Proton-Coupled Erythromycin Antiiport at Rat Blood-Placenta Barrier

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ABSTRACT:

The aim of the present study was to characterize the mechanism of erythromycin transport at the blood-placenta barrier, using TR-TBT 18d-1 cells as a model of rat syncytiotrophoblasts. [14C]Erythromycin was taken up by TR-TBT 18d-1 cells with a Michaelis constant of 466 μM. Although the uptake was not dependent on extracellular Na⁺ or Cl⁻, it was increased at weakly alkaline pH. Significant overshoot of [14C]erythromycin uptake by placental brush-border membrane vesicles was observed in the presence of an outwardly directed proton gradient. These results indicate that erythromycin is transferred by the H⁺-coupled transport system in syncytiotrophoblasts. To address the physiological transport of erythromycin in rat placenta, fetal-to-maternal transport clearance was estimated by means of the single placental perfusion technique. Clearance of [14C]erythromycin was higher than that of [14C]inulin, a paracellular pathway marker, and was decreased by the addition of 5 mM erythromycin, indicating that saturable efflux system from fetus to mother is involved. The effect of various transporter inhibitors on [14C]erythromycin efflux from TR-TBT 18d-1 cells was evaluated. Cyclosporin A, fumitremorgin C, and probenecid had no effect, whereas ethylisopropylamiloride, a specific inhibitor of Na⁺/H⁺ exchangers (NHEs), was significantly inhibitory. These results suggest that erythromycin efflux transport at the rat blood-placenta barrier is mediated by an erythromycin/H⁺ antiiport system, driven by H⁺ supplied by NHEs.

Introduction

Erythromycin transport across the placenta is restricted by the blood-placenta barrier. The fetal plasma concentration of erythromycin is 3 to 10% of the maternal concentration in humans (Kiefer et al., 1955; Heikkinen et al., 2000), and the placental and fetal distribution of erythromycin is also quite low in rats (Kibwage et al., 1989). Therefore, it is likely that an efflux transporter(s) is involved in erythromycin transport at the placenta. Erythromycin is used to treat pregnant women because it is considered to present little risk to fetal development. However, it has been reported that some infants exposed to erythromycin as fetuses showed pylorostenosis (Källén et al., 2005). Because the fetal toxicity of erythromycin is unclear, it is important to clarify the molecular mechanism of erythromycin transport at the blood-placenta barrier.

Several drug transporters can transport erythromycin. For example, erythromycin is a substrate and an inhibitor of P-gp (Hofsi and Nissen-Meyer, 1989; Dey et al., 2004). Although P-gp function at the blood-placenta barrier is an important determinant of fetal drug exposure (Smit et al., 1999), functional involvement of P-gp in erythromycin transfer in the placenta has not been reported. MRP2 (ABCC2) also recognizes erythromycin (Karla et al., 2007). Furthermore, some solute carriers (SLCs) function as efflux transporters. Therefore, possible involvement of SLC and ABC transporters in erythromycin transport in the placenta should be clarified.

The aim of the present study was to characterize erythromycin transport at the blood-placenta barrier by using TR-TBT 18d-1 cells as a model of rat syncytiotrophoblasts, which compose the blood-placenta barrier. It has been established that TR-TBT 18d-1 cells show transport function for nucleosides, antiretroviral drugs, and β-amino acids (Kitano et al., 2002; Chishu et al., 2008; Nishimura et al., 2008; Sai et al., 2008; Sato et al., 2009). Therefore, TR-TBT 18d-1 cells should be a suitable model to charac-
elize the molecular mechanism of drug transport at the blood-placenta barrier. Fetal-to-maternal clearance of erythromycin in vivo in rats and erythromycin uptake by placental brush-border membrane vesicles were also examined.

Materials and Methods

Chemicals and Animals. [14C]Erythromycin (2.0 GBq/mmol) and [14C]julin (74 MBq/g) were purchased from American Radiolabeled Chemicals (St. Louis, MO). [3-(6-Isobutyl-9-methoxy-1,4-dioxo-1,2,3,4,6,7,12,13-octahydroprazinyl][1,2',1'-6]pyridin][3,4-b]indol-3-yl)-propionic acid tert-butyl ester] (Koi143) was synthesized as described previously (Asadima et al., 2006). All other chemicals were commercial products of analytical grade. Wistar female rats were purchased from Japan SLC (Hamamatsu, Japan). Animal studies were performed in accordance with the guideline for the care and use of laboratory animals of Keio University School of Medicine.

Cell Culture. TR-TBT 18d-1 cells were cultured in Dulbecco’s modified Eagle’s medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (SAFC Biosciences, St. Louis, MO), 100 U/ml benzylenecillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (In-vitrogen, Carlsbad, CA) on 100-mm culture dishes (Corning Life Sciences, Lowell, MA) in a humidified incubator at 33°C under an atmosphere of 5% CO2 in air. For the uptake and efflux studies, TR-TBT 18d-1 cells were seeded on four-well plates (Nalge Nunc International, Rochester, NY) coated with porcine skin collagen type I (Nitta Gelatin Canada Inc., Toronto, ON, Canada) at a density of 1 × 104 cells/well. After incubation for 3 days at 33°C, the cells were cultured at 37°C for a further 4 days.

Uptake and Efflux of [14C]Erythromycin by TR-TBT 18d-1 Cells and Effect of Other Macromolecules Drugs on Uptake. Transport medium contained 122 mM NaCl, 25 mM NaHCO3, 3.4 mM CaCl2, 1 mM MgSO4, 0.4 mM K2HPO4, 10 mM d-glucose, and 10 mM Hepes at pH 7.4. Na+-free transport medium was prepared by replacing Na+ with choline+, Li+, K+, or N-methyl-d-glucamine+. Cl–-free transport medium was prepared by replacing Cl– with Br–, NO3–, and thiocyanate (SCN–). Medium pH was adjusted at 6.4 to 8.4 with 1 N HCl or 1 N NaOH.

For the uptake study, TR-TBT 18d-1 cells were washed twice with the transport medium. After a 10-min preincubation in the above medium, the transport medium was replaced with drug solution containing [14C]erythromycin (10 μM) in the absence or presence of an inhibitor to initiate the uptake reaction. For the efflux study, TR-TBT 18d-1 cells were incubated with the transport medium containing [14C]erythromycin (10 μM) for 15 min. These cells were washed twice with the transport medium and further incubated in fresh medium (pH 7.4). At the designated time, both the uptake and efflux reaction was terminated by addition of ice-cold transport medium, and the cells were washed twice in the same medium.

The cells were solubilized in 500 μl of 0.1 M NaOH/1% Triton X-100 solution. Then 400 μl of the cell lysate was mixed with 3 ml of scintillation cocktail (Closarel-I; Nacalai Tesque, Kyoto, Japan), and radioactivity was measured with a liquid scintillation counter (Tri-Carb 317TR/SRL; Packard Instrument Company, Waltham, MA). Cellular protein content was quantified by means of the BCA method using a protein assay kit (Pierce handled on ice during all procedures).

Preparation of Rat Placental BBMVs. Rat placental BBMVs were prepared according to the reported method with some modifications (Alonso de la Torre et al., 1991). The placentas were isolated from pregnant rats at gestation day 18 under anesthesia with ether and were homogenized [first homogenate (H1)] in 10 volumes of buffer containing 300 mM sorbitol, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, and 20 mM Tris/MES, pH 7.4, in a Teflon-glass homogenizer (Iwaki, Tokyo, Japan) with 10 strokes at 2500 rpm. MgCl2 was added at the final concentration of 10 mM, and the homogenate was incubated for 30 min. Supernatant (S1) and pellet (P1) were obtained by centrifugation at 800g for 10 min. P1 was homogenized again in the 10 mM MgCl2 buffer. Supernatant (S1) was obtained by centrifugation at 800g for 10 min and was mixed with S1, and the mixture was centrifuged at 10,400g for 20 min. The resulting supernatant was further centrifuged at 110,000g for 45 min. The final pellet was suspended in the buffer containing 300 mM sorbitol and 20 mM Tris/MES (adjusted to pH 7.4) via a 25-gauge needle, and 100-μl aliquots were frozen in liquid nitrogen and stored at −80°C until use. The tissue was handled on ice during all procedures.

Protein content was measured by the SDS-Lowry method with bovine serum albumin as a standard (Lowry et al., 1951). Lowry reagent (1 ml) containing 2% Na2CO3, 0.4% NaOH, 1% CuSO4, and 2% sodium potassium tartrate was added to a 200-μl aliquot of diluted of BBMVs, and the mixture was incubated for 10 min at room temperature. Then 100 μl of 2-fold diluted Folin-Ciocalteu’s phenol reagent (Sigma-Aldrich) was added. The absorbance was measured at 750 nm after incubation for 30 min at room temperature.

For assay of ALP, the reported method was used (Hirohashi et al., 2000). In brief, 45 μl of the diluted BBMV solution and H1 was mixed with 5 μl of Triton X-100, and the mixture was incubated for 30 min at room temperature. Then, 900 μl of the buffer ALP containing 50 mM CaCl2, 5 mM MgCl2, 300 mM sorbitol, and 50 μl of substrate solution containing 100 mM p-nitrophenyl phosphate in buffer ALP were added to the sample, and the whole mixture was incubated for 30 min at 37°C. To terminate the reaction, the sample was mixed with 1 ml of 10% trichloroacetic acid. The mixture was centrifuged at 12,000g for 5 min, and the absorbance of the supernatant was measured.

A ouabain-sensitive Na+-K+-ATPase assay was conducted according to the reported method (Scharschmidt et al., 1979). A 10-μl aliquot of the diluted BBMV solution and H1 was mixed with 500 μl of the buffer containing 200 mM MgCl2, 126 mM NaCl, 13 mM KCl, 5.2 mM Na2SO4, 5 mM ATP, 1.1 mM EGTA, and 131 mM Tris, pH 7.4, in the absence or presence of 1.1 mM ouabain. The mixture was incubated for 10 min at 37°C. To terminate the reaction, 200 μl of 35% trichloroacetic acid was added. The supernatant was obtained by centrifugation at 12,000g for 5 min, and a 200-μl aliquot was mixed with 40 μl of 10 N H2SO4, 40 μl of ammonium molybdate, and 40 μl of filtered reagent containing 0.25% l-2-aminooxyoctane-4-sulfonic acid, 15% sodium bisulfite anhydrous, 0.5% sodium sulfate anhydrous, and 680 μl of distilled water. The mixture was incubated for 7 min at 37°C, and the absorbance at 660 nm was measured. BBMV solution that showed a 10× greater ALP activity and a 2× lower Na+-K+-ATPase activity than H1 was used for the drug uptake study.

Rapid Filtration Technique for Evaluating Drug Uptake by BBMVs. A 20-μl aliquot of the BBMV solution was preincubated for 10 min at 37°C. The reaction was initiated by adding 80 μl of the transport medium, 300 mM sorbitol, and 10 mM Hepes/Tris, pH 7.4 or 8.4, containing a designated concentration of radiolabeled compound to the BBMV solution. To terminate the uptake, 900 μl of ice-cold transport medium containing 1 mM erythromycin was added; the unlabeled erythromycin served to reduce nonspecific binding of the radiolabeled compound to the filter. The mixture was immediately filtered through a HAWP 0.45-μm filter (Millipore Corporation, Billerica, MA). The filter was washed with 8 ml of the ice-cold transport medium.
and solubilized in 4 ml of Clearsol I. The radioactivity was measured with a liquid scintillation counter.

mRNA Expression of Na+/H+ Exchangers, NHE1 and NHE3. Total RNA was isolated from the placenta of a rat at gestational day 18 and from TR-TBT 18d-1 cells cultured as described for the uptake study, using a RNeasy mini kit (QIAGEN, Valencia, CA). cDNA was synthesized by reverse transcriptase (ReverTra Ace; Toyobo Co., Ltd., Osaka, Japan). Polymerase chain reaction was performed using Platinum PCR SuperMix (Invitrogen). Specific primers were designed for rat NHE1 and NHE3 as follows: 5'-GA-ACATCCACCCCAAGTCTG-3' for NHE1 forward, 5'-CAGTGGGCTCTAGGC-CATTCG-3' for NHE1 reverse, 5'-ACTGCTTAAAGCAGGTTGACGT-3' for NHE3 forward, and 5'-AAAGCAGAGGCGGTCTGATGAT-3' for NHE3 reverse.

Data Analysis. Kinetic analysis was done using the following Michaelis-Menten equation:

\[
v = \frac{V_{\text{max}} S}{K_m + S} + \frac{V_{\text{max}} K_m}{K_m + S}
\]

where \( v \), \( S \), \( V_{\text{max}} \), \( K_m \), and \( K_{\text{ns}} \) represent initial uptake velocity, substrate concentration, maximum uptake velocity, Michaelis constant, and nonsaturable uptake constant, respectively. The fitting was performed by nonlinear least-square regression analysis using Delta Graph (version 5.5.5; RedRock Software Inc., Salt Lake City, UT).

Statistical analysis was performed by the use of Student’s t test or analysis of variance with Dunnett’s post hoc test. \( P < 0.05 \) was considered significant.

Results

Cellular Uptake of [14C]Erythromycin by TR-TBT 18d-1 Cells. To address the transporter-mediated cellular uptake of [14C]erythromycin across the blood-placenta barrier, [14C]erythromycin uptake by TR-TBT 18d-1 cells was measured. [14C]Erythromycin was strongly taken up by TR-TBT 18d-1 cells, and the uptake was reduced by the addition of an excess of unlabeled erythromycin, indicating that the [14C]erythromycin uptake was predominantly mediated by a transport system(s) (Fig. 1A). To characterize the transport system, its Na\(^+\) and Cl\(^-\) dependence was examined. No significant difference was observed upon replacement of these ions with other monocations and monoanions, respectively (Fig. 1B). To further characterize [14C]erythromycin uptake, the uptake kinetics was analyzed based on the Michaelis-Menten equation (Fig. 2). Erythromycin uptake showed a clear saturable curve, and the Eadie-Hofstee plot was linear.

FIG. 1. [14C]Erythromycin uptake by TR-TBT 18d-1 cells. A, [14C]erythromycin (10 \( \mu \)M) uptake by TR-TBT 18d-1 cells as measured for 30 min in the absence (○) and presence (●) of an excess concentration (5 mM) of unlabeled erythromycin. B, Na\(^+\) and Cl\(^-\) dependence of initial [14C]erythromycin uptake was measured by replacing Na\(^+\) and Cl\(^-\) with other monocations and monoanions as shown. Data represent the mean ± S.E.M. of four determinations. * indicates significant difference from the corresponding control \( P < 0.05 \).

Specificity of the Erythromycin Transport System in Syncytiotrophoblasts. To examine whether or not the erythromycin transport system is specific for erythromycin, the inhibitory effect of other macrolide drugs on erythromycin uptake by TR-TBT 18d-1 cells was investigated. Josamycin was ineffective, whereas spiramycin, azithromycin, clarithromycin, and erythromycin significantly inhibited [14C]erythromycin uptake to approximately 25 to 40% of the control respectively. These results indicate that a single transporter is involved in [14C]erythromycin uptake.

Response to pH Gradient of Erythromycin Transport in Syncytiotrophoblasts. To investigate the response to extracellular pH, [14C]erythromycin uptake was measured in medium at various pH values in the range of 6.4 to 8.4. Compared with the uptake at pH 6.4, saturable uptake was gradually increased at higher pH, suggesting that the saturable transport of [14C]erythromycin is enhanced by an outwardly directed proton gradient (Fig. 3A). To clarify the effect of such a gradient on erythromycin transport in syncytiotrophoblasts, [14C]erythromycin transport by BBMVs was measured in the presence and absence of an outwardly directed proton gradient. Significant overshoot was observed only in the presence of the proton gradient, indicating that [14C]erythromycin is countertransported with protons (Fig. 3B).

FIG. 2. Michaelis-Menten type kinetics of erythromycin uptake by TR-TBT 18d-1 cells. A, initial uptake of [14C]erythromycin (10 \( \mu \)M) by TR-TBT 18d-1 cells was measured in the presence of various concentrations of unlabeled erythromycin up to 5 mM. The fitting curve to the Michaelis-Menten equation is shown as a solid line, and nonsaturable uptake estimated from the fitting is shown as a dotted line. B, saturable [14C]erythromycin uptake (○) was calculated by subtracting nonsaturable uptake from total uptake (●); an Eadie-Hofstee plot is shown. Fitted saturable uptake is indicated by a dashed line. Data represent the mean ± S.E.M. of four determinations.

FIG. 3. pH dependence of [14C]erythromycin uptake by TR-TBT 18d-1 cells and rat placental brush-border membrane. A, initial uptake of [14C]erythromycin (10 \( \mu \)M) by TR-TBT 18d-1 cells was measured in the uptake buffer adjusted to pH 6.4 to 8.4 in the presence (○) and absence (●) of [14C]erythromycin. Data represent the mean ± S.E.M. of four determinations. * indicates significant difference from the corresponding control \( P < 0.05 \). B, [14C]erythromycin (10 \( \mu \)M) uptake by rat placental brush-border membrane vesicles was measured in the absence (○), pH 7.4/7.4 \( \pm \) 0.4 of an outwardly directed pH gradient. Data represent the mean ± S.E.M. of three determinations.
The cellular pathway transport (Fig. 5). Compared with [14C]inulin clearance, corresponding to clearance via the paracellular transport (Fig. 6A). Erythromycin itself did not inhibit [14C]erythromycin efflux, and probenecid or a V-type ATPase inhibitor, bafilomycin A1 (data not shown). Therefore, efflux of [14C]erythromycin was characterized at 1 min. [14C]Erythromycin efflux was not inhibited by representative ABC efflux transporter inhibitors (cyclosporin A, fumitremorgin, and probenecid) or a V-type ATPase inhibitor, bafilomycin A1 (Fig. 6A). Erythromycin itself did not inhibit [14C]erythromycin efflux but rather tended to increase the efflux, although this was not statistically significant. On the other hand, ethylisopropylamiloride, a specific inhibitor of NHEs, was significantly inhibitory (Fig. 6A).

(Fig. 4). These results suggest that the transport system has broad specificity for an erythromycin-like structure.

**Fetal-to-Maternal Transport of Erythromycin across Rat Placenta.** To address the physiological transport of erythromycin in rat placenta, fetal-to-maternal transport clearance was estimated by means of the single placental perfusion technique. [14C]Inulin showed minimal transport clearance, corresponding to clearance via the paracellular pathway transport (Fig. 5). Compared with [14C]inulin clearance, clearance of [14C]erythromycin was quite high and was saturated by the addition of 5 mM erythromycin (Fig. 5). These results suggest that a fetus-to-mother-oriented efflux transport system is involved in physiological transplacental transport of erythromycin.

**Erythromycin Efflux from TR-TBT 18d-1 Cells.** To address the efflux transport of erythromycin in TR-TBT 18d-1 cells, the effect of transporter inhibitors was investigated. TR-TBT 18d-1 cells were preincubated with [14C]erythromycin, and the time course of efflux was followed for 30 min. [14C]Erythromycin was 90% eliminated from the cells within 30 min, and 60% was eliminated within 1 min (data not shown). Therefore, efflux of [14C]erythromycin was characterized at 1 min. [14C]Erythromycin efflux was not inhibited by representative ABC efflux transporter inhibitors (cyclosporin A, fumitremorgin, and probenecid) or a V-type ATPase inhibitor, bafilomycin A1 (Fig. 6A). Erythromycin itself did not inhibit [14C]erythromycin efflux but rather tended to increase the efflux, although this was not statistically significant. On the other hand, ethylisopropylamiloride, a specific inhibitor of NHEs, was significantly inhibitory (Fig. 6A).

Discussion

P-gp and BCRP are expressed at the blood-placenta barrier, and consequently the transfer of their substrates, such as digoxin, saquinavir, paclitaxel, topotecan, and mitoxantrone, from mother to fetus is limited (Smit et al., 1999; Jonker et al., 2000; Kolwankar et al., 2005), although administration of these drugs in early pregnancy, i.e., before development the blood-placenta barrier, presents a high risk to the fetus. However, other drugs can be transported through the blood-placenta barrier and are transferred to the fetus. Thus, drug therapy for pregnant women is usually to be avoided. Our findings here indicate that the limited fetal transfer of erythromycin involves a proton antiport system cooperating with a sodium/proton exchanger(s). This proton antiport efflux system, which is distinct from ABC transporters as mentioned above, represents a novel mechanism restricting drug transfer at the blood-placenta barrier, especially in the brush-border membrane of syncytiotrophoblast. These findings imply that low-risk drug therapy for pregnant women may be possible using drugs that are transported by this system. Therefore, it is important to identify other substrates of this efflux system and to fully characterize the transport protein.

TR-TBT 18d-1 cells showed clear erythromycin uptake activity (Fig. 1). The concentrative cell-to-medium ratio indicated the involvement of uphill transport. Although membrane transporters driven by Na+ and Cl− gradients across the plasma membrane generally mediate such transport, the erythromycin transport system did not require a Na+ or Cl− gradient. Kinetic study revealed relatively low-affinity transport, compared with clinically and experimentally achievable

![Fig. 4. Inhibitory effect of macrolide drugs on [14C]erythromycin uptake by TR-TBT 18d-1 cells. Initial uptake of [14C]erythromycin (10 μM) by TR-TBT 18d-1 cells was measured in the uptake buffer containing an inhibitor and 1% DMSO. Inhibitor concentrations were 1 mM except for clarithromycin (0.4 mM). Data represent the mean ± S.E.M. of five determinations, and those are normalized by control uptake. * = significant difference from the corresponding control (P < 0.05).](image)

![Fig. 5. Fetal-to-maternal clearance of [14C]erythromycin in rat placenta. Fetal-to-maternal clearance of [14C]erythromycin (0.2 μM, closed column) was measured by rat placental perfusion for 30 min. [14C]Inulin (1.2 μM, open column) was used as a control of paracellular transport. Clearance was estimated in terms of disappearance from the perfusion medium. Data represent the mean ± S.E.M. of four to nine determinations. * = significant difference from the corresponding control (P < 0.05).](image)

![Fig. 6. Effect of drug transporter inhibitors on [14C]erythromycin efflux by TR-TBT 18d-1 cells and mRNA expression of NHEs. A, the initial efflux of [14C]erythromycin (10 μM) by TR-TBT 18d-1 cells was measured for 1 min in the uptake buffer containing 1% DMSO (pH 7.4) in the absence and presence of a designated inhibitor. Inhibitor concentrations are shown in the figure. Data represent the mean ± S.E.M. of four determinations. * = significant difference from the corresponding control (P < 0.05). B, mRNA expression of NHEs in rat placenta and TR-TBT 18d-1 cells was determined. Reaction mixture without reverse transcriptase, RT (−), was used as a negative control. EIPA, ethylisopropylamiloride; CsA, cyclosporin A; FTC, fumitremorgin C.](image)
plasma concentrations of erythromycin (Fig. 2), indicating that the transport system is likely to be involved in physiological placental disposition of erythromycin, at least in rats. The transport process appeared to involve a single transporter (Fig. 2B).

Known SLCs seem unlikely to be involved in placental erythromycin transport. OAT2 does transport erythromycin (Kobayashi et al., 2005a), but the reported affinity (K_m = 18.5 μM) is different from that found in the present study. In addition, OAT2 mRNA is only weakly expressed in the placenta (Leazer and Klaassen, 2003; Nishimura and Naito, 2005). Organic anion-transporting polypeptides 1A2 and 2B1 recognize erythromycin at least as an inhibitor, but the affinity was lower than that found in the present study (EC_50 > 1600 and 1100 μM, respectively) (Lan et al., 2009). It has also been reported that OSCP1 accepts erythromycin as a substrate (Izuno et al., 2007; Kobayashi et al., 2007). We investigated the expression of OSCP1 in rat placenta. Placenta isolated at gestational day 18 and TR-TBT 18d-1 cells, as well as the testis (positive control), showed protein expression of OSCP1, but placental BBMVs did not contain OSCP1 (data not shown). These results indicate that OSCP1 is expressed in the placenta but not in the brush-border membrane of rat syncytiotrophoblasts, in accordance with the subcellular localization in basolateral membrane of human syncytiotrophoblasts (Kobayashi et al., 2005b). Consequently, the contribution of these SLC transporters to erythromycin transport in brush-border membranes in syncytiotrophoblasts is considered to be negligible.

Erythromycin transport was significantly elevated under weakly alkaline conditions (Fig. 3A). This result can be interpreted in two ways: 1) the optimal pH is in the alkaline range or 2) the transport process is driven by an outwardly directed H^+ gradient. To examine these possibilities, we prepared rat placental BBMVs, because BBMVs are useful for analysis of the driving force of transporters. An outwardly directed H^+ gradient showed overshoot transport of erythromycin, indicating that an H^+ antiporter contributes to erythromycin uptake in rat syncytiotrophoblasts (Fig. 3B). An erythromycin/H^+ antiport system has previously been found only in bacteria, such as Staphylococcus aureus and Escherichia coli (Kodama et al., 1998; Zgurskaya and Nikaide, 1999).

It has been reported that OCTN1 shows organic cation/H^+ antiport function (Tamai et al., 2004). mRNA expression of OCTN1 in placenta is similar in rats and 4 times lower in humans compared with that in kidney, which shows the maximum expression (Leazer and Klaassen, 2003; Nishimura and Naito, 2005). OCTN1 generally transports compounds with lower molecular weights than erythromycin (mol. wt. 734), such as tetraethylammonium (mol. wt. 130) and ergothioneine (mol. wt. 229) (Tamai et al., 2004; Gründemann et al., 2005). However, we cannot exclude the involvement of OCTN1 in erythromycin transport in the placenta. MATE1 is also known to be an organic cation/H^+ antiporter (Otsuka et al., 2005). However, the mRNA expression of MATE1 is quite low in the placenta, at least in humans (Kobayashi et al., 2005b). It has also been reported that OSCP1 accepts erythromycin as a substrate (Izuno et al., 2007; Kobayashi et al., 2007). To conclude, we have found an H^+ gradient-driven erythromycin transport system in rat placenta. Although TR-TBT 18d-1 cells showed [14C]erythromycin uptake under weakly alkaline conditions, it has been supposed that [14C]erythromycin transfer from mother to fetus is restricted. Therefore, it is possible that the present transport system acts as a part of the blood-placenta barrier, mediating erythromycin efflux by using H^+ supplied by NHE1 or NHE3 as the driving force.

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