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A low-affinity nucleobase transporter in the protozoan parasite *Giardia intestinalis*

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A membrane transporter with general affinity for purine and pyrimidine bases has been identified in *Giardia intestinalis* trophozoites by measuring cellular influx of [^{3}H]adenine, [^{3}H]guanine and [^{3}H]thymine at 0°C. The base transporter is distinct from the thymine/uracil-specific (type 1) and broad-specificity (type 2) nucleoside transporters of *G. intestinalis*. Influx of each labelled base was retarded by unlabelled bases, with inhibition in the order: hypoxanthine > adenine > thymine > uracil. The IC_{50} values for these bases (measured for [^{3}H]adenine influx) were 0.46 mM, 1.15 mM, 1.52 mM and 2.28 mM, respectively. Nucleosides did not inhibit base influx ($\leq 15\%$ inhibition at 2 mM, a 400-fold molar excess, at which concentration [^{3}H]nucleoside influx was inhibited by > 95%). The Michaelis-Menten constant (K_m), calculated for adenine and thymine influx at 0°C, was 1.44 ± 0.08 mM and 1.61 ± 0.37 mM, respectively, with corresponding V_{max} of 383 ± 16 and 498 ± 112 pmol min^{-1} (10^6 cells)$^{-1}$. The data demonstrate the existence of a low-affinity, facilitative base transporter with no detectable affinity for nucleosides. The inability of uridine or thymidine to significantly reduce the rate of thymine influx indicates that the previously described thymine/uracil-specific (type 1) thymidine transporter cannot transport thymine, despite its affinity for the base.

Introduction

Trophozoites of the gastrointestinal parasite *Giardia intestinalis* (syn. *G. lamblia*) take up nucleosides through two distinct membrane permeases, referred to herein as type 1 and type 2 [1,2]. Type 1 transporters, detected by measuring [^{3}H]thymidine flux [1], exhibit high affinity at 0°C for uracil and thymine (K_i = 30–45 μM), thymidine (K_m = 50 μM), uridine and 2′-deoxyuridine (K_i range = 64–96 μM). Cytosine, adenine and guanine, as well as their corresponding nucleosides, do not inhibit this carrier. Type 2 transporters, identified using [^{3}H]deoxycytidine, [^{3}H]adenosine and [^{3}H]guanosine, are of broad specificity. These transporters require the presence of the sugar moiety for recognition of ribonucleosides and 2′-deoxyribonucleosides (K_m range = 25–150 μM) and they discriminate only marginally between nucleosides containing different base moieties [2]. Both types of transporters were characterized by measuring cellular influx at 0°C. At ambient temperatures, the trophozoites adhere (by a Venturi effect) to the surfaces of tubes and pipettes, causing non-random sampling errors. The use of 0°C eliminates adherence and has the advantage of slowing transport, making it easier to measure initial uptake velocities. Intracellular metabolism of labelled nucleoside to non-permeable phosphorylated derivatives is also minimized at this temperature.

The existence of the two nucleoside transporters is consistent with the apparent reliance of *G. intestinalis* on exogenous ribo-and 2′-deoxyribonucleosides. It had been shown previously that axenically cultured trophozoites of two independent isolates (WB and Portland-1) lack the enzymes necessary for de novo synthesis of purines [3] and pyrimidines [4–6] and that ribonucleosides are not utilized directly. At physiological temperatures, intracellular catabolism of isotopically-labelled nucleosides by trophozoites is rapid and precedes the incorporation of uracil, adenine and guanine into the nucleotide pool by specific phosphoribosyltransferases [6–10]. Hypoxanthine is not utilized [3,8], although trophozoite lysates possess substantial inosine hydrolase activity (Ey, P.L. and Andrews, R.H., unpublished data).

The enzymic pathways involved in nucleotide metabolism in *G. intestinalis* are unusual in two respects. First, the organism is unable to interconvert adenine and guanine nucleotides [3,9,10], thus requiring a source of both purines. Secondly, the cells appar-

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ently lack ribonucleotide reductase [11], the enzyme responsible in all eukaryotic organisms (the only other known exception being *Trichomonas vaginalis*; Ref. 12) for the conversion of ribonucleoside diphosphates to 2′-deoxyribonucleotides. Thus, it appears likely that the parasite has an absolute dependence on exogenous deoxynucleosides, which it can phosphorylate directly [6,10,11] for incorporation into DNA.

The broad-specificity (type 2) nucleoside transporters should facilitate the uptake by trophozoites of all nucleosides, including 2′-deoxynucleosides, although it is likely that at low (< 0.2 mM) concentration uridine, and especially thymidine, will enter the cells preferentially through type 1 transporters if the kinetics measured at 0°C for these substrates show equivalent specificity under physiological conditions at 37°C. It could be proposed, however, that the principal role of type 1 transporters may be to facilitate uracil salvage if pyrimidine nucleosides are scarce and to enable thymine, generated by phosphorolysis of thymidine [7], as well as excess uracil, to leave the cell. A similar situation may apply to the purine bases, i.e., a purine base transporter may be necessary to allow the efflux/influx of adenine and guanine, which are utilized via separate enzymic pathways in this organism [3,8–11]. To examine this possibility, we have studied the transport of labelled adenine, guanine and thymine into trophozoites.

Materials and Methods

Giardia cultures

Giardia intestinalis trophozoites (Adelaide-1 isolate) [13] were grown axenically as described [1]. Adherent cells were harvested and washed thoroughly in ice-cold modified phosphate-buffered saline (PBS$_m$: 2.68 mM KCl, 214 mM NaCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.2), resuspended at $1 \cdot 10^8$ cells ml^{-1} in ice-cold assay buffer (PBS$_m$ containing 20 mM D-glucose, 2 mM L-cysteine-HCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 15 mg ml^{-1} BSA) and were used within 1 h.

[^{3}H]-labelled base and nucleoside solutions

[2-^{3}H]Adenosine (TRK.423; 810 GBq mmol^{-1}) and [*methyl*-^{3}H]thymidine (TRK.300; 925 GBq mmol^{-1}) were obtained from Amersham. [2-^{3}H]Guanosine (32217–2; 130 GBq mmol^{-1}) was obtained from Sigma. Each was checked for purity by thin-layer chromatography on silica gel sheets (Kieselgel 60 F$_{254}$, Merck, Darmstadt) in butanol/acetic acid/water (5 : 1 : 3, v/v) or butanol/aqueous ammonia (15 M)/water (6 : 1 : 2, v/v). [^{3}H]Adenine, [^{3}H]guanine and [^{3}H]thymine were generated by enzymatic hydrolysis of the respective labelled nucleosides using a hypotonic lysate of *G. intestinalis* trophozoites. The latter are rich in uridine/thymidine phosphorylase [4,5,7] and adenosine/guanosine hydrolase [3] activity but lack adenosine deaminase and cannot interconvert guanine and adenine nucleotides [3]. To prepare the lysate, $2 \cdot 10^8$ trophozoites were vigorously suspended in 1.5 ml of ice-cold 12.5 mM potassium phosphate, 5 mM EDTA, 5 μg ml^{-1} bovine serum albumin (pH 7.4). After 10 min, the lysate was spun 2 min in a microcentrifuge, the supernatant was filtered (0.22 μm) and the filtrate was dialysed for 2.5 h at 4°C against 1.2 litre of 25 mM potassium phosphate, 5 mM EDTA (pH 7.4) (changed after 1 h). The dialysate was used immediately: 0.25 ml was mixed with 0.3 ml of labelled nucleoside and after reacting for 2.5 h at 25°C and 18 h at 4°C, each mixture was acidified with 30 μl 2 M $HClO_4$, neutralised with KOH, and the supernatants obtained after centrifugation were lyophilized. Each labelled base was purified (to $\geq 99\%$) by thin-layer chromatography and its identity confirmed. For each uptake experiment, fresh 2 × -concentrated stock solutions containing ^{3}H-labelled base or nucleoside, unlabelled homologous carrier (sufficient to bring the total concentration to 10 μM) and 5 mg ml^{-1} (1-^{14}C)-acetylated bovine serum albumin [1] were prepared in assay buffer. In inhibition studies, the concentration of unlabelled base or nucleoside was varied (for K_m estimations) or additional competitors were included (for IC$_{50}$ measurements). Inhibitor and carrier concentrations were confirmed spectrophotometrically.

Rapid sampling assay

The detailed protocol is described elsewhere [1,14]. All steps up to and including microcentrifugation were carried out in a 4°C coldroom, using ice-cold tubes and reagents. Briefly, trophozoites were mixed with freshly-prepared 2 × -concentrated (^{3}H/^{14}C) stock solution and left on ice. 10 s prior to the end of the uptake period (45 s, except where indicated otherwise), a 50 μl sample ($2.5 \cdot 10^6$ cells) was layered over a 230 μl oil phase and the cells were then sedimented by microcentrifugation (30 s) into a 50 μl aqueous underlay. The overlying medium was removed, the tubes were rinsed and excess oil was aspirated. The underlay was then frozen in solid CO_2/ethanol, residual oil was dissolved in mineral turpentine, aspirated, and the cells in the underlay were solubilised in 1% Triton X-100. Radioactivity (^{3}H and ^{14}C) was measured by liquid scintillation counting. Extracellular fluid contamination ('drag') was estimated from the [^{14}C]albumin content of each underlay.

Analysis of intracellular metabolites

Trophozoites were incubated at 0°C with labelled base or nucleoside (final concentration = 5 μM) for 45 s, then sedimented into a 1 M KOH underlay containing 1 mM unlabelled markers (e.g., adenine plus adenosine). After recovery, each underlay was neutralised with $HClO_4$ and the labelled metabolites and added standards were separated by thin-layer chromatography (see above). Phosphorylated derivatives remained at the origin. The radioactivity associated with each component was measured and compared with labelled standards (bases and nucleosides) that had been subjected to parallel treatment with KOH and $HClO_4$. Hydrolysis of nucleosides in these controls was insignificant ($\leq 2.5\%$).

Liquid scintillation counting and data analysis

Samples (0.1 ml) were mixed with 1.5 ml of Optiphase 'HiSafe' II scintillation fluid (LKB, FSA Laboratory Supplies, England) and counted in a Beckman LS-6000TA counter employing ^3H and ^{14}C channel separation and quench monitoring by 'H' number. Calculations utilized the measured ratio of ^3H to ^{14}C in each assay mixture to determine the extracellular ^3H content, which was then subtracted from the total cell-associated ^3H content.

Analysis of transport kinetics

(a) Inhibition of type 1 and type 2 nucleoside transporters. Selective and virtually complete inhibition of influx of 5 μM labelled probes through type 1 nucleoside transporters was attained with 1–2 mM uridine, thymidine, uracil or thymine [1]. Type 2 transporters were blocked with ribo- or 2′-deoxyribonucleosides at 1–2 mM [2].

(b) Calculation of relative inhibitor constants. The concentration (IC_{50}) of inhibitor that halved the rate of influx of 5 μM [^{3}H]adenine was determined by measuring influx at 0°C for 45 s over a range of inhibitor concentrations (as indicated). IC_{50} values were calculated from rectangular hyperbolas fitted by non-linear regression using Inplot (V3.1; GraphPAd Software Inc., San Diego, CA).

(c) Calculation of Michaelis-Menten constants. It was assumed that influx was a saturable process following Michaelis-Menten kinetics [15]. Influx was measured in duplicate or triplicate tubes over a 45 s period at 0°C to obtain near-initial velocities. Michaelis-Menten constants were calculated for adenine and thymine by fitting the data to rectangular hyperbolas by non-linear regression using Inplot.

Results

Time course of adenine uptake at 0°C

Adenine was taken up readily by trophozoites at 0°C, at a rate slightly faster than adenosine (Fig. 1), the time required to reach half the extrapolated equilibrium level being 1.13 ± 0.15 and 2.42 ± 0.38 min, respectively. These rates are considerably faster than can be attributed to passive diffusion across the plasma membrane [16]. Both sets of data fitted rectangular hyperbolas with calculated steady-state levels of approx. 2.2 pmol(10^6 cells)$^{-1}$, equivalent (assuming identical intra- and extracellular concentrations) to an available cell volume of about 0.45 pl per cell. Thymine, which is not metabolized by *G. intestinalis* trophozoites [4,5], exhibited similar uptake kinetics (data not shown). The lack of metabolism of thymine was confirmed by analysis of cell-associated label ($\geq 99\%$ base after 45 s uptake).

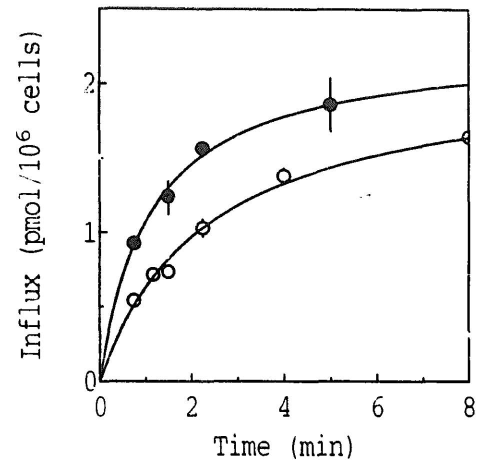

Fig. 1. Uptake of labelled adenine and adenosine. Cells were incubated at 0°C with 5 μM ^{3}H-labelled base or nucleoside for the indicated times and then sedimented without dilution through oil (triplicate samples). Curves were fitted by non-linear regression. ●, adenine (means from two experiments); ○, adenosine.

Analysis of intracellular metabolites

Chromatographic analysis of intracellular metabolites after 45 s uptake at 0°C with 5 μM [^{3}H]adenosine ($\geq 98\%$ pure) revealed that 63% had been hydrolyzed to adenine, with 34% remaining intact and $\leq 2.5\%$ present as phosphorylated derivatives (data not shown). This result was consistent with the known high level of adenosine hydrolase in *G. intestinalis* trophozoites [3]. Cells similarly incubated with [^{3}H]adenine for 45 s yielded $\geq 96\%$ of the label as free base (data not shown). At this time point, therefore, the uptake of labelled adenine represented influx only. Identical results were obtained with guanine ($\geq 98\%$ free base after 45 s uptake).

Taking into consideration the amount of label needed for accurate measurements and the need for estimates of initial influx rates, a 45 s fixed-time uptake period was chosen for subsequent kinetic experiments. At this time, influx was still essentially linear, with mean influx rates $\geq 85\%$ of initial rates calculated by extrapolation from timed measurements.

Specificity of adenine and adenosine influx

Preliminary experiments using [^{3}H]adenine and [^{3}H]adenosine indicated that distinct transporters were responsible for the uptake of each compound (Fig. 2).

Using 2 mM competitors, the influx of labelled adenine was reduced significantly ($\approx 50\%$) by unlabelled adenine, but not at all ($\leq 5\%$) by adenosine, guanosine, cytidine or deoxycytidine. In contrast, the influx of labelled adenosine was less sensitive to inhibition by adenine ($\approx 26\%$) but was strongly inhibited by all four nucleosides. Adenosine was the most effective inhibitor of adenosine influx (98% inhibition), but the other nucleosides were only slightly less active (85–96% inhibition). It was concluded that adenosine enters trophozoites only through the broad-specificity (type 2) nucleoside transporter and that adenine entry occurs through a different transporter that has no affinity for adenosine.

Unlabelled adenine, guanine, hypoxanthine, xanthine, uracil and uridine were each tested at various concentrations for their capacity to inhibit influx of 5 μM $[^3H]$adenine (Fig. 3). The best inhibitor was hypoxanthine ($IC_{50} = 0.46 \pm 0.03$ mM), with adenine ($IC_{50} = 1.15 \pm 0.07$ mM), thymine (1.52 ± 0.07 mM) and uracil (2.28 ± 0.24 mM) significantly less effective. Uridine exhibited only marginal inhibition ($\leq 10\%$ at 2 mM), consistent with the previously observed inactivity of adenosine and cytidine. No useful values were obtained for guanine and xanthine, due to their poor solubility. Cytosine was not tested. This base cannot be utilized by *G. intestinalis* trophozoites, and it is unlikely to be produced by catabolism since cytidine and deoxycytidine are rapidly deaminated to uridine and deoxyuridine [2,4–6].

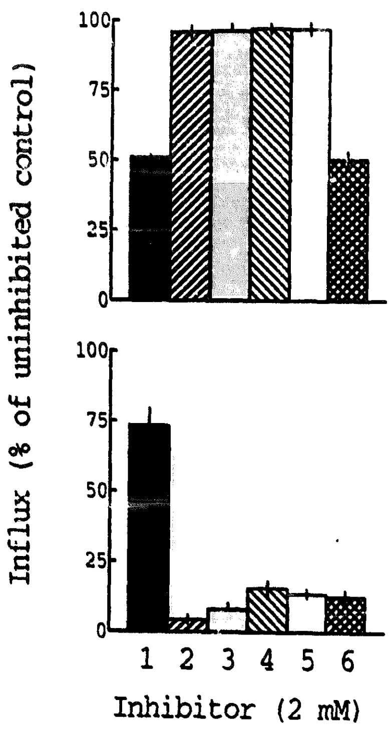

Fig. 2. Inhibition of $[^3H]$adenine (top) and $[^3H]$adenosine (bottom) influx by potential competitors. Influx was measured in duplicate over a 45 s period at 0°C, with 5 μM adenine or adenosine and 2 mM competitor. Error bars depict 2 S.E. 1, adenine; 2, adenosine; 3, guanosine; 4, cytidine; 5, deoxycytidine; 6, cytidine + adenine (each 2 mM).

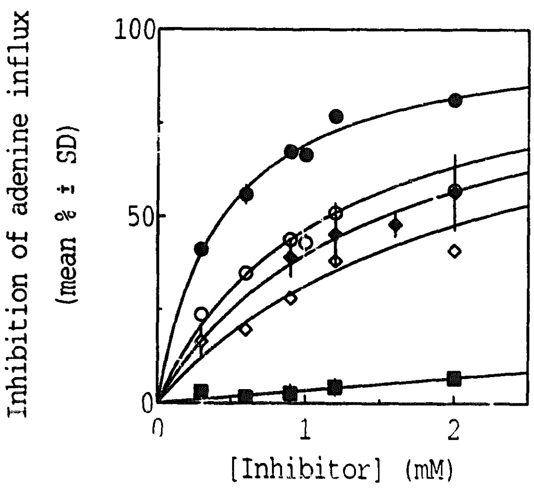

Fig. 3. IC_{50} curves depicting inhibition of $[^3H]$adenine influx by nucleobases and lack of inhibition by uridine. Uptake was for 45 s at 0°C, using 5 μM $[^3H]$adenine with additional competitor as indicated: ●, hypoxanthine; ○, adenine; ◆, thymine; ◇, uracil; ■, uridine.

Comparison of adenine, guanine and thymine influx

To determine whether a single permease was responsible for the influx of purine and pyrimidine bases by *G. intestinalis* trophozoites, the specificity of uptake of labelled adenine, guanine and thymine was examined by comparing the inhibitory activity of various base and nucleoside analogues, each tested at a final concentration of 2 mM. Table I lists the results of these experiments. The uptake of all three bases was affected maximally by hypoxanthine (range 67 to 82% inhibition). Adenine (49 to 62% inhibition), thymine (56 to 58%) and uracil (47 to 55%) were less effective competitors, although each still inhibited the influx of all three bases. Measurement of the IC_{50} for adenine against $[^3H]$guanine influx (data not shown) yielded a value of 1.17 ± 0.15 mM, identical to that derived using $[^3H]$adenine.

Nucleosides had little if any effect on base influx (inhibition range 2 to 18%). Thymine influx was only marginally inhibited (10–12%) by 2 mM uridine or thymidine (Table I). The effect of 2 mM uridine on thymine transport was tested repeatedly in a variety of experiments (some with quadruplicate control and test samples) and the inhibition was consistently within the 9–10% range.

Michaelis-Menten constants of the base transporter

Calculations were made from data obtained for the influx of $[^3H]$adenine and $[^3H]$thymine (Fig. 4). Both

TABLE I

Inhibition of base influx by unlabelled competitors

The influx of labelled adenine, guanine or thymine over 45 s at 0°C was measured in the presence or absence of various unlabelled competitors (2 mM). Uninhibited influx rates (pmol min^{-1} $(10^6\ \text{cells})^{-1}$) were 1.24 (adenine), 1.31 (guanine), 2.21 (thymine, unblocked) and 1.99 (thymine, blocked with 2 mM uridine)). The values represent pooled data from eight separate experiments.

Competitor (2 mM)	Inhibition of influx (mean % ± S.D.) for:			
	[^{3}H]adenine	[^{3}H]guanine	[^{3}H]thymine	
			no block [a]	uridine block [a]
None	0 ± 2.8	0 ± 1.8	0 ± 1.6	0 ± 1.5
Adenosine	4.8 ± 2.3	3.9 ± 2.1	1.8 ± 1.5	–
Inosine	5.4 ± 1.5	6.7 ± 1.0	–	–
Guanosine	10.6 ± 3.3	–	–	–
Cytidine	6.5 ± 0.3	7.0 ± 0.4	12.6 ± 6.7	–
Uridine	12.8 ± 5.1	9.4 ± 0.7	9.6 ± 1.4	–
Deoxycytidine	17.6 ± 12.3	1.7 ± 2.6	–	–
Thymidine	–	–	11.7 ± 2.8	–
Adenine	57.2 ± 5.9	62.3 ± 4.8	49.1 ± 2.8	52.1 ± 3.7
Hypoxanthine	81.8 ± 2.5	74.6 ± 9.8	66.7 ± 2.3	68.7 ± 2.4
Uracil	47.5 ± 6.6	54.9 ± 7.1	51.7 ± 1.5	46.6 ± 2.1
Thymine	56.0 ± 1.9	57.9 ± 2.0	57.9 ± 0.1	56.9 ± 0.9

[a] Thymine/uracil-specific thymidine transporter blocked ⩾ 96% by 2 mM uridine. Values in this column do not include the 10% inhibition by uridine evident in the unblocked column.

bases showed saturable uptake kinetics. K_m values, calculated from duplicate experiments for each base, were 1.44 ± 0.08 mM (adenine) and 1.61 ± 0.37 mM (thymine), with V_{max} = 0.38 ± 0.02 and 0.50 ± 0.11 nmol min^{-1} $(10^6\ \text{cells})^{-1}$, respectively (means ± S.E.). The K_m values are very similar to the IC_{50} values derived for adenine influx and are consistent with passage through a common carrier.

Discussion

The findings presented reveal that *G. intestinalis* trophozoites take up purine and pyrimidine bases via a previously undescribed membrane transporter. At 0°C, the initial rate of uptake of thymine, which the organism is unable to metabolize [4,5], represented influx. The kinetics and specificity of thymine influx were essentially identical to the uptake of adenine and guanine. Although the latter bases can be converted at ambient temperatures to AMP and GMP by phosphoribosyltransferases within *G. intestinalis*, it was shown for both that the cell-associated radioactivity recovered at the end of the chosen fixed-time uptake period (45 s) at 0°C was almost entirely (≥ 98%) unmetabolized free base. The uptake kinetics of adenine and guanine therefore also reflected influx only. The use of 0°C facilitated the measurement of influx by slowing transport to a rate that can be measured accurately (e.g., Ref. 17), as well as preventing adherence of trophozoites to surfaces [1].

The pattern of inhibition of influx by different bases and nucleosides indicates that a single base transporter is responsible for transporting adenine, guanine and thymine. In each case, hypoxanthine was the most effective competitor and uracil and thymine were the least effective of the bases. The degree of inhibition by a given compound was similar for each labelled base. The affinity of the transporter at 0°C is very low (K_m 1.5 to 2 mM), consistent with its apparent poor specificity in transporting both purines and pyrimidines. Base transport was unaffected by nucleosides, even at high concentration, indicating that attachment of the ribofuranosyl moiety abolishes recognition of the base and that the transporter does not mediate the passage

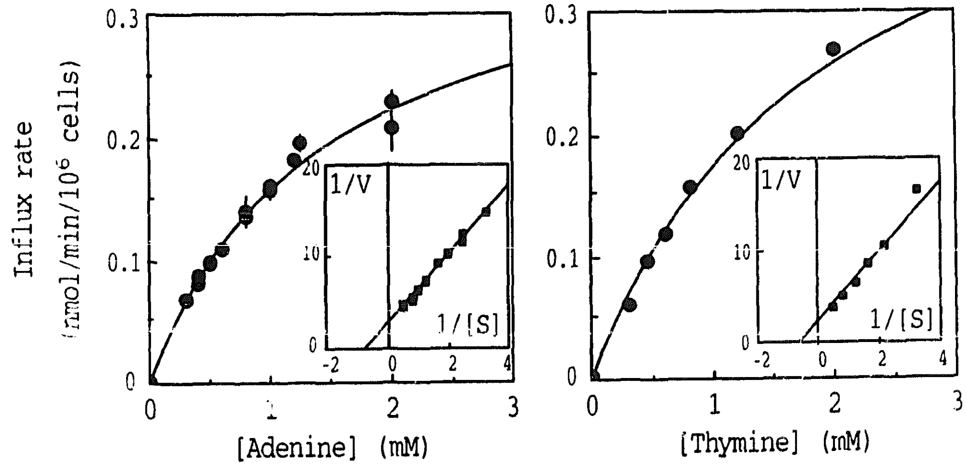

Fig. 4. Plots of initial influx velocity (nmol min^{-1} $(10^6\ \text{cells})^{-1}$) versus substrate concentration for adenine (left panel) and thymine (right panel). Thymine influx was measured in the presence of 0.2 mM uridine to exclude possible entry through the type 1 nucleoside transporter (see Fig. 5). Measurements were done in triplicate (two experiments for each base) and the data were fitted to rectangular hyperbolas by non-linear regression to derive K_m and V_{max} values. The insets depict double reciprocal (Lineweaver-Burk) plots of the same data.

of nucleosides. The existence of this common base transporter may account for the observation [3] that 0.4 mM unlabelled guanine could partially inhibit the incorporation at 37°C of 0.04 mM labelled adenine, despite the distinct enzymic pathways involved in the utilization of these two bases [3,8–11].

Because a thymine/uracil-specific transporter had been detected previously by measuring [^{3}H]thymidine flux at 0°C [1], efforts were made to determine whether or not this carrier could facilitate the influx of thymine as well as thymidine. If thymine was transported with an efficiency similar to that of thymidine and with a K_m similar to the measured K_i for thymine of 30 μM [1], one can calculate for an external concentration of 5 μM that the influx of thymine through the thymidine transporter would be about 10 pmol min^{-1}(10^6 cells)$^{-1}$. However, the observed influx rate was only about 2 pmol min^{-1}(10^6 cells)$^{-1}$ and virtually all of this was attributable to the base transporter. The minor inhibitory effect (10 ± 2% at 2 mM) of uridine (K_i for thymidine transport = 64 μM; Ref. 1) and thymidine (K_m = 50 μM) indicates that the thymidine transporter could have contributed no more than 10% of the gross thymine influx, i.e., $\leq$ 0.2 pmol min^{-1}(10^6 cells)$^{-1}$. It therefore appears that the thymidine transporter plays no major role in the influx of thymine, despite its high affinity for the base, and that thymine transport occurs predominantly, if not exclusively, through the base transporter.

The specificities of the nucleobase and nucleoside transporters described in this and other studies [1,2] are not compatible with the 'transport pathway(s)' postulated by Jarroll et al. [18] (reviewed in Refs. 19 and 20) on the basis of uptake data derived from experiments performed at 37°C. As discussed previously [1], the conditions and protocols used for our uptake measurements minimized metabolism so that the uptake kinetics reflected mainly influx. This was not the case in the earlier study [18], when uptake was examined at 37°C and the cells were subsequently washed at 0°C over a 10–20 min period. From our experience, nonphosphorylated bases and nucleosides efflux rapidly even at 0°C from trophozoites upon dilution or washing, leaving only phosphorylated metabolites to be counted. Similar rates of exchange have been described for the transmembrane equilibration of uridine in human erythrocytes [17]. The uptake kinetics of Jarroll et al. [18] would therefore have reflected purely enzymic metabolism and indeed, their inhibition and uptake data are entirely consistent with the known metabolic pathways of *G. intestinalis* [4,5].

The information available in the literature on nucleobase transporters is insufficient to make more than a superficial comparison with the systems involved in other organisms. Mammalian cells possess facilitative transporters for hypoxanthine, adenine and uracil (reviewed in Ref. 16). In some cells (Group I), hypoxanthine transport (K_m range at 25°C, 0.5–3 mM) can be inhibited with uridine, indicating that it may be mediated by a nucleoside carrier. In other (Group II) cells, hypoxanthine transport ($K_m \approx 0.4$ mM) is not inhibited by uridine. Guanine, as well as adenine, may be transported by the same carrier as hypoxanthine in both groups of cells [16]. Mosquito cells, in contrast, lack a hypoxanthine transporter but take up adenine efficiently [21]. Cytosine apparently is not transported by mammalian or mosquito cells, whereas uracil is transported by a simple carrier that exhibits a very high K_m (5–15 mM). Hypoxanthine is taken up actively in yeast [22], apparently by a permease that transports adenine, guanine and cytosine [23,24]. Permeases that transport more than one purine may also occur in fungi [25]. *Escherichia coli* possess several active transport systems for the uptake of purine bases and uracil [26], transport being linked intimately to nucleotide formation.

Two observations indicate that the *Giardia* base transporter is a facilitative diffusion carrier. Firstly, the influx of both thymine and adenine approaches a steady-state level that is equivalent (assuming identical intra- and extracellular concentrations at infinite time) to an available volume of about 0.45 pl per cell, a value similar to the physical dimensions of a trophozoite (Ref. 27: length 12–15 μm, width 5–9 μm; for a 2–3 μm thickness, volume = 0.3–0.4 pl per cell). Thus, bases are not transported against a concentration gradient. Secondly, the active transport of Ca^{2+} ions in *G. intestinalis* trophozoites virtually ceases at 0°C [28].

Our present view of the importance of the *G. intestinalis* nucleoside and nucleobase transporters is summarized in Fig. 5. The thymine/uracil-specific thymidine transporter (Site 1) constitutes an uptake system

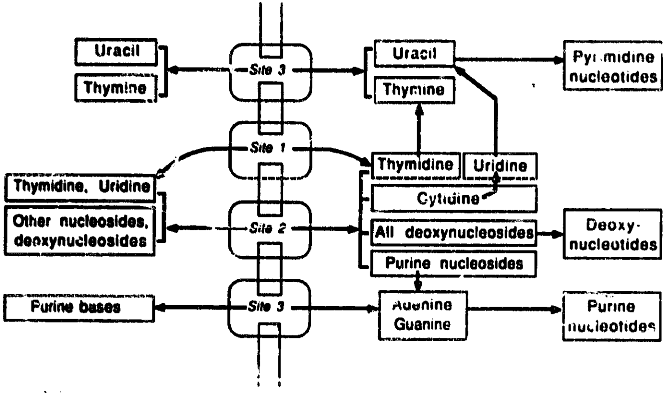

Fig. 5. Schematic representation of the thymine/uracil-specific (type 1) and broad-specificity (type 2) nucleoside transport pathways (sites 1 and 2), and the nucleobase (site 3) transport pathway of *G. intestinalis* trophozoites. The putative role of each carrier in the demonstrated influx and presumed efflux of nucleosides and nucleobases is indicated.

for thymidine, and probably also for uridine. Despite the high affinity of this carrier for thymine and uracil ($K_i = 30$ and 45 μM at 0°C, respectively, Ref. 1), the data indicate that it does not transport thymine. If the base and furanosyl moieties are both required by this transporter to effect transmembrane passage, then uracil also would not be transported. The broad-specificity transporter (Site 2) recognizes structural features on the β-furanosyl moiety of nucleosides and seems vital for the deoxycytidine, deoxyadenosine and deoxyguanosine requirements of the parasite, and to allow entry of ribonucleosides. The high level of cytidine deaminase observed in trophozoites [5] may be required if in vivo the cells derive most of their uracil by catabolism of cytidine and deoxycytidine [4–7].

The precise role of the base transporter (Fig. 5, Site 3) is less obvious. Clearly, this carrier constitutes a point of entry for adenine, guanine and uracil, although the availablility of these bases (as compared with nucleosides) within the gastrointestinal tract is not well documented and may vary with intestinal flora. However, a more important function may be to mediate the cellular efflux of thymine and excess adenine, guanine and uracil which are produced by nucleoside catabolism. Without such an escape route, it is very likely that these bases would accumulate intracellularly, perhaps to toxic levels. This is especially true for thymine, which is produced from thymidine by uridine/thymidine phosphorylase [7] but cannot be utilized by *G. intestinalis* [4,5]. If the transporter has a low affinity at 37°C similar to that measured at 0°C, it may operate efficiently only at high (millimolar) concentrations of base, consistent with an efflux role. For example, using the Michaelis-Menten constants determined for the influx of adenine ($K_m = 1.44$ mM; $V_{max} = 383$ pmol min^{-1} (10^6 cells)$^{-1}$) and adenosine ($K_m = 0.045$ mM; $V_{max} = 11$ pmol min^{-1} (10^6 cells)$^{-1}$, Ref. 2), one can calculate that the relative rates of transport (base/nucleoside) across the trophozoite membrane at 0°C would be approx. 1 : 1 at 0.01 mM, 4 : 1 at 0.1 mM, 19 : 1 at 1 mM, and 26 : 1 at 2 mM, assuming directional symmetry and similar mobility of the carrier when empty and loaded. Similar ratios are obtained for thymine and thymidine. Thus, if the relative transport kinetics of these substrates are similar at 37°C, base efflux may not (depending on phosphoribosyltransferase activity) exceed the rate of nucleoside influx and hydrolysis at low nucleoside concentrations (e.g., ≤ 0.1 mM). Under conditions of unlimited nucleoside availability, however, the intracellular concentrations of free bases would increase to a steady-state level with a net outflow from the cell.

The applicability of the calculations to transport at 37°C depends on whether or not the temperature dependence of transporter affinity and mobility is similar for each of the substrates considered. Although the Michaelis-Menten constants for zero-trans influx of uridine and thymidine in mammalian erythrocytes were found to increase 5–20-fold over the range 5–37°C [17,29], it is not clear if the transport kinetics of other substrates vary similarly. A detailed analysis of transporter affinity and mobility under various transport situations [16] at different temperatures will be required to fully characterize the kinetic properties of the nucleoside and nucleobase transporters of *Giardia* trophozoites and to determine how these properties vary with temperature. Such studies, based on the comprehensive kinetic analyses of nucleoside transport in erythrocytes [16,17,29], are a logical extension of our work which has unambiguously demonstrated the existence and substrate specificity of these transporters in this ubiquitous protozoan parasite. Given the apparently essential nature of these transporters in *Giardia*, it seems very likely that their expression is constitutive. Their identification and further characterization should facilitate efforts to develop toxic nucleobase or nucleoside analogues that are selective for this parasite.

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