View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by CiteSeerX

DRUG METABOLISM AND DISPOSITION
Vol. 36, No. 10
Copyright © 2008 by The American Society for Pharmacology and Experimental Therapeutics
21345/3389454
DMD 36:2080–2085, 2008
Printed in U.S.A.

Enhancement of Zidovudine Uptake by Dehydroepiandrosterone Sulfate in Rat Syncytiotrophoblast Cell Line TR-TBT 18d-1

Tomohiro Nishimura, Yoshiaki Seki, Kazuko Sato, Takuya Chishu, Noriko Kose, Tetsuya Terasaki, Young-Sook Kang, Yoshimichi Sai, and Emi Nakashima

Faculty of Pharmacy, Keio University, Tokyo, Japan (T.N., Y.Se., K.S., T.C., N.K., Y.Sa., E.N.); Division of Membrane Transport and Drug Targeting, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan (T.T.); Solution-Oriented Research for Science and Technology, Japan Science and Technology Agency, Kawaguchi, Japan (T.T.); and College of Pharmacy, Sookmyung Women's University, Seoul, Korea (Y.-S.K.)

Received March 4, 2008; accepted July 22, 2008

ABSTRACT:

AZT (3′-azido-3′-deoxythymidine; zidovudine), which is used for the prevention of mother-to-child transmission of HIV-1, is transplacentally transferred to the fetus across the blood-placenta barrier, which is composed of syncytiotrophoblasts. We recently showed that apical uptake of AZT by syncytiotrophoblasts is mediated by saturable transport system(s) in the TR-TBT 18d-1 cell line, and the cellular accumulation of AZT was increased in the presence of dehydroepiandrosterone sulfate (DHEAS). Here, we aimed to clarify the mechanism of this effect of DHEAS. Inhibitors of efflux transporters, including breast cancer resistance protein, P-glycoprotein, and multidrug resistance proteins, had little effect on the cellular accumulation of AZT in TR-TBT 18d-1. Kinetic study revealed that the rate constant for AZT uptake was greatly increased in the presence of 1 mM DHEAS. These results suggested that the effect of DHEAS was because of enhancement of the uptake process(es), rather than inhibition of efflux. When AZT uptake was analyzed according to the Michaelis-Menten equation, the estimated Michaelis constant, K_m, for AZT uptake in the presence of 1 mM DHEAS was lower than that in its absence, whereas maximum uptake velocity, V_{max}, and nonsaturable uptake clearance, k_{ns}, were similar in the presence and absence of DHEAS, indicating that DHEAS may change the recognition characteristics of the transporter for AZT in TR-TBT 18d-1. Thus, the increase of AZT uptake in TR-TBT 18d-1 cells in the presence of DHEAS was concluded to be because of a DHEAS-induced change in the affinity of AZT uptake system, although the transporter responsible for AZT uptake has not been identified.

Transport of nutrients and xenobiotics across the placenta is regulated by the blood-placenta barrier, which is composed of syncytiotrophoblast cells. Many transporters are expressed in various tissues and play important roles in absorption, distribution, and excretion of their substrates. For example, transport of nucleosides is primarily mediated by members of the concentrative nucleoside transporter family (CNTs, SLC28) and the equilibrative nucleoside transporter family (ENTs, SLC29). TR-TBT 18d-1 cells, established by our group, are derived from the placenta of a rat at gestational age 18 days and have a similar transporter expression pattern to syncytiotrophoblast I cells (Kitano et al., 2002, 2004). We recently showed that apical uptake of uridine and adenosine is mediated by ENT1 and ENT2 in TR-TBT 18d-1 cells (Chishu et al., 2008). Therefore, transfer of nucleosides between mother and fetus should be partly regulated by these transporters in the placenta, at least in the late stage of pregnancy.

AZT (zidovudine) and ddI (didanosine) are nucleoside reverse transcriptase inhibitors (NRTIs) and are used to treat pregnant women infected with HIV-1 to prevent mother-to-child transmission. Although it was reported that thymidine shares a transporter with adenosine in the placenta, it has been believed that AZT, a thymidine analog, passively diffuses across the placenta (Dancis et al., 1993). Some influx transporters, such as CNTs (Yao et al., 1996; Ritzel et al., 2001), ENTs (Yao et al., 2001), organic anion transporters (Wada et al., 2000; Morita et al., 2001; Takeda et al., 2002; Hasegawa et al., 2003), organic cation transporters (Chen and Nelson, 2000), and OATPs (Takeuchi et al., 2001), can take up AZT, and several efflux transporters, such as P-gp (Antonelli et al., 1992), MRP4 (Schuetz et al., 1999), and BCRP (Wang et al., 2004; Pan et al., 2007), could also transport it. Although it is known that some of these transporters are expressed in human and rat placenta, their contribution to AZT

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, by a grant from the "High-Tech Research Center" and "Open Research Center" Project for Private Universities matching fund subsidy, by the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan, and by a grant from the Joint Research Project under the Japan-Korea Basic Scientific Cooperation Program of the Japan Society for the Promotion of Science and Korea Science and Engineering Foundation.

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.

doi:10.1124/dmd.108.021345.

ABBREVIATIONS: CNT, concentrative nucleoside transporter; SLC, solute carrier; ENT, equilibrative nucleoside transporter; AZT, 3′-azido-3′-deoxythymidine (zidovudine); ddI, 2′,3′-dideoxyinosine (didanosine); NRTI, nucleoside reverse transcriptase inhibitor; OATP, organic anion-transporting polypeptide; P-gp, P-glycoprotein; MRP, multidrug resistance protein; BCRP, breast cancer resistance protein; DHEAS, dehydroepiandrosterone sulfate; CsA, cyclosporin A; FTC, fumitremorgin C.

transport has hardly been examined. Recently, though, we indicated at least that apical uptake of AZT in TR-TBT 18d-1 cells was transporter-mediated, although the transport could not be explained by the contributions of identified transporters (Sai et al., 2008). In the case of ddI, apical uptake by TR-TBT 18d-1 cells was sensitive to NBMPR, a well known inhibitor of ENT family members, but kinetic analysis implied that the transport characteristics were not consistent with those of ENT2, indicating that the transporter responsible for ddI uptake was an ENT2-like transporter (unpublished data). Therefore, transporters contribute to the disposition of these NRTIs.

In the previous study, we found that AZT uptake by TR-TBT 18d-1 cells was enhanced by addition of 1 mM DHEAS (Sai et al., 2008). Possible explanations for the increased cellular AZT accumulation might be: 1) DHEAS inhibited the efflux pump responsible for AZT excretion from the cells or 2) DHEAS facilitated the uptake process of AZT by altering the membrane fluidity, transporter expression level, or affinity of the transporter for AZT. In the present study, we aimed to clarify the mechanism of enhancement of AZT accumulation by DHEAS in TR-TBT 18d-1 cells. This is of interest because it may allow better placental targeting of AZT, resulting in an improvement of the pharmacological effect of AZT.

Materials and Methods

Chemicals. [^{3}H]AZT (0.47 TBq/mmol), [^{3}H]ddI (1.55 TBq/mmol), and [^{3}H]thymidine (2.53 TBq/mmol) were purchased from Moravek Biochemicals (Brea, CA). Cyclosporin A (CsA) and probenecid were purchased from Wako Pure Chemicals (Osaka, Japan). Fumitremorgin C (FTC) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were commercial products of analytical grade.

Cell Culture. TR-TBT 18d-1 cells were cultured in Dulbecco's modified Eagle's medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (SAFC Biosciences, Lenexa, KS), 100 U/ml benzylpenicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (Invitrogen, San Diego, CA) on 100-mm culture dishes (Corning Life Sciences, Acton, MA) in a humidified incubator at 33°C under an atmosphere of 5% CO_2 in air. For the uptake study, TR-TBT 18d-1 cells were seeded on four-well plates (Nalge Nunc International, Rockville, NY) coated with porcine skin collagen type I (Nitta Gelatin Canada Inc., Toronto, ON, Canada) at a density of 1×10^5 cells/well. After incubation for 3 days at 33°C, the cells were cultured at 37°C for a further 4 days.

Uptake Study. TR-TBT 18d-1 cells were washed twice with transport medium containing 122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgSO_4$, 0.4 mM K_2HPO_4, 10 mM D-glucose, and 10 mM Hepes adjusted to pH 7.4 with Tris. Sodium-free transport medium was prepared by replacing 122 mM NaCl and 25 mM $NaHCO_3$ with 122 mM *N*-methyl-D-glucamine and 25 mM $KHCO_3$. After a 10-min preincubation in the above medium, the transport medium was replaced with drug solution containing a radiolabeled compound with or without DHEAS (~5 mM) to initiate the uptake reaction. At the designated time (1, 2, 5, 15 min), the reaction was terminated by addition of ice-cold transport medium, and the cells were washed twice in the same medium. The cells were solubilized by incubation overnight in 500 μl of 0.1 M NaOH/1% Triton X-100 solution at 37°C. Next, 400 μl of the cell lysate was mixed with 3 ml of scintillation cocktail (Clearsol-I; Nacalai Tesque, Kyoto, Japan), and radioactivity was measured with a liquid scintillation counter (TRI-CARB 317TR/SL; PerkinElmer Life and Analytical Sciences, Waltham, MA). Cellular protein was quantified by BCA method using a protein assay kit (Pierce Chemical, Rockford, IL) with bovine serum albumin as a standard.

Data Analysis. The following simple model was designed to describe the kinetics of cellular uptake of AZT.

$$\frac{dX(t)}{dt} = -k_{uptake} \times X(t) + k_{efflux} \times X_{cell}(t) \tag{1}$$

$$\frac{dX_{cell}(t)}{dt} = k_{uptake} \times X(t) - k_{efflux} \times X_{cell}(t) \tag{2}$$

where X(t), X_{cell}(t), k_{uptake}, and k_{efflux} indicate the drug amount in transport medium as a function of time, the drug amount in cells as a function of time, the first order rate constant for drug transport from medium to cells and the first order rate constant for drug transport from cells to medium, respectively. Initial values of X(t) and X_{cell}(t) are assumed to be D and A, respectively, where the sum of D and A is equal to the dose experimentally added. Because a part of the drug is expected to be immediately adsorbed on the cells, A represents the background adsorption of the drug, that is, $X_{cell}(t = 0)$ is A. Then, the following simultaneous differential equation gives X_{cell}(t).

$$X_{cell}(t) = \frac{k_{uptake} \cdot (A + D)}{k_{uptake} + k_{efflux}} \{1 - e^{-(k_{uptake}+k_{efflux}) \cdot t}\} + A \cdot e^{-(k_{uptake}+k_{efflux}) \cdot t} \tag{3}$$

After this equation is normalized by C, which is the concentration of drug in the solution at the end of uptake experiment, the time course of cell/medium ratio can be represented as follows:

$$\frac{X_{cell}(t)}{C(t)} = \left[\frac{k_{uptake} \cdot (A + D)}{k_{uptake} + k_{efflux}} \{1 - e^{-(k_{uptake}+k_{efflux}) \cdot t}\} + A \cdot e^{-(k_{uptake}+k_{efflux}) \cdot t}\right] \cdot \frac{V_o}{D} \tag{4}$$

Because C(t) can be approximated by a constant, and the drug amount in cells is quite low compared with that in the medium, C(t) can be written as D/V_o, where V_o is the volume of drug solution in the transport experiment. $A + D$ can be approximated to be D because A is generally much smaller than D. Consequently, we obtained the following equation, which was employed for kinetic analysis:

$$\frac{X_{cell}(t)}{C(t)} = \frac{k_{uptake} \cdot V_o}{k_{uptake} + k_{efflux}} \{1 - e^{-(k_{uptake}+k_{efflux}) \cdot t}\} + V_o' \cdot e^{-(k_{uptake}+k_{efflux}) \cdot t} \tag{5}$$

where the background adsorbed volume ($V_o \cdot A/D$) is represented as V_o'. Each parameter (k_{uptake}, k_{efflux}, V_o, V_o') was calculated from the experimental values [time series of X_{cell}(t) (disintegrations per minute per milligram of protein)/C(t) (disintegrations per minute per microliter)] by nonlinear least-squares regression analysis using MULTI software (Yamaoka et al., 1981).

To characterize the effect of DHEAS on [^{3}H]AZT uptake by TR-TBT 18d-1 cells, the uptake parameters were determined based on the Michelis-Menten equation by nonlinear least-squares regression analysis:

$$V = \frac{V_{max} \times S}{K_m + S} + k_{ns} \times S$$

where V, V_{max}, K_m, S, and k_{ns} represent initial uptake velocity, maximum uptake velocity, Michaelis constant, substrate concentration, and nonsaturable uptake clearance, respectively. Data showed that Michaelis-Menten equation of single saturable component fit better than other parameters (data not shown). Statistical analyses were performed by one-way analysis of variance with Dunnett's post-hoc test.

Results

Transporter Involvement in AZT Accumulation in TR-TBT 18d-1 Cells and Effect of DHEAS. The effects of several known inhibitors of efflux transporters P-gp, Bcrp1, and Mrp(s) on [^{3}H]AZT uptake were investigated to clarify the involvement of these transporters in AZT uptake. Compared with the enhancement by DHEAS, addition of CsA, FTC, or probenecid had little effect (Fig. 1). Thus, it is unlikely that the above efflux transporters are involved in the uptake of AZT. To examine whether the effect of DHEAS on apical uptake of AZT is enhancement of uptake rather than inhibition of efflux, the time course of [^{3}H]AZT uptake by TR-TBT 18d-1 cells was kinetically analyzed and compared with that of [^{3}H]ddI uptake. We have shown that AZT and ddI are taken up by TR-TBT 18d-1 cells through transporter-mediated system(s), although the transporter responsible has not been identified (Sai et al., 2008). [^{3}H]AZT uptake was increased by DHEAS in a time-dependent manner, whereas [^{3}H]ddI uptake was slightly decreased (Fig. 2). We developed a simple kinetic

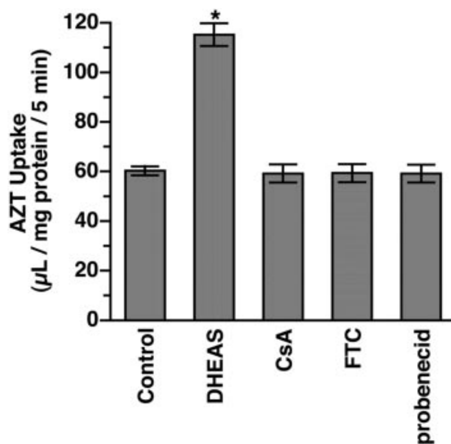

FIG. 1. Effect of efflux transporter inhibitors on [^{3}H]AZT uptake by TR-TBT 18d-1 cells. The effect of DHEAS (1 mM), CsA (10 μM), FTC (10 μM), or probenecid (5 mM) on [^{3}H]AZT (143 nM) uptake was measured at 37°C for 5 min. Each point represents the mean ± S.E.M. of four determinations. *, significant difference from the corresponding control ($p < 0.05$).

model for cellular drug uptake (see *Materials and Methods*), and the values of kinetic parameters of uptake and efflux were calculated by fitting the kinetic model into time-dependent uptake of [^{3}H]AZT and [^{3}H]ddI shown in Fig. 2. The first order rate constant for the uptake (k_{uptake}) of [^{3}H]AZT was increased by DHEAS, whereas that for efflux (k_{efflux}) was little affected (Table 1). On the other hand, k_{uptake} of ddI was decreased, and k_{efflux} was little affected (Table 1). In accordance, we hypothesized that DHEAS enhances the uptake process of [^{3}H]AZT in TR-TBT 18d-1 cells.

Kinetic Analysis for [^{3}H]AZT Uptake by Michaelis-Menten Equation. To clarify the nature of the enhancing effect of DHEAS on [^{3}H]AZT uptake by TR-TBT 18d-1 cells, initial uptake of [^{3}H]AZT was analyzed according to the Michaelis-Menten equation. [^{3}H]AZT uptake velocity in the presence of 1 mM DHEAS was significantly higher than that in its absence, especially at concentrations well below the K_{m} value (Fig. 3, A and B). The obtained parameters are listed in Table 2. The Eadie-Hofstee plot clearly showed that the K_{m} value was much smaller in the presence of DHEAS, whereas the V_{max} value was similar in the presence and absence of DHEAS (Fig. 3C). These results indicate that DHEAS might change the affinity of the transporter for AZT. Indeed, the intrinsic uptake clearance of AZT in TR-TBT 18d-1 cells was 2 times higher in the presence of DHEAS than in its absence.

Concentration Response of Enhancement Effect on AZT Uptake. Although [^{3}H]AZT uptake by TR-TBT 18d-1 is almost completely saturable, as shown previously (Sai et al., 2008), the enhanced uptake of [^{3}H]AZT in the presence of DHEAS should also be saturable if DHEAS changes the affinity between the influx transporter and AZT. Indeed, the enhanced uptake was almost completely inhibited by an excess of AZT (Fig. 4A). To further address the mechanism of the enhancement by DHEAS, we measured the concentration dependence of the effect of DHEAS on AZT uptake. [^{3}H]AZT uptake was increased with increasing concentration of DHEAS (Fig. 4B), whereas nonsaturable uptake was only slightly changed, suggesting that cytotoxicity could be excluded from the mechanism of uptake enhancement. Although the maximum enhancement could not be measured because of the limited solubility of DHEAS, AZT uptake was at least 2.5 times higher in the presence of 5 mM DHEAS than in its absence.

Effect of DHEAS on [^{3}H]Thymidine Uptake. Thymidine, an endogenous structural analog of AZT, may share the uptake transporter with AZT because an excess of thymidine could inhibit [^{3}H]AZT as much as AZT itself (Sai et al., 2008). Therefore, the effect of DHEAS on [^{3}H]thymidine uptake was measured. [^{3}H]Thymidine uptake was, however, decreased in the presence of 1 mM DHEAS, although most of the uptake was inhibited by the addition of an excess thymidine or AZT (Fig. 4C).

Discussion

The placenta is one of the pharmacological target tissues of NRTIs, including AZT. Transport of AZT across the blood-placenta barrier has been thought to occur by simple diffusion, but we recently showed that it is transporter-mediated, at least in TR-TBT 18d-1 cells (Sai et al., 2008). In that study, it was found that DHEAS stimulated cellular AZT accumulation, although the mechanism was not addressed. Here, we show that DHEAS enhances saturable influx transport of AZT by changing the affinity of the transporter for AZT.

TR-TBT 18d-1, a conditionally immortalized syncytiotrophoblast cell line established by our group, retains placental transport characteristics for nucleosides, including nucleobase- and nucleoside-like drugs. The mRNAs of several nucleoside transporters, such as CNTs (SLC28) and ENTs (SLC29), are expressed in TR-TBT 18d-1 cells (Kitano et al., 2004). Uptake of uridine and adenosine by TR-TBT 18d-1 could be mediated primarily through ENT1 and ENT2, which are NBMPR-sensitive transporters (Chishu et al., 2008). Although AZT is structurally similar to nucleoside, i.e., 3′-OH of thymidine is replaced with $-N_3$, ENT1, and ENT2 seem unlikely to be involved in AZT uptake by TR-TBT 18d-1 cells because NBMPR did not inhibit AZT uptake (Sai et al., 2008). Therefore, transporter(s) involved in the placental transport of nucleosides remain to be identified. Although DHEAS induced AZT uptake by TR-TBT 18d-1 cells, it inhibited uridine uptake (Chishu et al., 2008). DHEAS also slightly

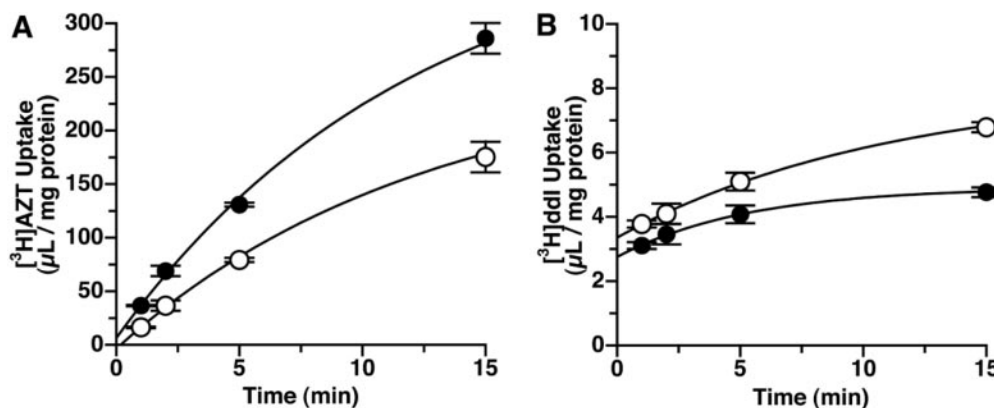

FIG. 2. Time course of radiolabeled NRTI uptake by TR-TBT 18d-1 cells in the presence of DHEAS. [^{3}H]AZT (143 nM) and [^{3}H]ddI (43.2 nM) uptake was measured at 37°C in the absence (○) and presence (●) of 1 mM DHEAS. Cellular uptake of [^{3}H]AZT and [^{3}H]ddI was represented by the cell/medium ratio, obtained by dividing the uptake amount by the drug concentration in the transport medium. The line was fitted using eq. 5, and the obtained parameters are shown in Table 1. Each point represents the mean ± S.E.M. of four determinations.

TABLE 1

Kinetics of cellular AZT and ddI accumulation in TR-TBT 18d-1 cells

Kinetic parameters were analyzed according to the model in *Materials and Methods*. Data are presented as means ± S.D., estimated by nonlinear least-squares regression analysis using the MULTI program.

Parameter	AZT Uptake	DHEAS		ddI Uptake	DHEAS	
		(−)	(+)		(−)	(+)
k_{uptake} (10^{-2} min^{-1})		0.772 ± 0.114	1.54 ± 0.15		0.0264 ± 0.0057	0.0111 ± 0.0049
k_{efflux} (10^{-2} min^{-1})		6.38 ± 2.81	9.86 ± 2.46		16.0 ± 4.5	13.0 ± 7.7

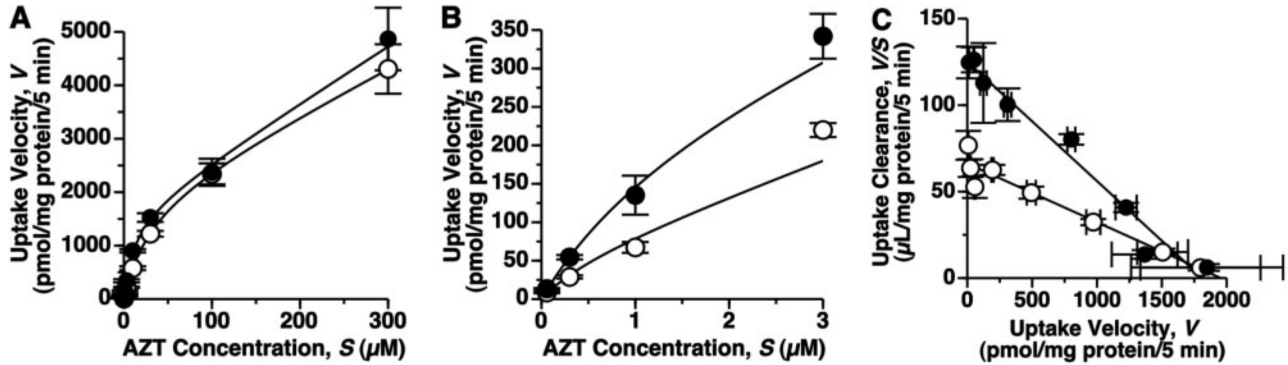

FIG. 3. Kinetic analysis of the effect of DHEAS on [^{3}H]AZT uptake. The concentration dependence of [^{3}H]AZT (143 nM) uptake with (●) or without (○) 1 mM DHEAS was measured at 37°C for 5 min in the presence of unlabeled AZT (0~300 μM). A, kinetic analysis was conducted by fitting to the Michaelis-Menten equation as described under *Materials and Methods*. Results obtained in the range of relatively low concentration are expanded in B. C, Eadie-Hofstee plot. Each point represents the mean ± S.E.M. of four determinations. Parameters fitted by nonlinear least-squares regression analysis are listed in Table 2.

inhibited ddI uptake, which is mediated by an unidentified ENT2-like protein (unpublished data). Thus, it appears that DHEAS can preferentially stimulate the transporter(s) responsible for AZT uptake.

When cellular drug accumulation is increased by drug-drug interaction, it is reasonable to consider that inhibition of efflux transport may be involved. To distinguish whether the enhancing effect of DHEAS on AZT accumulation is because of stimulation of influx transporter(s) or inhibition of efflux transporter(s), we first investigated the effect of DHEAS on AZT uptake by TR-TBT 18d-1 cells in comparison with that of representative efflux transporter inhibitors. However, various efflux transporter inhibitors, including BCRP, P-gp, and MRPs, had little effect on the cellular accumulation of AZT in TR-TBT 18d-1 cells, although DHEAS caused a significant increase of AZT uptake (Fig. 1), indicating that the increased accumulation of AZT by DHEAS is unlikely to be because of inhibition of the above efflux transport. These results rather suggest that DHEAS may enhance uptake of AZT by TR-TBT 18d-1 cells, although possible involvement of unidentified efflux transporter(s) cannot be excluded. Therefore, we analyzed the time course of AZT uptake by using the kinetic model described under *Materials and Methods* (Fig. 2). The

TABLE 2

Parameters of initial uptake of AZT by TR-TBT 18d-1 cells obtained by fitting to the Michaelis-Menten equation

K_m, V_{max}, and k_{ns} are transport affinity, maximum transport velocity, and nonsaturable transport clearance, respectively. V_{max}/K_m indicates intrinsic transport clearance. Data are presented as mean ± S.D. estimated by nonlinear least-squares regression analysis using the MULTI program.

Parameters	DHEAS	
	(−)	(+)
K_m (μM)	30.5 ± 8.5	13.9 ± 1.6
V_{max} (10^3 pmol/mg protein/5 min)	1.97 ± 0.56	1.75 ± 0.18
k_{ns} (μl/mg protein/5 min)	8.36 ± 2.51	10.1 ± 1.2
V_{max}/K_m (μl/mg protein/5 min)	64.6	126

first order rate constant for AZT uptake was greatly increased by the addition of DHEAS, although the first order rate constant for efflux was little changed (Table 1). In contrast, both of the rate constants for ddI tended to be rather decreased (Table 1). Taken together, these results indicate that DHEAS enhances the apical uptake process of AZT in TR-TBT 18d-1 cells.

Possible mechanisms underlying this observation include the following: 1) increased plasma membrane viscosity, 2) induction of uptake transporter expression on plasma membrane, and 3) enhancement of the transporter activity by changing the affinity for substrate(s). By analyzing the initial uptake of AZT in the presence and absence of DHEAS according to the Michaelis-Menten equation, we can distinguish among these mechanisms, based on the k_{ns}, V_{max}, and K_m values. The estimated K_m value for AZT uptake in the presence of DHEAS was lower than that in its absence, whereas the k_{ns} and V_{max} values were similar in the presence and absence of DHEAS (Table 2). These results indicate that 3) is more likely than 1) or 2). This is consistent with the report that DHEAS has little effect on the membrane fluidity in rat brain, although DHEA and its analogs did alter it (Morissette et al., 1999).

It has been reported that several steroids, such as estrone, can facilitate the uptake activity of OATP2B1 (Tamai et al., 2001; Grube et al., 2006). In addition, several steroid sulfates can facilitate the transport activity of MRP8 (ABCC11) (Chen et al., 2005). Thus, steroids and their conjugates appear to enhance the activity of certain types of drug transporters, although little is known about the mechanism involved. The mechanism may not be simple because, for instance, nonconjugated estrone inhibits estrone-3-sulfate uptake by OATP2B1 but enhances DHEAS uptake by OATP2B1 (Tamai et al., 2001; Grube et al., 2006). The results of the present study and Grube et al. showed that the enhanced uptake activity was caused by a change of the affinity of the transporter for its substrate, indicating that the steroids may directly or indirectly interact with the

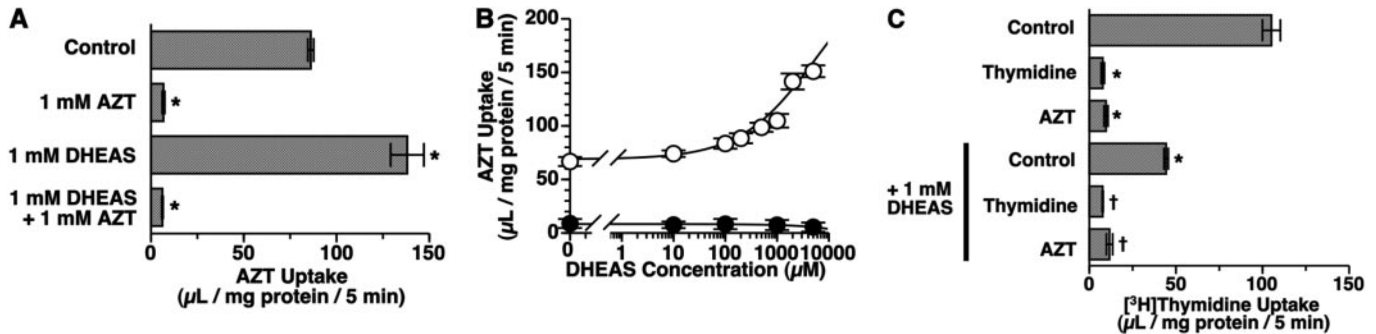

FIG. 4. Effect of DHEAS on saturable [^{3}H]AZT uptake by TR-TBT 18d-1 cells and the specificity compared with [^{3}H]thymidine uptake. A, [^{3}H]AZT (143 nM) uptake with or without 1 mM DHEAS was measured in the presence and absence of an excess of unlabeled AZT (1 mM). B, [^{3}H]AZT (143 nM) uptake was measured with designated concentration of DHEAS (0~5 mM) in the absence (○) and presence (●) of 1 mM AZT. C, [^{3}H]thymidine (57.2 nM) uptake with or without DHEAS was measured in the presence and absence of the unlabeled compounds (1 mM). Uptake of [^{3}H]AZT and [^{3}H]thymidine was performed at 37°C for 5 min. Each point represents the mean ± S.E.M. of four determinations.* and †, significant difference from the control in the absence and presence of 1 mM DHEAS, respectively ($p < 0.05$).

transporter at target region(s) other than the primary site of action of the protein (allosteric activation).

In pregnancy, DHEAS is synthesized in the adrenal gland of the fetus and is taken up into the placenta from fetal circulating blood. It has been suggested that OATP2B1 localized in the basolateral membrane of syncytiotrophoblast is involved in the uptake of DHEAS, and BCRP (ABCG2) localized in the apical membrane secretes it into the maternal blood (Grube et al., 2007). Actually, basolateral uptake of DHEAS was higher than apical uptake in TR-TBT 18d-1 cells (Kitano et al., 2004). In agreement with our findings, it was reported that DHEAS uptake from apical membrane is saturable in HRP-1 cells, although the molecular mechanism has not been clarified (Zhou et al., 2003). Therefore, although DHEAS is physiologically taken up from fetal blood in syncytiotrophoblast, it is possible that the transporter responsible for apical uptake of DHEAS is also involved directly or indirectly in AZT uptake.

The effect of DHEAS on the transport substrates AZT and thymidine was different, although their chemical structures are similar (Fig. 4). Apparently, these results suggest that the uptake mechanisms of AZT and thymidine are different. However, it is also possible that the effect of DHEAS on the activity of the transporter is different for different substrates (inhibitory or stimulatory). Therefore, we cannot say whether AZT and thymidine share a transport system, even though DHEAS relatively specifically enhances AZT uptake.

Although the concentration of DHEAS required to enhance AZT uptake in TR-TBT 18d-1 cells (Fig. 4B) is relatively high compared with the physiological plasma concentration (<10 μM), our findings suggest that AZT uptake by the placenta could be increased by the simultaneous use of DHEAS or drugs that have similar characteristics for DHEAS to improve the therapeutic effect on mother-to-child transmission of HIV-1 or to reduce the oral dose without decreasing the therapeutic effect. Because protein biding of DHEAS is 70 to 80% in the millimolar range and more than 90% in physiological concentration range (Plager, 1965), we could expect only slight enhancement of AZT uptake in physiological condition. Further studies are required to clarify whether increased AZT uptake can improve the pharmacological effect and whether AZT uptake is enhanced by DHEAS in vivo.

In conclusion, our findings show that the saturable AZT uptake in TR-TBT 18d-1 that we previously observed was enhanced by DHEAS through a change in the affinity of the uptake system for AZT. Although the transport molecule responsible for AZT uptake has not yet been identified, it might be possible to apply our results to improve delivery of AZT into the placenta in the clinical context.

References

Antonelli G, Turriziani O, Cianfriglia M, Riva E, Dong G, Fattorossi A, and Dianzani F (1992) Resistance of HIV-1 to AZT might also involve the cellular expression of multidrug resistance P-glycoprotein. *AIDS Res Hum Retroviruses* **8:**1839–1844.

Chen R and Nelson JA (2000) Role of organic cation transporters in the renal secretion of nucleosides. *Biochem Pharmacol* **60:**215–219.

Chen ZS, Guo Y, Belinsky MG, Kotova E, and Kruh GD (2005) Transport of bile acids, sulfated steroids, estradiol 17-beta-D-glucuronide, and leukotriene C4 by human multidrug resistance protein 8 (ABCC11). *Mol Pharmacol* **67:**545–557.

Chishu T, Sai Y, Nishimura T, Sato K, Kose N, and Nakashima E (2008) Potential of various drugs to inhibit nucleoside uptake in rat syncytiotrophoblast cell line, TR-TBT 18d-1. *Placenta* **29:**461–467.

Dancis J, Lee J, Mendoza S, and Liebes L (1993) Nucleoside transport by perfused human placenta. *Placenta* **14:**547–554.

Grube M, Köck K, Karner S, Reuther S, Ritter CA, Jedlitschky G, and Kroemer HK (2006) Modification of OATP2B1-mediated transport by steroid hormones. *Mol Pharmacol* **70:**1735–1741.

Grube M, Reuther S, Meyer Zu Schwabedissen H, Köck K, Draber K, Ritter CA, Fusch C, Jedlitschky G, and Kroemer HK (2007) Organic anion transporting polypeptide 2B1 and breast cancer resistance protein interact in the transepithelial transport of steroid sulfates in human placenta. *Drug Metab Dispos* **35:**30–35.

Hasegawa M, Kusuhara H, Endou H, and Sugiyama Y (2003) Contribution of organic anion transporters to the renal uptake of anionic compounds and nucleoside derivatives in rat. *J Pharmacol Exp Ther* **305:**1087–1097.

Kitano T, Iizasa H, Hwang IW, Hirose Y, Morita T, Maeda T, and Nakashima E (2004) Conditionally immortalized syncytiotrophoblast cell lines as new tools for study of the blood-placenta barrier. *Biol Pharm Bull* **27:**753–759.

Kitano T, Iizasa H, Terasaki T, Asashima T, Matsunaga N, Utoguchi N, Watanabe Y, Obinata M, Ueda M, and Nakashima E (2002) Polarized glucose transporters and mRNA expression properties in newly developed rat syncytiotrophoblast cell lines, TR-TBTs. *J Cell Physiol* **193:**208–218.

Morissette M, Dicko A, Pézolet M, Callier S, and Di Paolo T (1999) Effect of dehydroepiandrosterone and its sulfate and fatty acid ester derivatives on rat brain membranes. *Steroids* **64:**796–803.

Morita N, Kusuhara H, Sekine T, Endou H, and Sugiyama Y (2001) Functional characterization of rat organic anion transporter 2 in LLC-PK1 cells. *J Pharmacol Exp Ther* **298:**1179–1184.

Pan G, Giri N, and Elmquist WF (2007) Abcg2/Bcrp1 mediates the polarized transport of antiretroviral nucleosides abacavir and zidovudine. *Drug Metab Dispos* **35:**1165–1173.

Plager JE (1965) The binding of androsterone sulfate, etiocholanolone sulfate, and dehydroisoandrosterone sulfate by human plasma protein. *J Clin Invest* **44:**1234–1239.

Ritzel MW, Ng AM, Yao SY, Graham K, Loewen SK, Smith KM, Ritzel RG, Mowles DA, Carpenter P, Chen XZ, et al. (2001) Molecular identification and characterization of novel human and mouse concentrative Na^+-nucleoside cotransporter proteins (hCNT3 and mCNT3) broadly selective for purine and pyrimidine nucleosides (system cib). *J Biol Chem* **276:**2914–2927.

Sai Y, Nishimura T, Shimpo S, Chishu T, Sato K, Kose N, Terasaki T, Mukai C, Kitagaki S, Miyakoshi N, et al. (2008) Characterization of the mechanism of zidovudine uptake by rat conditionally immortalized syncytiotrophoblast cell line TR-TBT. *Pharm Res* **25:**1647–1653.

Schuetz JD, Connelly MC, Sun D, Paibir SG, Flynn PM, Srinivas RV, Kumar A, and Fridland A (1999) MRP4: a previously unidentified factor in resistance to nucleoside-based antiviral drugs. *Nat Med* **5:**1048–1051.

Takeda M, Khamdang S, Narikawa S, Kimura H, Kobayashi Y, Yamamoto T, Cha SH, Sekine T, and Endou H (2002) Human organic anion transporters and human organic cation transporters mediate renal antiviral transport. *J Pharmacol Exp Ther* **300:**918–924.

Takeuchi A, Masuda S, Saito H, Abe T, and Inui K (2001) Multispecific substrate recognition of kidney-specific organic anion transporters OAT-K1 and OAT-K2. *J Pharmacol Exp Ther* **299:**261–267.

Tamai I, Nozawa T, Koshida M, Nezu J, Sai Y, and Tsuji A (2001) Functional characterization

of human organic anion transporting polypeptide B (OATP-B) in comparison with liver-specific OATP-C. *Pharm Res* **18:**1262–1269.

Wada S, Tsuda M, Sekine T, Cha SH, Kimura M, Kanai Y, and Endou H (2000) Rat multispecific organic anion transporter 1 (rOAT1) transports zidovudine, acyclovir, and other antiviral nucleoside analogs. *J Pharmacol Exp Ther* **294:**844–849.

Wang X, Nitanda T, Shi M, Okamoto M, Furukawa T, Sugimoto Y, Akiyama S, and Baba M (2004) Induction of cellular resistance to nucleoside reverse transcriptase inhibitors by the wild-type breast cancer resistance protein. *Biochem Pharmacol* **68:**1363–1370.

Yao SY, Cass CE, and Young JD (1996) Transport of the antiviral nucleoside analogs 3′-azido-3′-deoxythymidine and 2′,3′-dideoxycytidine by a recombinant nucleoside transporter (rCNT) expressed in *Xenopus laevis* oocytes. *Mol Pharmacol* **50:**388–393.

Yao SY, Ng AM, Sundaram M, Cass CE, Baldwin SA, and Young JD (2001) Transport of antiviral 3′-deoxy-nucleoside drugs by recombinant human and rat equilibrative, nitrobenzylthioinosine (NBMPR)-insensitive (ENT2) nucleoside transporter proteins produced in *Xenopus* oocytes. *Mol Membr Biol* **18:**161–167.

Yamaoka K, Tanigawara Y, Nakagawa T, and Uno T (1981) A pharmacokinetic analysis program (multi) for microcomputer. *J Pharmacodyn* **4:**879–885.

Zhou F, Tanaka K, Soares MJ, and You G (2003) Characterization of an organic anion transport system in a placental cell line. *Am J Physiol Endocrinol Metab* **285:**E1103–E1109.

Address correspondence to: Dr. Yoshimichi Sai, Faculty of Pharmacy, Keio University of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan. E-mail: sai-ys@pha.keio.ac.jp