A purine-selective nucleobase/nucleoside transporter in PK15NTD cells

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Hoque KM, Chen L, Leung GP, Tse C-M. A purine-selective nucleobase/nucleoside transporter in PK15NTD cells. Am J Physiol Regul Integr Comp Physiol 294: R1988–R1995, 2008. First published April 16, 2008; doi:10.1152/ajpregu.00016.2008.—Nucleoside and nucleobase transporters are important for salvage of purines and pyrimidines and for transport of their analog drugs into cells. However, the pathways for nucleobase translocation in mammalian cells are not well characterized. We identified an Na-independent purine-selective nucleobase/nucleoside transport system in the nucleoside transporter-deficient PK15NTD cells. This transport system has 1,000-fold higher affinity for nucleobases than nucleosides with $K_m$ values of 2.5 ± 0.7 μM for $[^3]$Hadenine, 6.4 ± 0.5 μM for $[^3]$Hguanine, 1.1 ± 0.1 mM for $[^3]$Hguanosine, and 4.2 ± 0.5 mM for $[^3]$Hdeoxyadenosine. The uptake of $[^3]$Hguanine (0.05 μM) was inhibited by other nucleobases and nucleobase analog drugs (at 0.5–1 mM in the order of potency): 6-mercaptopurine = thioguanine = guanine > adenine >> thymine = fluorouracil = uracil. Cytosine and methylcytosine had no effect. Nucleoside analog drugs with modification at 2’ and/or 5’ positions (all at 1 mM) were more potent than adenosine in competing the uptake of $[^3]$Hguanine: 2-chloro-2’-deoxyadenosine > 2-chloroadenosine > 2,3’-dideoxyadenosine = 2’,3’-dideoxyadenosine > 5-deoxyadenosine > adenosine. 2-Chloro-2’-deoxyadenosine and 2-chloroadenosine inhibited $[^3]$Hguanine uptake with $IC_{50}$ values of 68 ± 5 and 99 ± 10 μM, respectively. The nucleobase/nucleoside transporter was resistant to nitrobenzylthioinosine {6-[(4-nitrobenzyl)thiol]-9-β-D-ribofuranosylpurine} (NBMPR), dipyrindamole, and dilazep.

Little is known about the molecular basis of nucleobase transport in mammalian cells, and no functional mammalian nucleobase transporter has been cloned. Although the human and rat Na-dependent ascorbic transporters SVCT1 and SVCT2 have been defined as orthologs of bacterial nucleobase transporters in mammals, these proteins do not transport nucleobases (20, 23). On the other hand, of the cloned equilibrative nucleobase transporters, ENT2 is capable of transporting nucleobases and nucleosides, although the affinity for nucleobase transport of ENT2 has not been defined (4, 8, 24). The multifunctional transporter mouse ENT4/plasma membrane monoamine transporter (PMAT) is also found to transport adenine with a $K_m$ of 2.6 mM (2). However, its human ortholog lacks the adenine transport activity (2).

Our laboratory has previously generated nucleoside transporter-deficient (PK15NTD) cells (24). Although these cells are deficient in $[^3]$Huridine uptake, they exhibit a measurable amount of $[^3]$Hadenosine and $[^3]$Hguanosine uptake, which is resistant to inhibition by NBMPR and cannot be accounted for by simple diffusion of these nucleosides into cells. The present study is to characterize this residual $[^3]$Hadenosine and $[^3]$Hguanosine uptake pathway. We now demonstrate in these cells the presence of a purine-selective nucleobase/nucleoside transport system, which is resistant to ENT inhibitors but is inhibited by the organic cation transporter inhibitor decynium-22 and by papaverine.

METHODS

Cell culture. PK15NTD cells were derived from the porcine PK15 cells, which were obtained from American Type Culture Collection

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grown on 24-well culture plates. Media were changed every other day, simultaneously with [3H]nucleosides (10^3 M) solution. For the time course of [3H]nucleobase/nucleoside uptake, uptake) or 5 min ([3H]nucleoside uptake), and were rapidly washed three times with ice-cold PBS containing (in mM) 137 NaCl, 2.68 KCl, 3.33 NaH_2PO_4, 0.83 Na_2HPO_4, 1.0 CaCl_2, 1.0 MgCl_2, 10 glucose, and 5 HEPES (pH 7.4). Confluent monolayers of cells were washed three times in HEPES-buffered solution. For the time course of [3H]nucleobase/nucleoside uptake, HEPES-buffered solution containing [3H]adenosine (10 μM, 2 μCi/ml), [3H]guanosine (10 μM, 2 μCi/ml), [3H]adenine (0.1 μM, 0.5 μCi/ml), or [3H]guanine (0.1 μM, 0.5 μCi/ml) was added, and the plates were incubated for the time as indicated. When the effects of drugs, nucleosides, and nucleobases were studied, these regents were simultaneously added to the cells along with [3H]nucleobases/nucleosides. For concentration dependence of [3H]nucleobase/nucleoside uptake, HEPES-buffered solution containing varying concentrations of [3H]nucleobase/nucleoside (±30 μM decynium-22) was added. The plates were then incubated either for 2 min ([3H]nucleobase uptake) or 5 min ([3H]nucleoside uptake), and were rapidly washed three times with ice-cold PBS containing (in mM) 137 NaCl, 2.68 KCl, 1.47 KH_2PO_4, and 8.1 NaH_2PO_4 (pH 7.4). The cells were solubilized in 0.5 ml of 5% Triton X-100, and radioactivity was measured in a β-scintillation counter. The protein contents of representative monolayers were determined spectrophotometrically by means of a commercial BCA assay (Sigma). Briefly, reagent A (BCA solution) and reagent B (copper sulfate solution) were mixed in a proportion of 50:1. Then 0.9 ml of resulting solution was added to 0.1 ml of protein sample, and the resulting mixture was incubated at 60°C for 1 h. The samples were read at OD_{562nm}, and the protein concentration was determined from the standard curve (5 to 100 μg).

Data analysis. Nucleobase/nucleoside uptake data was expressed as means ± SE of at least three experiments performed in triplicate.

Concentration response curves were fitted with a logistic function curve, and IC_{50} values were determined. Apparent K_m and V_{max} values were calculated by regression analysis of velocity vs. velocity substrate (v vs. V/s) plots using Origin software. Student’s t-test and analysis of the variance were used for paired and multiple variants, respectively. P < 0.05 was considered as statistically significant.

Chemicals. Chemicals were purchased from Sigma-Aldrich, Fisher Scientific, or Invitrogen. Cell culture media and supplements were obtained from Invitrogen (Grand Island, NY), [3H]Nucleobases and [3H]nucleosides were purchased from Moravek Biochemicals (Brea, CA).

RESULTS

Uridine is a well-characterized substrate of the equilibrative nucleoside transporters ENT1 and ENT2. PK15NTD cells lack endogenous NBMPR-inhibitable uridine uptake, confirming the absence of ENT1 and ENT2 in these cells (24). However, these cells demonstrated uptake of the purine nucleosides (10 μM) [3H]guanosine and [3H]adenosine. There was no difference in the uptake of [3H]adenosine and [3H]guanosine in the presence or the absence of Na, and the uptake was linear for up to 10 min (data not shown) with a rate of 14.7 ± 0.8 pmol·mg^{-1}·min^{-1} (n = 20) and 3.5 ± 0.1 pmol·mg^{-1}·min^{-1} (n = 18), respectively. As shown in Fig. 1A, nonradioactive adenosine inhibited [3H]adenosine (10 μM) uptake in a concentration-dependent manner with 17 ± 6% inhibition at 1 mM and 50 ± 6% inhibition at 5 mM. Similarly, guanosine (1 mM) inhibited the [3H]adenosine uptake by 45 ± 5%. This uptake of [3H]adenosine was inhibited by 15% with 100 μM NBMPR, but not by 100 μM dipyridamole and dilazep, the concentration that is sufficient to completely inhibit ENT1 and ENT2.

Recently, the function of ENT4/PMAT has been reported (2, 10). ENT4/PMAT is a multifunctional transporter that accepts adenosine, monoamines, and organic cations as substrates and

![A](https://example.com/image1.png)

![B](https://example.com/image2.png)

**Fig. 1.** [3H]nucleobase uptake by porcine nucleoside transporter-deficient (PK15NTD) cells. A: [3H]adenosine was measured in the absence (control) or simultaneous addition of competing nucleosides and inhibitors of equilibrative nucleoside transporters (ENTS) and organic cation transporters (OCTs). Each value is the mean ± SE of 4 experiments. NBMPR, [6-[4-nitrobenzyl]thioli]-9-β-d-ribofuranosylpurine; GBR12935, dopamine transporter inhibitor. *P < 0.05, **P < 0.01, and ***P < 0.001, NS; not significant. B: inhibition of [3H]adenosine (○) and [3H]guanosine (●) uptake by decynium-22. Inhibitor was added simultaneously with [3H]nucleosides (10 μM, 5-min uptake). IC_{50} = 1.2 ± 0.2 μM and 1.4 ± 0.3 μM for [3H]adenosine and [3H]guanosine, respectively.
is inhibited by the organic cation transport inhibitor decynium-22 and by the dopamine transporter inhibitor GBR12935 (2, 9, 10, 27). Therefore, we tested whether adenosine uptake was inhibited by decynium-22 and GBR12935 (2, 9, 10). As shown in Fig. 1A, decynium-22 (10 μM) inhibited 90 ± 2% of [3H]adenosine uptake, while GBR12935 (100 μM) inhibited 50 ± 3% of [3H]adenosine uptake. Furthermore, decynium-22 inhibited [3H]adenosine and [3H]guanosine uptake at a concentration-dependent manner with similar IC50 values of 1.2 ± 0.2 μM and 1.4 ± 0.3 μM, respectively, (Fig. 1B).

The complete inhibition of [3H]adenosine and [3H]guanosine uptake by decynium-22 provided a convenient way to define the kinetic properties of the transport system. The concentration dependence of [3H]adenosine and [3H]guanosine uptake was measured in the absence and the presence of 30 μM decynium-22. As shown in Fig. 2, the decynium-22-sensitive [3H]adenosine and [3H]guanosine uptake was saturable and conformed to Michaelis-Menton kinetics. Kinetic parameters (apparent K_m and V_max) were calculated by the v vs. v/s plot (graph insets). The K_m and V_max values for [3H]adenosine uptake were 4.2 ± 0.5 mM and 2.379 ± 234 pmol·mg⁻¹·min⁻¹, respectively, and those for [3H]guanosine uptake were 1.1 ± 0.1 mM and 193 ± 6 pmol·mg⁻¹·min⁻¹, respectively.

The [3H]adenosine uptake was not inhibited by pyrimidine nucleosides: thymidine, cytidine, and uridine (all at 1 mM) (Fig. 3). Unexpectedly, the purine nucleobases adenine and guanine inhibited [3H]adenosine uptake. Adenine (1 mM) inhibited [3H]adenosine uptake by 98 ± 5%. Similarly, guanine (0.5 mM) inhibited [3H]adenosine uptake by 66 ± 2%. In contrast, the pyrimidine bases inhibited [3H]adenosine uptake with a rank order of potency thymine (1 mM, 31 ± 3%) > uracil (1 mM, 20 ± 2%) > cytosine (1 mM, 10 ± 5%), which did not significantly inhibit [3H]adenosine uptake.

The ability of the purine nucleobases to inhibit [3H]adenosine uptake suggested the presence of a novel purine nucleobase/nucleoside transporter in PK15NTD cells. To test this hypothesis, we measured the time course of uptake of [3H]adenine (0.1 μM) and [3H]guanine (0.1 μM) in the presence and the absence of decynium-22 (30 μM) (Fig. 4A). Decynium-22 inhibited [3H]adenine and [3H]guanine uptake at a concentration-dependent manner with similar IC50 values of 0.35 ± 0.05 μM and 0.58 ± 0.04 μM, respectively (Fig. 4B). The decynium-22-sensitive [3H]adenine and [3H]guanine uptake was saturable and conformed to Michaelis-Menton kinetics with K_m values of 2.5 ± 0.7 μM and 6.4 ± 0.5 μM, respectively, and V_max values of 184 ± 33 pmol·mg⁻¹·min⁻¹ and 260 ± 12 pmol·mg⁻¹·min⁻¹, respectively (Fig. 5).

The ability of nucleobases [1 mM except guanine (0.5 mM)], nucleosides (1 mM), monoamines (1 mM), and organic cations

![Fig. 2](http://ajpregu.physiology.org/)

**Fig. 2.** Kinetic analysis of decynium-22-sensitive [3H]adenosine (A) and [3H]guanosine (B) uptake of PK15NTD cells. Concentration dependence of adenosine and guanosine uptake was determined by measuring [3H]nucleoside uptake (± 30 μM decynium-22) for 5 min. Insets show the Eadie-Hofstee plots (v vs. v/s) that were used for determination of the apparent K_m and V_max values. The K_m and V_max values for [3H]adenosine were 4.2 ± 0.5 mM and 2.379 ± 234 pmol·mg⁻¹·min⁻¹, respectively, and those for [3H]guanosine were 1.1 ± 0.1 mM and 193 ± 6 pmol·mg⁻¹·min⁻¹, respectively. N = 4 cells.

![Fig. 3](http://ajpregu.physiology.org/)

**Fig. 3.** Inhibition of decynium-22-sensitive [3H]adenosine uptake (10 μM) of PK15NTD cells by nucleosides and nucleobases. Competing nucleosides and nucleobases were at 1 mM except guanine (0.5 mM). These nucleosides and nucleobases were added simultaneously with [3H]adenosine (10 μM, 2 μCi/ml, 5 min uptake). Each value is the mean ± SE of 4 experiments. *P < 0.05, **P < 0.01, and ***P < 0.001; NS, not significant.
(1 mM) to inhibit [3H]guanine uptake (0.05 μM) was tested. As shown in Fig. 6, the nonradioactive nucleobases inhibited [3H]guanine uptake with a rank order of potency guanine (98 ± 2%) > adenine (93 ± 3%) >> thymine (58 ± 2%) > uracil (42 ± 3%) >> cytosine (10 ± 4%), which did not significantly inhibit the [3H]guanine uptake. This order of potency strongly suggests the presence of a purine-selective, decynium-22-sensitive nucleobase/nucleoside transport system in PK15NTD cells. For nucleosides, only guanosine (43 ± 2%) and adenosine (29 ± 3%) significantly inhibited the [3H]guanine uptake, whereas inosine, thymidine, uridine, and cytidine had no effect. While the organic cations, 1-methyl-4-phenylpyridinium iodide (MPP), and TEA did not inhibit the [3H]guanine uptake, serotonin inhibited [3H]guanine uptake by 20 ± 4%. Papaverine is an inhibitor of nucleobase transport in erythrocytes (16). At 1 mM, it inhibited 83 ± 2% of [3H]guanine uptake.

To evaluate the ability of the nucleobase/nucleoside transporter in the uptake of nucleobase and nucleoside analog drugs, we tested the ability of these drugs in competing the uptake of [3H]guanine (0.05 μM). The guanine analogs, 6-mercaptopurine and thioguanine, inhibited 95%, while the uracil analog 5-fluorouracil inhibited 46 ± 6% and the cytosine analog, methylcytosine had no effect. While 1 mM adenosine inhibited 20% (Fig. 1), adenosine analog drugs with modification at 2' and/or 5 positions (all at 1 mM) were more potent than adenosine in competing the uptake of [3H]guanine: 2-chloro-2'-deoxyadenosine (> 95%) > 2-chloroadenosine (90 ± 1%) >> 2',3'-dideoxyadenosine (70 ± 3%) = 2'-deoxyadenosine (68 ± 4%) >> 5-deoxyadenosine (35 ±
drugs in inhibiting [3H]guanine uptake. As shown in Fig. 8, the transport of purine nucleobases/nucleosides was resistant to inhibition by NBMPR, dilazep, and dipyridamole. Second, adenosine, guanosine, and guanosine inhibited the uptake of each other (Figs. 3 and 6). Third, the IC50 values of decynium-22 inhibition of [3H]adenine (0.35 ± 0.05 μM), [3H]guanine (0.58 ± 0.04 μM), [3H]adenosine (1.2 ± 0.2 μM), and [3H]guanosine (1.4 ± 0.3 μM) were similar (Figs. 1B and 4B).

The nucleobase/nucleoside transport in PK15NTD cells had a high affinity (numerically low Km values) for purine nucleobases, [3H]adenine (2.5 ± 0.7 μM) and [3H]guanine (6.4 ± 0.5 μM), but a low affinity (numerically high Km values) for purine nucleosides, [3H]adenosine (4.2 ± 0.5 mM) and [3H]guanosine (1.1 ± 0.1 mM). Furthermore, the adenosine-deaminase-resistant nucleoside analog drugs 2-chloro-2'-deoxyadenosine and 2-chloroadenosine potently inhibited [3H]guanine uptake (Figs. 7 and 8), and this further supports that the transporter accepts both purine nucleobases and nucleosides. Wild-type

3% > adenosine. Because 2-chloro-2'-deoxyadenosine and 2-chloroadenosine potently inhibited the [3H]guanine uptake, we further determined the IC50 values of these nucleoside drugs in inhibiting [3H]guanine uptake. As shown in Fig. 8, 2-chloro-2'-deoxyadenosine and 2-chloroadenosine inhibited [3H]guanine uptake with IC50 values of 68 ± 5 μM and 99 ± 10 μM, respectively.

The plasma membrane adenosine and monoamine transporter ENT4 is stimulated by acidic pH. In contrast, the nucleobase/nucleoside transporter was not affected by the alkaline pH (pH = 8.0) but was inhibited by acidic pH (pH = 5.5) (Fig. 9).

PK15NTD cells are derived from porcine PK15 cells by mutagenesis with ethylmethanesulfonate (24). To eliminate the possibility that the decyanium-22-sensitive nucleobase/nucleoside transport resulted from modification of transporter functions by ethylmethanesulfonate in PK15 cells, we tested whether PK15 cells expressed the decyanium-22-sensitive nucleobase/nucleoside transport identical to that of PK15NTD cells. Since ENT1 is present in PK15 cells (24), the concentration dependence of decyanium-22-sensitive uptake of [3H]adenosine, [3H]guanosine, [3H]adenosine, and [3H]guanine was measured in the presence of 100 nM NBMPR, which completely inhibited the endogenous ENT1. Table 1 compares the apparent Km values for adenine, guanine, and adenosine of the wild-type PK15 and the PK15NTD cells.

**DISCUSSION**

Nucleosides and nucleobase transporters are important for salvage of purines and pyrimidines. Although the nucleoside transporting pathways have been defined and the proteins responsible for transport of nucleosides have been cloned (8), little is known about mammalian nucleobase transporters and nucleobase translocation pathways. In the present study, we identified a novel Na-independent purine-selective nucleobase/nucleoside transporter that is inhibited by papaverine and decyanium-22. Our results suggest that adenosine, guanosine, adenosine, and guanosine are transported by the same carrier. First, the transport of purine nucleobases/nucleosides was resistant to inhibition by NBMPR, dilazep, and dipyridamole. Second, adenosine, guanine, adenosine, and guanosine inhibited the uptake of each other (Figs. 3 and 6). Third, the IC50 values of decyanium-22 inhibition of [3H]adenine (0.35 ± 0.05 μM), [3H]guanine (0.58 ± 0.04 μM), [3H]adenosine (1.2 ± 0.2 μM), and [3H]guanosine (1.4 ± 0.3 μM) were similar (Figs. 1B and 4B).

The nucleobase/nucleoside transport in PK15NTD cells had a high affinity (numerically low Km values) for purine nucleobases, [3H]adenine (2.5 ± 0.7 μM) and [3H]guanine (6.4 ± 0.5 μM), but a low affinity (numerically high Km values) for purine nucleosides, [3H]adenosine (4.2 ± 0.5 mM) and [3H]guanosine (1.1 ± 0.1 mM). Furthermore, the adenosine-deaminase-resistant nucleoside analog drugs 2-chloro-2'-deoxyadenosine and 2-chloroadenosine potently inhibited [3H]guanine uptake (Figs. 7 and 8), and this further supports that the transporter accepts both purine nucleobases and nucleosides. Wild-type

![Fig. 6. Inhibition of decyanium-22-sensitive [3H]guanine uptake (0.05 μM) of PK15NTD cells by nucleobases, nucleosides, monoamines, organic cations, and papaverine. Competing nucleobases, nucleobases, monoamines, and organic cations were at 1 mM except guanine and papaverine, which were at 0.5 mM. The competing compounds were added simultaneously with [3H]guanine (0.05 μM, 0.5 μCi/ml, 2-min uptake). MPP, 1-methyl-4-phenyl-pyridinium iodide. Each value is the mean ± SE of 4 experiments. *P < 0.05, **P < 0.01, ***P < 0.001.](http://ajpregu.physiology.org/)

![Fig. 7. Inhibition of decyanium-22-sensitive [3H]guanine uptake (0.05 μM) of PK15NTD cells by nucleobase and nucleoside analog drugs. Competing nucleobase and nucleoside analog drugs were at 1 mM except 6-mercaptopurine (6-MP) and thioguanine (TG), which were at 0.5 mM. The competing compounds were added simultaneously with [3H]guanine (0.05 μM, 0.5 μCi/ml, 2-min uptake). Each value is the mean ± SE of 4 experiments. *P < 0.05, **P < 0.01, ***P < 0.001. Methyl-C, methylcytosine; 5 FU, 5-fluorouracil; 5-deoxyA, 5-deoxyadenosine; 2-deoxyA, 2-deoxyadenosine; 2'-deoxyA, 2'-deoxyadenosine; 2'-Cl-deoxyA, 2'- chloride-2-deoxyadenosine; 2'-Cl-2-deoxyA, 2'-chlorodenoxyadenosine; and 2'-Cl-2'-deoxyA, 2'-chloro-2'-deoxyadenosine.)
PK15 cells also exhibited decynium-22-sensitive uptake of adenine, guanine, and adenosine with similar affinities (Table 1), confirming that the decynium-22-sensitive nucleobase/nucleoside transporter is not due to the artifact of mutagenesis of the PK15 cells with ethylmethanesulfonate, which modifies DNA by alkylation (18, 27). When comparing these $K_m$ values of adenosine and guanosine uptake by the nucleobase/nucleoside transporter with those of ENT1 and ENT2, the $K_m$ value for adenosine of the nucleobase/nucleoside transporter is numerically two orders of magnitudes higher than that of ENT1 ($K_m = 0.04$ mM) and 30-fold higher than that of ENT2 ($K_m = 0.14$ mM) (8, 24). While the $K_m$ value for guanosine of the nucleobase/nucleoside transporter is 30-fold higher than that of ENT1 ($K_m = 0.14$ mM), it is threefold lower than that of ENT2 ($K_m = 2.7$ mM), suggesting that the nucleobase/nucleoside transporter has a higher affinity for guanosine than that of ENT2 and may play a more physiological relevant role than ENT2 in regulating the physiological functions of guanosine.

Purine-selective nucleobase transporters have also been described in mammalian erythrocytes, T-lymphoblast JPA2, CCRF-CEM, OK, primary human cardiac microvascular endothelial cells, and LLC-PK1 cells (1, 4, 12, 13, 19). Although none of these nucleobase transporters is shown to transport nucleosides, adenosine inhibits $[^3H]$hypoxanthine uptake in cardiac microvascular cells with a $K_i$ of 1.2 mM, suggesting that the nucleobase transporter in human cardiac microvascular cells might also transport adenosine with low affinity (4). The affinity constants ($K_m$ or $K_i$) of these nucleobase transporters are $13–30\times10^{-6}$ M for adenine, and $18–37\times10^{-6}$ M for guanine, which are five- to twelvefold and three- to fivefold, respectively, numerically higher than those of the nucleobase/nucleoside transporter described in the present study. Papaverine ($500\times10^{-6}$ M) inhibits the nucleobase/nucleoside transport in PK15NTD cells. It also inhibits the adenine uptake in erythrocytes and LLC-PK1 cells (13, 16), although it is not known whether papaverine inhibits the nucleobase transporter in JAP2, human cardiac microvascular cells and CCRF-CEM cells.

The nucleobase/nucleoside transporter shares many properties similar to the recently characterized multifunctional ENT4/PMAT (2, 9, 10). First, both the uptake of $[^3H]$adenosine by the

Table 1. Comparison of the apparent $K_m$ values of wild-type PK15 and PK15NTD cells for adenine, guanine, and adenosine

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<th>PK15 Cells</th>
<th>PK15NTD Cells</th>
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<tr>
<td>Adenine</td>
<td>$1.7\pm0.3$ μM</td>
<td>$2.6\pm0.6$ μM</td>
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<tr>
<td>Guanine</td>
<td>$7.3\pm0.9$ μM</td>
<td>$6.8\pm1.4$ μM</td>
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<tr>
<td>Adenosine</td>
<td>$5.7\pm0.7$ mM</td>
<td>$4.2\pm0.5$ mM</td>
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PK15NTD, procine nucleoside transporter-deficient cells.

ENT1 ($K_m = 0.14$ mM), it is threefold lower than that of ENT2 ($K_m = 2.7$ mM), suggesting that the nucleobase/nucleoside transporter has a higher affinity for guanosine than that of ENT2 and may play a more physiological relevant role than ENT2 in regulating the physiological functions of guanosine.

Purine-selective nucleobase transporters have also been described in mammalian erythrocytes, T-lymphoblast JPA2, CCRF-CEM, OK, primary human cardiac microvascular endothelial cells, and LLC-PK1 cells (1, 4, 12, 13, 19). Although none of these nucleobase transporters is shown to transport nucleosides, adenosine inhibits $[^3H]$hypoxanthine uptake in cardiac microvascular cells with a $K_i$ of 1.2 mM, suggesting that the nucleobase transporter in human cardiac microvascular cells might also transport adenosine with low affinity (4). The affinity constants ($K_m$ or $K_i$) of these nucleobase transporters are $13–30\times10^{-6}$ M for adenine, and $18–37\times10^{-6}$ M for guanine, which are five- to twelvefold and three- to fivefold, respectively, numerically higher than those of the nucleobase/nucleoside transporter described in the present study. Papaverine ($500\times10^{-6}$ M) inhibits the nucleobase/nucleoside transport in PK15NTD cells. It also inhibits the adenine uptake in erythrocytes and LLC-PK1 cells (13, 16), although it is not known whether papaverine inhibits the nucleobase transporter in JAP2, human cardiac microvascular cells and CCRF-CEM cells.

The nucleobase/nucleoside transporter shares many properties similar to the recently characterized multifunctional ENT4/PMAT (2, 9, 10). First, both the uptake of $[^3H]$adenosine by the
nucleobase/nucleoside transporter and by the cloned ENT4/PMAT are Na-independent and inhibited by decynium-22 and GBR12935 (2, 9, 10). Second, serotonin inhibits the uptake of [3H]adenosine by the nucleobase/nucleoside transporter and by the cloned ENT4/PMAT, suggesting that serotonin is a substrate of both transport systems (2, 9, 10). Third, adenine is a substrate of both mouse ENT4/PMAT and the nucleobase/nucleoside transporter (2). However, the nucleobase/nucleoside transporter has characteristics that are distinct from ENT4/PMAT: 1) the uptake of [3H]guanine, [3H]adenine, [3H]guanosine, and [3H]adenosine by the nucleobase/nucleoside transporter is inhibited by acidic pH (Fig. 9), while the uptake of [3H]adenosine by ENT4/PMAT is stimulated (2, 26); 2) mouse ENT4/PMAT mediates [3H]adenosine uptake with low affinity (a numerically high K_m value of 2.6 mM), whereas the nucleobase/nucleoside transporter mediates the uptake of [3H]adenosine with high affinity (a numerically low K_m value of 2.5 µM), a difference of 1,040-fold (2); 3) guanosine is a substrate of the nucleobase/nucleoside transporter but is not a substrate of ENT4/PMAT (9, 10); 4) the K_i for decynium-22 inhibition of MPP uptake by ENT4/PMAT is 0.1 µM, and that for the inhibition of adenosine by the nucleobase/nucleoside transporter is 1.2 µM, a 10-fold difference (9); 5) although GBR12935 inhibits both ENT4/PMAT and the nucleobase/nucleoside transporter, it inhibits ENT4/PMAT with a K_i of 7.9 µM, whereas 100 µM GBR12935 inhibits only 50% of the nucleobase/nucleoside transporter (Fig. 1A); 6) while the K_m for MPP of ENT4 is 33 µM, MPP (1 mM) has no effect on the [3H]guanine uptake (Fig. 6). These differences in pharmacological and functional properties between the nucleobase/nucleoside transporter and ENT4/PMAT suggest that these transporters are not identical. Unfortunately, porcine ENT4/PMAT has not been identified even though the pig genome-wide sequencing project is currently ongoing. We have attempted, but failed to use degenerate primers to amplify ENT4/PMAT mRNA from PK15NTD cells (data not shown). Therefore, it is unclear whether ENT4/PMAT is expressed in PK15NTD cells.

A nucleoside-sensitive organic cation transporter, which is Na-independent, pH-independent, and inhibited by decynium-22 (K_i = 0.01 µM) has been described in OK cells (7). It is believed that this nucleoside-sensitive organic cation transporter mediates secretion of deoxytubercidine from the kidney. The molecular identity of this nucleoside-sensitive organic cation transporter is not known or consistent with OCT1 or OCT2 (5, 6). Whether this organic cation transporter is a prototype of ENT4/PMAT is not conclusive (26). On the other hand, although the IC_{50} value of adenosine for inhibiting the TEA efflux by OK cells is 3 mM, which is similar to the K_m value of the nucleobase/nucleoside transporter of the PK15NTD cells, the nucleoside-sensitive organic cation transport is inhibited by NBMPR with an IC_{50} value of 25 µM (7). This NBMPR sensitivity does not fit the nucleobase/nucleoside transporter. It is therefore unlikely that the porcine nucleobase/nucleoside transporter is the same as the nucleoside-sensitive organic cation transporter in OK cells.

**Perspective and Significance**

In the present study, we demonstrate a mammalian purine nucleobase/nucleoside transporter in PK15NTD cells with high affinity for nucleobases and low affinity for nucleosides. This transporter is potentially important in salvage of purines and in the regulation of the local concentrations of purines in the vicinity of purine receptors, each specific for adenine, guanine, or guanosine (3, 11, 22, 25). Pharmacologically, this nucleobase/nucleoside transporter is potentially important in the transport of nucleobase and nucleoside analog drugs, such as 6-mercaptopurine, thioguanine, 5-fluorouracil, 2-chloro-2′-deoxyadenosine (cladribine), and acycloguanosine (acyclovir). The molecular identity of this nucleoside/nucleobase transporter remains to be determined.

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**REFERENCES**


