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Mobility of nucleoside transporter of human erythrocytes differs greatly when loaded with different nucleosides

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Time courses of transmembrane equilibration of 2-chloroadenosine, 2′-deoxyadenosine, 3′-deoxyadenosine, cytidine and 2′-deoxycytidine were measured by rapid kinetic techniques in human erythrocytes under equilibrium exchange and zero-*trans* conditions. The kinetic parameters for transport were computed by fitting appropriate integrated rate equations to the data pooled for seven concentrations and compared to the kinetic parameters for uridine, adenosine, thymidine and formycin B transport determined previously for human erythrocytes under comparable experimental conditions. The transport of all nucleosides conformed to the simple carrier model and was directionally symmetric. The Michaelis-Menten constants for equilibrium exchange (K^{ee}) ranged from 22 μM for 2-chloroadenosine to about 4 mM for cytidine and the maximum velocities (V^{ee}) differed in a similar manner, so that the first-order rate constants (V^{ee}/K^{ee}) were similar for all nucleosides. The kinetic parameters for 2′-deoxyadenosine transport were similar to those for adenosine transport, whereas the lack of the 3′-OH group greatly reduced the affinity of 3′-deoxyadenosine (cordycepin) for the carrier. 2′, 3′-Dideoxynucleosides were transported < 1% as efficiently as 2′- and 3′-deoxynucleosides. Thus, the 2′- and 3′-OH groups play an important role in nucleoside transport. The mobility of the carrier when loaded with pyrimidine nucleosides (reflected by V^{ee}) was 5–10-times greater than that of the empty carrier, whereas the mobility of the adenosine-loaded or 2′-deoxyadenosine-loaded carrier was about equal to that of the empty carrier. Loading the carrier with 2-chloroadenosine or 3′-deoxyadenosine actually decreased its mobility. Thus, the differential mobility of the loaded and empty carrier differs greatly with the nucleoside substrate. The mobility of the loaded carrier as well as K^{ee} increased with a decrease in lipid solubility of the nucleoside substrate, but the relationship was complex.

Introduction

Mammalian cells in general possess a facilitated nucleoside transporter with broad substrate specificity [1–3]. Where investigated in detail, nucleoside transport has been found to be well-described by the simple carrier model and to exhibit directional symmetry [1–3]. In cultured mammalian cells, the mobility * of the carrier is the same whether or not it is loaded with a variety of nucleosides [1,2]. This is not the case for the carriers of human and pig erythrocytes [4–6]. At least when loaded with uridine or thymidine, the carriers move at 25 °C about 6-times more rapidly than when empty, and the differential mobility of loaded and empty carrier is increased to about 30-fold at 5 °C [5,7]. This differential mobility of substrate-loaded and empty carrier is most clearly recognized and quantified by a greater maximum velocity of equilibrium exchange than of zero-*trans* flux of a nucleoside [2,5]. It can also be reponsible for the *trans*-stimulation of the entry or exit of a radiolabeled nucleoside by an excess of the same or another nucleoside in unlabeled form on the *trans*-side of the membrane [1,8,9]. However, inhibition of the backflow of the radiolabeled nucleoside by the excess of unlabeled nucleoside on the *trans*-side may have an effect (generally referred to as 'countertransport'), which can resemble true *trans*-stimulation [1,4,10].

Detailed kinetic analyses of nucleoside transport in erythrocytes have been largely confined to uridine and thymidine. These nucleosides have been the primary substrates used, because they are not metabolized in these cells so that their transmembrane flux can be measured uncomplicated by metabolic conversions.

* Mobility is defined in kinetic terms [1,3] and connotes some macromolecular movement, if only a conformational shift.

Abbreviation: NBTI, nitrobenzylthioinosine.

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However, it has been observed that the presence of adenosine in the medium did not stimulate the efflux of uridine from preloaded human erythrocytes, and 2-chloroadenosine in the medium actually inhibited uridine efflux [9]. Similarly, a complete kinetic analysis of adenosine transport in ATP-depleted, deoxycoformycin-treated human erythrocytes indicated that the mobilities of the adenosine-loaded and empty carrier are about the same [1,2,11]. For ATP depletion, the cells were incubated in a medium containing 50 mM deoxyglucose and 5 mM KF for 4 h [12], but this treatment was probably not the cause of the different behavior of the nucleoside carrier when loaded with uridine or adenosine, since it did not seem to affect the differential mobility of uridine-loaded and empty carrier [12]. The results suggest, therefore, that the mobility of the nucleoside-loaded transporter may greatly depend on the nature of the nucleoside that is transported. This conclusion has been confirmed in the present study in which we have extended our detailed kinetic analysis of nucleoside transport in human erythrocytes to additional substrates, namely 2-chloroadenosine, 2′-deoxyadenosine, 3′-deoxyadenosine, 2′-deoxycytidine and cytidine. The data also illustrate the important role of the 2′- and 3′-OH groups in nucleoside transport.

Experimental procedures

Cells. Erythrocytes from freshly drawn human blood were kindly supplied by Dr. J. Kersey (Department of Pathology, University of Minnesota) as a by-product of lymphocyte isolation. The cells were thrice washed in cold saline containing 5 mM Tris-HCl (pH 7.4) (Tris-saline) and suspended in the same to $4 \cdot 10^8$ to $9 \cdot 10^8$ cells/ml, if not indicated otherwise.

Nucleoside transport measurements. Time courses of uptake of ^{3}H-labeled nucleosides were measured at 25° C using a dual syringe apparatus combined with manual sampling for longer time points (15 time points/time course) as described previously [1,4]. Transport was measured under zero-*trans* and equilibrium exchange conditions. For inward equilibrium exchange measurements, samples of a cell suspension were incubated with appropriate concentrations of unlabeled nucleoside at 37° C for 1 h and then the equilibrium exchange of radiolabeled nucleoside at the preloading concentration was measured. The mixing/sampling procedure involves separating the cells from the medium by rapid centrifugation through an oil layer and analyzing the cell pellet for radioactivity. Radioactivity/cell pellet was corrected for that attributable to trapping in extracellular space as estimated with [^{14}C]inulin [13]. Intracellular H_2O space was measured with ^3H_2O [13].

For the analysis of the kinetics of transport, inward equilibrium exchange and zero-*trans* influx were measured each at seven nucleoside concentrations in the same population of cells [4]. The concentration of radiolabeled nucleoside was kept constant in all samples, while the specific radioactivity was altered by addition of unlabeled nucleoside. The following equation was fitted to the pooled equilibrium exchange data

$$N_{2,t} = N_1\left[1-\exp\left(\frac{V^{ee}\cdot t}{K^{ee}+S}\right)\right] \quad (1)$$

where $N_{2,t}$ is the intracellular concentration of radiolabel at time t ($N_{2,0}=0$); N_1 is the extracelluar concentration of radioactivity; S is the chemical concentration of substrate; and K^{ee} and V^{ee} are the apparent Michaelis-Menten constant and maximum velocity, respectively. $N_{2,t}$ was calculated on the basis of the experimentally determined cell water volume or, in the case of 2-chloroadenosine, 2′-deoxyadenosine and 3′-deoxyadenosine on the basis of the apparent nucleoside accessible intracellular space [14]. The analysis yields best fitting parameters of K^{ee} and V^{ee}. Then the following equation was fitted to the pooled zero-*trans* data with R_{ee} ($=1/V^{ee}$) fixed at the value experimentally determined for the cell population being analyzed with R_{12} held equal to R_{12} (directional symmetry):

$$S_{2,t} = S_1\left[1-\exp\left(-\frac{t+(R_{21}+R_{ee}R_1/K)S_{2,t}}{KR_{00}+R_{12}S_1+R_{21}S_1+S_1^2R_{ee}/K}\right)\right] \quad (2)$$

where $S_{2,t}$ is the concentration of intracellular substrate at time t($S_{2,0}=0$); S_1 is the extracellular concentration of substrate (taken as a constant). The R terms are resistance factors, proportional to the round trip time of the carrier in four modes [1,3,15]; empty in both directions (R_{00}), loaded in both directions (R_{ee}), empty inward and substrate-loaded outward (R_{21}) and loaded inwards and empty outwards (R_{12}). The latter analysis yields best fitting parameters of K, the limit Michaelis-Menten constant, and $R_{12}=R_{21}$ ($=1/V^{zt}$, the maximum velocity of zero-*trans* entry and exit). The values permit calculation of the zero-*trans* entry Michaelis-Menten constant $K^{zt}=KR_{00}/R_{12}$ and of $R_{00}=R_{12}+R_{21}-R_{ee}$ [1,3,15], and consequently of the R_{00}/R_{ee} ratio, which quantifies the differential mobility of loaded and empty carrier [2,4]. Only when loaded and empty carrier mobilities are equal does $R_{ee}=R_{oo}$. R_{oo}/R_{ee} is also given by K^{ee}/K [1,15], two other fitted parameters.

Initial zero-*trans* entry (v^{zt}) and equilibrium exchange velocities (v^{ee}) were calculated for given substrate concentrations as the slopes of the curves described by Eqns. 2 and 1, respectively, for $t=0$ [1,4]. For measuring adenosine, 2′-deoxyadenosine and 3′-deoxyadenosine transport, the cells were pretreated with 20 or 25 μM deoxycoformycin and, where indicated, depleted of ATP by preincubation at 37° C for 3–4 h in Tris-saline containing 5 mM KF and 50 mM deoxyglucose [11,12].

For nucleoside exit measurements, undiluted suspensions of erythrocytes (about $5 \cdot 10^9$ cells/ml) were equilibrated with ^{3}H-labeled nucleoside. Samples of the suspension of preloaded cells were mixed with the dual syringe apparatus in short time intervals in a ratio of 1 : 7.3 (opposite to that in entry measurements) with Tris-saline devoid of nucleosides ('zero-*trans* exit'), Tris-saline containing the nucleoside used for preloading in unlabeled form at the preloading concentration ('outward equilibrium exchange').

Materials. [5-^{3}H]Uridine, [2,8-^{3}H]adenosine, 2'-deoxy[2,8-^{3}H]adenosine, 3'-deoxy[G-^{3}H]adenosine, 2-chloro[8-^{3}H]adenosine, [*methyl* – ^{3}H]thymidine, [5-^{3}H]cytidine, 2'-deoxy[5-^{3}H]cytidine, and 2', 3'-dideoxy[5'-^{3}H]cytidine were purchased from Moravek Biochemicals (Brea, CA). Their chemical purity was at least 95% as assessed by chromatographic analysis with three solvent systems and no significant contamination with ^3H_2O (volatile radioactivity) was detected. Unlabeled nucleosides were obtained from Sigma (St. Louis, MO), and ^3H_2O and [^{14}C]inulin from NEN (Boston, MA). Octanol partition coefficients (concentration in octanol/concentration in aqueous solution) of ^{3}H-labeled nucleosides were determined as described earlier (see Ref. 2).

Results and Discussion

Directional symmetry of the carrier

Directional symmetry of the nucleoside transporter of human erythrocytes has previously been demonstrated with uridine [4] and formycin B [14] as substrates. The data in Fig. 1 extend this conclusion to adenosine transport in deoxycoformycin-treated red blood cells. The initial velocities of zero-*trans* entry and exit of 2.5 mM adenosine were the same within experimental errors. At this high concentration, the rate of adenosine phosphorylation is insignificant relative to the rate of its transport [11], so that its transmembrane equilibration can be measured uncomplicated by metabolism. Furthermore, initial zero-*trans* and equilibrium exchange transport rates in either direction were about the same (Fig. 1A), which supports our earlier results obtained with ATP-depleted human erythrocytes [11] indicating about equal mobility of the empty and adenosine-loaded carrier. As indicated by the computer simulation in Fig. 1C, the differences in the time courses of transmembrane equilibration of 2.5 mM adenosine under zero-*trans* and equilibrium conditions (Fig. 1A) are predicted by the simple carrier model for a symmetrical transporter with equal mobility when empty and substrate-loaded and a Michaelis-Menten constant of 60 μM (see Table II). Only when the substrate concentration approaches K_m, do the equilibrium exchange and zero-*trans* time courses of uptake begin to coincide (Fig. 1B).

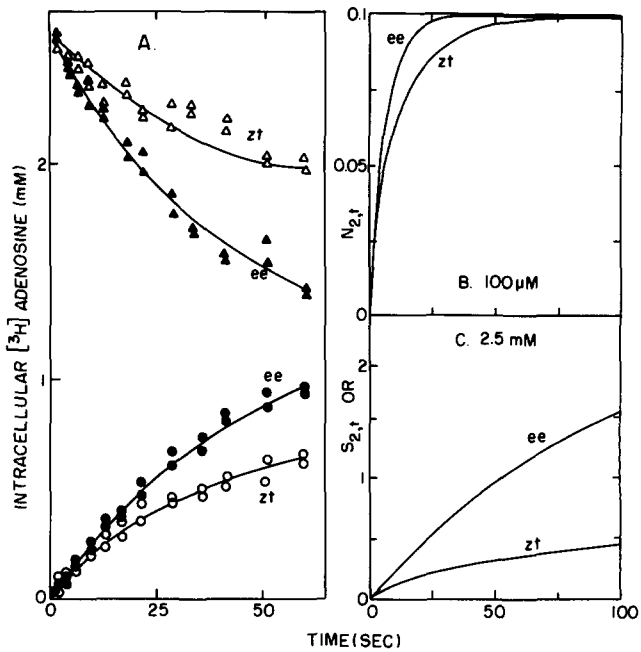

Fig. 1. Zero-*trans* entry and exit and inward and outward equilibrium exchange of adenosine in deoxycoformycin-treated human erythrocytes at 25° C (A). Time courses of zero-*trans* entry (○———○) and exit (△———△) and inward (●———●) and outward (▲———▲) equilibrium exchange of 2.5 mM [^{3}H]adenosine were measured in duplicate in samples of a suspension of $5 \cdot 10^8$ deoxycoformycin-treated red cells per ml by rapid kinetic techniques as described under Experimental procedures. (B and C) Simulated time courses of zero-*trans* entry (zt) and inward equilibrium exchange (ee) for a simple transporter with directional symmetry and equal mobility when substrate-loaded and empty. The progress curves for $S_{2,t}$ and $N_{2,t}$ were generated for the indicated substrate concentrations (S_1 and N_1) by numerical solutions of Eqns. 2 and 1, respectively, with $K = K^{ee} = 60$ μM, $V = V^{ee} = 25$ pmol/μl cell water per s.

Kinetic parameters for the transport of various nucleosides

We have extended our complete kinetic analysis of nucleoside transport in human erythrocytes to 2-chloroadenosine, 2'-deoxyadenosine, 3'-deoxyadenosine, cytidine and 2'-deoxycytidine. In each case, equilibrium exchange and zero-*trans* influx were measured by rapid kinetic techniques at seven concentrations in the same population of cells [4] and each substrate was assayed in at least two separately prepared cell populations. Representative time courses of equilibration of cytidine under zero-*trans* and equilibrium exchange conditions, which illustrate the general approach, are shown in Fig. 2. The transport of 2'- and 3'-deoxyadenosine was measured in deoxycoformycin-treated cells. Complete inhibition of deamination by deoxycoformycin was verified by chromatographic analysis of the culture fluid and cell extracts [16,17]. 2-Chloroadenosine is resistant to deamination, which was similarly verified experimentally (data not shown). For all nucleosides analyzed, metabolism was insignificant under the conditions of the transport assays (data not shown). Phosphorylation of 2-chloroadenosine, 2'-deoxyadenosine or 3'-deoxy-

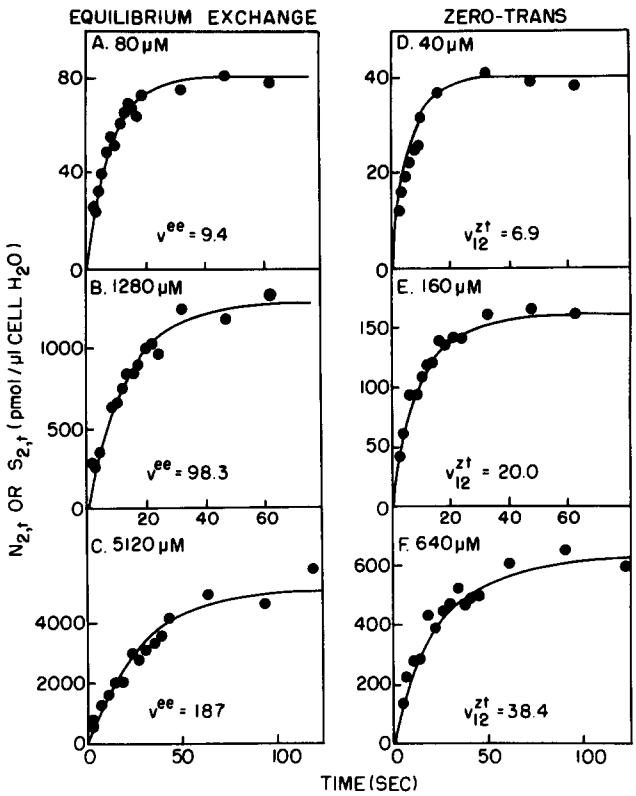

Fig. 2. Representative time courses of transmembrane equilibration of various concentrations of [^{3}H]cytidine by human erythrocytes under equilibrium exchange (frames A–C) and zero-*trans* (frames D–F) conditions at 25 °C. The experiment was conducted as described under Experimental procedures and the text. The initial velocities (v^{ee} and v^{zt}) were calculated from the fitted kinetic parameters for the given substrate concentrations as slopes of the theoretical curves at $t = 0$.

adenosine was insignificant over the time period required for transport assays whether or not the erythrocytes were ATP-depleted (data not shown).

In each experiment, the integrated rate equation for equilibrium exchange (Eqn. 1) was fitted to the equilibrium exchange data pooled for the seven concentrations. This yielded estimates of the Michaelis-Menten constant (K^{ee}) and maximum velocity (V^{ee}) for equilibrium exchange. Then Eqn. 2 was fitted to the pooled zero-*trans* data with R_{ee} ($= 1/V^{ee}$) fixed at the value determined for the population of cells under investigation and R_{12} and R_{21} held equal (as justified by the directional symmetry of the carrier). This analysis yielded estimates of K, the limit Michaelis-Menten constant, and $R_{12} = R_{21}$ from which R_{00} was calculated, as well as the R_{00}/R_{ee} ratio, which quantifies the differential mobility of loaded and empty carrier.

Complete sets of best fitting and calculated parameters for the transport of 2-chloroadenosine, 2′-deoxyadenosine and 3′-deoxyadenosine obtained in duplicate independent experiments are compared in Table I to illustrate the validity of the assays and the extent to which the transport kinetics of adenosine change due to modifications at the 2′- and 3′-positions and in the nucleobase. The kinetic parameters for 2′-deoxyadenosine transport were similar to those of adenosine transport determined previously (Ref. 11; see Table II), indicating that the lack of the OH group at the 2′-position had little effect on the affinity of the substrate for the carrier or the efficiency of its transport. Furthermore, the kinetic parameters for 2′-deoxyadenosine transport were about the same whether or not the

TABLE I

Kinetic parameters for the equilibrium exchange (ee) and zero-trans (zt) influx of various nucleosides in freshly isolated human erythrocytes at 25 °C

Nucleoside exchange and influx were measured each at seven concentrations in duplicate independent experiments (1 and 2). The data were subjected to integrated rate analysis as described under Experimental Procedures and the text. The best fitting parameters are listed ± S.E. of the estimate. The test concentration ranges were 5–320 μM for 2-chloroadenosine and 20–1280 μM for 2′-deoxyadenosine and 3′-deoxyadenosine. 2′-Deoxyadenosine and 3′-deoxyadenosine transport was measured in deoxycoformycin-treated cells. In one 2′-deoxyadenosine transport experiment (No. 2) the cells were also ATP-depleted. In all experiments the cell density fell between $4 \cdot 10^8$ and $6 \cdot 10^8$/ml and the radioactivity concentration between 150 and 350 cpm/μl, irrespective of nucleoside concentration.

Expt. Protocol	Kinetic parameter	Substrate					
		2-chloroadenosine		2′-deoxyadenosine		3′-deoxyadenosine	
		1	2	1	2	1	2
ee	K^{ee} (μM)	24.6 ± 3.6	21.9 ± 2.8	75.3 ± 17.8	111 ± 25	355 ± 54	323 ± 47
	V_{ee} (μM/s)	10.5 ± 0.35	10.6 ± 0.3	46.3 ± 2.2	21.9 ± 1.5	27.0 ± 2.2	23.0 ± 1.3
	V^{ee}/K^{ee} (s^{-1})	0.41	0.52	0.62	0.20	0.076	0.076
	R_{ee} (s/mM)	98	95	22	46	36	43
zt	$R_{12} = R_{21}$ (s/mM)	67 ± 1	81 ± 1	44 ± 1	59 ± 2	21 ± 1	29 ± 1
	K (μM)	4.3 ± 1.9	27 ± 0.7	18 ± 1.0	35 ± 1.9	2277 ± 1057	875 ± 55
	$K^{zt}_{12} = K^{zt}_{21}$ (μM)	22.8	22.6	27.4	42.4	638	428
	$V^{zt}_{12} = V^{zt}_{21}$ (μM/s)	14.8	12.0	22.7	17.1	47.8	34.9
	V^{zt}/K^{zt} (s^{-1})	0.61	0.46	0.83	0.40	0.075	0.081
	R_{00} (s/mM)	36	67	101	71	6	14
	R_{00}/R_{ee}	0.36	0.71	1.6	1.5	0.16	0.37

erythrocytes were ATP depleted (Expts. 2 and 1, respectively; Table I), indicating that the long-term incubation with deoxyglucose and KF required to deplete the cells of ATP for adenosine transport measurements [11] had no significant effect on the kinetics of transport.

In contrast, the lack of a OH group at the 3′-position greatly reduced the affinity of the 3′-deoxyadenosine for the carrier and, overall, greatly affected the kinetics of transport (Table I). In fact, the limit Michaelis-Menten constant (K) for 3′-deoxyadenosine transport was about 100-times higher than that for 2′-deoxyadenosine transport. Furthermore, as discussed in more detail below, the loading of the carrier with 2′-deoxyadenosine slightly increased the mobility of the carrier ($R_{00}/R_{ee} > 1$), whereas loading the carrier with 3′-deoxyadenosine or with 2-chloroadenosine [9] lowered its mobility ($R_{00}/R_{ee} < 1$). Modifications at the 3′-position have also been shown previously to lower the affinity of pyrimidine nucleosides for the human erythrocyte carrier [18]. The validity of the kinetic analyses presented in Table I is indicated by the equality, within experimental errors, of the V^{ee}/K^{ee} and V^{zt}/K^{zt} ratios in each experiment, which is required by the simple carrier model. Furthermore, comparable kinetic parameters were obtained for each substrate in duplicate independent experiments.

We have shown previously [19,20], that the lack of both the 2′- and 3′-OH groups further reduces the affinity of nucleosides for the carrier, but that in addition it decreases the efficiency of transport $\leq 1\%$ of that observed for 2′- or 3′-deoxynucleosides (see Fig. 3); equilibration of 2′,3′-dideoxycytidine across the plasma membrane requires > 60 min at 25 or 37 °C. The relatively high lipid solubility of 3′-deoxyadenosine and the consequently high rate of its non-mediated permeation precludes analysis of its facilitated transport [19]. On the other hand, dideoxycytidine has been shown to enter cells mainly via facilitated transport [20]. Influx

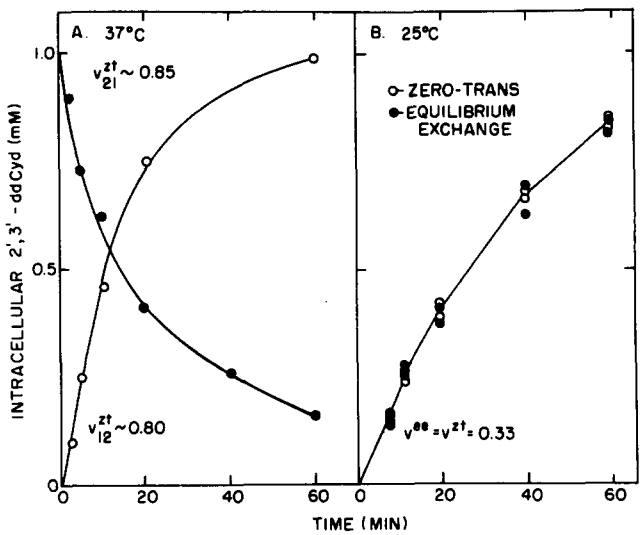

Fig. 3. Comparison of zero-*trans* (zt) influx and efflux (A) and of zero-*trans* influx and inward equilibrium exchange (ee) (B) of 2′, 3′-dideoxycytidine. In A, a suspension of $1 \cdot 10^9$ erythrocytes per ml was supplemented with 1 mM dideoxy[^{3}H]cytidine (0.17 cpm/pmol) and incubated at 37 °C. At the indicated times of incubation the cells from duplicate 0.5-samples of suspension were collected by centrifugation through an oil layer and analyzed for radioactivity (influx; Ref. 19). After 60 min of incubation, the remainder of cells were collected by centrifugation, suspended to the same cell density in fresh medium and at various times of incubation samples of cells were analyzed for radioactivity (efflux). All points are averages of the duplicate samples. In B, one sample of a suspension of $9 \cdot 10^8$ cells/ml was incubated with 1 mM unlabeled dideoxycytidine at 37 °C for 90 min, then equilibrated to 25 °C and supplemented with dideoxy[^{3}H]cytidine to 146 cpm/μl (equilibrium exchange). A second sample of the cell suspension was preincubated in the same manner without dideoxycytidine and then supplemented with 1 mM dideoxy[^{3}H]cytidine (146 cpm/μl; zero-*trans* influx). Both suspensions were incubated at 25 °C and monitored for cell-associated radioactivity as described above. Initial velocities of zero-*trans* entry (v_{12}^{zt}) and exit (v_{21}^{zt}) and equilibrium exchange (v^{ee}) were estimated from the initial linear portions of the progress curves and are expressed in pmol/μl cell water per s.

TABLE II

Summary of the kinetic parameters for the equilibrium exchange and zero-trans entry of various nucleosides in human erythrocytes at 25 °C.

The kinetic parameters listed for each nucleoside are averages of values obtained in duplicate independent experiments in the present study, reported previously in the case of adenosine [11] and formycin B [14] or a combination of these sources in the case of thymidine [4]. The values for 2′-deoxyadenosine transport are means from four independent experiments; detailed analyses of two of these are presented in Table I. The kinetic parameters for uridine transport are means obtained in 13 equilibrium exchange and four zero-*trans* experiments in the present and earlier studies [4 and 14]. Z_{oct} = octanol partition coefficient [2].

Substrate	K^{ee} (μM)	K (μM)	V^{ee}/K^{ee} (s^{-1})	V_{12}^{zt}/K_{12}^{zt} (s^{-1})	R_{ee} (s/mM)	R_{00} (s/mM)	R_{00}/R_{ee}	Z_{oct}
2-Chloroadenosine	22	35	0.46	0.53	96	51	0.53	0.510
Adenosine	60	61	0.32	0.28	35	38	1.1	0.123
2′-Deoxyadenosine	71	31	0.63	0.57	38	69	2.1	0.219
3′-Deoxyadenosine	339	1576	0.076	0.078	40	10	0.26	0.287
Formycin B	502	223	0.12	0.12	19	40	2.1	0.0307
Thymidine	338	51	0.43	0.55	8.1	42	5.2	0.0753
Uridine	574	69	0.37	0.32	8.0	48	6.0	0.0149
2′-Deoxycytidine	1580	58	0.21	0.59	4.0	32	8.0	0.0220
Cytidine	4039	72	0.12	0.30	2.3	31	11	0.0120

showed no sign of saturation up to a concentration of 1 mM dideoxycytidine, but transport had not been further characterized [20]. Fig. 3A shows that influx and efflux of 1 mM dideoxycytidine at 37 °C were about equally slow ($v_{12}^{zt} = v_{21}^{zt} = \approx 0.8$ pmol/μl cell water per s). Furthermore, the finding that time courses of inward equilibrium exchange and zero-*trans* entry of 1 mM dideoxycytidine were the same (Fig. 3B), indicates that the Michaelis-Menten constant for dideoxycytidine transport must be $\gg$ 1 mM (Refs. 1 and 2; see Figs. 1B and C).

The average critical kinetic parameters for all nucleosides analyzed in the present study are summarized in Table II and compared to those determined previously for adenosine, formycin B, thymidine, and uridine transport [4,11,14]. The kinetic parameters and the differential mobilities of nucleoside-loaded and empty carrier differed greatly for different nucleosides. The Michaelis-Menten constants for equilibrium exchange (K^{ee}) ranged from 22 μM for 2-chloroadenosine to 4000 μM for cytidine, whereas the values for the limit Michaelis-Menten constant (or for K^{zt}, not shown) generally fell into a much more narrow range. For 2-chloroadenosine transport K^{ee} was slightly lower than K and K^{zt} (Table I) and similar to the K^{zt} values reported previously for human erythrocytes [10] and guinea pig myocytes [21]. The 20-fold higher K^{ee} for cytidine than uridine transport suggests that a change from an hydroxyl to an amino group at the 4-position greatly lowers the affinity of pyrimidine nucleosides for the carrier. The opposite seems to be the case for purine nucleosides, since K^{ee} for the transport of adenosine with an amino group at a position equivalent to that in cytidine is lower than K^{ee} for the transport of inosine [1,22] and formycin B, a C-nucleoside analog of inosine [14]. On the other hand, as discussed already, the hydroxyl groups at the 2′- or 3′-position of ribose play a major role in the transport of both purine and pyrimidine nucleosides.

The resistance factors for the nucleoside-loaded carrier (R_{ee}) varied about 40 fold in an inverse manner as K^{ee} (Table II). This inverse relationship between K^{ee} and R_{ee} ($= 1/V^{ee}$) explains the finding that the first order rate constants for equilibrium exchange (V^{ee}/K^{ee}) and zero-*trans* flux (V^{zt}/K^{zt}) were similar for all nucleosides in spite of the 40-fold variation in maximum velocity (Table II). Thus, at concentrations $\ll K_m$, as for example at physiological concentrations below 10 μM, all nucleosides are transported by the carrier with comparable efficiency either in the equilibrium exchange or zero-*trans* mode.

The first-order rate constants for 3′-deoxyadenosine and formycin B transport were lower than those for the transport of the other nucleosides (Table II). The reason for this difference is unknown, since low values for 3′-deoxyadenosine and formycin transport were obtained in two independent experiments (Table I; Ref. 14). The mobility of the empty carrier (R_{00}) must be the same regardless of the nucleoside, whose transport is measured. Within experimental errors this was probably the case (Table II). Two factors may contribute to variability in observed R_{00} values. One reflects general experimental variation, the other may relate to the use of different populations of erythrocytes. Since the R_{00} value is calculated from transport velocities expressed on the basis of cell volume rather than per carrier molecule, which is an unknown value, some variations in R_{00} as well as in R_{ee} may reflect differences between the number of carriers per cell volume in populations of cells obtained from different individuals and/or at different times [4]. Again, however, the R_{00} value for 3′-deoxyadenosine transport was lower in two independent experiments than those observed for the transport of the other nucleosides (Tables I and II).

The marked differences in the mobility of the carrier when loaded with different nucleosides was reflected in similar differences in the R_{00}/R_{ee} ratio (Table II), which quantifies the differential mobility of loaded and empty carriers [1,4]. As reported earlier [11], the mobility of the adenosine-loaded and empty carrier is about the same. In contrast, loading the carrier with 2-chloroadenosine and 3′-deoxyadenosine markedly decreased its mobility, whereas the mobility of the pyrimidine nucleoside-loaded carrier exceeded that of the empty carrier 5–10-fold. In general, the mobility of the carrier when loaded with different nucleosides was inversely related to the lipid solubility of the transported nucleoside (estimated by its solubility in octanol) but the relationship differed for ribo- and deoxyribonucleosides (Table II) and did not seem to apply to 3′-deoxyadenosine.

The R_{00}/R_{ee} value is also given by the ratio of the Michaelis-Menten constant for equilibrium exchange/the limit Michaelis-Menten constant, two other fitted parameters ($R_{00}/R_{ee} = K^{ee}/K$; Ref. 1 and 15). The R_{00}/R_{ee} values given by this relationship were generally comparable to those derived from the fitted resistance factors listed in Table II, indicating internal consistency in these analyses. Exceptions were the values for cytidine and 2′-deoxycytidine transport. In these cases higher R_{00}/R_{ee} values were derived from K^{ee}/K than from R_{ee} and $R_{12} = R_{21}$. The discrepancies probably reflect inaccuracies in the estimation of the K^{ee} values, which fall in the upper range of the substrate concentrations that could be assayed, due to the limited solubility of these nucleosides. An overestimation of K^{ee} would also explain why V^{ee}/K^{ee} was lower than V_{12}^{zt}/K_{12}^{zt} for cytidine and deoxycytidine transport (Table II) contrary to the requirement of the simple carrier model that the first-order rate constants for equilibrium exchange and zero-*trans* flux are the same [1,15].

In summary, the mobility of the nucleoside trans-

porter of human erythrocytes differs greatly depending on the nucleoside it is loaded with. An increase in mobility correlates with a decrease in lipid solubility of the nucleoside and a decrease in affinity of the nucleoside for the carrier. Thus, at physiological concentrations all nucleosides are transported at comparable rates. Loading the carrier with some nucleosides, such as adenosine or 2′-deoxyadenosine, does not affect its mobility signficantly, whereas loading with some other nucleosides, such as 2-chloroadenosine or 3′-deoxyadenosine, actually lowers its mobility. Our data also yield new information on the function of the 2′- and 3′-OH groups in determining the affinity of nucleosides to the carrier and their translocation across the membrane. Our results also underscore the methodological point of Jarvis [9] that the property of *trans*-stimulation of uridine transport in human red cells in an inadequate criterion to decide whether or not a nucleoside is a substrate for this carrier. A more reliable criterion is the inhibition of the equilibrium exchange of nucleosides by each other at concentrations $> K^{ee}$.

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