-S</td>
<td>19.9 ± 7.0</td>
</tr>
<tr>
<td>[3H]estrone-sulfate</td>
<td>17.7 ± 4.3</td>
</tr>
<tr>
<td>[3H]estradiol-17β-glucuronide</td>
<td>9.0 ± 0.8*</td>
</tr>
<tr>
<td>[3H]taurocholate</td>
<td>0.4 ± 0.3*</td>
</tr>
<tr>
<td>[3H]ouabain</td>
<td>0.4 ± 0.7*</td>
</tr>
<tr>
<td>[3H]PAH</td>
<td>0.3 ± 0.3*</td>
</tr>
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</table>

Values are means ± SE from n different cell preparations. [3H]-DHEA-S, [3H]-labeled dehydroepiandrosterone-3-sulfate; [3H]PAH, [3H]-labeled p-aminohippurate. Suspended cells (1.5 × 10⁶ cells/ml) were incubated with 1 µM radiolabeled substrate at 37°C. After 15, 30, 45, and 60 s, aliquots were taken, and cells were centrifuged through silicone oil. Radioactivity of the pellet was measured, and initial slope of the specific amount of substrate, which was sedimented with the cells, was calculated by linear regression analysis. *Value not significantly different from zero (P < 0.05).
OATP-D and OATP-E with the RT-PCR technique (10, 33). Our results indicate that uptake of steroid sulfates by isolated trophoblast cells is predominantly mediated by OATP-B and OAT-4. However, the uptake of steroid sulfates by OATP-D, OATP-E, and other so-far-unknown transporting polypeptides with a similar substrate specificity cannot be excluded. The function of the very low expression of other transporting polypeptides in isolated trophoblast cells is unclear and may be due to a meaningless “illegitimate” physiological transcription of the respective genes (5). Independently, the fact that OATP-C was lowly expressed in placenta tissue but not detectable in isolated trophoblasts may indicate that OATP-C is only expressed in nontropho-

Table 2. Summary of substrate specificity of cloned human steroid sulfate carriers and isolated trophoblasts

<table>
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<tbody>
<tr>
<td>BSP</td>
<td>(+)³</td>
<td>+1</td>
<td>+1,5,10</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>i²</td>
<td>i²</td>
<td>i²</td>
<td>+14</td>
</tr>
<tr>
<td>Estrone sulfate</td>
<td>(+)³</td>
<td>+1</td>
<td>+1,5,10,9,10</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>i²</td>
<td>i²</td>
<td>i²</td>
<td>+14</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>NT</td>
<td>+1</td>
<td>+1,5,10,1,9,13</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>i²</td>
<td>i²</td>
<td>i²</td>
<td>+14</td>
</tr>
<tr>
<td>Taurocholate</td>
<td>+3</td>
<td>+1</td>
<td>+1,5,10,1,9,13</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>i²</td>
<td>i²</td>
<td>i²</td>
<td>+14</td>
</tr>
<tr>
<td>Ouabain</td>
<td>−3</td>
<td>+1</td>
<td>+1,5,10,1,9,13</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>i²</td>
<td>i²</td>
<td>i²</td>
<td>+14</td>
</tr>
<tr>
<td>E17βG</td>
<td>−3</td>
<td>+1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>i²</td>
<td>i²</td>
<td>i²</td>
<td>+14</td>
</tr>
<tr>
<td>PAH</td>
<td>NT</td>
<td>+1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>i²</td>
<td>i²</td>
<td>i²</td>
<td>+14</td>
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Uptake of different radiolabeled substrates by cloned transporting polypeptides was determined either after injection of cRNA in *Xenopus laevis* oocytes or after expression of cDNA in eukaryotic cell lines. BSP, sulfobromophthalein DHEA-S, dehydroepiandrosterone-3-sulfate; E17βG, estradiol-17β-glucuronide; PAH, p-aminobiphenyl. See text for transporter abbreviations. +, significant uptake; (+), significant but very low uptake; −, no uptake; NT, not tested; i, inhibits uptake of estrone sulfate or DHEA-S; (i), low inhibition of estrone sulfate or DHEA-S uptake. Superscript nos. show relevance to reference nos. in parentheses: ¹(18), ²(4), ³(24), ⁴(3), ⁵(34), ⁶(28), ⁷(15), ⁸(6), ⁹(1), ¹⁰(33), ¹¹(10), ¹²(11), ¹³(14), ¹⁴this study.

**Fig. 5.** Immunohistochemical detection of OAT-4 in human first- and third-trimester placentas. A–C, third-trimester placentas; D, first-trimester placenta. Staining for OAT-4 was strong at the basal surface of the syncytiotrophoblast (arrow in C). Staining decreased to background levels after adsorption of the antibody with the antigenic peptide (B). Cytotrophoblasts of first-trimester placenta, which have not fused with the syncytiotrophoblasts, reveal distinct staining of the cytoplasmic membrane (D, arrowhead) and of the perinuclear region. Bars, 25 μm.
With use of the Xenopus oocyte expression system, the $K_m$ value of OAT-4 was calculated to be 0.63 μM for DHEA-S and 1.26 μM for E1S (4) and that of OATP-B to be 9.0 or 6.3 μM for E1S (18, 34). Thus it seems that, compared with OATP-B, the affinity of OAT-4 to steroid sulfates is ~5–10 times higher. However, with use of isolated MT, a kinetic discrimination of these two transporting polypeptides was not possible. The calculated apparent $K_m$ value of DHEA-S uptake by isolated MT was higher (26 μM) than that of the transporting polypeptides expressed in Xenopus oocytes but similar to that of E1S uptake by basal membrane vesicles from the placental syncytiotrophoblast (30).

Uptake of DHEA-S by MT was strongly inhibited by compounds with one or more sulfate residues (see Figs. 2 and 3). Thus the sulfate residue independent of the position in the molecule, e.g., C-3 or C-17 of the steroid ring, seems to be essential for inhibition and may also be for transport. On the other hand, uptake of DHEA-S by MT was not influenced by 1.2 mM SO$_4^{2-}$ of the transport buffer, indicating that SO$_4$ is not an inhibitor/substrate of the steroid sulfate transporters and that the carbon backbone of the steroid sulfates is a prerequisite for inhibition/transport.

Uptake of E1S (data not shown) and DHEA-S by isolated MT was partly dependent on extracellular Na$^+$ ions. In contrast, uptake of E1S by OAT-4 expressed in Xenopus oocytes was sodium independent (4). Kullak-Ublick et al. (17) showed that uptake of DHEA-S by human OATP-A was also partly sodium dependent, whereas, to our knowledge, sodium dependency of steroid sulfate uptake by OATP-B remains to be tested. Thus the interpretation that the Na$^+$-dependent part of DHEA-S uptake by MT is mediated by OATP-B is highly speculative.

In cultured trophoblasts, the expression of OATP-B decreased after 1 day and then increased after 4 days compared with freshly isolated cells. These changes occur in parallel with syncytia formation. The former observation may be explained by an adaptation of the cells to the culture condition; the latter observation may indicate an increase during differentiation of MT to the syncytiotrophoblast. Expression of OAT-4 and the other more highly expressed transporting polypeptides did not change significantly during syncytium formation in vitro. Thus our results demonstrate that OATP-B and OAT-4 are highly expressed not only in MT, i.e., cytrophoblast, but also in the multinucleated syncytiotrophoblast formed in vitro and may also contribute to the uptake of steroid sulfates of the syncytiotrophoblast in vivo. The difference in expression of OATP-B and OAT-4 during cultivation may indicate a different regulation of transcription of the respective genes.

Very recently, we have shown that immunohistochemical staining for OATP-B is abundant in the placenta throughout gestation, with strong reactivity in the cytrophoblast membranes and at the basolateral surface of the syncytiotrophoblast (30). A very similar staining pattern was observed for OAT-4 in the present study (see Fig. 5). These results demonstrate a physiological role of OAT-4 and OATP-B in the placental uptake of fetus-derived steroid sulfates only. In contrast, the identity and characteristics of carriers for uptake of maternal steroid sulfates at the microvillus membrane of the syncytiotrophoblast (see introduction) are still unknown. Our results probably indicate either that these microvillus carriers show a substrate specificity similar to OATP-B and OAT-4, or that these transporter polypeptides are not expressed in isolated MT, or that they have been destroyed during the enzymatic isolation procedure. The binding of the OAT-4 antibodies to the perinuclear region in cytrophoblasts may reflect a strong synthesis (translation) of the OAT-4 polypeptide in the rough endoplasmatic reticulum of cytrophoblasts but not in syncytiotrophoblasts. Intracellular transport to the plasma membrane of the cytrophoblast and fusing of these cells with the preexisting syncytiotrophoblast add the OAT-4 polypeptide to the basal membrane of the syncytiotrophoblast. However, to test this hypothesis, further experiments are necessary.

The preponderance of 16α-hydroxyestrogens during pregnancy is greater than might be expected from the ratio of 16α-OH- to 16-deoxy-C-19 steroid sulfates in fetal plasma (for review see Ref. 26). Thus, presumably, precursor abundance is not the sole regulator of the relative amounts of the 16α-OH- and 16-deoxyestrogen formed in the placenta. An explanation of the discrepancy between the ratio of 16α-OH- and 16-deoxy-C-19 substrates in fetal plasma and that of the products in the urine is that the 16α-hydroxy-C-19 sulfate precursors might be converted more efficiently to estrogens than the corresponding 16-deoxy precursors. In the placenta, both sulfated precursors follow the same pathway, because the tissue lacks steroid 16α-hydroxylase. This pathway involves sequential desulfation at C-3β, oxidation and isomerization to the corresponding 4-en-3-oxosteroids, and aromatization. Reduction at C-17 catalyzed by 17β-hydroxysteroid dehydrogenase yields a mixture of estrone and estradiol-17β from DHEA-S and 16α-hydroxyestrone and estriol from 16α-OH-DHEA-S. The utilization of these precursors has been investigated using purified enzymes (9, 27), microsomal preparation, and tissue slices (26). However, the results of these studies have demonstrated that formation by the intracellular enzymatic pathway of 16-deoxyestrone from the sulfate precursors is more efficient than that of 16α-hydroxyestrogens. In contrast, nothing is known about the efficiency of the transport systems responsible for the uptake of the 16α-hydroxy-C-19 and 16-deoxy-C-19 sulfate precursors. Now, one may speculate whether the discrepancy of the placental synthesis of 16-hydroxyestrogens vs. 16-deoxyestrogens mentioned above is due to different efficient
uptake of the respective sulfated precursor from fetal and maternal blood. It will take further studies to characterize the uptake of these substances by isolated and cultured trophoblasts and cells expressing cloned placent al carrier proteins, which have been identified to be involved in the uptake steroid sulfates in the present study.

In conclusion, we have shown that uptake of steroid sulfates by isolated mononucleated trophoblasts is mediated by OATP-B and OAT-4. Our results suggest a physiological role of both carrier polypeptides in placental uptake of fetus-derived steroid sulfates.

We acknowledge the significant scientific contributions to this work of Professor Dr. Erich Kuss. We also thank Karin Regemann and Angelika Bolle for excellent technical assistance and Jasmin Winiker for help in preparing the manuscript. This work was supported in part by Friedrich-Baur-Stiftung der Medizinischen Fakultät, Munich, Germany, Grant no. 26/95 (to B. Ugele) and the National Science Foundation Switzerland, Grant no. 31–056020.98 (to M. V. St-Pierre).

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